Genetic Variation in the Growth Hormone Synthesis Pathway in Relation to Circulating Insulin-Like Growth Factor-I, Insulin-Like Growth Factor Binding Protein-3, and Breast Cancer Risk: Results from the European Prospective Investigation into Cancer and Nutrition Study

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Abstract

Insulin-like growth factor-I (IGF-I) stimulates cell proliferation and can enhance the development of tumors in different organs. Epidemiologic studies have shown that an elevated level of circulating IGF-I is associated to increased risk of breast cancer as well as other cancers. Genetic variants affecting the release or biological action of growth hormone (GH), the main stimulator of IGF-I production, may predict circulating levels of IGF-I and have an effect on cancer risk. We tested this hypothesis with a large case-control study of 807 breast cancer patients and 1,588 matched control subjects nested within the European Prospective Investigation into Cancer and Nutrition. We genotyped 22 common single nucleotide polymorphisms in 10 genes involved in GH production and action (*GHRH, GHRHR, SST, SSTR1-SSTR5, POU1F1*, and *GH1*), and in parallel, we measured serum levels of IGF-I and IGFBP-3, its major binding protein, in samples of cases and controls. *SST* and *SSTR2* polymorphisms showed weak but statistically significant associations with breast cancer risk. *SSTR5* polymorphisms were associated with IGF-I levels, whereas one polymorphism in *GHRHR* and one in *POU1F1* were associated with IGFBP-3 levels. Our conclusion is that common genetic variation in the GH synthesis pathway, as measured by single nucleotide polymorphisms selected in the present study, is not a major determinant of IGF-I and IGFBP-3 circulating levels, and it does not play a major role in altering breast cancer risk. (Cancer Epidemiol Biomarkers Prev 2005;14(10):2316–25)

Introduction

There is substantial evidence that insulin-like growth factor-I (IGF-I) can enhance the development of tumors in different organs. Studies *in vitro* have shown that IGF-I inhibits apoptosis and stimulates cell proliferation in a variety of cell types (1). Furthermore, tumor development can be strongly enhanced in animals or organs that have been genetically or

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otherwise manipulated to either overexpress IGF-I or the IGF-I receptor, whereas animals made deficient in IGF-I are protected (2). In humans, several epidemiologic studies have shown increased risks of cancers of the breast (3-7), colon (8-11), prostate (12-15), and possibly other organs (16) among women and men who have comparatively elevated blood levels of IGF-I, measured either as absolute concentrations or relative to its principal plasma binding protein, IGFBP-3. The association of circulating IGF-I levels with breast cancer risk thus far has been particularly apparent among women of premenopausal or early menopausal age (3-5).

Nutrition, especially the availability of energy and amino acids, is a key determinant of circulating IGF-I levels (17, 18). Besides nutrition, however, heritability studies have shown that in Western populations a large part (40-60%) of variation in IGF-I is determined by genetic factors (19-21). Although current research to identify genetic determinants of circulating IGF-I and IGFBP-3 is intensifying (22-25), thus far, few studies have been conducted to search comprehensively for polymorphisms in genes directly or indirectly involved in regulating IGF-I synthesis and to correlate these with intersubject variations in IGF-I and IGFBP-3 levels or cancer risk.

The main endocrine stimulus of hepatic and tissue production of IGF-I and IGFBP-3 is growth hormone (GH). Therefore, examination of genetic variants, which could affect the pituitary release or biological action of GH, may be one way of predicting circulating levels of IGF-I (22). In addition to the gene encoding human GH itself (*GH1*), major candidate genes to be examined are those involved in controlling the pituitary synthesis and release of GH. The latter include GH releasing hormone (*GHRH*) and its receptor (*GHRHR*) as well as somatostatin (*SST*) and its receptors (*SSTR1-SSTR5*), which enhance or inhibit the synthesis and release of GH, respectively. A pituitary-specific transcription factor, called POU domain class 1 transcription factor 1 (*POU1F1*), is also centrally involved in regulating GH synthesis.

For each of these genes, polymorphisms that change gene expression or protein function might result in a relative increase or decrease in circulating IGF-I or IGFBP-3 levels. In several of these genes, rare genetic mutations have been identified that result in radically altered hormone levels and in growth-related diseases, such as acromegaly or familial dwarfism (26-29). However, only a few studies have shown associations between more common polymorphisms and variation of IGF-I levels compatible with normal physiology (23, 25).

To examine whether common genetic variants of *GHRH, GHRHR, SST, SSTR1-SSTR5, POU1F1*, and *GH1* were associated with variations in circulating IGF-I and IGFBP-3 levels and possibly also with breast cancer risk, we conducted a large case-control study of 807 breast cancer patients and 1,588 matched control subjects nested within the cohorts of the European Prospective Investigation into Cancer and Nutrition (EPIC; refs. 30, 31). For the present study, an attempt was made to include all known, common polymorphisms that have the highest chance of having an effect on gene expression or function of the gene product.

Materials and Methods

The EPIC Study. The EPIC cohort consists of ~370,000 women and 150,000 men, ages 35 to 69 years, recruited between 1992 and 1998 in 23 research centers in 10 Western European countries. The vast majority (>97%) of subjects recruited in the EPIC cohort are of European (Caucasian) origin. EPIC study subjects provided anthropometric measurements (height, weight, waist, and hip circumferences) and extensive, standardized questionnaire information about medical history, diet, physical activity, smoking, and other lifestyle factors. Women also answered questions about menstrual and reproductive history, hysterectomy, ovariectomy, and use of exogenous hormones for contraception or treatment of menopausal symptoms. In addition, ~240,000 women and 140,000 men provided a blood sample, and plasma, serum, red cells, and a buffy coat were stored for future analyses on cancer cases and controls. Cohort members are contacted periodically to renew information on smoking, alcohol drinking, physical activity, weight, menstrual history, pregnancies, use of medications and exogenous hormones, hysterectomy, and first development of major diseases other than cancer (e.g., diabetes).

Cases of cancer occurring after recruitment into the cohort are identified through local and national cancer registries in 7 of the 10 countries and in France, Germany, and Greece by a combination of contacts with national health insurances and/or active follow-up through the study subjects or their next of kin. Follow-up on vital status, to monitor the population remaining at risk for cancer, is achieved through record linkage with mortality registries. In all EPIC study centers, cancer diagnosis is confirmed through comprehensive review of pathology reports, and checks for completeness of follow-up are made periodically. A fully detailed description of the EPIC study has been published elsewhere (30, 31).

Selection of Case and Control Subjects. Cases and controls from the present study were from 16 of the 23 EPIC recruitment centers in 7 of the 10 countries (United Kingdom, Germany, the Netherlands, France, Spain, Italy, and Greece), and most were also part of nested case-control studies on serum hormones and breast cancer risk reported in detail elsewhere[22,23] (32).

Case subjects were selected among women who developed breast cancer after their recruitment into the EPIC study, and before the end of the study period, for each study center defined by the latest end-date of follow-up. Women who used any hormone replacement therapy at the time of blood donation or any exogenous hormones for contraception or medical purposes and who had previous diagnosis of cancer (except nonmelanoma skin cancer) were excluded from the study, because each of these various factors could have altered circulating hormone levels.

For each case subject with breast cancer, two control subjects were chosen at random from among cohort members alive and free of cancer (except nonmelanoma skin cancer) at the time of diagnosis of the index case. Control subjects were matched to the cases by study center where the subjects were enrolled in the cohort as well as by menopausal status (premenopausal, postmenopausal, or perimenopausal/undefined), age (±6 months) at enrollment, follow-up time, fasting status, time of the day of blood donation, and phase of the menstrual cycle for premenopausal women[22] (32).

Approval for the study was given by the relevant ethical committees both at the IARC and in the EPIC recruitment centers.

Identification and Selection of Single Nucleotide Polymorphisms. We collected data on polymorphisms from publicly available databases, such as dbSNP (http://www.ncbi.nlm.nih.gov/SNP/), SNPper (http://snpper.chip.org/), and Frequency Finder (http://bluegenes.bsd.uchicago.edu/frequencyfinder/). We complemented database searches with literature review and for some genes (SST, SSTR1-SSTR5, GHRH, GHRHR, and *POU1F1*) with analysis of 95 subjects from the EPIC population by denaturing high-performance liquid chromatography (DHPLC; ref. 33).

To be included in the study, polymorphisms had to be located in exons (including untranslated regions), exon-intron junctions, or promoter regions of a gene of interest or otherwise

[22] R. Kaaks et al. Postmenopausal serum androgens, oestrogens and breast cancer risk: the European Prospective Investigation into Cancer and Nutrition (EPIC), in press.
[23] Rinaldi et al., in preparation.

should be within intronic regions that showed >80% homology between human and mouse (as reported by the University of California at Santa Cruz Genome Browser, http://genome.ucsc.edu/) and thus were likely to harbor regulatory sequences. In addition, we also included polymorphisms with documented evidence of their existence in Caucasians according either to literature data or to our own experimental analysis by DHPLC. Among all polymorphisms thus identified, we only retained those with a minor allele frequency ≥5% in Caucasians or those that result in an amino acid change and had a minor allele frequency ≥1%. Finally, we particularly favored the inclusion of all polymorphisms reported previously to be associated with cancer and/or levels of circulating hormones.

Collecting information on polymorphisms from the literature, public databases, and our own experimental analyses by DHPLC provided a list of 74 single nucleotide polymorphisms (SNP). All new SNPs identified in our laboratory by DHPLC searches have been deposited in dbSNP (http://www.ncbi.nlm.nih.gov/SNP/). By applying the selection criteria outlined above, we selected 32 SNPs for genotyping. For 2 SNPs, genotyping assays could not be designed (i.e., specialized algorithms were unable to find suitable PCR primers and/or TaqMan probes), and for 8 more SNPs, TaqMan assays were generated but provided poor genotyping results (i.e., insufficient amplification and/or insufficient separation of genotype clusters). This left 22 polymorphisms that were genotyped on the DNAs of cases and controls (Table 1). The number of SNPs typed per gene ranged from one for the small somatostatin receptors *SSTR1, SSTR3*, and *SSTR4* to five for *GHRHR*.

Genotyping. Buffy coat samples for the study subjects were retrieved from the EPIC biorepository and DNAs were extracted on an Autopure instrument (Gentra Systems, Minneapolis, MN) with Puregene chemistry (Gentra Systems).

Genotyping was done by the 5′ nuclease assay (TaqMan). The order of DNAs from cases and controls was randomized on PCR plates to assure that an equal number of cases and controls could be analyzed simultaneously. TaqMan probes were synthesized by either Applied Biosystems [with minor groove binder (MGB) chemistry] or Proligo (Paris, France) [with or without locked nucleic acid (LNA) chemistry]. Sequences of primers and probes are reported in Appendix 1. The reaction mix included 10 ng genomic DNA, 5 pmol of each primer, 1 pmol of each probe, and 2.5 μL of 2× Master Mix (Applied Biosystems Foster City, CA) in a final volume of 5 μL. The thermocycling included 50 cycles with 30 seconds at 95°C followed by 60 seconds at 60°C. PCR plates were read on an ABI PRISM 7900HT instrument (Applied Biosystems). To validate genotype identification, we repeated 8% of all genotypes. Laboratory personnel was kept blinded to case-control status throughout the study.

Table 1. Polymorphisms used in the present study

Polymorphism*	Alleles (major>minor)	Position in gene	Codon
GHRH			
rs4988491	A>G	Downstream of 3′ untranslated region	—
GHRHR			
rs4988495	C>T	Intron 2	—
rs4988496	G>A	Exon 3	$Ala^{57}Thr$
rs4988501	T>C	Intron 5	—
rs4988503	C>T	Intron 10	—
rs4988505	C>G	Intron 12	—
GH1			
rs2005172	G>T	Promoter	—
rs6171	A>G	Promoter	—
rs6173	A>C	Exon 1, 5′ untranslated region	—
rs2665802	T>A	Intron 4	—
SST			
rs4988513	T>C	Intron 1	—
P0689†	T>C	Intron 1	—
SSTR1			
rs2228497	T>C	Exon 1	$Val^{293}Val$
SSTR2			
rs998571	A>G	Promoter	—
rs1466113	G>C	Promoter	—
SSTR3			
rs229569	G>A	Exon 2	$Ala^{264}Ala$
SSTR4			
rs3746726	T>G	Exon 1	$Phe^{284}Val$
SSTR5			
rs4988483	C>A	Exon1	$Leu^{48}Met$
rs4988487	C>T	Exon 1	$Pro^{109}Ser$
rs169068	T>C	Exon 1	$Leu^{335}Pro$
rs642249	G>A	Exon 1	$Pro^{348}Pro$
POU1F1			
rs300982	C>T	Promoter	—

*Polymorphisms are identified by their dbSNP accession no. dbSNP is accessible at http://www.ncbi.nlm.nih.gov/SNP/.
†P0689 is an internal reference. This polymorphism is not represented in dbSNP (46).

Hormone Measurements. Measurements of IGF-I and IGFBP-3 were done in the laboratory of the Hormones and Cancer Team at IARC using ELISAs from Diagnostic System Laboratories (Webster, TX). The IGF-I assays included an acid-ethanol precipitation step to eliminate IGF-I binding proteins to avoid their interference with the IGF-I measurement. Measurements were done on never-thawed serum sample aliquots. The mean intrabatch and interbatch coefficients of variation were 6.2% and 16.2%, respectively, for IGF-I and 7.2% and 9.7%, respectively, for IGFBP-3.

Statistical Analysis. We reconstructed individuals' haplotype frequencies (i.e., estimated numbers of copies of haplotypes) using the program ''tagSNPs'' (http://www-rcf.usc.edu/~stram/tagSNPs.html; refs. 34, 35). This program calculates, for each individual, the expected numbers of copies (''dosages'') of each of the haplotypes compatible with the individual's SNP genotypes. This method takes into account uncertainties in the haplotype reconstruction for individuals that are heterozygote for two or more of the SNPs within a given gene. Haplotype dosages are estimated from the individuals' SNP genotype data and from overall haplotype frequency estimates for the full study population (cases and controls combined) estimated by a maximum likelihood method. For each haplotype, the dosage values range from 0 to 2.0 (alleles), and for each individual, these dosage values add up to a total value of 2.0 across all possible haplotypes.

All association analyses, at the level of individual SNPs or gene loci, were done under different assumed modes of inheritance of effect (dominant, recessive, or codominant) associated with alleles. In the ''dominant'' model, circulating peptide levels or disease risks were compared between subjects carrying at least one copy of the rare allele and those who had none; in the ''recessive'' model, the comparison was between those who were homozygous for the rare allele and all others; in the ''codominant'' model, individuals' peptide levels or the logarithm of disease risk were linearly related to the number of copies of an allele (0, 1, or 2 for SNP alleles or dosages for the haplotype) carried by the individuals. For rare alleles, with a frequency <20% (i.e., a prevalence of homozygous recessive allele carriers <4.0%), only the dominant model was used.

Relationships of polymorphic gene variants with serum levels of IGF-I and IGFBP-3 were estimated by standard regression models, stratified by EPIC recruitment center, and further adjusted for age. These analyses were done both using all the study subjects and only the controls, who represent the population giving rise to the cases. Relationships of polymorphic variants with breast cancer risk [odds ratios (OR)] were estimated using conditional logistic regression models

applied on the matched case-control sets. Both series of analyses were done at the level of single SNP loci as well as at the level of haplotypes (using the haplotype dosage values). Haplotype analyses were done at the level of full gene loci (i.e., including haplotypes based on all of the SNPs in that gene). In all haplotype analyses, the most common haplotype was used as the reference category.

Subgroup analyses on women with a breast cancer diagnosis either before (45% of the subjects) or after age 55 years were done, and possible heterogeneity of effect between these two age groups was tested using a χ^2 test.

Results

Eight hundred seven incident cases of breast cancer from the EPIC cohort and 1,588 matched controls were included in our study. Table 2 summarizes the baseline characteristics of cases and controls. The mean age of study subjects was 55 years. For cases, the mean age at diagnosis was 57 years. Based on the questionnaire data, 32% of the subjects were premenopausal at blood donation, 10% were perimenopausal or of unknown menopausal status, and 58% were postmenopausal. Cases had a significantly lower number of full-term pregnancies than controls (mean, 2.35 versus 2.47; $P = 0.02$) and were significantly older at first full-term pregnancy (26 versus 25.5 years in controls; $P = 0.02$). Age at menarche did not differ between cases and controls, nor did body mass index or height. Serum levels of IGF-I adjusted for age and center were not significantly different between cases and controls (mean, 248.7 versus 244.4 ng/mL; $P = 0.15$), nor for the subgroups of subjects with cancer diagnosis before or at age 55 years (272.0 versus 270.7; $P = 0.79$) or after age 55 years (224.0 versus 217.5; $P = 0.08$). Case subjects did show higher mean levels of serum IGFBP-3 than controls (mean, 3,422 versus 3,361 ng/mL; $P = 0.04$). The latter difference, however, was due mostly to the subgroup with cancer diagnosis after age 55 years (3,473 versus 3,378; $P = 0.02$) and was not clearly visible among the younger women (3,190 versus 3,173; $P = 0.66$). IGF-I and IGFBP-3 levels were significantly correlated (Pearson partial correlation coefficient on log-transformed data = 0.43; $P < 0.0001$). Details on the relationships of IGF-I and IGFBP-3 with breast cancer risk, with an extended series of 1,081 breast cancer cases and 2,098 control subjects, are reported elsewhere.[23]

Genotyping call rates ranged between 95.36% and 99.53%. The distributions of genotypes of all polymorphisms were in agreement with Hardy-Weinberg equilibrium (calculated in the control group). Repeated quality control genotypes (8% of the total) showed >99% concordance for all assays.

Results of associations between individual SNPs and cancer risk and circulating hormone levels are reported in Table 3. Table 4A to E reports results of analyses of haplotypes of genes for which two or more polymorphisms have been typed.

Serum concentrations of IGF-I showed a nominally significant association only with one SNP (rs642249; $P = 0.002$) in *SSTR5* but not with any polymorphisms in the other genes studied. Haplotype analysis did not add anything to this finding, as there was a significant association with the only *SSTR5* haplotype (hCCCA), which includes the A allele of rs642249. When we stratified the data by age at diagnosis (before or after age 55 years), the association was observed only in women ages <55 years, but the heterogeneity test was not significant (data not shown). Significant associations with reduced levels of IGF-I were also observed for two haplotypes of *GH1* (i.e., hTAAT and hGGAT), although no single SNP of this gene was associated with IGF-I level. We observed the same associations when we restricted analysis to the controls.

Serum IGFBP-3 concentrations were significantly increased among carriers of the rs300982 C allele in *POU1F1* ($P = 0.01$ and 0.01 for codominant and dominant models, respectively) both when we analyzed all the study subjects together and when we analyzed only the controls. A stratified analysis showed that the association was present only in the older women, although a test for heterogeneity was not significant.

In addition, analyses restricted to the controls identified also a weak ($P = 0.04$ for the dominant model) association with SNP rs2228487 of *SSTR1*.

Breast cancer risk showed statistically significant associations ($P < 0.05$) with polymorphic variants in the *SST, SSTR2*, and *GH1* genes.

For the *SST* gene, carriers of two different SNPs showed an increase in breast cancer risk, with relative risks of ~1.3 for both rs4988513 C and P0689 C alleles. Reflecting these two associations, the hCC haplotype of *SST* also showed an effect on risk [OR, 1.27; 95% CI (95% CI),1.02-1.59 for the dominant model]. In analyses stratified by age at diagnosis, a statistically significant increase in risk was observed only in the higher age group (OR, 1.53; 95% CI, 1.17-2.01 for carriers of the rs4988513 C allele; OR, 1.41; 95% CI, 1.07-1.88 for carriers of the P0689 C allele) but not among women with a breast cancer diagnosis at age <55 years. However, interaction tests showed no statistically significant heterogeneity of effect between the two age groups.

For the *SSTR2* gene, breast cancer risk was decreased among homozygous carriers of the C allele of SNP rs1466113 (OR, 0.74; 95% CI, 0.57-0.96). This reduction in risk was mirrored by an increased risk associated with the haplotypes bearing the other allele (hGG: OR, 1.24; 95% CI, 1.03-1.51; hAG: OR, 1.19; 95% CI, 0.99-1.45). Heterozygosity at the other *SSTR2* polymorphism we typed (rs998571) was associated with a nearly significant increase in breast cancer risk (OR, 1.20; 95% CI, 0.99-1.45).

For the *GH1* gene, only subjects who were heterozygous for the rs6171 allele showed an association with reduced cancer risk (OR, 0.77; 95% CI, 0.63-0.94), which was compatible, however, with a dominant effect of the G allele toward a reduction in risk ($P = 0.03$).

Table 2. Baseline characteristics of breast cancer cases and control subjects

Variable	Cases	Controls	P_{diff}
n	807	1,588	
Premenopausal women, *n*	260	510	
Perimenopausal women, *n*	79	151	
Postmenopausal women, *n*	468	927	
Age at blood donation (y), mean (5th-95th percentiles)	55.0 (40.0-68.7)	55.0 (39.9-68.7)	0.26
Age at diagnosis (y), mean (5th-95th percentiles)	57.0 (42.0-71.0)	—	—
Age at first full-term pregnancy (y), mean (5th-95th percentiles)	26.0 (20.0-34.0)	25.5 (20.0-33.0)	0.02
Age at menarche (y), mean (5th-95th percentiles)	13.0 (11.0-16.0)	13.1 (11.0-16.0)	0.11
No. full-term pregnancies (y), mean (5th-95th percentiles)	2.35 (1.00-4.00)	2.47 (1.00-5.00)	0.02
Height, mean (5th-95th percentiles)	160.9 (150.9-172.0)	160.6 (149.5-172.0)	0.22
Body mass index, mean (5th-95th percentiles)	26.3 (20.3-34.6)	26.3 (20.2-35.0)	0.88

Table 3. Associations between SNPs and breast cancer risk and mean IGF-I and IGFBP-3 levels adjusted for age and center

Gene	SNP		Genotype			$P_{codominant}$*	$P_{dominant}$†	$P_{recessive}$‡
			Homozygous major	Heterozygous	Homozygous minor			
GH1	rs2005172	Cases/controls	333/657	352/682	96/186			
		OR (95% CI)	1.00	1.02 (0.85-1.23)	1.01 (0.76-1.34)	0.88	0.84	1.00
		Mean IGF-I§	247.5	244.3	240.6	0.11	0.16	0.24
		Mean IGFBP-3§	3,385	3,357	3,372	0.54	0.39	0.98
	rs6171	Cases/controls	268/450	333/722	156/286			
		OR (95% CI)	1.00	0.77 (0.63-0.94)	0.90 (0.70-1.16)	0.22	0.03	0.20
		Mean IGF-I§	242.6	247.6	247.3	0.20	0.12	0.63
		Mean IGFBP-3§	3,355	3,379	3,396	0.30	0.34	0.45
	rs6173	Cases/controls	787/1,540	11/25	0/0			
		OR (95% CI)	1.00	0.86 (0.42-1.76)	—	0.68	0.68	—
		Mean IGF-I§	250.1	261.4	—	0.33	0.33	—
		Mean IGFBP-3§	3,404	3,633	—	0.05	0.05	—
	rs2665802	Cases/controls	276/495	350/728	148/278			
		OR (95% CI)	1.00	0.86 (0.70-1.04)	0.95 (0.74-1.22)	0.48	0.18	0.12
		Mean IGF-I§	244.0	245.1	248.8	0.28	0.49	0.26
		Mean IGFBP-3§	3,345	3,361	3,399	0.21	0.38	0.22
POU1F1	rs300982	Cases/controls	715/1,416	61/118	3/2			
		OR (95% CI)	1.00	1.04 (0.75-1.45)	3.01 (0.50-18.01)	0.53	0.66	0.23
		Mean IGF-I§	254.4	242.5	291.8	0.09	0.05	0.22
		Mean IGFBP-3§	3,248	3,125	2,841	0.01	0.01	0.19
GHRH	rs4988491	Cases/controls	745/1,468	35/60	0/0			
		OR (95% CI)	1.00	1.16 (0.75-1.79)	—	0.50	0.50	—
		Mean IGF-I§	244.9	250.2	—	0.47	0.47	—
		Mean IGFBP-3§	3,369	3,382	—	0.86	0.86	—
GHRHR	rs4988495	Cases/controls	719/1,383	76/66	1/4			
		OR (95% CI)	1.00	0.87 (0.65-1.16)	0.42 (0.05-3.77)	0.27	0.31	0.45
		Mean IGF-I§	249.9	252.4	270.9	0.49	0.55	0.50
		Mean IGFBP-3§	3,403	3,424	3,770	0.45	0.55	0.24
	rs4988496	Cases/controls	719/1,401	86/175	1/7			
		OR (95% CI)	1.00	0.95 (0.72-1.24)	0.26 (0.03-2.09)	0.41	0.54	0.21
		Mean IGF-I§	246.0	247.6	249.7	0.70	0.71	0.88
		Mean IGFBP-3§	3,386	3,385	3,554	0.85	0.94	0.49
	rs4988501	Cases/controls	434/824	305/612	47/111			
		OR (95% CI)	1.00	0.94 (0.79-1.13)	0.81 (0.57-1.15)	0.24	0.36	0.29
		Mean IGF-I§	245.9	244.8	240.9	0.42	0.55	0.43
		Mean IGFBP-3§	3,370	3,358	3,466	0.42	0.92	0.07
	rs4988503	Cases/controls	612/1,226	157/292	10/13			
		OR (95% CI)	1.00	1.08 (0.87-1.34)	1.56 (0.68-3.56)	0.29	0.38	0.31
		Mean IGF-I§	246.0	243.0	226.2	0.19	0.29	0.18
		Mean IGFBP-3§	3,369	3,383	3,240	0.98	0.84	0.35
	rs4988505	Cases/controls	393/724	325/656	61/152			
		OR (95% CI)	1.00	0.91 (0.76-1.09)	0.75 (0.54-1.03)	0.07	0.14	0.11
		Mean IGF-I§	245.8	245.4	242.7	0.63	0.76	0.57
		Mean IGFBP-3§	3,363	3,361	3,455	0.21	0.60	0.06
SST	rs4988513	Cases/controls	584/1,196	181/282	11/24			
		OR (95% CI)	1.00	1.31 (1.06-1.61)	0.95 (0.46-1.94)	0.04	0.02	0.72
		Mean IGF-I§	2.54	251.0	252.0	0.37	0.34	0.89
		Mean IGFBP-3§	3,233	3,279	3,167	0.42	0.27	0.51
	P0689	Cases/controls	609/1,236	158/252	9/14			
		OR (95% CI)	1.00	1.27 (1.02-1.59)	1.28 (0.55-2.97)	0.04	0.03	0.66
		Mean IGF-I§	254.5	249.3	263.6	0.36	0.24	0.49
		Mean IGFBP-3§	3,233	3,289	3,182	0.24	0.17	0.66
SSTR1	rs2228497	Cases/controls	348/661	317/632	81/140			
		OR (95% CI)	1.00	0.95 (0.78-1.14)	1.10 (0.81-1.49)	0.87	0.78	0.42
		Mean IGF-I§	256.1	250.0	250.6	0.08	0.04	0.60
		Mean IGFBP-3§	3,259	3,207	3,248	0.32	0.14	0.77
SSTR2	rs998571	Cases/controls	314/661	339/599	83/158			
		OR (95% CI)	1.00	1.20 (0.99-1.45)	1.12 (0.83-1.50)	0.16	0.08	0.87
		Mean IGF-I§	255.5	251.3	252.5	0.29	0.85	0.85
		Mean IGFBP-3§	3,260	3,235	3,190	0.15	0.22	0.22
	rs1466113	Cases/controls	249/444	376/697	111/277			
		OR (95% CI)	1.00	0.98 (0.80-1.19)	0.74 (0.57-0.96)	0.04	0.31	0.02
		Mean IGF-I§	255.3	259.6	259.7	0.25	0.66	0.66
		Mean IGFBP-3§	3,253	3,257	3,285	0.52	0.45	0.45
SSTR3	rs229569	Cases/controls	492/951	244/483	39/73			
		OR (95% CI)	1.00	0.98 (0.81-1.18)	1.03 (0.69-1.54)	0.94	0.88	0.86
		Mean IGF-I§	254.0	254.4	252.5	0.96	0.97	0.81
		Mean IGFBP-3§	3,241	3,244	3,248	0.90	0.91	0.93
SSTR4	rs3746726	Cases/controls	280/549	377/715	112/243			
		OR (95% CI)	1.00	1.04 (0.86-1.25)	0.89 (0.68-1.17)	0.59	0.98	0.28
		Mean IGF-I§	243.7	247.1	246.6	0.38	0.29	0.81
		Mean IGFBP-3§	3,091	3,096	3,090	0.98	0.91	0.92

(*Continued on the following page*)

Table 3. Associations between SNPs and breast cancer risk and mean IGF-I and IGFBP-3 levels adjusted for age and center (Cont'd)

Gene	SNP		Genotype			$P_{codominant}$*	$P_{dominant}$†	$P_{recessive}$‡
			Homozygous major	Heterozygous	Homozygous minor			
SSTR5	rs4988483	Cases/controls	631/1,190	83/135	1/2			
		OR (95% CI)	1.00	1.16 (0.87-1.55)	1.00 (0.09-11.03)	0.33	0.32	1.00
		Mean IGF-I§	245.1	239.3	204.8	0.17	0.21	0.32
		Mean IGFBP-3§	2,954	2,963	2,416	0.89	0.98	0.17
	rs4988487	Cases/controls	654/1,187	59/135	2/5			
		OR (95% CI)	1.00	0.81 (0.59-1.11)	0.76 (0.13-4.40)	0.19	0.18	0.77
		Mean IGF-I§	243.5	249.7	252.4	0.22	0.22	0.75
		Mean IGFBP-3§	2,960	2,922	3,268	0.80	0.61	0.23
	rs169068	Cases/controls	210/394	339/643	166/290			
		OR (95% CI)	1.00	1.00 (0.81-1.24)	1.09 (0.84-1.41)	0.56	0.80	0.48
		Mean IGF-I§	242.6	245.5	245.4	0.48	0.39	0.78
		Mean IGFBP-3§	2,967	2,951	2,943	0.57	0.59	0.70
	rs642249	Cases/controls	680/1,255	33/69	2/3			
		OR (95% CI)	1.00	0.86 (0.56-1.33)	1.14 (0.19-6.93)	0.58	0.53	0.87
		Mean IGF-I§	243.4	263.9	277.4	0.002	0.002	0.29
		Mean IGFBP-3§	2,954	2,952	3,092	0.88	0.95	0.65

*P_{trend} for codominant model.
†P for dominant model.
‡P for recessive model.
§Mean of hormone levels (in ng/mL) for subjects (cases + controls) belonging to each genotype category adjusted for age and center.

Discussion

We have done a large-scale association study, nested in the EPIC cohort, to assess the role of genetic variation of genes involved in the GH synthesis pathway on risk of breast cancer and on circulating levels of IGF-I and IGFBP-3. The present study was conducted on a large subset of the breast cancer cases and matched control subjects that were included in a parallel study on breast cancer risk in relation to IGF-I and IGFBP-3[23] as well to circulating sex steroids[22] (32). To our knowledge, this is the first study to look in a comprehensive way at breast cancer risk and genetic variation in the GH synthesis pathway, including a total of 10 different genes, and the first study to simultaneously analyze associations of genetic variants with circulating levels of IGF-I and IGFBP-3 in the European population.

Our objective was to include into our analyses all SNPs that would have a minimum allele frequency of 5% or otherwise a high chance of having an effect on gene expression or function of the gene product (e.g., known coding variants). We therefore did extensive searches through the literature and public databases. Although the DHPLC technique approach may be somewhat less sensitive than a systematic resequencing for the identification of new SNPs, it is a quite reliable method for SNP detection (reviewed in ref. 33). Although systematic resequencing would have probably led to the identification of further polymorphisms, this approach did not fall within the financial scope of our project. We believe, however, that most of the additional SNPs that could have been identified by such more stringent approach probably will have an allele frequency <5% and that resequencing would have led to the identification of only very few additional common polymorphisms, with higher allele frequencies. Overall, therefore, we are confident that we have included in our study most of the common variants existing in Caucasians in the 10 candidate genes examined in this study. Because the SNPs in our study were not specifically selected according to a haplotype-tagging approach, it has to be noted that our haplotype analysis could miss associations with unknown genetic variants of the candidate genes that are not in linkage disequilibrium (LD) with the SNPs we selected.

Although we have tried to have a fair representation of all common variants, we took great care in retaining for our study those polymorphisms for which evidence of experimental validation was available. This was particularly relevant for SNPs of the *GH1* gene, given that it belongs to a cluster of five genes that all share very high degree of homology even for intronic and promoter regions (36). Although many polymorphisms have been reported in the *GH1* gene, several of these may have been actually artifacts due to nonspecific amplification of target sequences in one of the other *GH* homologues. In this regard, it is important to note that none of polymorphisms we have typed in the *GH1* gene showed any significant departure from Hardy-Weinberg equilibrium, which suggests that our genotyping assays for *GH1* gene polymorphisms have been properly designed.

Our study population included women from 7 of the 10 countries participating in the EPIC project ranging from southern Europe (Greece, Italy, and Spain) to the United Kingdom. Over 97% of EPIC subjects are estimated to be of Caucasian origin. Nevertheless, there is substantial regional difference in breast cancer incidence rates across Europe (most likely due to differences in lifestyle). Thus, spurious associations of risk with allelic variants could be found if allelic frequencies varied substantially between regions. This potential bias was avoided, however, by matching the control subjects to the breast cancer cases by EPIC recruitment center and by performing a conditional logistic regression analysis. Regression models relating IGF-I or IGFBP-3 to polymorphic variants were also systematically adjusted for the factor ''recruitment center.''

Serum levels of IGF-I and IGFBP-3 showed statistically significant associations with variants (SNPs and haplotypes) in several of the candidate genes studied. However, in spite of the large size of this cross-sectional study component, most of these associations were not highly statistically significant (Ps = 0.01-0.05). The only exception was the association between a synonymous polymorphism in exon 1 of *SSTR5* and IGF-I level supported by a P of 0.002. This novel finding will have to be confirmed by further epidemiologic and/or functional studies. Furthermore, for none of the SNPs found to be associated with serum peptide levels was there any previous evidence of a similar association or any experimental evidence for a possible direct, functional role. This makes it difficult to assess whether the associations observed in our

Table 4.

A. Associations between haplotypes in *GH1* and breast cancer risk and IGF-I and IGFBP-3 levels adjusted for age and center

GH1 haplotypes*	Haplotype frequency		Model		
			Codominant	Dominant	Recessive
hGGAA	0.33	OR (95% CI)†	1.00	1.00	1.00
		α IGF-I (*P*)‡	434.39	433.82	428.42
		α IGFBP-3 (*P*)‡	2,523	2,521	2,480
hTAAT	0.29	OR (95% CI)†	1.03 (0.88-1.21)	1.05 (0.86-1.28)	1.00 (0.74-1.36)
		β IGF-I (*P*)‡	−5.31 (0.04)	−6.44 (0.05)	−1.27 (0.81)
		β IGFBP-3 (*P*)‡	−40.88 (0.11)	−57.86 (0.07)	22.66 (0.66)
hGAAT	0.17	OR (95% CI)†	1.13 (0.94-1.36)	1.13 (0.92-1.40)	1.29 (0.80-2.09)
		β IGF-I (*P*)‡	−0.88 (0.77)	0.50 (0.88)	0.68 (0.94)
		β IGFBP-3 (*P*)‡	−36.66 (0.23)	−19.12 (0.58)	−119.97 (0.14)
hGGAT	0.09	OR (95% CI)†	0.94 (0.73-1.22)	0.96 (0.73-1.26)	0.35 (0.08-1.54)
		β IGF-I (*P*)‡	−7.00 (0.10)	−4.78 (0.29)	−41.98 (0.02)
		β IGFBP-3 (*P*)‡	−81.60 (0.05)	−71.75 (0.11)	−272.79 (0.14)
hGAAA	0.06	OR (95% CI)†	0.97 (0.71-1.34)	0.98 (0.70-1.38)	0.76 (0.20-2.90)
		β IGF-I (*P*)‡	−13.42 (0.01)	−13.59 (0.02)	−17.80 (0.42)
		β IGFBP-3 (*P*)‡	−71.15 (0.17)	−71.47 (0.20)	−109.74 (0.61)
hTAAA	0.03	OR (95% CI)†	1.01 (0.66-1.54)	1.03 (0.67-1.60)	—
		β IGF-I (*P*)‡	−3.94 (0.58)	−1.36 (0.85)	−32.42 (0.51)
		β IGFBP-3 (*P*)‡	−23.85 (0.73)	−0.95 (0.99)	−216.23 (0.66)
hTGAT	0.02	OR (95% CI)†	1.02 (0.56-1.86)	1.01 (0.56-1.84)	—
		β IGF-I (*P*)‡	−10.66 (0.29)	−9.60 (0.34)	—
		β IGFBP-3 (*P*)‡	77.03 (0.44)	91.97 (0.35)	—

B. Associations between haplotypes in *GHRHR* and breast cancer risk and IGF-I and IGFBP-3 levels adjusted for age and center

GHRHR haplotypes§	Haplotype frequency		Model		
			Codominant	Dominant	Recessive
hCGTCC	0.57	OR (95% CI)‖	1.00	1.00	1.00
		α IGF-I (*P*)‡	430.56	430.14	428.99
		α IGFBP-3 (*P*)‡	2,459	2,465	2,466
hCGCCG	0.24	OR (95% CI)‖	0.94 (0.81-1.10)	0.98 (0.81-1.19)	0.73 (0.49-1.09)
		β IGF-I (*P*)‡	−1.36 (0.59)	−0.84 (0.78)	−2.91 (0.64)
		β IGFBP-3 (*P*)‡	30.35 (0.22)	23.01 (0.44)	99.56 (0.11)
hCGTTC	0.10	OR (95% CI)‖	1.19 (0.95-1.49)	1.23 (0.95-1.59)	1.51 (0.66-3.45)
		β IGF-I (*P*)‡	−4.15 (0.23)	−3.61 (0.33)	−15.08 (0.30)
		β IGFBP-3 (*P*)‡	17.24 (0.62)	20.91 (0.57)	−91.26 (0.52)
hTATCG	0.05	OR (95% CI)‖	0.78 (0.59-1.04)	0.79 (0.59-1.07)	0.67 (0.07-6.41)
		β IGF-I (*P*)‡	1.61 (0.73)	1.18 (0.80)	8.01 (0.82)
		β IGFBP-3 (*P*)‡	46.45 (0.30)	37.44 (0.42)	435.63 (0.20)
hCGCCC	0.02	OR (95% CI)‖	1.14 (0.72-1.82)	1.15 (0.72-1.84)	—
		β IGF-I (*P*)‡	−10.20 (0.19)	−9.98 (0.20)	—
		β IGFBP-3 (*P*)‡	−20.06 (0.79)	−23.03 (0.76)	—

C. Associations between haplotypes in *SST* and breast cancer risk and IGF-I and IGFBP-3 levels adjusted for age and center

SST haplotypes¶	Haplotype frequency		Model		
			Codominant	Dominant	Recessive
hTT	0.88	OR (95% CI)**	1.00	1.00	1.00
		α IGF-I (*P*)‡	428.40	428.49	428.19
		α IGFBP-3 (*P*)‡	2,491	2,490	2,496
hCC	0.10	OR (95% CI)**	1.24 (1.01-1.52)	1.27 (1.02-1.59)	1.21 (0.52-2.80)
		β IGF-I (*P*)‡	−2.97 (0.39)	−4.13 (0.27)	10.08 (0.49)
		β IGFBP-3 (*P*)‡	41.17 (0.23)	52.56 (0.15)	−62.19 (0.66)
hCT	0.02	OR (95% CI)**	1.06 (0.66-1.70)	1.08 (0.67-1.75)	—
		β IGF-I (*P*)‡	−2.62 (0.74)	−2.42 (0.76)	−23.52 (0.74)
		β IGFBP-3 (*P*)‡	−62.23 (0.43)	−63.18 (0.43)	−113.09 (0.87)

D. Associations between haplotypes in *SSTR2* and breast cancer risk and IGF-I and IGFBP-3 levels adjusted for age and center

SSTR2 haplotypes††	Haplotype frequency		Model		
			Codominant	Dominant	Recessive
hAC	0.43	OR (95% CI)‡‡	1.00	1.00	1.00
		α IGF-I (*P*)‡	426.30	426.05	423.80
		α IGFBP-3 (*P*)‡	2,467	2,459	2,460
hGG	0.33	OR (95% CI)‡‡	1.15 (0.99-1.33)	1.24 (1.03-1.51)	1.03 (0.78-1.36)
		β IGF-I (*P*)‡	−2.49 (0.31)	−3.16 (0.32)	−0.73 (0.88)
		β IGFBP-3 (*P*)‡	−26.07 (0.29)	−27.42 (0.39)	−45.40 (0.34)

(*Continued on the following page*)

Table 4. (Cont'd)

D. Associations between haplotypes in *SSTR2* and breast cancer risk and IGF-I and IGFBP-3 levels adjusted for age and center

SSTR2 haplotypes[††]	Haplotype frequency		Model		
			Codominant	Dominant	Recessive
hAG	0.24	OR (95% CI)[‡‡]	1.13 (0.96-1.33)	1.19 (0.99-1.45)	0.96 (0.66-1.40)
		β IGF-I (P)[‡]	−2.44 (0.37)	−1.13 (0.73)	−8.17 (0.20)
		β IGFBP-3 (P)[‡]	13.67 (0.61)	35.75 (0.26)	−31.47 (0.61)

E. Associations between haplotypes in *SSTR5* and breast cancer risk and IGF-I and IGFBP-3 levels adjusted for age and center

SSTR5 haplotypes[§§]	Haplotype frequency		Model		
			Codominant	Dominant	Recessive
hCCTG	0.54	OR (95% CI)[‖‖]	1.00	1.00	1.00
		α IGF-I (P)[‡]	422.66	422.52	425.75
		α IGFBP-3 (P)[‡]	2,428	2,439	2,412
hCCCG	0.33	OR (95% CI)[‖‖]	1.05 (0.91-1.22)	1.05 (0.86-1.27)	1.12 (0.85-1.49)
		β IGF-I (P)[‡]	−0.32 (0.89)	0.16 (0.96)	−2.99 (0.53)
		β IGFBP-3 (P)[‡]	−15.32 (0.51)	−26.41 (0.40)	−0.45 (0.99)
hACCG	0.06	OR (95% CI)[‖‖]	1.29 (0.85-1.96)	1.32 (0.85-2.05)	1.00 (0.09-11.03)
		β IGF-I (P)[‡]	−4.32 (0.39)	−3.74 (0.46)	−40.04 (0.32)
		β IGFBP-3 (P)[‡]	−3.77 (0.94)	3.13 (0.95)	−537.71 (0.17)
hCTCG	0.05	OR (95% CI)[‖‖]	0.82 (0.60-1.13)	0.81 (0.58-1.11)	0.78 (0.13-4.53)
		β IGF-I (P)[‡]	6.22 (0.21)	6.73 (0.20)	7.92 (0.76)
		β IGFBP-3 (P)[‡]	−18.35 (0.71)	−31.90 (0.54)	311.84 (0.23)
hCCCA	0.03	OR (95% CI)[‖‖]	0.90 (0.60-1.34)	0.87 (0.57-1.33)	1.21 (0.20-7.42)
		β IGF-I (P)[‡]	19.75 (0.002)	21.10 (0.002)	32.61 (0.29)
		β IGFBP-3 (P)[‡]	2.53 (0.97)	−3.51 (0.96)	138.88 (0.65)

*The order of the SNPs defining the haplotypes is rs2005172, rs6171, rs6173, rs2665802.
†Reference group = hGGAA.
‡β estimate for IGF-I and IGFBP-3 levels (in ng/mL) for a one-unit change in haplotype dosage adjusted for age and center.
§The order of the SNPs defining the haplotypes is rs4988495, rs4988496, rs4988501, rs4988503, rs4988505.
‖Reference group = hCGTCC.
¶The order of the SNPs defining the haplotypes is rs4988513, P0689.
**Reference group = hTT.
††The order of the SNPs defining the haplotypes is rs998571, rs1466113.
‡‡Reference group = hAC.
§§The order of the SNPs defining the haplotypes is rs4988483, rs4988487, rs169068, rs642249.
‖‖Reference group = hCCTG.

study represent a true effect on serum peptide levels (directly or through LD with other, functional polymorphisms) or whether they were merely chance findings. Additional large association studies will be needed to confirm our findings.

With regard to breast cancer risk, the only relatively consistent pattern of associations was with variants of the *SST* and *SSTR2* genes. It is possible that associations of breast cancer risk with polymorphisms in the *SST* and *SSTR* genes reflect autocrine or paracrine mechanisms of cellular proliferation active at the level of the breast epithelium. Both somatostatin (37) and its receptors (38) have been found to be produced by breast cancer cells, and all five somatostatin receptors have been found in human breast tumors, with subtype 2 occurring more frequently (39). The presence of somatostatin receptors in human breast cancers has been correlated with well-differentiated tumors and more favorable prognosis (38), and treatment with somatostatin or its synthetic analogues has been shown to have antiproliferative effects on breast tumors, both *in vitro* and *in vivo* (40), and to result in a positive tumor response in >40% of patients (41).

In view of the above observations, it is possible that polymorphisms, which affect expression of the somatostatin gene *SST* and/or its receptor *SSTR2*, could modulate the antiproliferative effect that somatostatin exerts on breast tumors. The *SST* SNPs that we found to be associated with cancer risk are located in intron 1 of this gene, and nothing is known of their possible function. The association we report either may reflect a direct functional effect of the polymorphisms studied or may be due to LD with unknown functional polymorphisms. On the other hand, the *SSTR2* SNPs we have typed are located in the promoter, respectively, at positions −57 and −83 upstream of the start of transcription. Interestingly, the *SSTR2* haplotype (hGG) that shows an increased risk of breast cancer has been also reported to be associated with a 60% to 70% reduction of *SSTR2* transcription in pancreatic cancer cells by use of site-directed mutagenesis and a luciferase reporter gene assay (42). It has to be noted that, at the individual SNP level, it was the polymorphism at position −83 that was found to be responsible for this decrease (42). We found a borderline, nonsignificant association with the SNP at the −83 position and a significant association with the SNP at −57 position. In our sample, there is a complete but not perfect LD between these two polymorphisms ($D' = 1$, $r^2 = 0.36$), reflecting the fact that their frequencies are different (minor allele frequencies of 32% and 44%, respectively). It is difficult therefore to say whether the associations we observe and the previously reported functional role for one of the *SSTR2* promoter SNPs are in relation or not.

Only one SNP included in our work has been studied previously in relation to breast cancer or circulating levels of IGF-I and IGFBP-3. SNP rs2665802, located in intron 4 of *GH1*, has been found to be associated with level of IGF-I and risk of colorectal neoplasia (43). Another study found an association of this polymorphism with secretion of GH and IGF-I and with stature in a group of Japanese prepubertal short children (24). The same polymorphism did not show any association with breast cancer risk, however, in a large case-control study done in a Chinese population, where IGF-I levels were not measured (44). Likewise, we did not observe any association of this polymorphism with cancer risk or hormone level in our study.

Nevertheless, we have found two *GH1* haplotypes that showed a weak association with reduced IGF-I level. This leaves the possibility that the previously reported associations reflected LD between SNP rs2665802 and other polymorphisms in *GH1* or possibly in neighboring genes.

Previous prospective cohort studies have shown increased prediagnostic IGF-I levels among women who developed breast cancer, especially when the cancer was diagnosed at a relatively young (premenopausal or early menopausal) age. Contrary to these previous findings, however, our data from the EPIC cohort, with an extended series of breast cancer cases (n = 1,081) and control subjects (n = 2,098), showed a weak, direct association of serum IGF-I with breast cancer risk only among women of postmenopausal age but not among the younger women (although mean levels did not differ between the two groups).[23] In the present study, we also did analyses of genetic variants in relation to breast cancer risk stratified by age at breast cancer diagnosis (age <55 and >55 years). Our study was not large enough, however, to allow for statistically powerful tests for differences in associations between older

Appendix 1. PCR primers, probes, and labels for TaqMan genotyping assays

Polymorphism*	Probes †	PCR primers
GHRH1		
rs4988491	VIC-MGB-ATGTTAGCTTTAAAAAA	AGCTGCCTGGTTTGCACTCT
	FAM-MGB-TGTTAGCTTCAAAAAA	TCCCAAAGTGGTATTACGAGGTGTA
GHRHR		
rs4988495	HEX-LNA-TCCTCCAGCTTCACCC	TAGGAGGCAGGTGGTGGCTT
	FAM-LNA-CCTCCAGCCTCACCC	CCAGGAGCAGCAGAGACTCAG
rs4988496	HEX-LNA-AGGCTGCCCTGCGACCTGGGA	CTCTGCTGCTCCTGGCTCTC
	FAM-LNA-AGGCTGCCCTACGACCTGGGA	GCACCCCTCACCTGACTCTG
rs4988501	VIC-MGB-AGTTTGATTCGATTCAC	TCAAGTTCAGCTCAATTCAATTCAG
	FAM-MGB-ATTCGGTTCACCTCC	GGATTCTGGTGTGCTGCAACT
rs4988503	VIC-MGB-CTTGGGAGCCTAGGA	AAGTGCACACGACAGTTTCTAATCC
	FAM-MGB-AGTCTTGGAAGCCTAG	CGCCTGCAGGAAAGACAAA
rs4988505	VIC-MGB-TGAACCGGAATGTT	CTGTCCTGAGCTTCTGGATCAAG
	FAM-MGB-TGAACCCGAATGTT	TCAGAGGAAGGATGGATAAGAGATG
GH1		
rs2005172	HEX-TCCCACTGTTGACCCCACCTGTTT	CATTAGCACAAGCCCGTCAGT
	FAM-TCCCACTGTTGCCCCCACCTGTTT	CCTTTTTATACCCTGGCCCCTTC
rs6171	HEX-LNA-AC+AA+GAG+ACC+AG+CTC	AGAGAAGGGGCCAGGGTATAAA
	FAM-LNA-AC+AA+GAG+ACC+GG+CTC	CTAGGTGAGCGTCCACAGGA
rs6173	HEX-LNA-CCATTGCCGCTAGGTGA	GACACATTGTGCCCAAAGG
	FAM-LNA-CCATTGCAGCTAGGTGA	CAGCTCCAGCATCCCAAG
rs2665802	HEX-LNA-TAG+CAG+ACC+AGG+CCCTG	ACTTTGAGAGCTGTGTTAGAGAAAC
	FAM-LNA-TAG+CAG+TCC+AGG+CCCTG	TTCACGAGGGGAAATGAAGAATAAG
GHR		
rs6179	VIC-MGB-CATCCATCCTTTCTG	ATATCCAAGTGAGATGGGAAGCA
	FAM-MGB-ATCCACCCTTTCTG	CCATTTAGTTTCATTTACTTCTTTGTATTGA
rs6182	HEX-CATGATGCTTTCCCTGCTACTCAGC	TTATGCCTTGACCAGAAGAATCAAA
	FAM-CATGATGCTTGCCCTGCTACTCAGC	GTTTTTCTCTGCTTGGATAACACTG
rs6180	VIC-MGB-AGTGCCTCCCTGTGG	TTATGGACAATGCCTACTTCTGTGA
	FAM-MGB-ATCCACCCTTTCTG	GCTGTATGTGTGATTCAACCTTGAT
SST		
rs4988513	HEX-LNA-AGCCCTCCCTAAGCCTTG	TCTTTAGAAGGACTGAGCATCCCT
	FAM-LNA-CCCTCCCTGAGCCTTGC	CAGGTAAGGAGACTCCCTCGAC
P0689 ‡	VIC-MGB-CTGGAGAATCCGGG	GGTAAGGAGACTCCCTCGACGT
	FAM-MGB-CTGGAGGATCCGGG	GGCAGGAGCAAGGCTTAGG
SSTR1		
rs2228497	HEX-AGCTGGTCAACGTGTTTGCTG	CGCAAGATCACCTTAATGG
	FAM-AGCTGGTTAACGTGTTTGC	CGAGGATGACCGACAG
SSTR2		
rs998571	VIC-MGB-CTGAGAGGCTAAACCAGAAA	AACTCTAGAGCTTAATGTTGATGTGGAAA
	FAM-MGB-AGAGGCTAAGCCAGAAA	CGTCGGTCACAGGCTGTTTAA
rs1466113	HEX-LNA-TGGTGAGACTTTAAACA	AGAGCTTAATGTTGATGTGG
	FAM-LNA-TGGTGAGAGTTTAAACA	CCTTAATGGACCCTGGAG
SSTR3		
rs229569	VIC-MGB-CGAAGAGCGCCACC	GGGTCACGCGCATGGT
	FAM-MGB-CGAAGAGTGCCACCAC	CACCACGTTGACGATGTTGAG
SSTR4		
rs3746726	VIC-MGB-CACGAAGAGGTTCAG	TCTGCTGGATGCCTTTCTACGT
	FAM-MGB-CACGACGAGGTTC	TGGTTGACGGTGGCATCA
SSTR5		
rs4988483	VIC-MGB-CAGCAGGTACAGCAC	CTGGAGGCGGTGACAACAG
	FAM-MGB-CAGCATGTACAGCAC	CCCGGCCGCACACA
rs4988487	HEX-LNA-CCCTTCGGCTCCGT	CTACATTCTCAACCTGGC
	FAM-LNA-CCTTCGGCCCCGT	GCAGAAGACACTGGTGAAC
rs169068	HEX-CGCCACGGAGCTGCGTCCAGACA	AAGGGCTCTGGTGCCAAGGA
	FAM-CGCCACGGAGCCGCGTCCAGACA	CACTCTCACAGCTTGCTGGTCT
rs642249	HEX-AGGCCACGCCGCCCGCGCAC	CTGCGTCCAGACAGGATCCG
	FAM-AGGCCACGCCACCCGCGCAC	CACTCTCACAGCTTGCTGGTCT
POU1F1		
rs300982	HEX-LNA-AC+TAGCG+TGCA+CC	AAAGGGATTTCCTTGCAGTA
	FAM-LNA-TACT+AGCG+CGC+ACCC	AGCTTGGCAACTCATTCC

*Polymorphisms are identified by their dbSNP accession no. dbSNP is accessible at http://www.ncbi.nlm.nih.gov/SNP/.
†Fluorescent dye and presence of stabilizing molecules (MGB or LNA) are indicated. For LNA probes, a plus sign indicates bases modified with a LNA molecule.
‡P0689 is an internal reference. This polymorphism is not represented in dbSNP (46).

and younger women, especially in relation to the rarer polymorphisms.

This large study, for the first time, investigated the role of genetic variation across 10 different genes belonging to the same pathway. This obviously entailed a large number of statistical tests, which may have led to several spurious associations due to chance. One approach to account for the multiple comparisons is to use Bonferroni's method, applying a more stringent criterion for statistical significance, at the level of each gene studied. This method is conservative, however, as it is difficult to account for dependence between statistical tests due to LD between SNPs. An alternative is to apply a Bayesian approach to calculate false-positive response probabilities (FPRP; ref. 45). We have computed FPRPs for the nominally significant associations we have observed between SNPs and breast cancer risk. Use of a prior probability of 0.1 resulted in noteworthy FPRPs for the association with breast cancer risk of polymorphisms rs6171 in *GH1* (FPRP = 0.16) and rs4988513 in *SST* (FPRP = 0.16). Wacholder et al. suggest that for a large study like ours FPRP < 0.2 might be an appropriate threshold for noteworthiness (45). When using a prior probability of true association of ≤0.01, which is more likely to be correct, we obtained high FPRP values, all >0.67.

In conclusion, the associations we report here do not have a strong statistical support making replication the key to confirm or dismiss these results. Given that associations with individual genetic variants seem to be of a relatively small magnitude, even larger studies will be needed to confirm our findings and to allow for association studies on rarer polymorphic variants as well.

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