Null Results in Brief

IGF2R Missense Single-Nucleotide Polymorphisms and Breast Cancer Risk: The Multiethnic Cohort Study

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Abstract

***IGF2R* has been proposed to be a tumor suppressor gene given its antagonist role on cellular growth and evidence of loss of heterozygosity in several cancers, including breast cancer. To investigate whether inherited differences in potentially functional *IGF2R* variants influence the risk of breast cancer, we sequenced 46 exons of *IGF2R* to identify novel missense single-nucleotide polymorphisms (SNP) and tested 12 missense SNPs for their associations with breast cancer risk among 1,614 breast cancer cases and 1,960 controls from the Multiethnic Cohort. None of these missense SNPs were significantly associated with breast cancer risk. Our findings provide no evidence that missense SNPs in *IGF2R* influence breast cancer susceptibility.** (Cancer Epidemiol Biomarkers Prev 2009;18(6):1922–4)

Introduction

The insulin-like growth factor-II receptor (IGF2R) is a transmembrane receptor that primarily binds IGF-II, resulting in the degradation of IGF-II by internalization and transport to the lysosomes. By removing IGF-II from the extracellular environment and precluding its activation of the insulin-like growth factor-I receptor (IGF1R), IGF2R is believed to reduce the mitogenic effects of IGF-II (1). Loss of heterozygosity at the *IGF2R* locus has been reported in breast carcinomas, and somatic missense mutations of the remaining allele have shown alteration in ligand binding (2-4). *IGF2R* has been proposed to be a tumor suppressor gene given its antagonist role on cellular growth and evidence of loss-of-heterozygosity and loss-of-function mutations in several cancers (5-10), including breast cancer (2-4). To investigate whether inherited differences in potentially functional *IGF2R* variants influence the risk of breast cancer, we sequenced 46 exons of *IGF2R* to identify novel missense single-nucleotide polymorphisms (SNP) and tested 12 missense SNPs for their associations with breast cancer risk among 1,614 breast cancer cases and 1,960 controls from the Multiethnic Cohort.

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Materials and Methods

Study Subjects. The Multiethnic Cohort study is a large population-based cohort study of more than 215,000 individuals from Hawaii and California. The cohort is composed of predominantly African Americans, Native Hawaiians, Japanese Americans, Latinos, and Whites, who were between the ages of 45 and 75 y when recruited from 1993 to 1996. Further methodologic details of this study are provided elsewhere (11).

The present case-control study was nested in the Multiethnic Cohort, as previously described (12). It includes 1,614 breast cancer cases and 1,960 controls that were frequency matched on age (within 5 y) and race/ethnicity. The majority of these women were postmenopausal at baseline (87% of cases and 82% of controls). This study was approved by the Institutional Review Boards at the University of Hawaii and the University of Southern California.

Sequencing and Validation Genotyping. To identify novel missense SNPs, we successfully sequenced 46 of the 48 exons of *IGF2R* in 95 advanced breast cancer cases (n = 19 per racial/ethnic group). Two exons (exon 1 and exon 30) did not meet our sequencing criteria of >80% of samples with Phred (base-calling) scores >20 for >80% of the target bases; thus, for these we relied on publicly available SNP information. Further details on sequencing methods are described elsewhere (13). Nineteen *IGF2R* missense SNPs were identified by sequencing. For validation, these 19 SNPs and 2 additional common missense SNPs (minor allele frequency >0.05) in dbSNP (rs8191754 and rs629849) were genotyped in an independent multiethnic panel of 349 control subjects (n = 69-70 per racial/ethnic group).

Table 1. Twelve *IGF2R* missense SNPs used for association testing

SNP	Exon no.	Position*	Nucleotide change†	Amino acid change	PolyPhen prediction‡	Minor allele frequency (%)				
						African Americans	Native Hawaiians	Japanese Americans	Latinos	Whites
rs8191746	5	160365688	C>T	Pro203Leu	Probably damaging	2.1	0	0	0	0
rs8191754	6	160368314	C>G	Leu252Val	Benign	12.1	18.4	31.6	6.4	14.5
rs6413491	16	160388299	G>A	Ala724Thr	Benign	3.9	0	0	0.8	0.8
IGF2R_C722T§	17	160388898	C>T	Pro772Leu	Probably damaging	2.9	0	0	0.70	0
rs8191808	18	160389500	G>C	Val817Leu	Benign	0	0	0.7	0.7	0.7
rs8191844	25	160402919	C>G	Thr1184Ser	Possibly damaging	3.6	0	0	0	0
IGF2R_C1194T§	25	160402949	C>T	Ser1194Leu	Benign	0	2.2	0	0	0
rs8191859	28	160405480	G>A	Gly1315Glu	Possibly damaging	0	0	8.0	0	0
rs629849	34	160414399	G>A	Gly1619Arg	Benign	2.9	11.0	9.4	6.5	10.7
IGF2R_C1822T§	37	160419371	C>T	Thr1822Met	—	3.6	0	0	1.43	0
rs8191904	38	160420618	G>A	Arg1832His	Benign	4.8	0	0	0	0.91
rs8191955	48	160446006	C>T	Ala2459Val	Benign	0	2.9	0.7	0	0.7

*SNP position based on dbSNP reference assembly (Build 36.3).
†Minor allele based on all groups combined.
‡PolyPhen: http://genetics.bwh.harvard.edu/pph/.
§Novel missense SNP identified by sequencing.

Genotyping of Breast Cancer Cases and Controls. Table 1 lists the 12 *IGF2R* missense SNPs that were genotyped in the breast case-control study. Ten SNPs were genotyped using the Sequenom genotyping platform; two SNPs (rs8191754 and rs629849) that failed Sequenom assay design or genotyping were genotyped by the TaqMan platform. The average concordance rate for ~5% quality control repeats was 99.7% and the average genotyping success was 97.1%. There were no deviations from Hardy-Weinberg equilibrium ($P > 0.01 > 1$ racial/ethnic group).

***In silico* Analysis.** The Polymorphism Phenotype (PolyPhen)[4] algorithm (14) was used to predict the potential effect of each missense SNP on IGF2R protein structure and function (Table 1). Predictions are based on sequence, phylogenetic, and structural information to evaluate the degree of damage a variant may have on the structural properties of the protein. A score is assigned to each SNP indicating either "probably damaging," "possibly damaging," or "benign" effects on protein function and/or structure.

Statistical Analysis. Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated by unconditional logistic regression to estimate genotype-specific risks, adjusting for age and ethnicity in any analysis that combined racial/ethnic groups. All results were similar when adjusted for established breast cancer risk factors (15). In addition, given that missense SNPs generally have low minor allele frequencies, we examined the association between the aggregate number of variant missense alleles and breast cancer risk to assess whether there was an overrepresentation of variant alleles among cases versus controls. The aggregate number of variant alleles was determined by counting the total number of minor alleles across all 12 missense SNPs (38.5% of controls had >1 variant allele). All reported *P* values are two-sided.

Results

Nineteen *IGF2R* missense SNPs were identified by sequencing 46 exons. With genotyping of these 19 SNPs in a multiethnic panel for validation, 3 SNPs were identified to be novel (Supplementary Table S1) because they are not present in the dbSNP database[5]; 7 SNPs were already present in dbSNP; and 9 SNPs were monomorphic (minor allele frequency <0.01 in all of the five racial/ethnic groups).

Ten validated missense SNPs identified by sequencing and two additional missense SNPs from dbSNP (rs8191746 and rs8191808) were tested in our breast cancer case-control study. There were no associations between these missense SNPs and breast cancer risk ($P > 0.06$; Table 2). In addition, no association with breast cancer risk was observed for the aggregate number of variant missense alleles in comparison with no variant alleles (OR, 1.07; 95% CI, 0.93-1.25). Similarly, no association was observed for an increment of one variant allele (OR, 1.03; 95% CI, 0.95-1.12). For the aggregate number of variant alleles of the four missense SNPs (rs8191746, IGF2R_C722T, rs8191844, and rs8191859) predicted to have "probably/possibly damaging effects," no association was observed (OR, 0.97; 95% CI, 0.66-1.42).

Discussion

In summary, our multiethnic study does not support the influence of *IGF2R* missense SNPs on breast cancer risk. Our study had 80% power to detect a minimum OR of 1.35 and 1.57 for SNPs at 5% and 2% allele frequencies, respectively ($\alpha = 0.05$, two-sided hypothesis test, log-linear model; ref. 16). In addition, we had 80% power to detect a minimum OR of 1.16 for missense SNPs in aggregate (38.5% of controls had >1 variant allele) under similar parameters. Our study cannot exclude the possibility that common genetic variation in *IGF2R* may have

[4] http://genetics.bwh.harvard.edu/pph/

[5] http://www.ncbi.nlm.nih.gov/projects/SNP/

Table 2. Associations between *IGF2R* missense variants and breast cancer risk

SNP	Genotype	All		
		Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)*
rs8191746	CC	1584 (99.5)	1862 (99.4)	1.00
	CT	8 (0.5)	11 (0.6)	0.76 (0.30-1.91)
rs8191754	CC	1096 (71.1)	1354 (71.2)	1.00
	CG/GG	445 (28.9)	547 (28.8)	1.14 (0.95-1.30)
rs6413491	GG	1568 (98.6)	1833 (98.4)	1.00
	GA/AA	23 (1.4)	29 (1.6)	0.85 (0.48-1.48)
IGF2R_C722T	CC	1572 (99.9)	1923 (100.0)	1.00
	CT	1 (0.06)	1 (0.1)	1.18 (0.07-18.97)
rs8191808	CC	1572 (99.1)	1846 (99.3)	1.00
	CG	14 (0.9)	14 (0.8)	1.28 (0.60-2.73)
rs8191844	CC	1562 (98.3)	1839 (98.6)	1.00
	CG/GG	27 (1.7)	26 (1.4)	1.12 (0.64-1.96)
IGF2R_C1194T	CC	1574 (97.9)	1851 (98.7)	1.00
	CT/TT	34 (2.1)	25 (1.3)	1.40 (0.82-2.39)
rs8191859	GG	1557 (98.9)	1877 (97.4)	1.00
	GA/AA	17 (1.1)	50 (2.6)	0.87 (0.48-1.59)
rs629849	AA	1283 (82.8)	1589 (83.7)	1.00
	AG/GG	267 (17.2)	309 (16.3)	1.06 (0.88-1.28)
IGF2R_C1822T	CC	1572 (98.7)	1839 (98.7)	1.00
	CT	21 (1.3)	24 (1.3)	0.92 (0.50-1.68)
rs8191904	GG	1551 (99.1)	1893 (98.4)	1.00
	GA/AA	14 (0.9)	31 (1.6)	0.54 (0.28-1.03)
rs8191955	CC	1550 (99.5)	1910 (99.5)	1.00
	CT	8 (0.5)	9 (0.5)	1.50 (0.55-4.14)

*Adjusted for age and racial/ethnic group.

weak effects on breast cancer risk. Moreover, larger studies, such as in the National Cancer Institute Breast and Prostate Cancer Consortium (17), are indicated to evaluate whether the combined effects of several genes in the IGF pathway are more likely to affect breast cancer susceptibility.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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