Null Results in Brief

No Breast Cancer Association for Transforming Growth Factor-β Pathway Colorectal Cancer Single Nucleotide Polymorphisms

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Abstract

SMAD7 and GREM1 are signaling components on the transforming growth factor-β pathway, which regulates normal mammary gland development and has been implicated in breast tumor invasion and metastasis. Three variants within *SMAD7* and two variants in *CRAC1* (a colorectal cancer–associated region on chromosome 15 in which *GREM1* is located) have been associated with colorectal cancer risks [odds ratios (OR), 0.85-1.26; all $P < 10^{-7}$]. We genotyped these five variants in a series of 1,267 bilateral breast cancer cases and 900 controls to determine whether they are associated with breast as well as colorectal cancer risk. None of these single nucleotide polymorphisms were associated with breast cancer risk in our study and the 95% confidence limits of our data, pooled with data from the Cancer Genetic Markers of Susceptibility study, exclude per allele ORs of <0.94 or >1.08. One or more of these variants may be associated with a very small OR for breast cancer, but our data suggest that the effects of these alleles are cancer site–specific. (Cancer Epidemiol Biomarkers Prev 2009;18(6):1934–6)

Introduction

A possible explanation for the surprising observation that familial cancer risks are predominantly site-specific (1) is tissue-specific regulation of some of the crucial genes underlying polygenic susceptibility. This is consistent with recent whole genome association studies of common epithelial cancers, which have identified single nucleotide polymorphisms (SNP) within a 0.5 Mb "gene desert" on chromosome 8q24, which was associated with several cancer sites (2-7). One variant (rs6983267) was associated with colorectal, prostate, ovarian, and possibly breast cancer, but others seem to be cancer site–specific (8, 9). The most significant SNPs identified by Broderick et al. (10) in their colorectal cancer genome-wide association study were rs6983267 at 8q24 and three variants (rs4939827, rs12953717, and rs4464148) that map to a 3 kb LD block within intron 3 of *SMAD7*. In a classic linkage study (11), Jaeger et al. identified a locus on chromosome 15q13.3-q14 (*CRAC1*) which was associated with colorectal cancer risk; genotyping of 145 SNPs within this region identified two SNPs (rs4779584 and rs10318) that were associated with colorectal cancer risk, one of which (rs10318) lies in the 3′-untranslated region of *GREM1*. We have genotyped these three variants within *SMAD7* and the two variants in *CRAC1* in a series of bilateral breast cancer cases and controls to determine whether they are associated with breast as well as colorectal cancer risk.

Materials and Methods

The British Breast Cancer Study

Cases. Eight hundred and eighty-two cases with two primary breast cancers were ascertained through the English and Scottish cancer registries as described previously (ref. 12; median age at first diagnosis, 48 years; range 25-65 years old). An additional 385 bilateral breast cancer cases were ascertained through the National Cancer Research Network as described previously (ref. 8; median age at first diagnosis, 50 years; range 24-70 years old).

Controls. Nine hundred controls who were friends or non–blood relatives of index cases were recruited through the cases (12). None of the controls had a personal history of malignancy at the time of ascertainment. All cases and controls were Caucasians resident in the United Kingdom. Written informed consent was obtained from all participants and the study was approved by the UK South East MREC.

[5] http://www.kbioscience.co.uk

Received 3/9/09; accepted 3/19/09; published online 6/8/09.

Grant support: Cancer Research UK and Breakthrough Breast Cancer. We acknowledge NHS funding to the NIHR Biomedical Research Centre.

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doi:10.1158/1055-9965.EPI-09-0214

Table 1. Risk of breast cancer associated with the *SMAD7* and *CRAC1* SNPs

Locus/SNP	Genotype	MAF controls	Cases		Controls		OR (95% CI)	*P* trend
			n	%	*n*	%		
rs4939827	TT	0.468	345	27.8	248	28.2	Reference	
18q21	TC		628	50.7	440	50.0	1.03 (0.84-1.26)	
	CC		266	21.5	192	21.8	1.00 (0.78-1.28)	
	Allelic OR						1.00 (0.88-1.13)	0.997
rs12953717	CC	0.445	375	30.0	277	31.2	Reference	
18q21	CT		630	50.4	431	48.6	1.08 (0.89-1.32)	
	TT		244	19.5	179	20.2	1.01 (0.79-1.29)	
	Allelic OR						1.01 (0.89-1.15)	0.856
rs4464148	TT	0.317	580	48.8	415	46.9	Reference	
18q21	TC		546	44.0	377	42.6	1.04 (0.86-1.24)	
	CC		114	9.2	92	10.4	0.89 (0.66-1.20)	
	Allelic OR						0.98 (0.85-1.12)	0.718
rs4779584	CC	0.185	825	66.8	575	66.2	Reference	
15q13	TC		364	29.5	267	30.7	0.95 (0.79-1.15)	
	TT		46	3.7	27	3.1	1.19 (0.73-1.93)	
	Allelic OR						1.00 (0.85-1.17)	0.995
rs10318	CC	0.180	808	66.7	582	67.4	Reference	
15q13	CT		359	29.6	252	29.2	1.03 (0.85-1.24)	
	TT		44	3.6	29	3.4	1.09 (0.68-1.77)	
	Allelic OR						1.03 (0.88-1.22)	0.684

Genotyping. DNA was extracted from blood samples and quantified using PicoGreen (Invitrogen). Genotyping of samples was done by KBioscience[5] using their proprietary in-house competitive allele-specific PCR SNP genotyping system (KASPar).

Statistical Methods. Genotype frequencies in controls were tested for departure from Hardy-Weinberg equilibrium using a χ^2 test. Unadjusted odds ratios (OR) with two-sided exact *P* values were calculated using standard methods. All five of these SNPs were genotyped in stage I of the Cancer Genetic Markers of Susceptibility (CGEMS) breast cancer genome-wide association study (13). Full details of CGEMS data available at the NCI CGEMS web site.[6] The OR for a second primary breast cancer is approximately the square of the OR for a first primary (14, 15), so we used a square root transformation of ORs from the British Breast Cancer (BBC) study for pooling with data from the CGEMS study (13). Pooled estimates are inverse variance-weighted averages of the log OR from the transformed BBC data and the CGEMS data. All statistical analyses were carried out using STATA 10 (Stata Corporation).

[6] http://cgems.cancer.gov/data/

Results and Conclusions

Call rates were 98%, 99%, 98%, 97%, and 96% for rs4939827, rs12953717, rs4464148, rs4779584, and rs10318, respectively. Genotype frequencies in controls did not deviate from Hardy-Weinberg equilibrium. We found no association between any of these SNPs and breast cancer in our data (Table 1). Meta-analyses of data from the BBC study and CGEMS are shown in Table 2.

Table 2. Summary ORs for *SMAD7* and *CRAC1* SNPs in breast cancer and colorectal cancer association studies

Locus/SNP	Study	Genotype	Allelic OR (95% CI)	*P* trend	*P* heterogeneity
rs4939827	BBC	T/C	1.00 (0.94-1.06)		
18q21	CGEMS*		1.01 (0.90-1.14)		
	BBC and CGEMS pooled		1.00 (0.95-1.06)	0.94	0.88
	Colorectal pooled (10)		0.85 (0.81-0.89)	1×10^{-12}	
rs12953717	BBC	C/T	1.01 (0.94-1.07)		
18q21	CGEMS*		1.02 (0.91-1.15)		
	BBC and CGEMS pooled		1.01 (0.95-1.07)	0.77	0.83
	Colorectal pooled (10)		1.17 (1.12-1.22)	9.1×10^{-12}	
rs4464148	BBC	T/C	0.99 (0.93-1.06)		
18q21	CGEMS*		1.03 (0.90-1.17)		
	BBC and CGEMS pooled		1.00 (0.94-1.06)	0.95	0.60
	Colorectal pooled (10)		1.15 (1.09-1.21)	6.68×10^{-8}	
rs4779584	BBC	T/C	1.00 (0.92-1.08)		
15q13	CGEMS*		1.04 (0.89-1.21)		
	BBC and CGEMS pooled		1.01 (0.94-1.08)	0.82	0.66
	Colorectal pooled (11)		1.26 (1.19-1.34)	4.44×10^{-14}	
rs10318	BBC	C/T	1.01 (0.94-1.10)		
15q13	CGEMS*		0.99 (0.85-1.15)		
	BBC and CGEMS pooled		1.01 (0.94-1.08)	0.80	0.78
	Colorectal pooled (11)		1.19 (1.12-1.26)	7.93×10^{-9}	

*CGEMS study comprises genotype data on 1,145 breast cancer cases and 1,142 controls (http://cgems.cancer.gov/data; ref. 13).

The 95% confidence limits exclude per allele ORs of <0.94 or >1.08 for these SNPs.

Both *SMAD7* and *GREM1* are signaling components on the transforming growth factor-β pathway, which regulates normal mammary gland development (16) and has been implicated in tumor invasion and metastasis (17). A nonsynonymous coding SNP (L10P) in transforming growth factor-β has previously been associated with a modest breast cancer risk (18). These five SNPs are therefore plausible candidates for association with breast as well as colorectal cancer, but our data and the CGEMS results show no associations with breast cancer risk. The confidence limits from the pooled data from this study and the CGEMS stage I study exclude a breast cancer OR of <0.95 for rs4989327 in *SMAD7* compared with an OR of 0.85 (95% CI, 0.81-0.89; Table 2) for colorectal cancer (10). The confidence limits for the other *SMAD7* and *GREM1* SNPs that we tested exclude breast cancer ORs of >1.08; in contrast, the ORs for colorectal cancer for these SNPs are all >1.15, with lower confidence limits of 1.09 or greater (Table 2; refs. 10, 11). One or more of these variants may be associated with a very small OR for breast cancer, but our data suggests that the effects of these alleles are cancer site–specific.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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