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Association Between Adiponectin Polymorphisms and the Risk of Colorectal Cancer

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Purpose: To discuss the association between adiponectin (*ADIPOQ*) gene rs2241766 and rs1501299 polymorphisms and the risk of colorectal cancer, and to analyze the role of the interaction between these two loci and environmental factors in colorectal cancer pathogenesis. *Methods:* The case–control study was performed with a 1:1 match. A self-designed questionnaire was used to perform a face-to-face survey with 600 new primary colorectal cancer cases confirmed by histopathology as well as 600 cases of people receiving a physical examination at the same time. The general information, lifestyle, and diet habits, etc. were collected from two groups of study subjects. Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) was used to identify *ADIPOQ* rs2241766 and rs1501299 genotypes. *Results:* After adjusting for factors such as colorectal cancer family history, body–mass index (BMI), daily sedentary time, weekly red meat intake frequency, as well regular tea drinking, conditional logistic regression analysis indicated that rs2241766 TG + GG carriers had a higher risk of colorectal cancer than TT carriers (OR = 1.433, 95% CI: 1.014–1.985); rs1501299 GT + TT carriers had a lower risk of colorectal cancer than GG carriers (OR = 0.723, 95% CI: 0.531–0.902). Generalized multifactor dimensionality reduction analysis showed that ADIPOQ rs2241766 and rs1501299 could have interaction with red meat intake ($p = 0.001$). *Conclusion: ADIPOQ* rs2241766 and rs1501299 single nucleotide polymorphisms (SNPs) could be associated with colorectal pathogenesis and could have interactions with red meat intake. Both factors impact colorectal cancer occurrence.

Introduction

Adiponectin is a recently discovered specific fat-derived collagen-like protein secreted by fat cells. Many studies have shown that the serum adiponectin concentration negatively correlates with colorectal cancer occurrence risk (Izadi *et al.*, 2012; Joshi *et al.*, 2014). Adiponectin is a product of the *ADIPOQ* gene, which spans ~16 kb with three exons on chromosome3q27 and has been linked to a susceptibility locus for metabolic syndrome (Suriyaprom *et al.*, 2014), type 2 diabetes (Ye *et al.*, 2014), cardiovascular disease (Zhou *et al.*, 2014), and cancers (Li *et al.*, 2014a, 2014b).

Previously, *ADIPOQ* gene exon 2 + 45T > G (rs2241766) and intron 2 + 276G > T (rs1501299) polymorphism were reported to be associated with changes in serum adiponectin concentrations (Li *et al.*, 2014c; Pineda-Tenor *et al.*, 2014). Also, several studies have shown that *ADIPOQ* rs266729 is associated with colorectal cancer occurrence risk (Pechlivanis *et al.*, 2009; Gornick *et al.*, 2011; Pham *et al.*, 2014). However, the association of *ADIPOQ* genetic polymorphisms with colorectal cancer in the Chinese population remains unclear.

In the present study, we used a 1:1 matching case–control study method to investigate the relationship between *ADIPOQ* rs2241766 and rs1501299 polymorphisms and colorectal cancer occurrence risk. We also studied the interaction between these two single nucleotide polymorphisms (SNPs) and environmental factors in colorectal cancer pathogenesis.

Materials and Methods

Study subjects

A total of 600 cases of primary and new colorectal cancer patients admitted to the First Affiliated Hospital and the Second Affiliated Hospital of Harbin Medical University from July 2008 to May 2013, confirmed by histopathology, were collected and considered the patient group. The age of the patient group ranged from 28 to 77 years, among which there were 350 male cases, 250 female cases, 402 colorectal cancer patients, and 198 colon cancer patients. The healthy individuals who received physical examinations in the community health center at the same time were chosen as the control group. The control group had no history of tumor and

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their ages were matched to the patient group. The gender was matched 1:1. All patients and controls were Han Chinese.

Methods

Survey content. A self-designed questionnaire was used, and the face-to-face survey was administered by trained investigators to collect the following information: (1) demographic characteristics, including age, gender, marital status, education, occupation, annual household income (Yuan), height, and health conditions; (2) diet within 10 years before the disease or the survey, including frequency of foods, such as vegetables, fruits, meat (red meat was defined as pork, beef, and lamb; according to the daily 50–75 g standard recommended by the Chinese dietary pagoda, our study uses 7 times/week as the cutoff), bean products, and pickles; (3) personal hobby and life habits, including smoking (regular smoking was defined as ≥ 1 cigarette per day, continuous or cumulative >6 months), drinking (regular drinking was defined as drinking ≥ 2 times per week for >6 months), drinking tea (regular drinking tea was defined as drinking tea ≥ 1 time daily for >3 months), exercise, and daily sedentary time etc.; and (4) tumor family history. The survey followed the principal of informed consent. Vacuum anticoagulant blood collection tubes were used to collect 2 mL of blood from the cubital vein of study subjects, and the blood samples were stored at -20°C for future use. Our study was approved by the Ethics Committee of the First Affiliated Hospital of Harbin Medical University.

Laboratory detection. A total of 0.5 mL of anticoagulant blood was used to extract genomic DNA using a DNA extraction kit (Omega Bio-Tek Company, Guangzhou, China). The DNA was stored at -20°C for future use.

There are 680 SNPs of the *ADIPOQ* gene listed in the NCBI database (www.ncbi.nlm.nih.gov/snp). Using the Haploview 4.2 software and the HapMap phrase II database, we obtained two tagging SNPs (rs2241766 and rs1501299) for the Han Chinese with the minor allele frequency ≥ 0.05 and $r^2 \geq 0.8$. The polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) technology was used to detect *ADIPOQ* rs2241766 and rs1501299 genotypes. The primer sequences were as follows: rs2241766 forward, 5′-GAA GTA GAC TCT GCT GAG ATGG-3′; rs2241766 reverse, 5′-TAT CAG TGT AGG AGG TCTGTG ATG-3′; rs1501299 forward, 5′-GGC CTC TTT CAT CAC AGA CC-3′; and rs1501299 reverse, 5′-AGA TGC AGC AAA GCC AAA GT-3′. The amplified product of rs2241766 was digested with endonuclease *Sma*I in a 25°C water bath for 10 h. The amplified product of rs1501299 was digested with endonuclease *Bsm*I in a 65°C water bath for 24 h. The digested products were then analyzed for genotypes using 2.5% agarose gel electrophoresis.

Statistical analysis

The original data were verified and organized. EpiData 3.1 software was used to enter the data, and SPSS 18.0 software was used to perform data analysis. The frequency distribution differences of general demographic characteristics, genotype, and allele between the patient group and the control group, as well as the Hardy–Weinberg equilibrium test of the control group were performed with the χ^2 test. The relationship between genes, environmental factors, and colorectal cancer occurrence risk, as well as the interaction between genes and environment were analyzed using a conditional logistic regression model. Multivariate analysis of high-level interaction used the combined method of generalized multifactor dimensionality reduction (GMDR) and logistic regression.

Results

ADIPOQ *rs2241766 and rs1501299 PCR amplification*

The amplification product of rs2241766 is a 372-bp fragment and has three genotypes after digestion: homozygous wild-type TT (372 bp; one fragment); homozygous mutant GG (219, 153 bp; two fragments); and heterozygous TG (372, 219, 153 bp; three fragments). The amplified rs1501299 product is the 196-bp fragment and has three genotypes after digestion: homozygous wild-type GG (150, 46 bp; two fragments); homozygous mutant TT (196 bp; one fragment); and heterozygous GT (196, 150, 46 bp; three fragments).

The relationship between lifestyle habits, diet, and colorectal cancer occurrence risk

The distribution differences of education, occupation, annual household income, etc. between the patient group and the control group were not significant ($p > 0.05$). Multivariate conditional logistic regression analysis results showed that regular tea drinking was a protective factor for colorectal cancer (OR: 0.455, 95% CI: 0.312–0.843), while daily sedentary time ≥ 8 h (OR: 1.743, 95% CI: 1.143–2.324), weekly red meat intake >7 times (OR: 1.543, 95% CI: 1.114–2.424), and colorectal cancer family history (OR: 2.122, 95% CI: 1.216–5.939) were risk factors for colorectal cancer. Body–mass index (BMI), drinking, smoking, and annual family income were not associated with colorectal cancer pathogenesis ($p > 0.05$; Table 1).

The relationship between ADIPOQ *rs2241766, rs1501299 polymorphisms, and colorectal cancer occurrence risks*

The Hardy–Weinberg equilibrium test showed that the *ADIPOQ* rs2241766 and rs1501299 genotype frequency of the control group complied with the Hardy–Weinberg equilibrium (both $p > 0.05$). The rs2241766 TG + GG genotype frequency in the patient group was higher than the control group ($p = 0.015$) and the rs1501299 GT + TT genotype frequency in the patient group was lower than the control group ($p = 0.031$).

Table 1. Logistic Regression Analysis of the Relation Between Risk Factors and Colorectal Cancer

Variables	*OR (95% CI)*	p-*Value*
BMI (kg/m^2)	0.987 (0.549–1.426)	0.426
Family income (Yuan)	0.865 (0.511–1.558)	0.142
Drinking	0.869 (0.431–1.433)	0.554
Smoking	1.433 (0.994–2.113)	0.103
Regular tea drinking	0.455 (0.312–0.843)	0.003
Daily sedentary time (h)	1.743 (1.143–2.324)	0.002
Red meat (time/week)	1.543 (1.114–2.424)	0.001
Cancer family history	2.122 (1.216–5.939)	0.004

BMI, body–mass index.

Table 2. The Relation Between *ADIPOQ* rs2241766 and rs1501299 and Colorectal Cancer

	Case group		*Control*				
SNPs	N	*(%)*	n	*(%)*	*OR (95% CI)*	*OR (95% CI)*[a]	p-*Value*
rs2241766							
TT	260	43.3	308	51.3	1.00	1.00	
TG	295	49.2	252	42.0	1.336 (1.064–1.677)	1.360 (0.996–1.898)	0.065
GG	45	7.5	40	6.7	1.135 (0.730–1.765)	1.433 (0.798–2.324)	0.024
TG + GG	340	56.7	292	48.7	1.379 (1.099–1.732)	1.433 (1.014–1.985)	0.015
rs1501299							
GG	297	49.5	255	42.5	1.000	1.00	
GT	266	44.3	304	50.7	0.775 (0.618–0.973)	0.674 (0.497–0.919)	0.034
TT	37	6.2	41	6.8	0.896 (0.565–1.418)	0.799 (0.544–1.211)	0.140
GT + TT	303	50.5	345	57.5	0.754 (0.600–0.946)	0.723 (0.531–0.902)	0.031

[a]Adjustment for other risk factors, such as cancer family history, BMI, regular tea drinking, daily sedentary time, and red meat.
SNPs, single nucleotide polymorphisms.

After adjusting for colorectal family history, BMI, daily sedentary time, weekly red meat intake frequency, and regular tea drinking factors, multivariate conditional logistic regression analysis showed that compared with rs2241766 homozygous wild-type TT, rs2241766 TG + GG carriers had a higher risk of colorectal cancer pathogenesis (OR = 1.433, 95% CI: 1.014–1.985). Compared with rs1501299 homozygous GG, rs1501299 GT + TT carriers had a lower risk of colorectal cancer pathogenesis (OR = 0.723, 95% CI: 0.531–0.902; Table 2).

The role of interaction between ADIPOQ rs2241766, rs1501299, and red meat in colorectal cancer pathogenesis

After adjusting for colorectal cancer family history, BMI, daily sedentary time, and regular tea drinking factors, conditional logistic regression results showed that rs2241766 and red meat, rs1501299 and red meat, rs2241766 and rs1501299 two–two interactions were not significant (all $p > 0.05$).

Analysis of higher order interaction among *ADIPOQ* rs2241766, rs1501299, and red meat using the GMDR method showed that the model with rs2241766, rs1501299, and red meat three factors with the lowest prediction error (39.83%) and the highest cross-validation consistency (10/10) was considered the best model, suggesting that rs2241766, rs1501299, and red meat may have interactions. rs2241766 TG + GG and rs1501299 GG were defined as risk genotypes. Trend χ^2 analysis results showed that the carried genotype number had a dose–response relationship with colorectal cancer occurrence risk ($p = 0.004$). Compared with an individual without a risk genotype, the individual carrying 2 risk genotypes had a higher colorectal cancer occurrence risk (OR = 1.876, 95% CI: 1.056–2.676). Conditional logistic regression analysis results showed that compared with an individual without a risk genotype and who had red meat intake ≤7 times/week, the individual carrying 2 risk genotypes and had red meat intake >7 times/week had a higher colorectal cancer risk (OR = 2.867, 95% CI: 1.123–4.765), suggesting that rs2241766, rs1501299, and red meat could have interactions (Table 3).

Discussion

Our results showed that after adjusting for colorectal cancer family history, BMI, daily sedentary time, weekly red meat

Table 3. Interactions Between *ADIPOQ* rs2241766 and rs1501299 and Red Meat Intake

Factor 1 (SNP)	*Factor 2 (red meat)*	*OR (95% CI)*	p-*Value*	*OR (95% CI)*[a]	p-*Value*[a]
rs2241766	Red meat (times/week)			1.231 (0.776–2.323)	0.165
TT	≤7	1.00			
TG + GG	≤7	1.312 (0.781–1.944)	0.132		
TT	>7	1.561 (0.994–2.421)	0.076		
TG + GG	>7	2.354 (1.456–3.212)	<0.001		
rs1501299	Red meat (times/week)			1.331 (0.765–2.608)	0.342
GT + TT	≤7	1.000			
GG	≤7	1.212 (0.756–1.987)	0.546		
GT + TT	>7	1.543 (0.987–2.955)	0.121		
GG	>7	2.656 (1.431–4.878)	<0.001		
rs2241766	rs1501299			0.868 (0.432–1.656)	0.377
TT	GT + TT	1.000			
TG + GG	GT + TT	1.655 (1.121–3.254)	0.031		
TT	GG	1.754 (1.132–2.656)	0.001		
TG + GG	GG	2.754 (1.653–5.527)	0.004		

[a]Interaction of two factors.

intake frequency, and regular tea drinking factors, the *ADIPOQ* rs2241 766 TG + GG carriers had a higher colorectal cancer risk than TT carriers. Furthermore, rs1501299 GT + TT carriers had a lower colorectal cancer risk than GG carriers, suggesting that the rs2241766 T → G mutation was associated with an increased risk of colorectal cancer occurrence, and the rs1501299 G → T mutation was associated with a decreased risk of colorectal cancer occurrence.

The GMDR method analysis results showed that *ADIPOQ* rs2241766, rs1501299, and red meat may have interactions. The conditional logistic regression method was used to analyze the combined effect between carried risk genotype numbers and red meat intake. The results showed a very good supplement to the above conclusion. It is generally considered that red meat has a large amount of saturated fatty acids as well as animal proteins and is also rich in iron. Currently, international studies all consider red meat a risk factor for colorectal cancer (Ley *et al.*, 2014; Oostindjer *et al.*, 2014). Studies have shown that a large intake of red meat can increase plasma TG and insulin levels (Kucera *et al.*, 2014). A high-fat diet can downregulate adiponectin RNA and protein expression in rats, inducing insulin resistance (IR) occurrence. Gao *et al.* (2014) also demonstrated that a high-fat diet can induce IR in rats. In addition, Simcox and McClain (2013) showed that an excessive iron overload can directly or indirectly mediate β-cell apoptosis and IR, further affecting diabetes occurrence, and red meat contains a large amount of iron. In summary, the interaction mechanism among *ADIPOQ* rs2241766, rs1501299, and red meat could together induce IR occurrence, therefore affecting colorectal cancer occurrence.

Currently, there are few studies regarding the association between *ADIPOQ* rs2241766 and rs1501299 polymorphisms and colorectal cancer occurrence, and the conclusions are not consistent. The studies by Kaklamani *et al.* (2008) and He *et al.* (2011) did not find that *ADIPOQ* rs2241766 and rs1501299 polymorphism were associated with colorectal cancer occurrence; however, one study in Saudi Arabia showed that rs2241766 TG + GG carriers had a lower colorectal cancer occurrence risk (Al-Harithy *et al.*, 2012). The association between *ADIPOQ* rs2241766 and rs1501299 polymorphisms and colorectal cancer still needs verification through large-sample studies.

Our study collected diet and life habit information of the study subjects from 10 years before the illness/survey; therefore, recall bias may play a role. Only two SNPs, *ADIPOQ* rs2241766 and rs1501299, were analyzed, and our future study will study additional SNPs of *ADIPOQ*.

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Author Disclosure Statement

No competing financial interests exist.

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