Original Article

Single-Nucleotide Polymorphism Associations for Colorectal Cancer in Southern Chinese Population

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DOI: 10.1007/s11670-012-0029-7

ABSTRACT

Objective: Genome-wide association studies (GWAS) have identified 11 loci that influence the risk of developing colorectal cancer (CRC). Given that these studies were conducted in European Caucasian populations, it is not clear whether the results are relevant for populations with different ethnicities. The aim of this study was to examine these associations in a southern Chinese population.

Methods: Eleven single-nucleotide polymorphisms (SNPs), rs12701937, rs16892766, rs7014346, rs6983267, rs719725, rs10795668, rs3802842, rs4444235, rs9929218, rs10411210, and rs961253, were genotyped in 229 CRC patients and 267 controls using the MassArray SNP genotyping system.

Results: Evidence of an association with CRC was found for four of the 11 loci. The strongest associations were with rs4444235 and rs961253, with significant odds ratios close to those reported in previous GWAS. Among these four loci, rs719725 and rs4444235 were significantly associated with female gender, rs3802842, rs961253, and rs4444235 with early disease onset, and rs3802842 with later disease onset. However, no associations with CRC risk were detected for six other loci (rs9929218, rs10411210, rs12701937, rs7014346, rs6983267, and rs10795668), and one SNP, rs16892766, was not polymorphic in any of the study participants.

Conclusion: The rs4444235 and rs961253 loci are strongly associated with the risk of CRC in southern Chinese.

Key words: Colorectal cancer; SNP; Association study; Chinese

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer and the fourth leading cause of cancer death worldwide. CRC is also known to aggregate in families, with the disease being two to three times more common among the first-degree relatives of these patients than among population controls. Nonetheless, the majority of CRC cases (up to 80%) are sporadic[1], indicating that both genetic and environmental factors contribute to the development of the disease. Unlike single-gene disorders, the genetic factors in CRC include many loci with relatively small effects, and few, if any, are absolutely required for the occurrence of CRC. However, most of the underlying susceptibility loci for CRC remain unknown.

In recent years, genome-wide association studies (GWAS) have emerged as a powerful new approach to identify susceptibility loci. By utilizing genotyping platforms that can type hundreds to thousands single-nucleotide polymorphisms (SNPs) simultaneously, GWAS can be conducted using sets of SNPs that tag the most widely known common variants in the genome and hence scan for associations independent of a gene's function or chromosomal position[2].

Over the last three years, a number of GWAS have examined CRC[3-7], identifying numerous loci associated with the disease. A meta-analysis based on GWAS also identified four new susceptibility loci for CRC[8]. However, all of these studies were conducted in Caucasians, with only one replicated in a Japanese population[5]. More recently, the association between

Received 2011–08–13; **Accepted** 2011–10–17
This work was supported by a grant from the Key Programs for Science and Technology Development of Guangzhou (No. 2008A1-E4151).
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several loci identified in GWAS for CRC was confirmed in a northern Han Chinese population[9] and in Hong Kong Chinese[10]. Here, we examined the impact of 11 previously reported loci in a Han Chinese population in Jiangxi Province, Southern China. Our results indicate that the rs4444235 and rs961253 loci are strongly associated with the risk of CRC in southern Chinese.

MATERIALS AND METHODS

Subjects

All subjects in this study comprised 229 CRC patients and 267 controls. Both groups were Han Chinese from Southern China. Patients were recruited from the First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi Province, China. All patients were histologically confirmed to have CRC. The mean age at diagnosis was 54.02 years (range 19–82 years); 60% were men, and 40% were women. Controls were cancer-free individuals randomly selected from the hospital's outpatients. The mean age was 56.43 years (range 19–82 years); 59% were men, and 41% were women. The details of the patients and controls are listed in Table 1. The study was approved by the Southern Medical University Ethics Committee, and written informed consent was obtained from all participants.

SNPs Selection

Eleven of the most significantly associated SNPs were selected, seven loci (rs12701937, rs16892766, rs7014346, rs6983267, rs719725, rs10795668, and rs3802842) had been identified in GWAS[3-10] and four (rs4444235, rs9929218, rs10411210, and rs961253) in meta-analyses based on GWAS data[11]. Table 2 shows these loci and the respective references.

Table 1. Characteristics of CRC cases and controls

Indices	CRC cases (*n*=229) (*n*, %)	Controls (*n*=267) (*n*, %)	*P**
Sex			
Male	134 (58.5)	159 (59.6)	0.971
Female	95 (41.5)	108 (40.4)	
Age (year)			
<50	76 (33.2)	85 (31.8)	0.047
50–59	68 (29.7)	50 (18.7)	
60–69	48 (21.0)	68 (25.5)	
≥70	37 (16.2)	64 (24.0)	
Site of tumor			
Colon	92 (40.2)		
Rectum	107 (46.7)		
Other and unknown	30 (13.1)		
Duke's stage			
A	6 (2.6)		
B	85 (37.1)		
C	72 (31.4)		
D	11 (4.8)		
Unknown	55 (24.0)		

*Two sides χ^2 test

Table 2. Eleven SNPs and their minor allele frequencies (MAFs) in southern Chinese population

SNP	Chromosome	Major allele	Minor allele	MAF control	MAF cases
rs12701937[3]	7p14.1	C	T	0.325	0.318
rs16892766[4]	8q23.3	A	C	-	-
rs7014346[5]	8q24	G	A	0.336	0.336
rs6983267[6]	8q24.21	T	G	0.441	0.460
rs719725[7]	9p24	A	C	0.329	0.283
rs10795668[4]	10p14	G	A	0.338	0.334
rs3802842[5]	11q23	A	C	0.430	0.444
rs4444235[8]	14q22.2	T	C	0.468	0.553
rs9929218[8]	16p22.1	G	A	0.193	0.176
rs10411210[8]	19q13.1	C	T	0.180	0.169
rs961253[8]	20p12.3	C	A	0.180	0.169

Table 3. PCR amplification and extension primers for each SNPs

SNP ID	Primer	Primer sequence
rs12701937	Forward	ACGTTGGATGTGACAATATAAGGCCAAGGG
	Reverse	ACGTTGGATGTTATTTCAACCATCGAAGCC
	Extension	ATATTGGTATTAGCAGGAATG
rs16892766	Forward	ACGTTGGATGGGGTGACATAAGGCATAACC
	Reverse	ACGTTGGATGCTACTTAGGGACTCAGAACG
	Extension	CTAAGGCATAACCTTTAACAGC
rs7014346	Forward	ACGTTGGATGCCTTCTAGCCTACAACATGG
	Reverse	ACGTTGGATGGCAGCTTCTGCCTAATGTTG
	Extension	CTTCTAGCCTACAACATGGATGTAA
rs6983267	Forward	ACGTTGGATGTCATCGTCCTTTGAGCTCAG
	Reverse	ACGTTGGATGCTCCCTCCCCCACATAAAAT
	Extension	AGCTCAGCAGATGAAAG
rs719725	Forward	ACGTTGGATGCTCTTAGTGAAGTTTGACAG
	Reverse	ACGTTGGATGCTGGAGAAATGATGATTATG
	Extension	AACTAATGTTTATTGATGCTATC
rs10795668	Forward	ACGTTGGATGCTATGAGCAGCAGCAGAAAG
	Reverse	ACGTTGGATGAATACTTGTACCTTGGTGGG
	Extension	GAAAGAGAAAAAGTTAGATTCTTA
rs3802842	Forward	ACGTTGGATGTATGTACAGCCCTTGCAGAC
	Reverse	ACGTTGGATGCACAGATGCTATCCTGGAAG
	Extension	TCCCCTGCAGACCCATAGAAAATCT
rs4444235	Forward	ACGTTGGATGTTTGGACATGATGCCCACAG
	Reverse	ACGTTGGATGTTGTGAGAAAGTTGGCTGGG
	Extension	CCACAGCCCTGATACTA
rs9929218	Forward	ACGTTGGATGGAATCTGGCTGCAAAAACAC
	Reverse	ACGTTGGATGGGTTTCCAGATCTTGTTCTTG
	Extension	CTGCAAAAACACAGGAAAGC
rs10411210	Forward	ACGTTGGATGTGTCAGAGGAAACCCTGAAG
	Reverse	ACGTTGGATGGAGCGGAGCTTGGCAAAATG
	Extension	CCAAGCACCAACGGTTTCCC
rs961253	Forward	ACGTTGGATGCCTTGATGCTCAGCAACTTC
	Reverse	ACGTTGGATGGATTGAAAGTGCATACCAAG
	Extension	CAACTTCAATTAATCTTTCTGAAT

Sample Genomic DNA (gDNA) Preparation and SNPs Detection

gDNA samples were extracted from the peripheral blood samples of all the participants using the Tiangen™ Genomic DNA Kit (Tiangen, China). The following primer sets, which included a pair of amplicon primers and an extension primer for each SNP, were designed using the Assay Design 3.1 software (Sequenom, San Diego, CA, USA) (Table 3). Genotyping was performed using the MassARRAY platform according to the manufacturer's instructions (Sequenom, San Diego, CA, USA). The genomic sequence containing the SNP is amplified by PCR, and the amplified product is cleaned using shrimp alkaline phosphatase (SAP) to neutralize any unincorporated dNTPs. Then a primer extension reaction was performed to introduce mass differences between alleles. Following the MassEXTEND reaction, SpectroCLEAN resin is added to the reaction mixture to remove extraneous salts that could interfere with matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry. The reaction mixture is then spotted onto a SpectroCHIP microarray and subjected to a Bruker Autoflex MALDI-TOF mass spectrometry. SpectroTYPER software identified the SNP-specific peaks according to their expected masses, and genotypes were assigned real-timely using Typer 3.1 software (Sequenom, San Diego, CA, USA).

Statistical Analysis

A significant departure of genotype frequency from Hardy-Weinberg equilibrium (HWE) for each SNP was estimated using Haploview 4.2 software (*www.broadinstitute.org/haploview/haploview*). Significant differences in genotype and allele frequency between CRC cases and controls were assessed using the χ^2 test for categorical variables. An association analysis based on unconditional binary logistic regression was carried out to determine the odds ratio (OR) and 95% confidence interval (95% CI) for each SNP, using age and sex as covariates. Stratified analysis by sex and age was also performed. All statistical analyses were carried out with SPSS 13.0 (SPSS Inc., Chicago, IL,

USA). The level of significance was set at $P<0.05$.

RESULTS

In this study, SNP rs16892766 was not found to be polymorphic in any of the participants, either patients or controls, and was thus excluded from further analysis. The genotype frequencies of the other 10 SNPs in patients and controls as well as the ORs and *P*-values are summarized in Table 4. Significant associations between two SNPs (rs4444235 and rs961253) and CRC risk were identified. SNP rs4444235, located at 14q22.2, showed a strong association, with an increased risk of CRC identified for homozygous, heterozygous, and allelic polymorphisms. For SNP rs961253, only two genotypes, CC and AC, were detected, similar to the Hapmap data reported for the Han Chinese population in Beijing. The increased risk of CRC associated with variant rs961253 was also confirmed, with a higher OR

Table 4. ORs for case-control study of 10 published CRC susceptibility loci

SNP	Genotypes	CRC cases (*n*, %)	Controls (*n*, %)	Adjusted OR (95% CI)	*P*
rs12701937	CC	108 (48.4)	124 (46.6)	1.00	
	CT	88 (39.5)	111 (41.7)	0.91 (0.62–1.34)	0.636
	TT	27 (12.1)	31 (11.7)	0.98 (0.55–1.76)	0.956
	CT+TT	115 (51.6)	142 (53.4)	0.93 (0.65–1.33)	0.927
	Allelic (T)			0.96 (0.74–1.26)	0.790
rs7014346	GG	99 (44.6)	119 (44.7)	1.00	
	GA	97 (43.7)	115 (43.2)	1.02 (0.69–1.49)	0.936
	AA	26 (11.7)	32 (12.0)	0.99 (0.55–1.78)	0.974
	GA+AA	123 (55.4)	147 (55.2)	1.01 (0.71–1.45)	0.956
	Allelic (A)			1.00 (0.77–1.31)	0.991
rs6983267	TT	71 (31.7)	74 (32.6)	1.00	
	GT	100 (44.6)	106 (46.7)	0.99 (0.65–1.53)	0.974
	GG	53 (23.7)	47 (20.7)	1.20 (0.71–2.00)	0.498
	GT+GG	153 (68.3)	153 (67.4)	1.06 (0.71–1.57)	0.794
	Allelic (G)			1.09 (0.84–1.42)	0.520
rs719725	AA	115 (51.6)	99 (44.0)	1.00	
	CA	90 (40.4)	104 (46.2)	0.71 (0.48–1.06)	0.091
	CC	18 (8.1)	22 (9.8)	0.70 (0.35–1.40)	0.316
	CA+CC	108 (48.5)	126 (56.0)	0.71 (0.49–1.04)	0.075
	Allelic (C)			0.79 (0.59–1.05)	0.106
rs10795668	GG	106 (47.5)	123 (46.4)	1.00	
	AG	85 (38.1)	105 (39.6)	0.96 (0.65–1.41)	0.819
	AA	32 (14.3)	37 (14.0)	0.96 (0.56–1.66)	0.887
	AG+AA	117 (52.4)	142 (53.6)	0.96 (0.67–1.37)	0.811
	Allelic (A)			0.97 (0.74–1.27)	0.829
rs3802842	AA	68 (32.9)	62 (30.0)	1.00	
	CA	94 (45.4)	112 (54.1)	0.74 (0.47–1.16)	0.189
	CC	45 (21.7)	33 (15.9)	1.24 (0.69–2.22)	0.470
	CA+CC	139 (67.1)	145 (70.0)	0.85 (0.55–1.30)	0.446
	Allelic (C)			1.05 (0.80–1.40)	0.717
rs4444235	TT	35 (16.3)	71 (26.7)	1.00	
	CT	122 (56.7)	141 (53.0)	1.82 (1.13–2.92)	0.014
	CC	58 (27.0)	54 (20.3)	2.27 (1.30–3.94)	0.004
	CT+CC	180 (83.7)	195 (73.3)	1.94 (1.23–3.06)	0.004
	Allelic (C)			1.43 (1.11–1.85)	0.006
rs9929218	GG	157 (69.8)	174 (65.2)	1.00	
	GA	57 (25.3)	83 (31.1)	0.77 (0.52–1.15)	0.203
	AA	11 (4.9)	10 (3.7)	1.78 (0.49–2.86)	0.716
	GA+AA	68 (30.2)	93 (33.8)	0.82 (0.56–1.19)	0.294
	Allelic (A)			0.89 (0.64–1.23)	0.484
rs10411210	CC	152 (68.5)	181 (68.0)	1.00	
	TC	65 (29.3)	74 (27.8)	1.05 (0.70–1.56)	0.830
	TT	5 (2.3)	11 (4.1)	0.55 (0.19–1.61)	0.274
	TC+TT	70 (31.6)	85 (31.9)	0.98 (0.67–1.44)	0.920
	Allelic (T)			0.92 (0.66–1.29)	0.643
rs961253	CC	179 (79.6)	230 (86.5)	1.00	
	AC	46 (20.4)	36 (13.5)	1.70 (1.05–2.76)	0.031
	Allelic (A)			1.62 (1.02–2.56)	0.040

Table 5. *P*-values from genotype stratified analysis of 10 CRC susceptibility loci

SNP/Loci phenotype	rs12701937	rs7014346	rs6983267	rs719725	rs10795668	rs3802842	rs9929218	rs10411210	rs961253	rs4444235
Sex										
Male	0.897	0.943	0.786	0.697	0.802	0.273	0.747	0.598	0.259	0.190
Female	0.390	0.921	0.357	0.003	0.928	0.354	0.214	0.572	0.053	0.001
Age of onset										
≤60 years	0.539	0.529	0.684	0.708	0.494	0.040	0.619	0.472	0.023	0.007
>60 years	0.174	0.258	0.070	0.054	0.444	0.028	0.529	0.950	0.294	0.577

Table 6. Stratified analysis for the six statistically significant results

SNP	Genotype	CRC cases (*n*, %)	Controls (*n*, %)	OR (95% CI)	*P*
rs719725	AA	57 (60.0)	33 (35.5)	1.00	
(Female)	CA	34 (35.8)	53 (57.0)	0.32 (0.17–0.61)	<0.001
	CC	4 (4.2)	7 (7.5)	0.30 (0.08–1.13)	0.075
rs4444235	TT	9 (10.3)	35 (32.7)	1.00	
(Female)	CT	53 (60.9)	45 (42.1)	4.94 (2.11–11.56)	<0.001
	CC	25 (28.7)	27 (25.2)	3.99 (1.57–10.15)	0.004
rs3802842	AA	36 (25.5)	31 (31.6)	1.00	
(age ≤60 years)	CA	69 (48.9)	55 (56.1)	1.05 (0.58–1.91)	0.877
	CC	36 (25.5)	12 (12.2)	2.42 (1.06–5.52)	0.035
rs961253	CC	124 (81.6)	128 (90.8)	1.00	
(age ≤60 years)	AC	28 (18.4)	13 (9.2)	2.29 (1.13–4.63)	0.022
rs4444235	TT	24 (16.7)	44 (31.2)	1.00	
(age ≤60 years)	CT	81 (56.3)	73 (51.8)	2.00 (1.11–3.61)	0.022
	CC	39 (27.1)	24 (17.0)	2.95 (1.45–6.02)	0.003
rs3802842	AA	32 (48.5)	31 (28.4)	1.00	
(age >60 years)	CA	25 (37.9)	57 (52.3)	0.44 (0.22–0.88)	0.020
	CC	9 (13.6)	21 (19.3)	0.46 (0.18–1.19)	0.110

of 1.70 (1.05–2.76) determined for the heterozygous state. Two other SNPs, rs719725, located on 9p24, and rs3802842, located at 11q23.1, showed weak trends, as reported previously[8]. By contrast, none of the other six SNPs (rs12701937, rs7014346, rs6983267, rs10795668, rs99292218, and rs10411210) was significantly associated with CRC risk.

Stratified analysis was performed on the 10 SNPs for sex and age of CRC onset (age ≤60 years and age >60 years). The *P*-values for the 10 SNPs are shown in Table 5. Six significant associations were found, two for female gender (>60 years), three for early onset (≤60 years), and one for later onset. SNPs rs719725 and rs4444235 were significantly associated with female gender (P=0.003 and 0.001, respectively), SNPs rs961253 and rs4444235 with early disease onset (P=0.023 and 0.007, respectively), and rs3802842 with both early and later disease onset (P=0.040 and 0.028, respectively). The details of these six significant results are presented in Table 6. Higher ORs and lower *P*-values were determined for SNP rs4444235 among females and early-onset patients; the same was true for SNP rs961253 among early-onset patients. The CA genotype of rs719725 had a highly significant protective effect in females (P<0.001). For SNP rs3802842, the CC genotype was associated with a risk effect in patients with early-onset disease (OR, 2.42; 95% CI, 1.06–5.52; P=0.035), whereas the CA genotype had a protective effect on those with later disease onset (OR, 0.44; 95% CI, 0.22–0.88; P=0.020).

DISCUSSION

Diseases of complex origin, in contrast to single-gene disorders, are caused by the concerted action of many genetic and environmental factors. Until several years ago, the candidate-gene approach was the only method available to identify potentially pathogenic genetic variants. GWAS, by which thousands of SNPs are tested for an association in hundreds or thousands of individuals, have revolutionized the search for these variants. However, GWAS have been criticized for the low effect size of the SNPs in contrast to their extremely significant *P*-values. Therefore, in addition to the discovery of rare variants, the re-validation of GWAS-derived SNPs in different populations and different diseases has become an important step. In the present study, we assessed the association of 11 previously reported SNPs with CRC susceptibility in a southern Chinese population.

Two statistically significant associations with CRC, both initially published in a meta-analysis of GWAS data[8], were identified: rs4444235 at 14q22.2 and rs961253 at 20p12.3. SNP rs4444235 is located 9.4 kb from the transcription start site of the gene encoding bone morphogenetic protein 4 preproprotein (BMP4). BMP4 is a member of the transforming growth factor-β family of signal-transduction molecules that plays an important role in CRC[11]. BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-β-catenin signaling[12]. SNP rs961253 maps to a region of 20p12.3, where there are no genes or predicted protein-encoding transcripts and no predicted genes or micro-RNAs in the vicinity. These two SNPs were also identified in studies of a northern Chinese population; however, whereas an association of rs961253 with CRC was confirmed, the association was only marginal for rs4444235[9].

For the loci rs719725 and rs3802842, no significant associations were detected in patients or controls, although stratifying associations based on sex and age were identified. An association of rs719725 with CRC was identified among female patients. This SNP was initially reported in cohorts from Canada, Newfoundland, Scotland, and France[7], and the association was later confirmed in cohorts from the USA, Canada, and Australia[13]. However, two subsequent studies failed to detect any association between CRC risk and rs719725[14, 15]. Our results suggest that there is a susceptibility locus among females that is not found in males. An association of CRC risk with SNP rs3802842 was first reported by Tenesa, et al.[5] and then confirmed by others[16-18]. In our study, this association was determined to be significant in patients with early disease onset (P=0.040) and even more so in those with later CRC onset (P=0.028).

No associations with a CRC risk were determined for six other loci (rs12701937, rs7014346, rs6983267, rs10795668, rs9929218, and rs10411210), and SNP rs16892766 was not polymorphic in any of the subjects, consistent with the results obtained in a northern Chinese population[9]. SNP rs12701937, located at 7p14.1, was recently reported to be associated with CRC[3], but the authors of that study were unable to replicate this result. No association with CRC risk was detected for either of two SNPs located on 8q24 (rs7014346 and rs6983267). An association between SNP rs7014346 and CRC risk, initially reported by Tenesa, et al.[5] and replicated in African and European Americans[19], was not detected in our subjects. The association of CRC with SNP rs6983267, also reported by Tenesa, et al.[5], was previously replicated in two Asian populations, one in Japan[20] and the other in northern China[9]. By contrast, this association was not significant in our subjects. For rs10795668, located on 10p14, there was no significant association with CRC risk, but its protective effect was similar to that described in previous studies[4, 10, 15]. Furthermore, this SNP was shown to be associated with CRC in a northern Chinese population[9] but not in our subjects. Additional studies are needed to characterize the role of rs10795668 in CRC. Neither SNP rs9929218 nor rs10411210, both chosen from a meta-analysis, was significantly associated with CRC in our study, consistent with the findings in Hong Kong and northern Chinese populations[9, 10].

In conclusion, despite the success of GWAS in identifying genetic associations of SNPs with complex traits, such as CRC risk, the results still need to be validated in different populations. This, in turn, will improve both understanding of the mechanisms of disease and the design of effective risk-assessment models. Results validated four previously identified SNP loci, showing evidence of an association with CRC in a southern Chinese population. The strongest associations were determined for rs4444235 and rs961253, with significant ORs close to those obtained in previous GWAS. Additional information on SNP associations with tumors may provide a basis for more effective cancer diagnosis, prognosis assessment, genetic determinations, and therapy.

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