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Functional Single-Nucleotide Polymorphisms in the *BRCA1* Gene and Risk of Salivary Gland Carcinoma

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Abstract

Objectives—Polymorphic *BRCA1* is a vital tumor suppressor gene within the DNA double-strand break repair pathways, but its association with salivary gland carcinoma (SGC) has yet to be investigated.

Materials and Methods—In a case-control study of 156 SGC patients and 511 controls, we used unconditional logistical regression analyses to investigate the association between SGC risk and seven common functional single-nucleotide polymorphisms (A1988G, A31875G, C33420T, A33921G, A34356G, T43893C and A55298G) in *BRCA1*.

Results—T43893C TC/CC genotype was associated with a reduction of SGC risk (adjusted odds ratio =0.55, 95% CI: 0.38–0.80, Bonferroni-adjusted $p=0.011$), which was more pronounced in women, non-Hispanic whites, and individuals with a family history of cancer in first-degree relatives. The interaction between T43893C and family history of cancer was significant ($p=0.009$). The GATGGCG and AACAACA haplotypes, both of which carry the T43893C minor allele, were also associated with reduced SGC risk.

Conclusion—Our results suggest that polymorphic *BRCA1*, particularly T43893C polymorphism, may protect against SGC.

Keywords

BRCA1 polymorphism; salivary gland carcinoma; genetic susceptibility; DNA repair; case-control study

Introduction

Salivary gland carcinoma (SGC) is a rare malignant tumor that accounts for 11.6% of head and neck cancers and 0.3% of cancers overall in the United States.¹ SGC is a

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morphologically and clinically diverse tumor type, encompassing more than 20 distinct histological subtypes, the most common of which are adenoid cystic carcinoma, mucoepidermoid carcinoma, and acinic cell carcinoma.^{1, 2} Exposure to ionizing radiation is the only established environmental risk factor for SGC.^{3, 4} Other potential risk factors have been implicated but remain unconfirmed, including smoking,^{5, 6} alcohol drinking,^{6, 7} hormonal factors,⁸ and diet.⁹

A number of subtypes of SGC have been associated with recurrent chromosomal abnormalities and resulting gene fusion.¹⁰ The somatic translocation t(11;19)(q21;p13) resulting in *MAML2-CRTC1* fusion has been documented in 80% of mucoepidermoid carcinomas, and has been significantly associated with ionizing radiation exposure.¹¹ The t(6;9)(q22-23;p23-24) translocation resulting in *MYBNFIB* fusion has been identified in 28% of primary and 35% of metastatic adenoid cystic carcinomas.¹² DNA double-strand breaks are required for creation of translocations. Therefore, it is biologically plausible that the individual efficiency of double-strand break repair pathways may affect the risk of somatic translocations and subsequent cancer risk. In support of this concept, we previously found reduced DNA repair capacity in SGC patients compared to cancer-free controls.¹³

The breast cancer 1, early onset (*BRCA1*) gene is a multifunctional tumor suppressor gene that is directly involved in the DNA double-strand break repair pathways.¹⁴ The importance of *BRCA1* in human cancers and the role of *BRCA1* in maintenance of genome stability have been well established. Previous studies have shown higher levels of chromosomal aberrations and chromosomal breaks in *BRCA1* mutation carriers than noncarriers.^{15, 16} Inherited genetic polymorphisms of *BRCA1* have been proposed to affect individual susceptibility to breast and ovarian cancer.¹⁷⁻¹⁹ Common variants of *BRCA1* are therefore candidate alleles for low- or moderate-penetrance susceptibility to SGC.

The aim of this hospital-based case-control study was to test the hypothesis that germline variants in *BRCA1* are associated with SGC risk. In order to ensure the study's potential, our study was restricted to common single-nucleotide polymorphisms (SNPs) (minor allele frequency $\geq 5\%$). In this exploratory analysis, seven common functional *BRCA1* SNPs were selected and the frequencies of their genotypes and the associated haplotypes between SGC cases and cancer-free controls were compared.

Materials and Methods

Study Subjects

A case-control study was conducted at The University of Texas MD Anderson Cancer Center. Case subjects were recruited between November 1999 and October 2008. Subjects were recruited prior to definitive surgery or any chemotherapy and/or radiation therapy, and the final diagnosis was confirmed through histopathological examination. Control subjects were cancer-free visitors to our institution who were recruited between November 1996 and March 2005 as controls for a molecular epidemiologic study of head and neck squamous cell carcinoma. Criteria for recruitment were the same for all subjects and were as follows: 1) at least 18 years of age; 2) no blood transfusion in the past 6 months; 3) no prior malignancy (except nonmelanoma skin cancer); and 4) not taking immunosuppressant medications. The study was approved by the Institutional Review Board.

After informed consent was obtained, each subject donated 20 ml of blood for laboratory analysis. A self-administrated questionnaire was used to collect information on demographic characteristics, history of exposure to possible carcinogens, and family history of cancer. Race and ethnicity were self-reported and categorized as non-Hispanic white or other. Subjects who had smoked more than 100 cigarettes in their lifetimes were classified as ever

smokers. Subjects who had used alcohol at least once a week for more than one year were classified as ever drinkers. Radiation exposure was defined as a history of radiotherapy for the treatment of any disease or condition, except for the treatment for the current illness. Clinicopathological information was obtained from the medical records.

BRCA1 SNP Selection and Genotype Analysis

Seven *BRCA1* functional SNPs with a minor allele frequency of 5% or greater were selected from SNP500Cancer.²⁰ A31875G (rs1799950), C33420T (rs799917), A33921G (rs16941), and A34356G (rs16942) are located within exon 11 that leads to amino-acid changes; T43893C (rs1060915) and A55298G (rs1799966) are located within exons 13 and 16, respectively; and A1988G is located within the promoter region. The genotypes of these seven SNPs were analyzed by polymerase chain reaction–restriction fragment length polymorphism assay using primers and methods published previously.²¹ Genotyping was performed by laboratory personnel blinded to case-control status, and a randomly selected subset consisting of 10% of the samples was subjected to duplicate genotyping.

Statistical Analysis

The observed genotype distributions in controls were tested for deviation from Hardy-Weinberg equilibrium using the chi-square test. Pairwise linkage disequilibrium measure D' was calculated between each pair of SNPs among controls.²²

Frequencies of demographic characteristics and environmental exposures in cases and controls were compared using the chi-square test. For genotype analysis, the Akaike information criterion was used to determine the best inheritance model. To calculate the odds ratios (ORs) and 95% CIs for the association between *BRCA1* genotypes and SGC risk, unconditional logistic regression analyses adjusted for age and sex were performed. In addition, number of minor alleles was coded as a continuous variable in a co-dominant model and was fitted in the regression model to test for an allele dose effect. The impact of other potential risk factors were also evaluated, but their inclusion in the model did not change the OR by 10% or more, and therefore these factors were not included in the final model.

In addition, genotype analyses stratified by age (<54 years and ≥54 years [median age]), sex (male, female), race and ethnicity (non-Hispanic white, other), smoking (never, ever), alcohol drinking (never, ever), and first-degree family history of cancer (yes, no) were conducted to examine the possible interactions between these factors and *BRCA1* SNPs. For the significance of interaction, a likelihood ratio test was used to compare the full model including the interaction term with the model containing only the main effects.

Haplotype frequencies were estimated using the expectation maximization algorithm in SNPStats.²³ Haplotypes with less than 3% frequency in both cases and controls were defined as rare; rare haplotypes were pooled. Unconditional logistic regression analysis, adjusted for age and sex, was used to calculate haplotype-specific ORs and corresponding 95% CIs.

To evaluate the potential for false-positive results due to multiple testing, two different approaches were applied: 1) adjust the p value using the Bonferroni correction and 2) calculate the false-positive report probability (FPRP) proposed by Wacholder et al.²⁴ FPRP was evaluated by incorporating the prior probability and the observed ORs and 95% CIs. The same value of prior probability was assumed as a recent publication²⁵—that is, a prior probability of 0.25 when the biological plausibility is high and existing epidemiological data are fair, a prior probability of 0.1 when the biological plausibility is high but existing epidemiological data are poor, and a prior probability of 0.01 when both are poor. An FPRP

value of <0.5 was considered to reflect a noteworthy association, and a value of <0.2 was considered to reflect a particularly noteworthy association. All statistical tests were two-sided, and $p<0.05$ was considered statistically significant. Statistical analysis was performed using SAS software, version 9.2 (SAS Institute Inc., Cary, NC).

Results

The study included 156 case patients with incident SGC and 511 cancer-free control subjects. Characteristics of the cases and controls are shown in Table 1. The median age at diagnosis for cases was 54 years (interquartile range, 45–65 years). The median age at recruitment for controls was 48 years (interquartile range, 42–56 years). Cases were more likely than controls to report a history of exposure to medical radiation ($p=0.024$), but most subjects did not have a medical radiation exposure history. The most common histological subtypes of SGC, in descending order of frequency, were adenoid cystic carcinoma (58/156, 37.2%), mucoepidermoid carcinoma (44/156, 28.2%), adenocarcinoma (14/156, 9.0%), and acinic cell carcinoma (11/156, 7.1%).

Genotypic distributions in controls were in accordance with Hardy-Weinberg equilibrium for all seven SNPs ($p>0.05$). The allele and genotype frequencies and corresponding genotype-specific risks of SGC are presented in Table 2. For all SNPs, the best inheritance model was a dominant model. A34356G and T43893C were associated with reduced SGC risk ($p=0.018$ and $p=0.002$, respectively); pairwise linkage disequilibrium analysis showed that these two SNPs were in strong correlation ($D'>0.90$). In a stepwise logistic regression including all seven SNPs and with entry and stay criteria set to $p=0.1$, only T43893C ($p=0.002$, dominant model) remained in the final model. Using a trend test for association, the T43893C was significantly associated with SGC ($p_{trend}=0.009$), and the TC/CC genotype was significantly associated with a 45% reduction of SGC risk (adjusted OR=0.55, 95% CI: 0.38–0.80), which remained significant after correction for multiple testing (Bonferroni-adjusted $p=0.011$). The association between A34356G (dominant model) and SGC risk disappeared after correction for multiple testing (Bonferroni-adjusted $p=0.126$).

Among SGC cases, there was no significant difference in genotype distributions of the investigated SNPs by histological subtype (chi-square test, data not shown). After adjustment for age and sex, T43893C TC/CC genotype was inversely associated with risk of adenoid cystic carcinoma (adjusted OR=0.59, 95% CI: 0.34–1.04, $p=0.068$), mucoepidermoid carcinoma (adjusted OR=0.45, 95% CI: 0.24–0.84, $p=0.013$), and adenocarcinoma (adjusted OR=0.20, 95% CI: 0.06–0.63, $p=0.006$), but only the last association remained significant after correction for multiple testing (Bonferroni-adjusted $p=0.042$).

The genotype-specific risks stratified by age, sex, race and ethnicity, first-degree family history of cancer, smoking status, and alcohol drinking status are shown in Table 3. All investigated SNPs except A31875G were associated with a statistically significant reduction of SGC risk in women and in subjects who reported a family history of cancer in first-degree relatives. These subgroup genotype-specific risks were somewhat stronger than the genotype-specific risks in overall sample. Significant interactions were detected between first-degree family history of cancer and SNPs A1988G, C33420T, A34356G, and T43893C ($p<0.05$). T43893C alone interacted with race and ethnicity ($p=0.014$).

Thirty-one different *BRCA1* haplotypes were found, and five haplotypes accounted for more than 90% of all haplotypes. Haplotype analysis revealed that the *BRCA1* gene was significantly associated with SGC risk (global association $p=0.013$), particularly in non-Hispanic whites (global association $p<0.001$). The age- and sex-adjusted haplotype-specific

risks are shown in Table 4. Overall, haplotypes 2 (GATGGCG) and 3 (AACAACA) were significantly associated with 31% and 61% reduction of SGC risk, respectively. In non-Hispanic whites, haplotypes 2 and 3 were significantly associated with 39% and 73% reduction of SGC risk, respectively.

FPRP estimates for the selected associations that were statistically significant ($p < 0.05$) under different prior probabilities are shown in Table 5. The FPRP estimates were below 0.5 given a prior probability of 0.1 for the two SNPs and two haplotypes. The most robust findings were for T43893C in non-Hispanic whites and individuals with a family history of cancer; the associations showed FPRP estimates below 0.2 given a prior probability below 0.001.

Discussion

BRCA1 is a critical component in maintenance of genomic stability and response to radiation-induced DNA damage. Previous findings from our group showed that apoptosis capacity and DNA repair capacity in response to gamma irradiation were significantly reduced in patients with SGC.^{13, 26} Experimental data revealed that BRCA1 modulates cellular susceptibility to apoptosis induced by gamma radiation,²⁷ and *BRCA1* mutations slow the rate of repair of radiation-induced double-strand breaks.²⁸ *BRCA1* is expressed in both acinar and ductal epithelial cells in the salivary gland, and its expression is correlated with proliferation.²⁹ In the current study, common functional polymorphisms in the *BRCA1* gene were examined to test our hypothesis that inherited variations in the *BRCA1* gene modulate individual susceptibility to SGC. To our knowledge, this is the first report to investigate common SNPs in the *BRCA1* gene in SGC patients.

In this study, a common functional SNP in the *BRCA1* gene, T43893C, was significantly associated with reduction of SGC risk, which was more pronounced in women, non-Hispanic whites, and most interestingly, individuals with a family history of cancer in first-degree relatives. In addition, two haplotypes, both carrying the minor allele of T43893C, were inversely associated with SGC risk, and the associations were more pronounced in non-Hispanic whites. T43893C (rs1060915, Ser1436Ser) is a synonymous SNP located next to a highly conserved coiled-coil motif (aa 1391-1424) in the AD1 domain which could activate *BRCA1* transcription.³⁰ Analysis of allele-specific expression showed that T43893C accounts for approximately 1.5-fold change in mRNA levels, and moreover, the allelic imbalance determined by the T-to-C ratios was significantly associated with risk of familial ovarian cancer and breast cancer.^{31, 32} Although synonymous SNPs have often been assumed to have no functional consequence, emerging evidence suggest such SNPs could affect protein activity by, for example, altering splicing and/or mRNA stability.³³ Another possibility is that T43893C may be in linkage disequilibrium with one or more nonsynonymous SNPs or promoter SNPs that exhibit functional effect. In our study, T43893C heterozygote and homozygote minor allele carriers had approximately half the SGC risk of individuals with the wild-type alleles. This result is intriguing because susceptibility to SGC is expected to be mediated through genetic variations in many genes, and each individual gene or variation is likely to contribute only a low or moderate impact on risk. The association appears to be consistent across SGC histological subtypes, and remains nominally significant for mucoepidermoid carcinoma. Although the association was more significant for the adenocarcinoma subtype, the potential mechanisms of such an association are more difficult to explain for adenocarcinoma is a group of tumors with different histogenesis and morphogenesis, and thus great diversity in genetics.

Because seven SNPs were tested for association with SGC and numerous analyses were performed, there exists a potential for false-positive results. In this study, the

nonsynonymous SNP, A34356G, was nominally significantly associated with reduced SGC risk. After applying Bonferroni correction, however, significance for this association disappeared. The Bonferroni correction is often seen as too conservative for SNP studies because it assumes independence between tests whereas many SNPs are in linkage disequilibrium and therefore correlated with each other. In our study, four SNPs in exon 11 and two SNPs in exon 13 and 16 were in linkage disequilibrium ($D' > 0.80$, data not shown). Thus, the FPRP approach was also applied. FPRP is the estimated probability that a significant result is false positive.²⁴ Given the absence of previous epidemiological data on *BRCA1* SNPs and SGC risk, a high prior probability of 0.25 is excluded. For A34356G, FPRP was 25.7% and 79.2% at a prior probability of 0.1 and 0.01, respectively, suggesting that this association is unlikely to be a false-positive finding but may not be noteworthy to replicate in other molecular epidemiological studies. However, our finding that T43893C was associated with SGC risk, particularly in non-Hispanic whites and individuals with a family history of cancer, was noteworthy at all the prior probabilities included in the analysis. Functional assays are needed to elucidate the biological significance of this *BRCA1* genetic variant.

An intriguing finding of this study was the interaction between *BRCA1* SNPs and family history of cancer. Significant or marginally significant interactions were detected between all *BRCA1* SNPs except A31875G and family history of cancer. These SNPs exhibited a stronger protective effect in individuals with a family history of cancer than in those without. A family history of cancer is a potential indicator of inherited cancer susceptibility and of shared environmental exposures and/or behaviors that are risk factors for cancer. A study comparing healthy individuals with and without a family history of cancer showed that a family history of cancer, not confined to a history of cancer in any specific organ, was associated with reduced DNA repair synthesis.³⁴ It is not clear what mechanisms are behind the DNA repair deficiency in individuals with a family history of cancer. DNA repair deficiency could be related to inherited individual differences in genes directly implicated in the DNA repair system or to metabolic differences that could suppress the normal DNA repair process.³⁴ An alternative possibility is that the individual defense system may be damaged by the attack of environmental risk factors that are shared within families. Either inherited or environmental factors may interact with *BRCA1* to play a complex role in cancer susceptibility.

Our study has several limitations. Both cases and controls were recruited at a single cancer center, and the population was primarily non-Hispanic whites; thus, our results are not generalizable to other racial and ethnic groups. In addition, the possibility of selection bias should be considered because of the limitation inherent in the case-control design. Viewed from a different perspective, the cancer patients' recruitment at a cancer center is an advantage of our study since diagnosis of SGC has often proved problematic because of the rarity of SGC and complex classification of tumor based on histopathology.

In conclusion, our results suggest for the first time that polymorphic *BRCA1* confers a protective effect against SGC risk. The significance of the reduced risk of SGC associated with *BRCA1* SNPs, particularly T43893C, the observed interaction between *BRCA1* SNPs and a family history of cancer, and the known importance of *BRCA1* in the DNA double-strand break repair pathways may indicate a true association. However, further confirmation in different populations with larger sample sizes is needed before definitive conclusions can be drawn.

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Table 1

Demographic and exposure characteristics of controls and SGC cases.

Variable	Controls (No. = 511)		Cases (No. = 156)		p [*]
	No.	(%)	No.	(%)	
Age, years					<0.001
<45	177	(34.6)	39	(25.0)	
45–65	289	(56.6)	81	(51.9)	
>65	45	(8.8)	36	(23.1)	
Sex					0.169
Male	245	(47.9)	65	(41.7)	
Female	266	(52.1)	91	(58.3)	
Race and ethnicity					0.943
Non-Hispanic white	401	(78.5)	122	(78.2)	
Other	110	(21.5)	34	(21.8)	
First-degree family history of cancer [†]					0.334
Yes	257	(51.4)	86	(55.8)	
No	243	(48.6)	68	(44.2)	
Smoking status [†]					0.307
Ever	213	(42.1)	72	(46.8)	
Never	293	(57.9)	82	(53.2)	
Alcohol drinking status [†]					0.764
Ever	246	(48.6)	77	(50.0)	
Never	260	(51.4)	77	(50.0)	
Radiation exposure [†]					0.024
No	504	(98.6)	147	(95.5)	
Yes	7	(1.4)	7	(4.5)	

* Chi-square analysis comparing case subjects to control subjects.

[†]Totals are less than the total number of patients because of missing data.

Table 2

BRCA1 allele and genotype frequencies in controls and SGC cases.

SNP	Minor allele frequency		Genotype	Controls (No. = 511)		Cases (No. = 156)		<i>p</i>	Adjusted OR* (95% CI)	<i>P_{trend}</i>
	Controls	Cases		No.	(%)	No.	(%)			
A1988G	0.33	0.29	AA	227	(44.4)	82	(52.6)	0.105	1.00	0.176
			AG	230	(45.0)	56	(35.9)		0.65 (0.44–0.97)	
			GG	54	(10.6)	18	(11.5)		0.87 (0.48–1.59)	
A31875G			AG/GG	284	(55.6)	74	(47.4)	0.052	0.70 (0.48–1.00)	
	0.06	0.08	AA	455	(89.0)	132	(84.6)	0.138	1.00	-
			AG	56	(11.0)	24	(15.4)		1.49 (0.88–2.52)	
C33420T			GG	0	(0.0)	0	(0.0)		-	
	0.39	0.35	CC	198	(38.8)	71	(45.5)	0.315	1.00	0.175
			CT	226	(44.2)	62	(39.7)		0.75 (0.51–1.12)	
A33921G			TT	87	(17.0)	23	(14.7)		0.74 (0.43–1.28)	
			CT/TT	313	(61.2)	85	(54.5)	0.129	0.75 (0.52–1.09)	
	0.33	0.29	AA	230	(45.0)	81	(51.9)	0.231	1.00	0.106
A34356G			AG	227	(44.4)	61	(39.1)		0.74 (0.50–1.09)	
			GG	54	(10.6)	14	(9.0)		0.69 (0.36–1.32)	
	0.33	0.28	AG/GG	281	(55.0)	75	(48.1)	0.089	0.73 (0.50–1.05)	
T43893C			AA	222	(43.4)	84	(53.9)	0.059	1.00	0.047
			AG	240	(47.0)	58	(37.2)		0.63 (0.43–0.93)	
			GG	49	(9.6)	14	(9.0)		0.70 (0.37–1.36)	
A55298G			AG/GG	289	(56.6)	72	(46.1)	0.018	0.64 (0.45–0.93)	
	0.41	0.34	TT	167	(32.7)	71	(45.5)	0.007	1.00	0.009
			TC	267	(52.2)	65	(41.7)		0.54 (0.36–0.80)	
			CC	77	(15.1)	20	(12.8)		0.58 (0.33–1.04)	
			TC/CC	344	(67.3)	85	(54.5)	0.002	0.55 (0.38–0.80)	
	0.32	0.29	AA	229	(44.8)	80	(51.3)	0.285	1.00	0.162
			AG	233	(45.6)	62	(39.7)		0.74 (0.50–1.09)	
			GG	49	(9.6)	14	(9.0)		0.76 (0.39–1.47)	
			AG/GG	282	(55.2)	76	(48.7)	0.114	0.74 (0.52–1.07)	

*Adjusted for age and sex.

Table 3

Stratification analysis of SGC risk associated with *BRCA1* genotypes.*

	A1988G	A31875G	C33420T	A33921G	A34356G	T43893C	A55298G
Age							
54 years	0.65 (0.40–1.05)	1.92 (1.00–3.69)	0.73 (0.45–1.19)	0.74 (0.46–1.21)	0.57 (0.35–0.92)	0.57 (0.35–0.93)	0.69 (0.42–1.12)
> 54 years	0.77 (0.44–1.35)	1.03 (0.43–2.45)	0.80 (0.46–1.41)	0.71 (0.41–1.24)	0.78 (0.45–1.36)	0.50 (0.28–0.90)	0.84 (0.48–1.46)
<i>P</i> _{interaction}	0.632	0.257	0.795	0.908	0.399	0.744	0.595
Sex							
Female	0.52 (0.32–0.85)	1.19 (0.57–2.47)	0.57 (0.35–0.93)	0.60 (0.37–0.98)	0.50 (0.30–0.81)	0.44 (0.27–0.72)	0.59 (0.36–0.97)
Male	1.01 (0.58–1.76)	1.94 (0.90–4.15)	1.08 (0.62–1.90)	0.94 (0.54–1.63)	0.90 (0.52–1.57)	0.73 (0.41–1.30)	1.00 (0.57–1.74)
<i>P</i> _{interaction}	0.079	0.365	0.091	0.234	0.113	0.192	0.167
Race and ethnicity							
Non-Hispanic white	0.63 (0.41–0.95)	1.58 (0.90–2.77)	0.66 (0.44–1.00)	0.67 (0.44–1.01)	0.58 (0.39–0.89)	0.43 (0.28–0.66)	0.70 (0.46–1.06)
Other	0.98 (0.45–2.15)	1.23 (0.23–6.73)	1.11 (0.43–2.87)	0.98 (0.45–2.15)	0.91 (0.41–1.98)	1.32 (0.59–2.93)	0.91 (0.41–1.98)
<i>P</i> _{interaction}	0.331	0.777	0.326	0.396	0.333	0.014	0.575
First-degree family history of cancer							
Yes	0.50 (0.30–0.82)	1.33 (0.63–2.80)	0.50 (0.30–0.83)	0.54 (0.32–0.89)	0.46 (0.28–0.76)	0.35 (0.21–0.58)	0.54 (0.33–0.89)
No	1.06 (0.61–1.84)	1.82 (0.86–3.86)	1.30 (0.73–2.32)	1.06 (0.61–1.84)	1.03 (0.59–1.79)	0.97 (0.55–1.72)	1.12 (0.64–1.94)
<i>P</i> _{interaction}	0.047	0.560	0.014	0.074	0.032	0.009	0.056
Smoking status							
Never	0.52 (0.31–0.87)	1.76 (0.87–3.55)	0.66 (0.40–1.09)	0.60 (0.37–1.00)	0.49 (0.30–0.82)	0.46 (0.28–0.76)	0.58 (0.35–0.96)
Ever	0.97	1.27	0.88	0.90	0.92	0.67	0.99

	A1988G	A31875G	C33420T	A33921G	A34356G	T43893C	A55298G
	(0.56–1.67)	(0.57–2.84)	(0.50–1.52)	(0.52–1.56)	(0.53–1.58)	(0.38–1.18)	(0.57–1.71)
<i>P</i> _{interaction}	0.109	0.639	0.488	0.296	0.110	0.311	0.171
Alcohol drinking status							
Never	0.55 (0.32–0.92)	1.94 (0.93–4.06)	0.72 (0.42–1.21)	0.69 (0.41–1.15)	0.51 (0.30–0.86)	0.53 (0.31–0.91)	0.62 (0.37–1.05)
Ever	0.90 (0.53–1.52)	1.22 (0.57–2.63)	0.82 (0.48–1.38)	0.79 (0.47–1.34)	0.87 (0.52–1.47)	0.57 (0.34–0.98)	0.91 (0.54–1.53)
<i>P</i> _{interaction}	0.188	0.397	0.723	0.692	0.151	0.851	0.318

* Values in table are adjusted ORs (95% CIs), under the dominant inheritance model, using wild-type as the reference group, adjusted for age and sex.

Table 4

Frequency of haplotypes in *BRCA1* gene and association with risk of SGC.

Haplotype	SNPs*							Frequency		p	Adjusted OR [†] (95% CI)
	1	2	3	4	5	6	7	Cases (No. = 156)	Controls (No. = 511)		
Overall											
1	A	A	C	A	A	T	A	0.516	0.446	-	1.00
2	G	A	T	G	G	C	G	0.253	0.294	0.026	0.69 (0.50–0.96)
3	A	A	C	A	A	C	A	0.037	0.076	0.007	0.39 (0.20–0.77)
4	A	A	T	A	A	T	A	0.046	0.065	0.229	0.72 (0.43–1.23)
5	A	G	C	A	A	T	A	0.063	0.045	0.395	1.30 (0.71–2.38)
Others [‡]	*	*	*	*	*	*	*	0.085	0.074	0.903	1.03 (0.63–1.66)
Non-Hispanic whites											
1	A	A	C	A	A	T	A	0.568	0.467	-	1.00
2	G	A	T	G	G	C	G	0.246	0.300	0.009	0.61 (0.43–0.88)
3	A	A	C	A	A	C	A	0.035	0.096	0.001	0.27 (0.12–0.58)
5	A	G	C	A	A	T	A	0.075	0.046	0.358	1.36 (0.70–2.62)
Others [‡]	*	*	*	*	*	*	*	0.076	0.091	0.235	0.71 (0.40–1.25)

* SNPs are as follows: 1, A1988G; 2, A31875G; 3, C33420T; 4, A33921G; 5, A34356G; 6, T43893C; and 7, A55298G.

[†] Adjusted for age and sex.

[‡] Rare haplotypes with frequencies <0.03.

Table 5

False-positive report probability for the results of SGC association analysis.

SNP or haplotype	OR (95% CI)	Prior probability			
		0.25	0.1	0.01	0.001
SNP*					
A34356G, overall	0.64 (0.45–0.93)	0.104	0.257	0.792	0.975
A34356G, female	0.50 (0.30–0.81)	0.028	0.080	0.490	0.907
A34356G, non-Hispanic white	0.58 (0.39–0.89)	0.071	0.185	0.715	0.962
A34356G, (+) family history of cancer	0.46 (0.28–0.76)	0.014	0.042	0.325	0.829
T43893C, overall	0.55 (0.38–0.80)	0.011	0.031	0.259	0.779
T43893C, female	0.44 (0.27–0.72)	0.007	0.019	0.177	0.684
T43893C, non-Hispanic white	0.43 (0.28–0.66)	0.001	0.002	0.022	0.184
T43893C, (+) family history of cancer	0.35 (0.21–0.58)	<0.001	<0.001	0.009	0.085
Haplotype					
Haplotype 2, overall	0.69 (0.50–0.96)	0.142	0.332	0.846	0.982
Haplotype 2, non-Hispanic white	0.61 (0.43–0.88)	0.047	0.129	0.619	0.943
Haplotype 3, overall	0.39 (0.20–0.77)	0.039	0.107	0.569	0.930
Haplotype 3, non-Hispanic white	0.27 (0.12–0.58)	0.005	0.014	0.135	0.612

* Dominant model.