GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Lack of association between an insertion/deletion polymorphism in *IL1A* and risk of colorectal cancer

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Genet. Mol. Res. 14 (3): 8490-8495 (2015)
Received December 9, 2014
Accepted April 28, 2015
Published July 28, 2015
DOI http://dx.doi.org/10.4238/2015.July.28.17

ABSTRACT. Previous study has shown that miR-122, miR-378, and their target gene *IL1A* may play crucial roles in the tumorigenesis of colorectal cancer (CRC). An insertion/deletion polymorphism

(rs3783553 TTCA/-) in the 3' untranslated region of *IL1A* has been identified as a contributing factor to the risk of developing several cancers. The aim of this study was to evaluate the effect of *IL1A* rs3783553 on CRC risk. CRC patients (N = 339) and healthy control subjects (N = 313) were enrolled and genomic DNA was extracted from peripheral venous blood. The *IL1A* rs3783553 polymorphism was genotyped using a polymerase chain reaction assay. No significant differences in *IL1A* rs3783553 genotype frequencies were found between CRC cases and controls in an analysis of the overall data [ins/del *vs* del/del: adjusted odds ratio (OR) = 0.96, 95% confidence interval (CI) = 0.69-1.34; ins/ins *vs* del/del: adjusted OR = 0.71, 95%CI = 0.43-1.16; ins *vs* del: adjusted OR = 0.86, 95%CI = 0.69-1.09], nor when data were stratified according to clinical parameters. These findings indicate that the *IL1A* rs3783553 polymorphism does not constitute a risk factor for the development of CRC.

Key words: Interleukin-1A; Polymorphism; Colorectal cancer

INTRODUCTION

Colorectal cancer (CRC) is a malignancy originating from the epithelial cells of the colon or rectum, with an estimated 1.2 million new cases and 608,700 deaths worldwide in 2008 (Jemal et al., 2011). Several factors have been shown to increase the risk of developing CRC, including male gender (Nguyen et al., 2009), high red meat, fat, or alcohol intake, and inadequate consumption of fresh fruit, vegetables, or fiber (Chao et al., 2005; Park et al., 2005; Cunningham et al., 2010; Watson and Collins, 2011). In addition to these environmental influences, genetic factors have also been linked to higher CRC rates. In our previous study, we found that *IL-16* rs11556218TG and *KRAS* rs712TT genotypes are associated with significantly increased risk of CRC (Gao et al., 2009; Pan et al., 2014).

MicroRNAs (miRNAs) are small non-coding RNA molecules that function in RNA silencing and post-transcriptional gene regulation by binding to the 3' untranslated region (3' UTR) of target mRNAs (Bartel, 2004, 2009). One miRNA can bind to multiple target mRNA transcripts, while one target gene can be bound by multiple miRNAs. Previous studies have shown that polymorphism in the 3' UTR may alter the strength with which miRNA binds mRNA and may regulate gene expression, thus influencing an individual's risk of developing cancer (Chin et al., 2008; Jazdzewski et al., 2008; Landi et al., 2008; Gao et al., 2009; Landi et al., 2011; Li et al., 2013). Gao et al. (2009) reported that a TTCA insertion/deletion polymorphism (rs3783553) may contribute to hepatocellular carcinoma susceptibility. Further analysis revealed that this insertion may disrupt binding of miR-122/miR-378 to *IL1A*, thereby increasing expression of interleukin-1 alpha (IL-1α; Gao et al., 2009). Subsequently, several groups have investigated the relationship between this polymorphism and hepatocellular carcinoma (Du et al., 2014; Wang et al., 2014), nasopharyngeal carcinoma (Yang et al., 2011), gastric cancer (Zeng et al., 2014), papillary thyroid carcinoma (Gao et al., 2014), cervical carcinoma (Pu et al., 2014b), and epithelial ovarian cancer (Zhang et al., 2014). However, no study to date has assessed its association with CRC risk. We performed a genetic association study to evaluate the effect of *IL1A* rs3783553 on CRC risk in a Chinese population.

MATERIAL AND METHODS

Study population

The study protocol was approved by the Ethics Committee of the Luoyang Central Hospital affiliated to the Zhengzhou University, and written informed consent was given by all subjects. The study population was composed of 339 CRC patients and 313 healthy controls, with all subjects being of Han Chinese descent. Detailed information concerning the study group has been described in our previous study (Pan et al., 2014). Briefly, all individuals were enrolled at the hospital between January 2011 and December 2012 and CRC diagnoses were confirmed by histopathology. Patient clinical data was obtained from medical records and included tumor differentiation status and clinical stage, and metastasis status. The CRC group consisted of 209 men and 130 women, with a mean age of 59.9 ± 13.4 years. Within this group, 68.1% of CRCs were well or moderately differentiated, with 31.9% being poorly differentiated or undifferentiated. Patients with stage I to II tumors represented 54.9% of the group, while those with stage III to IV malignancies made up 45.1%. Metastasis was observed in 41.9% of cases.

Healthy volunteers visiting the hospital for medical examination during the same period were selected as controls. The control group comprised 210 men and 103 women, with a mean age of 56.8 ± 11.2 years. The controls were frequency-matched to the CRC group according to age, gender, race, and geographic location. Individuals with a family history of cancer or inflammatory bowel diseases were excluded.

Genotyping

Genomic DNA was extracted from peripheral venous blood using a commercially available genome DNA extraction kit (BioTeke Corporation, Beijing, China). *IL1A* rs3783553 was genotyped using a polymerase chain reaction assay. Detailed information of the genotyping procedure, such as primer sequences and quality control, has been described in our previous study (Gao et al., 2014).

Statistical analysis

Comparisons of demographic characteristics between CRC cases and controls were analyzed using the chi-square test or the Student *t*-test. Hardy-Weinberg equilibrium was evaluated by the chi-square test. The effect of *IL1A* rs3783553 genotype on CRC risk was estimated by calculating odds ratios (ORs) and 95% confidence intervals (CIs). We also performed chi-square analysis to determine whether *IL1A* rs3783553 genotype was associated with clinical features, such as tumor differentiation status and clinical stage, and metastasis status. All statistical analyses were carried out using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA), and P values less than 0.05 were considered to be statistically significant.

RESULTS

Three *IL1A* rs3783553 genotypes (i.e., del/del, ins/del, and ins/ins) were observed and their distribution was found to be in accordance with Hardy-Weinberg equilibrium. Genotype

frequencies are presented in Table 1. No significant differences in allele and genotype frequencies were found between cases and controls in an analysis of the overall data (ins/del *vs* del/del: adjusted OR = 0.96, 95%CI = 0.69-1.34; ins/ins *vs* del/del: adjusted OR = 0.71, 95%CI = 0.43-1.16; ins *vs* del: adjusted OR = 0.86, 95%CI = 0.69-1.09). After stratification of the data according to tumor differentiation status and clinical stage, and metastasis status, no significant association was detected between *IL1A* rs3783553 genotype and clinical features (Table 2).

Table 1. Genotype distributions of the *IL1A* rs3783553 polymorphism in the CRC patients and healthy controls.

Genotypes	CRC [N (%)]	Controls [N (%)]	Adjusted OR (95%CI)[a]	P[a]
del/del (-/-)	142 (41.9)	124 (39.6)	1.00 (Ref)	
ins/del (ttca/-)	160 (47.2)	143 (45.7)	0.96 (0.69-1.34)	0.17
ins/ins (ttca/ttca)	37 (10.9)	46 (14.7)	0.71 (0.43-1.16)	0.81
del (-)	444 (65.5)	391 (62.5)	1.00 (Ref)	
ins (ttca)	234 (34.5)	235 (37.5)	0.86 (0.69-1.09)	0.21

[a]Adjusted for gender and age using the logistic regression model.

Table 2. Stratified analyses of the *IL1A* rs3783553 polymorphism with clinical features in CRC patients.

Clinical features	Genotype frequency		OR (95%CI)	P value
	N (%)	N (%)		
Differentiated status	Well-moderately	Poorly-undifferentiated		
del/del (-/-)	103 (44.6)	39 (36.1)	1.00 (Ref)	
ins/del (ttca/-)	104 (45.0)	56 (51.9)	1.42 (0.87-2.32)	0.16
ins/ins (ttca/ttca)	24 (10.4)	13 (12.0)	1.43 (0.66-3.09)	0.36
del (-)	310 (67.1)	134 (62.0)	1.00 (Ref)	
ins (ttca)	152 (32.9)	82 (38.0)	1.25 (0.89-1.75)	0.20
Clinical stages	I-II	III-IV		
del/del (-/-)	76 (40.9)	66 (43.1)	1.00 (Ref)	
ins/del (ttca/-)	88 (47.3)	72 (47.1)	0.94 (0.60-1.48)	0.80
ins/ins (ttca/ttca)	22 (11.8)	15 (9.8)	0.79 (0.38-1.64)	0.52
del (-)	240 (64.5)	204 (66.7)	1.00 (Ref)	
ins (ttca)	132 (35.5)	102 (33.3)	0.91 (0.66-1.25)	0.56
Metastasis	Yes	No		
del/del (-/-)	58 (40.8)	84 (42.6)	1.00 (Ref)	
ins/del (ttca/-)	68 (47.9)	92 (46.7)	0.93 (0.59-1.48)	0.77
ins/ins (ttca/ttca)	16 (11.3)	21 (10.7)	0.91 (0.44-1.88)	0.79
del (-)	184 (64.8)	260 (66.0)	1.00 (Ref)	
ins (ttca)	100 (35.2)	134 (34.0)	0.95 (0.69-1.31)	0.75

DISCUSSION

It has been well established that the expression of miRNAs differs according to cancer type. By binding to the 3' UTRs of target genes, miRNAs play important roles in tumor pathogenesis. The expression of miR-378 is known to be downregulated in both plasma and tumor tissue in CRC patients (Zanutto et al., 2014; Zhang et al., 2014a). Overexpression of miR-378 can inhibit cell growth and invasion, while its downregulation may promote these processes (Zhang et al., 2014a), suggesting that this miRNA may function as a tumor suppressor in CRC. Moreover, miR-122 is upregulated in CRC patients with liver metastasis. Its overexpression and the concomitant suppression of its target gene, *cationic amino acid transporter 1*, in the primary tumor has been shown to be involved in the development of colorectal liver metastasis

(Iino et al., 2013). The *IL1A* gene encoding IL-1α is targeted by miR-122 and miR-378 (Gao et al., 2009), and IL-1α mRNA and protein have been detected in highly metastatic colon cancer cells (Matsuo et al., 2009). In addition, knocking down the expression of IL-1α leads to a reduction of vascular endothelial growth factor secretion in colon cancer cells (Shao and Sheng, 2007). Taken together, these findings indicate that miR-122/miR-378 and their target gene *IL1A* potentially play a role in CRC tumorigenesis.

In 2009, a functional polymorphism in the *IL1A* 3' UTR (rs3783553 TTCA insertion/deletion) was discovered, involving a TTCA insertion that was found to alter the binding of miR-122/miR-378 to *IL1A*. Gao et al. (2009) reported that the *IL1A* rs3783553 ins/ins homozygous genotype is associated with a significantly reduced risk of hepatocellular carcinoma. Similarly, decreased risks were found for nasopharyngeal carcinoma (Yang et al., 2011), gastric cancer (Zeng et al., 2014), cervical carcinoma (Pu et al., 2014), and epithelial ovarian cancer (Zhang et al., 2014b). Furthermore, patients of this genotype demonstrated a reduced risk of developing stages T3 and T4 of papillary thyroid carcinoma (Gao et al., 2014) and poor cervical carcinoma tumor differentiation (Pu et al., 2014). Since *IL1A* rs3783553 may be used as a genetic biomarker for tumorigenesis, we hypothesized that it may be associated with CRC risk. However, no significant association was observed between this polymorphism and CRC risk in the overall analysis, nor when the data were stratified. One possibility for this contrary result may be that the role of *IL1A* rs3783553 varies according to cancer type. Moreover, the moderate sample size employed in this study cannot be ruled out in any attempt to explain these differing results. Therefore, further investigations involving larger numbers of samples are necessary to confirm these findings.

Some limitations regarding this study must be taken into account. The study design was hospital based, which may have influenced the observed effects of *IL1A* rs3783553 on CRC risk. Additionally, all participants enrolled in the study were Han Chinese, therefore any results obtained cannot be applied to other ethnicities. Further population-based association studies are warranted to verify our results, especially those including different ethnic groups.

In summary, no significant difference in *IL1A* rs3783553 was observed between CRC patients and controls, indicating that this TTCA insertion/deletion polymorphism does not constitute a risk factor for the development of CRC. However, other genetic risk factors for this disease may exist. Further research is needed to screen novel biomarkers and explore the mechanism behind CRC pathogenesis.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by grants from the National Natural Science Foundation of China (#81302149), the Ph.D. Programs Foundation of Ministry of Education of China (#20130181120011), the Distinguished Young Scientist Program of Sichuan University (#2013SCU04A38), and the Science & Technology Pillar Program of Sichuan Province (#2014SZ0001).

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