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The Association between Cyclin D1 (CCND1) rs9344 AA Genotype and Increased Risk of Colorectal Cancer in An Iranian Population

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Abstract

Background: Colorectal cancer (CRC) is a globally growing disease with a steady decrease in the age of incidence. Pathogenesis of this cancer stems from a complex interaction between environmental factors and genetic predisposition. Among genetic factors, high activity of cyclin D1gene is prominent. A polymorphism (G870A) in exon 4 of cyclin D1 is responsible for a variant transcript with longer half-life and may culminate in uncontrollable cellular growth, thereby contributing to cancer development.

Method: This case-control study evaluated the frequency of CCND1 G870A polymorphism and risk of sporadic CRC in an Iranian population. The study population comprised 50 CRC patients and 50 CRC-free controls selected on the basis of colonoscopy examination. For genotyping, we performed polymerase chain reaction – restriction fragment length polymorphism analysis (PCR-RFLP).

Result: AA genotype frequencies compared to GA+GG genotype frequencies between cases and controls showed that AA genotype frequency in the case group was significantly higher than the control group (AA vs. GG + GA: OR= 2.25, 95% CI: 1.13-5.54, *P*=0.04). Allele A frequency was 57% in patients and 46% in healthy subjects. Statistical analysis showed that the odds ratio of carriers with allele A for risk of CRC was 1.55 more than G allele carriers (OR=1.55, 95% CI: 0.856-2.828). Moreover, physical activity in cases was significantly less than controls (*P*=0.001). We further observed that the subjects in the case group used fewer non-steroidal anti-inflammatory drugs compared to healthy controls (*P*=0.02). Analysis of body mass index (BMI) between cases and controls revealed that the average of BMI in cases was higher than the controls (*P* =0.04).

Conclusion: Our results showed that individuals carrying the AA genotype ran a higher risk of developing CRC compared to GG genotype.

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Table 1. Primer sequences, restriction enzyme used and expected fragment length for each genotype after digestion

SNP ID	Ancestral allele	Primer sequence	PCR product length	Restriction enzyme	RFLP fragment size (bp)
Rs9344	G	Forward: ATTTCCAATCCGCCCTCCAT	251	MspI	GG:136+81+34
					AG:170+136+81+34
		Reverse: CCCCAACCTTGTCACCCTT			AA:170+81

PCR: Polymerase chain reaction, RFLP: Restiriction fragment length polymorphism

Introduction

Colorectal cancer (CRC) has recently become highly prevalent worldwide. The newly diagnosed CRC cases are estimated at approximately 1.2 million and there were 608,700 CRC-related deaths in 2008. Although the incidence of CRC is lower in Asian than in Western countries, recent studies have shown the increasing rates of CRC in Iran.[1,2] Previous studies have indicated that environmental factors such as gender, age, body mass index (BMI), physical activity, smoking status, and non-steroidal anti-inflammatory drugs (NSAIDs) consumption are correlated with change in CRC incidence in different populations. Although different individuals may be exposed to the same risk factors, not all develop CRC.[1,3] This fact shows that genetic variation can determine individual susceptibility to colorectal tumorigenesis. Among genetic factors, high activity of cyclin D1 gene is prominent. The polymorphism (G870A) in exon 4 of cyclin D1 is responsible for a variant transcript with longer half-life and may culminate in uncontrollable cellular growth, hence contributing to the development of cancer.[4] The G/A replacement corresponding to codon 241(pro-241-pro) in the conserved splice donor region of exon 4 generates an alternatively spliced transcript of CCND1 gene, called 'transcript b', which lacks exon 5.[5] This segment is an important regulatory motif for the degradation of cyclin D1. The G allele create a full transcript named 'transcript a' that inhibit the overexpression of cyclin D1.[6] Therefore; transcript b (A allele) has a longer half-life compared to transcript a (G allele) that results in more avoidance from G1-S cell cycle check point and higher cancer progression risks.[7] In this case-control study, we assessed the association of CCND1 G870A polymorphism with the risk of CRC in an Iranian population. No related study has been done in Iranian populations. We also investigated the association between CRC risk and a number of factors, including physical activity, use of NSAIDs, BMI, and smoking.

Materials and Methods

This case-control study comprised 50 sporadic CRC cases and 50 cancer-free controls from Isfahan province located in central Iran. Participants filled out a structured questionnaire regarding demographic data and other risk factors, including BMI, smoking, NSAIDs use, and physical activity. All subjects singed an informed consent form and the Ethics Committee of the University approved the study. We extracted DNA from peripheral blood leukocytes and assessed its quality and quantity by agarose gel electrophoresis and spectroscopy. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) performed the genotyping for the cyclin D1 G870A polymorphism. Table 1 shows the employed primers and restriction enzymes and the expected fragments generated following digestion. After digestion, we separated the products on 2.5% agarose gel containing 0.5 mg/ml DNA Green Viewer. To confirm RFLP genotyping results, we subjected around 10% of the randomly selected PCR products to direct sequencing and compared the outcome with RFLP results. $\chi 2$ test examined the genotype frequencies in case and controls for Hardy–Weinberg equilibrium. Odds ratios (ORs) and 95 % confidence intervals (CIs) assessed the correlation between this polymorphism and CRC. SPSS22 software (SPSS, Chicago, IL., USA) performed all statistical analyses. $P<0.05$ was considered statistically significant.

Table 2. Characteristics of study population

	Cases (N=50)	Controls (N=50)	*P* values
Age (mean±SD)	59/1±10/6	58/2± 10/8	0/67
Gender			
Male	26(52%)	23(46%)	0/54
Female	27(54%)	24(48%)	
BMI (mean±SD) (kg/m^2)	26/68±5/6	25/8±3/6	0/04*
Smoking			
Yes	5 (10%)	6 (12%)	0/75
No	45 (90%)	44(88%)	
Physical Activity			
Very low	13 (26%)	0 (0%)	0/001*
Low	21 (42%)	19 (38%)	
Moderate	15 (30%)	22 (44%)	
High	1 (2%)	9 (18%)	
NSAIDs or aspirin use			
Regular	4 (8%)	13 (26%)	0/02*
Irregular	46 (92%)	37 (74%)	

**P* values < 0.05; BMI: Body mass index; NSAIDs: Non-steroidal anti-inflammatory drugs

Results

Table 2 shows the characteristics of CRC patients and healthy controls. This study consisted of 50 patients (26 men and 24 women) plus 50 controls (23 men and 27 women). The mean age of cases and controls were 59.1±10.6 and 58.2±10.8 years, respectively. Statistical analysis showed no significant differences between the two groups regarding the distribution of age, gender, and smoking. The cases were less likely to have regularly used NSAIDs (8% vs. 26%); significant inverse associations existed between the risk of CRC and NSAIDs use (*P* value =0.02). There was a significant difference between cases and controls concerning physical activity; Mann-Whitney test showed that physical activity in cases was significantly lower than in controls (*P* value = 0.001). The average of BMI was higher in cases compared to the controls (*P* value =0.04). Table 3 shows the genotype and allele frequencies of CCND1 G870A polymorphism. Hardy-Weinberg analyses detected no deviation from equilibrium among cases or controls. Among the CRC cases, the frequency of the cyclin D1 G870A genotype was 22% for GG, 42% for GA, and 36% for AA; however, the frequency in the control population was 28% for GG, 52% for GA, and 20% for AA. The frequency of allele A was 57% in cases and 46% in healthy subjects. Odds ratio of allele A carrier individuals was 1.55 more than G allele carriers (OR=1.55, 95%CI: 0.856-2.828). We examined the frequency of AA genotype and GA+GG genotypes between the two groups. Results showed that AA genotype frequency in the cases was significantly higher than the control group (AA vs. GG + GA: OR= 2.25, 95% CI: 1.13-5.54, *P*=0.04). Moreover, individuals carrying AA genotype ran a higher risk of developing CRC compared to GG genotype (AA vs. GG: OR=2.29, 95% CI: 1.05-5.01).

Discussion

The purpose of the current study was to evaluate the frequency of CCND1 G870A single nucleotide polymorphism in CRC patients and healthy individuals in an Iranian population. Our results showed that AA genotype frequency in the case group was significantly higher than control group (AA vs. GG + GA: OR= 2.25, 95% CI: 1.13-5.54). Analysis showed that individuals carrying the AA genotype had more increased risk of CRC compared to GG genotype (AA vs. GG: OR=2.29, 95% CI: 1.05-5.01). Zhang et al. performed a meta-analysis in 2011; they showed

Table 3. Genotype and allele frequencies between cases and controls

	Case		Control	
	N	%	N	%
Allele frequency				
G	21.5	43%	27	54%
A	28.5	57%	23	46%
Genotype frequency				
GG	11	22%	14	28%
AG	21	42%	26	52%
AA	18	36%	10	20%

that 870A allele of cyclin D1 gene was a low-penetrant risk factor for progression of sporadic colorectal cancer, particularly among Caucasians (AA vs.GG: OR = 1.23, 95% CI = 1.04-1.44).[8] An extensive meta-analysis involving 13,642 subjects showed that the presence of the CCND1 rs9344 A allele, which increased CCND1 activity, conferred susceptibility to CRC. In a study performed by Sameer et al., A allele frequency was 58.5% in patients and 50.6% in healthy people; this frequency expressed statistically significant 2.01-fold increase in the odds ratio in a dominant model of inheritance; however, there was only a 1.25-fold increase in the OR regarding the A allele in a recessive model of inheritance (P<0.05).[10] We further analyzed the association between the incidence of CRC and several environmental factors such as NSAIDs, smoking, BMI, physical activity, sex, and age. We also examined the interaction of these factors with the cyclin D1 G870A polymorphism. Logistic regression analysis based on gender showed that the influence of GG, AG, and AA genotypes did not change according to gender. These findings suggest that the relationship between this polymorphism and the risk of sporadic CRC is not gender-specific. The participants were classified into two age groups, below 55 and above 55 years; analysis showed that AA genotype was more common in elderly people in comparison with GA and GG genotypes; however, generally, there was no significant association between these genotypes and CRC incidence by age (P value=0.092). Huang et al. conducted a study in 2006 and examined that the effect of AA / AG genotypes on the risk of CRC. On the other hand, they only observed that young men, and young men with CRC had significantly higher frequency of AA/AG genotypes compared to healthy controls (OR = 2.75; 95% CI, 1-7.9).[11] We also stratified the patients into two groups of smoking and non-smoking. The results showed no significant association between this factor and genotype polymorphism (P Value=0.181).

NSAID usage proved to be associated with the risk of CRC in the present population; however, there existed no significant association between the genotypes of this SNP and NSAIDs use.[12] In contrast, some studies have shown that those with GG genotype and consumers of NSAIDs are exposed to lower risks of CRC.[13,14] The relationship between CCND1 G870A polymorphism and the risk of several cancers, including breast, lung, colon, stomach, and bladder were studied. These studies revealed that this SNP behaved as a multicancer susceptibility marker. Numerous case-control studies have shown a positive correlation between CCND1 rs9344 polymorphism and the risk of CRC.[4,5] However, in many others, such positive correlations have not been confirmed.[6,7] Most studies in this area were conducted on the Caucasians and Asians. Their results have shown that G870A polymorphisms in cyclin D1 gene are a low-penetrance risk factor for CRC, especially among Caucasians.[8] It can be helpful to examine the effect of rs9344 on the incidence of CRC and its interaction with other genetic variants and environmental factors.

Conclusion

Based on the results, it can be concluded that AA genotype increases the risk of CRC compared to GG genotype. However, in general, there are inconsistent results about the relationship between this polymorphism and the risk of CRC. This shows that CRC depends on other factors, including ethnic and geographical differences, interaction of this polymorphism with other genetic factors or genetic background, and exposure to environmental factors.

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Conflict of Interest

None declared.

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