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ORIGINAL ARTICLE

LBP and CD14 polymorphisms correlate with increased colorectal carcinoma risk in Han Chinese

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Abstract

AIM: To explore the associations of polymorphisms of lipopolysaccharide binding protein (LBP), cluster of differentiation 14 (CD14), toll-like receptor 4 (TLR-4), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) with the colorectal carcinoma (CRC) risk in Han Chinese.

METHODS: Polymorphisms of LBP (rs1739654, rs2232596, rs2232618), CD14 (rs77083413, rs4914), TLR-4 (rs5030719), IL-6 (rs13306435) and TNF-α (rs35131721) were genotyped in 479 cases of sporadic colorectal carcinoma and 486 healthy controls of Han Chinese in a case-control study. Single-nucleotide polymorphisms (SNPs) between cases and controls were analyzed by unconditional logistic regression.

RESULTS: GA and GG genotypes of LBP rs2232596 were associated with a significantly increased risk of CRC [odds ratio (OR) = 1.51, 95% confidence interval (CI) 1.15-1.99, *P* = 0.003; OR = 2.49, 95% CI 1.16-5.38, *P* = 0.016, respectively]. A similar association was also observed for the CG genotype of CD14 rs4914 (OR= 1.69, 95% CI 1.20-2.36, *P* = 0.002). In addition, a combination of polymorphisms in LBP rs2232596 and CD14 rs4914 led to a 3.4-fold increased risk of CRC (OR = 3.44, 95% CI 1.94-6.10, *P* = 0.000).

CONCLUSION: This study highlights the LBP rs2232596 and CD14 rs4914 polymorphisms as biomarkers for elevated CRC susceptibility in the Chinese Han population.

Key words: Colorectal carcinoma; Cluster of differentiation 14; Lipopolysaccharide binding protein; Single-nucleotide polymorphisms

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INTRODUCTION

Colorectal carcinoma (CRC) is one of the major causes of cancer death throughout the world. In China, the incidence rate of newly diagnosed CRC cases is increasing rapidly[1]. Both environmental and genetic factors contribute to the tumorigenesis of CRC. The classical adenoma-carcinoma sequence model proposes that genetic mutations of K-ras, adenomatous polyposis coli (APC), the deleted in colorectal cancer (DCC), and p53 play important roles in the malignant transformation and cancer progression

of CRC[2]. Recent studies have demonstrated that chronic inflammation is also an important factor in the carcinogenesis of CRC[3,4]. In the tumor microenvironment, inflammatory cells, especially the so-called tumor-associated macrophages (TAMs), induce suppression of host antitumor activities, stimulate tumor cell growth, and promote malignant transformation, angiogenesis and metastasis[4-8].

TAMs are key regulatory components of cancer-related inflammation. TAMs mainly derive from monocytic precursors in blood circulation, and are recruited to the tumor sites by tumor-derived chemokines such as C-C motif ligand 2 (CCL2) as well as cytokines in the tumor microenvironment, including vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF) and macrophage colony stimulating factor (M-CSF). These chemokines and cytokines also regulate the survival and differentiation of TAMs[7,9]. Further studies have demonstrated that TAMs are defective in IFN-γ/lipopolysaccharide (LPS) responsiveness to bacterial invasion[10,11]. In solid tumors, TAMs are reprogrammed to have pro-tumor properties and therefore fail to respond to LPS stimulation that should have killed the cancer cells[12]. Whether LPS-induced signaling pathways play important roles in tumor progression warrants further investigation.

Lipopolysaccharide binding protein (LBP), cluster of differentiation 14 (CD14), and toll-like receptor 4 (TLR-4) are pattern-recognition receptors (PRRs) that mediate innate immune response to LPS challenge[13,14]. LBP is a secretory class I acute phase protein, which can drastically increase LPS-induced activation of immune cells by binding with LPS and transferring it to CD14. CD14 is a glycosylphosphatidylinositol (GPI)-linked LPS receptor which exists as either membrane-bound forms on the surface of immune cells or soluble forms in the serum. TLR4 belongs to a family of innate immune receptors expressed on the surface of monocytes and macrophages, recognizing pathogen-associated molecular patterns (PAMPs) such as LPS. In the LPS signaling pathway, TLR4 can specifically recognize LPS with the aid of LBP, CD14 and MD-2 molecular complex and activate macrophages in response to LPS-induced inflammation.

Genetic variations of inflammatory factor genes are correlated with increased risk in several malignant tumors. Previous studies have demonstrated a strong association between IL-1beta, IL-8 polymorphisms and gastric carcinoma[15,16], IL-6 polymorphism and cervical carcinoma[17], IL-8, IL-10, TLR4 polymorphisms and prostate carcinoma[18,19], TNF-α polymorphism and non-small cell lung cancer[20]. However, the association between polymorphisms of LPS-signaling-related genes and CRC susceptibility in the Chinese Han population remains elusive. In this study, we directly addressed this issue and investigated the association between polymorphisms of LBP, CD14, TLR4, IL-6 and TNF-α genes and the CRC risk in a case-control study.

MATERIALS AND METHODS

Study population

All subjects were genetically unrelated Chinese Han people living in the southwest region of China. The characteristics of CRC cancer patients and controls included in this study are summarized in Table 1. Patients were chosen from Chongqing Xinqiao Hospital, the second affiliated hospital of Third Military Medical University who were treated from 2008 to 2010. The diagnosis of CRC was confirmed histologically. Patients with histories of previous cancers other than CRC and radiotherapy or chemotherapy were excluded. The controls were healthy people matched to cases by age, sex and dietary habits. Informed consent was obtained from all subjects and the study was approved by the Ethical Committee of Third Military Medical University. Individuals who had smoked over 100 cigarettes were classified as smokers, including current smokers and former smokers who had stopped smoking for at least one year. Individuals who had been drinking alcohol at least once a week for more than 6 months were labeled as drinkers, including current drinkers and former drinkers. Former drinkers were those who had abstained from drinking for more than one year.

Table 1 Characteristics of colorectal carcinoma cases and controls *n* (%)

Parameters	Cases	Controls	*P* value
Age (mean ± SD, yr)	57.85 ± 10.05	58.10 ± 13.47	0.751
Sex			
Male	259 (54.1)	254 (52.3)	
Female	220 (45.9)	232 (47.7)	
Total	479	486	0.574
Smoking status			
Never	334 (69.7)	357 (73.5)	
Former	15 (3.1)	39 (8.0)	
Present	130 (27.1)	90 (18.5)	
Total	479	486	0.199
Drinking status			
Never	10 (2.1)	16 (3.3)	
Former	280 (58.5)	207 (42.6)	
Present	189 (39.5)	263 (54.1)	
Total	479	486	0.248
Histological grade			
High	11 (2.3)		
Intermediate	296 (61.8)		
Low	172 (35.9)		
Location			
Colon	233 (48.6)		
Rectum	242 (50.5)		
Colon and rectum	4 (0.8)		
Stage			
Ⅰ	142 (29.7)		
Ⅱ	110 (23.0)		
Ⅲ	142 (29.6)		
Ⅳ	85 (17.7)		

DNA extraction, polymorphism selection and genotyping

Genomic DNA was extracted from whole blood samples of subjects by the TIANamp Blood DNA Kit (Tiangen, China) according to the manufacturer's instructions. Eight polymorphisms in 5 genes (rs1739654, rs2232596 and rs2232618 in LBP; rs4914 and rs77083413 in CD14; rs5030719 in TLR4; rs13306435 in IL-6; and rs35131721 in TNF-α) examined in our study have been reported with

the minor allele frequency over 1%[21]. SNP genotyping was carried out by the two-step SNaPshot assay. The first step was amplification of gene fragments containing these polymorphic sites. The polymerase chain reaction (PCR) was performed in 25 μL reaction mixture containing 1 × master mix (Tiangen, China), 30 ng genomic DNA templates and 0.4 μmol/L primer sets. Primer sequences (5' to 3') were presented in the order of forward, reverse and SNaPshot sequences, including LBP rs1739654: ACAGAAATGCAGGGACACCTCT, CCTGAGGCTCTCTCTTCCTCAC, GCGGCCCAGGAGGGGCTATT; LBP rs2232596: TCCAACTGGACCCTAGTGGAGT, CGCCTGGCCCTTAATTTTTACT, CCATGTTTTTCAGATTTGCGAAATGATCCAGAAATC; LBP rs2232618: TATGTTGGCACACACAGAACCA, CACTTCCATGTGTCCCTCTGTC, GCTCCTCAACTATTACATCCTTAACACC; CD14 rs77083413: TGAATTGGTCGAAAAGTCCTCA, CCCTGAACTCCCTCAATCTGTC, TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGACCACGCCGGAGTTCATTGAGCCCTCGTG; CD14 rs4914: ACACGGACCCGTTGTTTAAGAT, TGGAACAGGTGCCTAAAGGACT, TTTTTTTTTTTTTTCTTGGATCTTAGGCAAAGCCCCGGGCCCCTTGGAG; TLR4 rs5030719: CAGAGTTGCTTTCAATGGCATC, TGCAGGAAACTCTGGTGTTCA, TTTTTTTTTTTTTTTTTTTTTTTTCCTGGACCTCTCTCAGTGTCAACTGGAGCA; IL-6 rs13306435: ATGGAAGGGTCCTACTCAGAGC, CATAAGTTCTGTGCCCAGTGGA, CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCACTTTCATTTTCCTTCAGGCAAAGAATCTAGA; TNF-α rs35131721: GCGGGAAATATGACAGCTAAGG, CTCCCCCAAGACCAAAACTTTA, TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTAACCATTCTCCTTCTCCCCAACAGTTCC. A similar amplification condition was used for all genes: one cycle of denaturation at 94℃ for 3 min; 30 cycles of denaturation at 94℃ for 30 s, annealing at 68℃ for 30 s, elongation at 72℃ for 40 s; and an eventual elongation at 72℃ for 5 min. The size of PCR products were confirmed by 1.5% agarose gel electrophoresis. The second step was genotyping using the SNaPshot Multiplex Kit (Applied Biosystems, USA). Three μL PCR amplification products were mixed with 5 μL SNaPshot Multiplex Kit and 1 μL 10 μmo/L SNaPshot primer. The SNaPshot PCR condition was 25 cycles of 10 s at 96℃, 5s at 50℃ and 30 s at 60℃. Subsequently, samples were mixed with Liz120 (Applied Biosystems, USA) and were electrophoresed using Genetic Analyzer 3130 instrument (Applied Biosystems, USA). The data were analyzed using the 4.0 Genemapper software (Applied Biosystems, USA).

Statistical analysis

Differences in demographic variables, smoking status, drinking status, grouped genotypic frequencies between cases and controls were evaluated by Student's *t* test and χ^2 test. Two-sided *P* values were considered significant at levels less than 0.05. The associations between polymorphisms of LPS-signaling-related genes and CRC risk were estimated from unconditional regression analysis using the SPSS 13.0 software (PASW, USA). All the eight SNPs were tested for the Hardy-Weinberg equilibrium.

RESULTS

The characteristics of 479 CRC cases and 486 healthy controls are summarized in Table 1. In this case-control study, eight polymorphisms of five genes involved in the LPS-signaling pathway were assayed, in which TLR4 rs5030719 and TNF-α rs35131721 SNPs were excluded due to data bias. All the other six polymorphisms satisfied the Hardy-Weinberg equilibrium ($P > 0.05$).

The effects of the polymorphisms of LPS-signaling-related genes on the risk of colorectal cancer are shown in Table 2. In the genetic model, the G allele of LBP rs2232596 SNP was significantly associated with CRC (GA genotype: odds ratio (OR) = 1.51, 95% confidence interval (CI) 1.15-1.99, $P = 0.003$; GG genotype: OR = 2.49, 95% CI 1.16-5.38, $P = 0.016$). Similarly, the G allele of CD14 rs4914 SNP showed a strong association with the risk of CRC (CG genotype: OR = 1.69, 95 %CI 1.20-2.36, $P = 0.002$). To examine the interaction between epidemiological factors and genetic variances, stratified analysis using logistic regression was performed and no significant difference was found in the genotype distribution of LBP rs2232596 and CD14 rs4914 with respect to age, sex, tumor location and stages (data not shown).

Gene-tobacco exposure interactions and gene-alcohol exposure interactions were also evaluated by stratification analysis using logistic regression (Tables 3 and 4). Smokers with GA genotype of LBP rs2232596 SNP had a significant association with the CRC risk (OR = 1.68, 95% CI 1.17-2.40, P =0.005), whereas in non-smokers, an increased CRC risk with CG genotype of CD14 rs4914 SNP (OR = 2.82, 95% CI 1.64-4.85, P =0.000) was observed. In alcohol drinkers, the presence of GA and GG genotypes of LBP rs2232596 SNP (OR = 1.61, 95% CI 1.23-2.11, $P = 0.001$) and CG genotype of CD14 rs4914 SNP (OR = 1.80, 95% CI 1.28-2.55, $P = 0.001$) was associated with increased risk of CRC.

Further evaluations of the combinatory effects of LBP rs2232596 and CD14 rs4914 SNPs were conducted using logistic regression analysis (Table 5). Subjects carrying risk genotypes in both LBP and CD14 (GA and GG in LBP rs2232596, CG in CD14 rs4914) showed synergistic effects on the associations with the CRC risk (OR = 1.46, 95% CI 1.11-1.92, $P = 0.007$ and OR = 3.44, 95% CI 1.93-6.10, $P = 0.000$).

DISCUSSION

Our study aimed to investigate the association of SNPs in LPS-signaling-related genes and the risk of CRC in the Chinese Han population. We found that the G alleles of both LBP rs2232596 and CD14 rs4914 were significantly associated with CRC. In addition, a combination of these two polymorphisms dramatically increased the CRC risk. To our knowledge, this is the first report on the relationship of these two polymorphisms with gastrointestinal malignancies.

Table 2 Genes, polymorphism and frequencies in colorectal carcinoma cases and controls

SNP	Genotype	Cases (n = 479)	Controls (n = 486)	Odds ratio (95% CI)	[1]P value
LBP					
rs1739654	GG	377 (78.7%)	360 (74.1%)		
	GA	93 (19.4%)	118 (24.3%)	0.75 (0.55-1.02)	0.07
	AA	9 (1.9%)	8 (1.6%)	1.07 (0.41-2.82)	0.88
	GA+AA	102 (21.3%)	126 (25.9%)	0.77 (0.57-1.04)	0.09
rs2232596	AA	289 (60.3%)	343 (70.6%)		
	GA	169 (35.3%)	133 (27.4%)	1.51 (1.15-1.99)	0.003
	GG	21 (4.4%)	10 (2%)	2.49 (1.16-5.38)	0.016
	GA+GG	190 (39.7%)	143 (29.4%)	1.58 (1.21-2.06)	0.001
rs2232618	TT	385 (80.4%)	396 (81.5%)		
	CT	93 (19.4%)	88 (18.1%)	1.09 (0.79-1.50)	0.613
	CC	1 (0.2%)	2 (0.4%)	0.51 (0.05-5.70)	0.581
	CT+CC	94 (19.6%)	90 (18.5%)	1.07 (0.78-1.48)	0.662
CD14					
rs77083413	GG	403 (84.1%)	400 (82.3%)		
	GC	70 (14.6%)	81 (16.7%)	0.858 (0.605-1.215)	0.388
	CC	6 (1.3%)	5 (1%)	1.19 (0.36-3.93)	0.774
	GC+CC	76 (15.9%)	86 (17.7%)	0.88 (0.63-1.23)	0.447
rs4914	CC	369 (77%)	415 (85.4%)		
	CG	102 (21.3%)	68 (14%)	1.69 (1.20-2.36)	0.002
	GG	8 (1.7%)	3 (0.6%)	3.00 (0.79-11.39)	0.091
	CG+GG	110 (23%)	71 (14.6%)	1.74 (1.25-2.42)	0.001
IL-6					
rs13306435	TT	415 (86.6%)	420 (86.4%)		
	AT	60 (12.5%)	65 (13.4%)	0.93 (0.64-1.36)	0.723
	AA	4 (0.9%)	1 (0.2%)	4.05 (0.45-36.37)	0.177
	AT+AA	64 (13.4%)	66 (13.6%)	0.98 (0.68-1.42)	0.921

[1]Adjusted for age, sex, smoking and drinking status.

Table 3 Stratification analyses for rs2232596 by smoking or drinking status

Genotype frequencies (%)	Status	Cases (n = 479)	Controls (n = 486)	[1]P value	Odds ratio (95% CI)
	Smoking				
AA	No	131 (27.3%)	155 (31.9%)		
GA	No	59 (12.3%)	55 (11.3%)	0.303	1.26 (0.81-1.95)
GG	No	10 (2.1%)	5 (1.0%)	0.292	1.86 (0.59-5.87)
GA/GG	No	69 (14.4%)	60 (12.3%)	0.190	1.32 (0.87-2.02)
AA	Yes	158 (33.0%)	188 (38.7%)		
GA	Yes	100 (20.9%)	78 (16.0%)	0.005	1.68 (1.17-2.40)
GG	Yes	11 (2.3%)	5 (1.0%)	0.084	2.59 (0.88-7.63)
GA/GG	Yes	111 (23.2%)	85 (17.0%)	0.002	1.73 (1.22-2.46)
	Drinking				
AA	No	6 (1.3%)	9 (1.9%)		
GA	No	4 (0.8%)	6 (1.2%)	0.892	0.89 (0.16-5.07)
GG	No	0 (0%)	1 (0.2%)	0.998	
GA/GG	No	4 (0.8%)	7 (1.4%)	0.739	0.75 (0.14-4.03)
AA	Yes	283 (59.1%)	334 (69.7%)		
GA	Yes	165 (34.4%)	127 (26.1%)	0.003	1.53 (1.16-2.03)
GG	Yes	21 (4.4%)	9 (1.9%)	0.015	2.68 (1.21-5.97)
GA/GG	Yes	186 (38.8%)	136 (28.0%)	0.001	1.61 (1.23-2.11)

[1]Adjusted for age, sex, and drinking or smoking status.

The LPS-signaling pathway is a crucial player in the innate immunity regulatory system, which includes LBP, CD14, TLR4/MD2 and other molecules involved in LPS-induced NF-κB activation such as MyD88, TIR, IRAK and TRAF6[22]. In intestinal mucosa, continuous exposure to LPS activates M1 type macrophages to perform tumoricidal tasks. TAMs in the cancer micro-environment belong to M2 macrophages and, on the contrary, promote tumor growth. In the presence of M2 macrophages, the LPS signaling pathway is down-regulated but the mechanisms remain unclear[10-12].

Previous studies in this field mainly focused on the effects of genetic variations of aforementioned genes in non-tumoral diseases such as bacterial infections[23,24], sepsis[25] and myocardial infarction[26]. Recently, how these genetic variations contribute to the risk of developing var-

Table 4 Stratification analyses for rs4914 by smoking or drinking status

Genotype frequencies (%)	Status	Cases (n = 479)	Controls (n = 486)	[1]P value	Odds ratio (95% CI)
	Smoking				
CC	No	146 (30.5%)	191 (39.3%)		
CG	No	50 (10.4%)	23 (4.7%)	0.000	2.820 (1.64-4.85)
GG	No	4 (0.8%)	1 (0.2%)	0.144	5.210 (0.57-47.55)
CG/GG	No	54 (11.2%)	24 (4.9%)	0.000	2.920 (1.72-4.96)
CC	Yes	223 (46.6%)	224 (46.1%)		
CG	Yes	52 (10.9%)	45 (9.3%)	0.524	1.155 (0.74-1.80)
GG	Yes	4 (0.8%)	2 (0.4%)	0.430	1.993 (0.36-11.05)
CG/GG	Yes	56 (11.7%)	47 (9.7%)	0.429	1.190 (0.77-1.83)
	Drinking				
CC	No	9 (1.9%)	10 (2.1%)		
CG	No	1 (0.2%)	5 (1.0%)	0.215	0.227 (0.02-2.37)
GG	No	0 (0%)	1 (0.2%)	0.998	
CG/GG	No	1 (0.2%)	6 (1.2%)	0.154	0.190 (0.02-1.88)
CC	Yes	360 (75.2%)	405 (83.3%)		
CG	Yes	101 (21.1%)	63 (13.0%)	0.001	1.800 (1.28-2.55)
GG	Yes	8 (1.7%)	2 (0.4%)	0.060	4.470 (0.94-21.18)
CG/GG	Yes	109 (22.8%)	65 (13.4%)	0.000	1.890 (1.34-2.64)

[1]Adjusted for age, sex and drinking or smoking status.

Table 5 Colorectal carcinoma risk with combined lipopolysaccharide binding protein rs2232596 and CD14 rs4914 SNPs

No. of risk genotype	Cases (n = 479)	Controls (n = 486)	[1]P value	Odds ratio (95% CI)
"0"	235 (49.1%)	296 (60.9%)		
"1"	194 (40.5%)	172 (35.4%)	0.007	1.46 (1.11-1.92)
"2"	50 (10.4%)	18 (3.7%)	0.000	3.44 (1.94-6.10)
"1+2"	244 (50.9%)	190 (39.1%)	0.000	1.66 (1.28-2.15)

[1]Adjusted for age, sex, smoking and drinking status.

ious cancers has drawn much attention. Polymorphisms in the CD14 promoter can affect the susceptibility to CRC[27] and *Helicobacter pylori* infection–related gastric carcinoma[28] in Chinese patients, and prostate cancer in African American men[29]. Effects of polymorphisms of TLR4 and other PRRs on cancer risk have also been reported[30,31].

Our study of the genetic variances in LBP rs2232596 and CD14 rs4914 provided strong evidence of interactions between LPS-signaling-related genes and the risk of CRC, indicating that the genetic modulation of LPS-induced inflammation may contribute to CRC development and progression. TAMs with defective LPS responsiveness are common components of the micro-environment of different cancers. In addition, the current study and several previous studies revealed that functional polymorphisms in LPS-signaling-related genes are associated with various cancer risks. More studies are needed to shed light on the underlying genetic mechanisms.

Tobacco and alcohol exposure have been identified as high-risk factors for CRC[32, 33]. However, our data failed to show any significant associations of tobacco and/or alcohol exposure with CRC susceptibility. We found that smokers and drinkers carrying LBP rs2232596 polymorphisms had a higher risk of CRC. But only drinkers carrying CD14 rs4914 polymorphism showed modest risk of CRC. One possible explanation is that different mechanisms regulate tobacco-gene and alcohol-gene interactions. This study lacked detailed information on the smoking and drinking status of the subjects. Further stratification analysis is needed to evaluate the risk of lifestyle factors.

What mediates the observed association between gene polymorphisms and CRC susceptibility still remains unknown. It would be interesting to compare the serum levels of LBP and CD14 from different genotypes to examine the relationship between gene polymorphisms and their expression levels.

In conclusion, the functional G alleles in both LBP rs2232596 and CD14 rs4914 SNPs showed significant associations with a high CRC risk. Further studies are needed to elucidate the effects of these genotypes on gene transcription, expression and functions in CRC and other types of malignancies.

COMMENTS

Background

Colorectal carcinoma (CRC) is a leading cause of cancer death in China and throughout the world. Chronic inflammation is considered to be important in the carcinogenesis of CRC. In this study, the authors examined the association between the gene polymorphisms of several lipopolysaccharide (LPS)-signaling factors and the risk of CRC to better elucidate the mechanism of inflammation in tumorigenesis.

Research frontiers

LPS-induced signaling is an important innate immune response that involves many different molecules, such as lipopolysaccharide binding protein (LBP), cluster of differentiation 14 (CD14), and toll-like receptor 4 (TLR-4). Recently, it has become a hot area to employ polymorphism analysis to identify genetic mutations in the immune system that are significantly correlated with tumor development.

Innovations and breakthroughs

To date, there has been no study on the polymorphisms of LPS-signaling-related genes and CRC susceptibility in the Chinese Han population. In this study, the authors directly addressed this issue and performed genetic analysis to screen for polymorphisms that are associated with increased CRC risk. The authors also explored the gene-environment interactions by studying the effects of smoking or drinking exposure on CRC susceptibility.

Applications

By demonstrating the association of LBP rs2232596 and CD14 rs4914 polymorphisms with increased CRC risk, the authors identified two important biomarkers for predicting CRC and further improved the understanding of the inflammation-related mechanisms in CRC development.

Terminology

Single-nucleotide polymorphism (SNP): a DNA sequence variation occurring when a single nucleotide in the genome differs between members of the same biological species or paired chromosomes in an individual. SNP analysis can shed light on how genetic variations affect disease development in humans.

Peer review

This is an interesting well-conducted and well-written study.

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