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Original Article

MPHOSPH6, ZNF208 and *RTEL1* polymorphisms in Chinese Han patients with colorectal cancer

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Abstract: National Cancer Center has reported that the male (5.2%) suffered from colorectal cancer (CRC) and the female is 4.8%, it is the second leading mortality of tumor. So far, few previous studies evaluated association between *RTEL1* and CRC risk in a Chinese Han population. We conducted a case-control study including 247 CRC cases and 300 controls. Fourteen SNPs were selected from previous genome-wide association studies and genotyped using Sequenom MassARRAY technology. Odds ratios and 95% confidence intervals (CIs) were calculated by unconditional logistic regression adjusting for age and gender. Using SPSS17.0 software and Microsoft Excel analyzed all the data. Dominant model (AA+AB vs. BB), Recessive model (BB vs. AB+AA), and Allele model (B vs. A) were used to evaluated the CRC risk. The rs2297441 in *RTEL1* may increase odds of developing CRC (OR=1.26, 95% CI=0.100-1.630, *P*=0.049) and the SNP was related to increase the CRC risk in a recessive model (OR=1.96, 95% CI=1.109-3.456, *P*=0.020). After adjusted by age and gender, the results showed that rs2297441 in the *RTEL1* increased 1.99-fold CRC risk (OR=1.99, 95% CI=1.115-3.563, *P*=0.020) in a recessive model. *RTEL1* variant rs2297441 may be a potentially valuable marker for CRC patients.

Keywords: Colorectal cancer, *RTEL1*, polymorphisms, case-control study

Introduction

Colorectal cancer (CRC) is the most aggressive gastrointestinal malignancy. According to cancer epidemiology survey, as a major death risk, CRC is second only to gastric cancer in developing countries (especially China) [1, 2]. Meanwhile, National Cancer Center has reported that the male (5.2%) suffered from CRC and the female is 4.8%, it is the second leading mortality of tumor [3]. Notwithstanding the surgical operation is an effective therapeutic method, the prognosis of patients with CRC remain poor and 5-year survival rates is no more than 47.2% [4]. Cell malignant transformation is a very complicated process and might be influenced by environmental factors such as high fat diet, low cellulose diet, lack trace elements, et al and genetic factors including polymorphisms of MPHOSPH6, ZNF208, RTEL1, et al. Due to people's daily lifestyle and living regions are very difficult to change, precision medicine (Single-nucleotide polymorphisms, SNPs) provides us with an effective method of preventing colorectal cancer at gene level.

Along with the development of precision medicine, genome-wide association studies (GWAS) of genetics and phenotypes can be performed by researchers. Many common SNPs related to CRC risk have been precisely identified by GWAS [5-7]. RNA transcription, which play a role in many signaling pathways, and Geurt Schilders' data indicated that *MPHOSPH6* was an exosome associated with RNA binding protein which participated in 5.8 s rRNA [8]. Zhou also reported that M-phase phosphoprotein 6 might be involved in cell cycle that the mitosis of metrocyte was divided into two daughter cells in M phase [9]. Hence, the SNPs of *MPHOSPH6*

may play important roles in cancer risk. Hirbe and co-workers collected blood, tumor, and other normal tissues of patients with neurofibroma to study by whole exome sequencing, the results revealed that the SNPs of *ZNF208* covered 78.26 kb were associated with the malignant transformation and metastasis of stem cells [10]. Lisa Mirabello et al found *RTEL1* was associated with telomere length [11], and Codd and Levy further demonstrated that *RTEL1* associated with cancer risk [12, 13]. However, studies about associations between the SNPs of *MPHOSPH6*, *ZNF208*, and *RTEL1* and CRC susceptibility are still rare.

In our study, fourteen SNPs in three genes (*MPHOSPH6*, *ZNF208*, and *RTE1*) were selected to perform a comprehensive association analysis with CRC. Establishing our analysis to the risk of CRC loci, some evidences are provided to share hereditary susceptibility between CRC and three genes at 14 new loci.

Materials and methods

Ethics statement

Our study was approved by the Ethics Committee of Northwest University. A standard statistical survey questionnaire and investigative agreement was drafted which follow World Medical Association Declaration of Helsinki. The primary coverage of our study was explained to each participant before signed informed consent.

Subjects

The venous blood samples of 547 participants (247 cases and 300 controls) from neurosurgery and medical examination center of Shaanxi Province Tangdu Hospital of the Fourth Military Medical University between 2010 and 2014. All participants were native residents in Xi'an, China and unrelated-individuals in genetics. We excluded the participants with history of cancer, or serious diseases, or unknown condition of chemotherapy.

Epidemiological and clinical data

Our questionnaire included age, sex, education status and other lifestyle factors from respondents. The data of standard statistical survey questionnaire were logged in Microsoft Excel worksheet 2010 software. The 547 blood samples (5 ml) were stored in accordance with our unified standards (-80°C). Clinical information was collected from medical records and radiological diagnosis.

SNP selection and genotyping

Fourteen SNPs from three genes were selected in DbSNP database (http://www.hapmap.org/index.html.en) and SNP Consortium database (http://snp.cshl.org/). SNP selection criteria followed standardization of Map Hap standard. The minor allele frequency (MAF) of each SNP is more than 5% in HapMap Chinese Han Beijing population. We used GoldMag-Mini Genomic DNA Purification Kits (GoldMag Co. Ltd. Xi'an City China) extracted from the venous blood samples of patients with CRC, and DNA concentrations were determined by high performance liquid chromatography using the NanoDrop 2000 (Thermo Scientific, Waltham, Massachusetts, USA). We designed the multiplexed SNP MassEXTENDED assay by Sequenom MassARRAY Assay Design3.0 software [14] (Sequenom Inc., San Diego, CA, United States) and site primers by Assay Design Suite v2.0 online software (http://www.mysequenom.com/Tools) in our study are listed in **Table 1**. Performing *genotyping* with the Sequenom MassARRAY RS1000 system [14], and data analysis with Sequenom Typer 4.0 Software [15].

Statistical analysis

Statistical analyses were conducted with the SPSS 17.0 statistical package. We used Fisher's test to assess whether the candidate SNPs of control match Hardy-Weinberg equilibrium (HWE) with algorithms in the Alrequin 3.1 program (L. Excoffier, CMPG, University of Bern, Switzerland), and Chi square test to determine the allelic frequencies (14 SNPs) were compared between the cases and the controls. The associations between the polymorphisms of *MPHOSPH6*, *ZNF208*, and *RTE1* and risk of CRC were assessed with odds ratios (ORs) and 95% confidence intervals (95% CI) which were calculated by unconditional logistic regression analysis with an adjustment for age and sex. Dominant model (AA+AB vs. BB), Recessive model (BB vs. AB+AA), and Allele model (B vs. A) were used to evaluated the CRC risk. All *P* values were two-sided and if *P* values were less-than 0.05, it would be considered statistically significant.

Table 1. Primers Used for this Study

SNP_ID	1st-PCRP	2nd-PCRP	UEP_SEQ
rs1056675	ACGTTGGATGACCTGGGGACAGGTATAGAG	ACGTTGGATGACAGGTGCCTGAATTCAAAA	TTGTTCCTCACTGTCTT
rs1056654	ACGTTGGATGCCTCCTTTCAATTCAGCAAC	ACGTTGGATGCTGATTACCGAGCAGATCAC	CAACTGGTTTGCTCACT
rs3751862	ACGTTGGATGTACAAACATGTACCCGGGTC	ACGTTGGATGGAAGGTTGGTGGAAATTGCG	AATTGCGTGAGGAAAAG
rs11859599	ACGTTGGATGAAGATTCATATGTGGCCAGG	ACGTTGGATGGGTCTCAAACTCCTGGGCTT	ACTCCTGGGCTTAAGCGA
rs2967361	ACGTTGGATGGGTTGTCCTCAAGATGCAGT	ACGTTGGATGTCAGTCACCAGACCGCCTTA	gACCCTGGCCCTTCATCTC
rs2188972	ACGTTGGATGTCTCAAGAATCCCCTCTTGC	ACGTTGGATGAAATATAGGTGGGCCCTGTC	AATCATGTGAAGGCTTGAA
rs2188971	ACGTTGGATGGGCTCTGATACCTGATTTGG	ACGTTGGATGAATCCACGTTACCTAAGCCC	ACCTAAGCCCAAATTATTAC
rs8103163	ACGTTGGATGAGACCCCAACCACACACTCT	ACGTTGGATGGACCTCCATCTTGGCTCAG	TCTTGGCTCAGGGCTCCTTG
rs7248488	ACGTTGGATGACTCACATGGACTCCCCATC	ACGTTGGATGATGTCTTCTGACCCGGAAGG	TGGGATGGATGGGAGTCCAC
rs8105767	ACGTTGGATGGCAAGTGGAGAATCAGAGTG	ACGTTGGATGGGTGAATTTCCAATCCAGTC	tttgTTGTCACTAGAGACCCG
rs6089953	ACGTTGGATGGCGTCTGTCATAAAAAGGGC	ACGTTGGATGCCCTTCAAAGGACGATCGTT	gaggTTTTACTTGTCAATCCTCTCTC
rs6010621	ACGTTGGATGCTGACAACCTCTTGACGACC	ACGTTGGATGAGCACGAGAACAGCACCGAG	cccaGAGAACAGCACCGAGGAAAAG
rs4809324	ACGTTGGATGAGTGTGCAGGTTTACCAGAG	ACGTTGGATGATTCACCTGTGGATGGGCTC	cATGGGCTCACGCGGG
rs2297441	ACGTTGGATGAACTCCCTGTCCCTCAACTC	ACGTTGGATGGTGTCCACTTTTAATCAGGG	GACAGGGCTCTCTAATAAA

Table 2. Characteristics of cases and controls in this study n (%)

Characteristics	Cases	Control	*P* Value
Number	247	300	
Age (mean ± SD)	60.4 ± 5.1	58.3 ± 12.8	0.015
Sex			<0.01
Male	107	180	
Female	140	120	

SD=standard deviation, P≤0.05 indicates statistical significance.

Results

This study included 247 CRC cases (107 men, 140 women; mean age 58.32 years) and 300 controls (180 men, 120 women; mean age 60.42 years). The clinical characteristics of cases and controls are summarized in **Table 2**. We observed significant differences in age and gender distribution between cases and controls (*P*<0.05).

We summarized chromosome position, base change and HWE test results for the 14 SNPs were presented in **Table 3**. All SNPs were in Hardy-Weinberg equilibrium in controls (P> 0.05). After did the Chi square test, the results showed that rs2297441 in *RTEL1* may increase odds of developing CRC (OR=1.26, 95% CI=0.100-1.630, *P*=0.049).

Table 4 showed the minor allele of 14 SNPs associate with CRC by multiple inheritance models. The results showed that SNP rs2297441 in the *RTEL1* was related to increase the CRC risk in a recessive model (OR=1.96, 95% CI=1.109-3.456, *P*=0.020). After adjusted by age and gender, the results showed (**Table 4**) that rs2297441 in the *RTEL1* increased 1.99-fold CRC risk (OR=1.99, 95% CI=1.115-3.563, *P*=0.020) in a recessive model.

Discussion

RTEL1 with 57 distinct introns and 24 cassette exons located on Chromosome 20q13.3 covers 41.56 kb from 62288836 to 62330395, which is a member of the tumor necrosis factor receptor superfamily. It was proposed to participate in the Cytokine-cytokine receptor interaction pathway and processes such as anti-apoptosis, DNA repair, telomere maintenance, et al (http://www.ncbi.nlm.nih.gov/IEB/ Research/Acembly/av.cgi). *RTEL1* also belongs to the Fe-S cluster helicase family such as xeroderma pigmentosum group D, Fanconi anemia complementation group J, cell adhesion molecule L1-like, et al [16], and the *RTEL1*'s proteins with an coordinated Fe-S cluster and the cytosolic ironsulfur protein assembly machinery can be assembled the target protein which has been implicated in DNA secondary structures and telomere homeostasis (genomic stability) [17, 18]. How to control telomere length is still elusive, but Ding reported that the *RTEL1* protein is lacking 6 amino acids at 3' terminus in M. spretus model (*RTEL1* knockout mouse), which resulted in the reduced proliferation and telomere length heterogeneity of embryonic stem cells [19, 20]. Because *RTEL1* has been implicated in G-rich DNA secondary structures, the phenotype of *RTEL1*-knockout cells might reflect the problem about unwinding DNA sec-

Table 3. Basic information on candidate SNPs

SNP ID	Gene name	Base change	MAF-case	MAF-control	*p* value for HWE test	ORs	95% CI		*p* value
rs1056675	MPHOSPH6	C/T	0.445	0.408	0.810	1.165	0.915	1.484	0.214
rs1056654	MPHOSPH6	A/G	0.278	0.304	0.584	0.882	0.678	1.149	0.350
rs3751862	MPHOSPH6	C/A	0.043	0.042	0.407	1.026	0.567	1.857	0.932
rs11859599	MPHOSPH6	C/G	0.231	0.248	0.089	0.908	0.687	1.200	0.499
rs2967361	MPHOSPH6	T/G	0.243	0.235	0.079	1.044	0.790	1.381	0.760
rs2188972	ZNF208	A/G	0.478	0.468	0.817	1.038	0.818	1.318	0.757
rs2188971	ZNF208	T/C	0.283	0.278	0.774	1.025	0.786	1.337	0.857
rs8103163	ZNF208	A/C	0.283	0.278	0.886	1.025	0.787	1.336	0.853
rs7248488	ZNF208	A/C	0.283	0.28	0.669	1.017	0.780	1.325	0.900
rs8105767	ZNF208	G/A	0.298	0.271	0.187	1.140	0.876	1.485	0.330
rs6089953	RTEL1	G/A	0.249	0.253	0.762	0.977	0.742	1.286	0.869
rs6010621	RTEL1	G/T	0.245	0.242	0.752	1.013	0.768	1.338	0.925
rs4809324	RTEL1	C/T	0.103	0.092	1.000	1.141	0.764	1.703	0.520
rs2297441	RTEL1	A/G	0.346	0.295	0.330	1.262	1.010	1.629	0.049*

MAF minor allele frequency, OR odds ratio, 95% CI 95% confidence interval. **p* indicate statistical significance

Table 4. Association between rs2297441, a polymorphism in RTEL1, and CRC under multiple models of inheritance

Model	Genotype	Cases	Controls	OR (95% CI)	*P* value	OR (95% CI)#	*P* value
Recessive	A/A-A/G	33	22	1	0.0201*	1	0.0199*
	G/G	213	278	1.958 (1.109-3.456)		1.993 (1.115-3.563)	
Dominant	A/A	137	109	1	0.3484	1	0.2827
	A/G-G/G	155	145	1.176 (0.838-1.649)		1.209 (0.855-1.710)	
Additive	A	170	177	1		1	
	G	322	432	1.263 1.633-1.781)		1.288 (1.676-1.888)	0.0590

#Adjusted by sex and age. *P<0.05 indicates statistical significance.

ondary structures resulted in the repair of telomere length [21].

Not all of chromosome ends remained the T-loop (lasso-like), which can invade the 3's TTAGGG of telomeric, resulted in forming D-loops to protect the degradation of chromosome end [22, 23]. *RTEL1* may play a role in the anti-homologous recombination to raise D-loop disassembly, and in other words *RTEL1* knockout might dismantle homologous recombination incomplete intermediates at telomeres [24]. Visualization of telomere length has revealed that *RTEL1* can promote rapid accumulation of telomere circles and T-loop disassembly to acquire telomere length. Agnel and co-workers analyzed association between the telomere phenotype and *RTEL1*, and the results also revealed that *RTEL1* might suppress telomere fragility [25], which hinder DNA intrinsic replication by repeated TTAGGG sequence. The SNP rs2297441 is located in the intron region of *RTEL1*, and a role for the variant was that the cryptic splice sites were formed [26]. However, until recently the studies about the potential mechanisms remained unclear. Therefore, pathologist, geneticist and clinician are required to make a further comparative examination of RTEL1 mRNA transcripts.

Despite our experimental results showed that RTEL1 rs2297441 related to the CRC risk, some limitations can be found in our study. First, the cases and control size was relatively small. Second, the histological type and tumor grade associated with genetic polymorphisms were not evaluated.

Conclusion

RTEL1 variant rs2297441 may be potentially valuable markers for CRC patients. The goal of

our study is to generate the genetic risk factors to move toward a brand period in which comprehensive therapy is individualized. Ultimately, through study in this field, we can inform a clearer understanding of CRC.

Disclosure of conflict of interest

None.

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