Original Article

Genetic variants identified by GWAS was associated with colorectal cancer in the Han Chinese population

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ABSTRACT

Aim of Study: Colorectal cancer (CRC), now the third most common cancer across the world, is known to aggregate in families. Recently, genome-wide association studies have identified two single nucleotide polymorphisms (SNP) associated with CRC in Caucasians.

Materials and Methods: To validate whether the same variations conferred risk to CRC in the Han Chinese population, we genotyped 760 individuals (380 controls and 380 cases samples) recruited from the Han Chinese origin.

Results: We found rs11987193 in 8p12 ($P = 0.0472$ after correction, OR = 0.751) was significantly associated with CRC but rs12080929 in 1p33 ($P = 0.0650$ after correction, OR = 0.750) was not.

Conclusion: Our findings supported that rs11987193 is a susceptibility locus for CRC, and gene *DUSP4* was possible to play a role in the pathology of CRC.

KEY WORDS: Case-control study, colorectal cancer, DUSP4, TRABD2B gene

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer and the fourth-leading cause of cancer death worldwide.[1] Among all causes for CRC, inherited genetic factors account for approximately 35% of the disease etiology.[2] High penetrant mutations account for less than 5% of cases in the pathogenesis of CRC.[3] Mildly or moderately penetrant alleles could explain about 8.3% of etiology in cases with familial aggregation.[4,5] The remaining genetic risk for CRC may be attributable to multiple common and low penetrant alleles.

Genome-wide association studies (GWAS), which made it possible to genotype thousands of single nucleotide polymorphisms (SNPs), have successfully identified potential susceptibility loci for CRC. Recent GWAS have identified SNPs at 20 genomic regions that were associated with CRC susceptibility, including 1q41, 3q26.2, 6p21, 8q23.3, 8q24.21, 10p14, 11q13.4, 11q23.1, 12q13.12, 14q22.2, 15q13.3, 16q22.1, 18q21.1, 19q13.11, 20p12.3, 20q13.33 and Xp22.2 in Caucasian populations ($P \leq 5 \times 10^{-8}$).[6-9] Recently, Ceres *et al*., have performed GWAS with Spanish cohort of 881 CRC cases and 667 controls and they successfully identified two new susceptible variants rs12080929 at 1p33 and rs11987193 at 8p12. Although two SNPs did not reach the genome -wide statistical significance due to limited sample size, two SNPs were successfully replicated at a nominal level of $P < 0.05$ in the second Phase ($P = 0.044$, OR (95% CI) =0.867 (0.722–0.994) and $P = 0.039$, OR (95% CI) =0.847 (0.724–0.992), respectively).[10] Therefore, larger sample size and different ethnic will be necessary to replicate the association between these two SNPs and CRC.

Until now, we have not found the association study of rs12080929 and rs11987193 in the CRC non-Caucasian population. The aim of study was to examine the association between the two SNPs in a case-control Han Chinese population.

MATERIALS AND METHODS

Subjects

In total, 380 colorectal cancer patients (205 males and 175 females, age: 61.23 ± 14.03 years) and 380 healthy controls (198 males and 182 females, age: 59.53 ± 7.94 years) were recruited for the case-control study. All patients were of ethnic Han Chinese origin. All the CRC patients had undergone curative resection between 1999 and 2007 at the surgical department of the first affiliated Hospital. Pathologic tumor staging was performed according to Duke's criteria. Patients with familial

adenomatous polyposis or hereditary nonpolyposis colorectal cancer were excluded from the study. Meanwhile, none of the patients had been treated with chemotherapy, and/or radiotherapy before enrolment into the study. Controls were randomly selected healthy individuals confirmed by physical examination, clinical and biochemical analyses during the same period of case recruitment, and matched with cases on age, gender and the same townships. Sample information was shown in Table 1. All subjects gave informed consent for the genetic analysis, which was reviewed and approved by the ethics committee.

Genotyping

Genomic DNA was extracted from peripheral blood sample using the RelaxGene Blood System (TianGen, Beijing, China) according to the manufacturer's instructions. All SNPs were genotyped on the ABI 7900 DNA detection system (Applied Bio-systems, Foster City, California) using TaqMan technology. All probes were designed by Applied Biosystems. The standard 5 μL PCR was performed using TaqMan Universal PCR Master Mix reagent kits according to the guidelines provided.

Statistical analysis

All the parameter calculations, including allele and genotype frequencies, Hardy–Weinberg equilibrium analysis were carried out online by SHEsis platform (http://analysis.bio-x.cn).[11] We corrected the *P* values of genotype and allele by Bonferroni correction in which the *P* values obtained are multiplied by the number of tests performed in the same dataset; the corrected values are usually considered significant if they are below 0.05.

Table 1: Characteristics of the study population

Variables	CRC patients	Controls	χ^2	*P* value
Age				
≥ 60	183	180	0.0475	0.828
< 60	197	200		
Sex				
Male	205	198	0.259	0.611
Female	175	182		
Smoking status				
Smokers	160	181	2.346	0.126
Nonsmoker	220	199		
Alcohol use				
Drinker	185	177	0.338	0.561
Nondrinker	195	203		

CRC=Colorectal cancer

RESULTS

A total of 380 colorectal cancer patients and 380 normal controls were studied in our experiment. Genotype distributions for the two SNPs were in Hardy-Weinberg equilibrium in either CRC or controls. The allele and genotype frequencies of the SNPs are listed in Table 2. CRC patients and controls showed statistically significant differences for two SNPs in the allelic distribution [rs12080929, $P = 0.0325$; OR = 0.750 (0.576–0.977); rs11987193, $P = 0.0236$, OR = 0.751 (0.586–0.963)]. After Bonferroni correction, rs11987193 continued to be significantly associated with CRC (corrected $P = 0.0472$).

DISCUSSION

rs12080929 showed nominal significant association with CRC in our study. rs12080929 is located on an intron position within *TRABD2B* on 1p33 [Figure 1]. This predicted gene is believed to code for a single-pass type I membrane protein. Interestingly, this transcript encoded TRABD2B is predominantly expressed in the colon but is also expressed in the tissues of the nervous system.[10] There is no known biological evidence to further imply that this gene has a role in CRC.

We successfully replicated the association between rs11987193 and CRC patients. rs11987193 SNP is located in the 8p12 locus, 128kb downstream this variant [Figure 2]. DUSP4 (also called MKP-2) is a member of dual kinase phosphatase family and potential tumor suppressor. High *DUSP4* expression is associated with microsatellite instability in colorectal. *DUSP4* overexpression *in vitro* causes alteration of expression of *MMR* genes, as well as genes involved in cell cycle control and proliferation.[12] Recently, *DUSP4* gene expression is upregulated in human CRC cells and correlated with nuclear ERK1/2 inhibition ERK1/2 activity controls the stability of *DUSP4*. *DUSP4* acts as an important regulator during colorectal oncogenesis in human.[13]

In previous GWAS study, rs11897193 showed nominal significant association with CRC in the Caucasian population. We further investigated the discrepancy between in the Chinese and Caucasian of CRC population. We notified the allele frequency of rs11897193 is different between these two populations, as the Minor allele Frequency (MAF) in Han Chinese is rare (0.162),

Table 2: Association between CRC and Genetic Variants at 1p33 and 8p12 in the Han Chinese population

SNP	Allele frequency		OR	*P* value	Adjusted *P* value	Genotype frequency			H-W *P* value
	T	C				TT	TC	CC	
rs12080929 (1p33)									
CRC	0.841	0.159	0.750 (0.576-0.977)	0.0325	0.0650	0.705	0.271	0.024	0.809
Control	0.798	0.202				0.642	0.312	0.046	0.545
rs11987193 (8p12)									
CRC	0.187	0.813	0.751 (0.586-0.963)	0.0236	0.0472	0.021	0.332	0.647	0.0752
Control	0.234	0.766				0.061	0.347	0.592	0.538

P<0.5 was in the bold; Adjusted *P* value is the *P* value modified by Bonferroni correction. CRC=Colorectal cancer, TT=Homozygous (TT), TC=Heterozygous (TC), CC=Homozygous (CC)

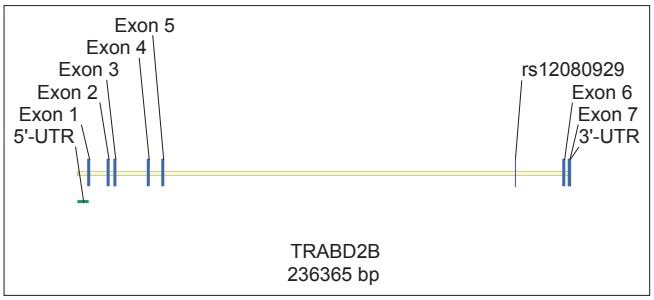

Figure 1: The structure of TRABD2B and location of rs12080929

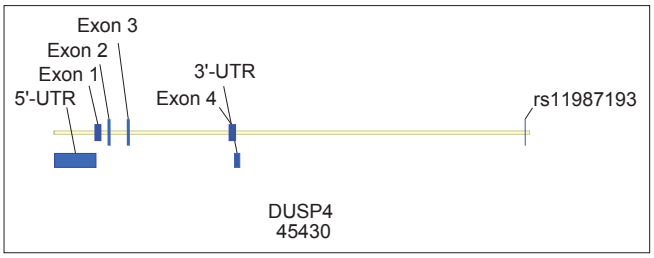

Figure 2: The structure of DUSP4 and location of rs11987193

but relatively common in Caucasia (0.283). To sum up, the MAF difference maybe the important reason for associations in Chinese/Caucasian CRC patients with the present sample size.

Some limitations in the case-control study need to be addressed. First, the sample size of our case-control study was relatively small, resulting in the relatively inadequate statistical power. Second, although significant association between rs11987193 and CRC has been confirmed in this study, function experiments have not been done, since the question of whether this variant is protecting remained uncertain.

In conclusion, our case–control study in the Han Chinese population verified that the variant rs11987193 was significantly associated with CRC risk. Further functional verification should be conducted to confirm the findings, which may unravel the underlying mechanisms of in *DUSP4* CRC development and progression.

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REFERENCES

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, *et al*. Cancer statistics, 2008. CA Cancer J Clin 2008;58:71-96.
2. Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, *et al*. Environmental and heritable factors in the causation of cancer-analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 2000;343:78-85.
3. Mates IN, Jinga V, Csiki IE, Mates D, Dinu D, Constantin A, *et al*. Single nucleotide polymorphisms in colorectal cancer: Associations with tumor site and TNM stage. J Gastrointestin Liver Dis 2012;21:45-52.
4. Lindgren G, Liljegren A, Jaramillo E, Rubio C, Lindblom A. Adenoma prevalence and cancer risk in familial non-polyposis colorectal cancer. Gut 2002;50:228-34.
5. Liljegren A, Lindblom A, Rotstein S, Nilsson B, Rubio C, Jaramillo E. Prevalence and incidence of hyperplastic polyps and adenomas in familial colorectal cancer: Correlation between the two types of colon polyps. Gut 2003;52:1140-7.
6. Houlston RS, Webb E, Broderick P, Pittman AM, Di Bernardo MC, Lubbe S, *et al*. Colorectal Cancer Association Study Consortium, CoRGI Consortium, International Colorectal Cancer Genetic Association Consortium. Meta-analysis of genome-wide association data identifies four new susceptibility loci for colorectal cancer. Nat Genet 2008;40:1426-35.
7. Houlston RS, Cheadle J, Dobbins SE, Tenesa A, Jones AM, Howarth K, *et al*. CORGI Consortium; COIN Collaborative Group; COINB Collaborative Group. Meta-analysis of three genome-wide association studies identifies susceptibility loci for colorectal cancer at 1q41, 3q26. 2, 12q13. 13 and 20q13. 33. Nat Genet 2010;42:973-7.
8. Tomlinson IP, Carvajal-Carmona LG, Dobbins SE, Tenesa A, Jones AM, Howarth K, *et al*. COGENT Consortium; CORGI Collaborators; EPICOLON Consortium. Multiple common susceptibility variants near BMP pathway loci GREM1, BMP4, and BMP2 explain part of the missing heritability of colorectal cancer. PLoS Genetics 2011;7:e1002105.
9. Dunlop MG, Dobbins SE, Farrington SM, Jones AM, Palles C, Whiffin N, *et al*. Colorectal Tumour Gene Identification (CORGI) Consortium. Common variation near CDKN1A, POLD3 and SHROOM2 influences colorectal cancer risk. Nat Genet 2012;44:770-6.
10. Fernandez-Rozadilla C, Cazier JB, Tomlinson IP, Carvajal-Carmona LG, Palles C, Lamas MJ, *et al*. A colorectal cancer genome-wide association study in a Spanish cohort identifies two variants associated with colorectal cancer risk at 1p33 and 8p12. BMC Genomics 2013;14:55.
11. Shi Y, Lin H. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005;15:97-8.
12. Gröschl B, Bettstetter M, Giedl C, Woenckhaus M, Edmonston T, Hofstädter F, *et al*. Expression of the MAP kinase phosphatase DUSP4 is associated with microsatellite instability in colorectal cancer (CRC) and causes increased cell proliferation. Int J Cancer 2013;132:1537-46.
13. Cagnol S, Rivard N. Oncogenic KRAS and BRAF activation of the MEK/ERK signaling pathway promotes expression of dual-specificity phosphatase 4 (DUSP4/MKP2) resulting in nuclear ERK1/2 inhibition. Oncogene 2012;32:564-76.

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