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Evaluation of Polymorphisms rs762624 and rs3176336 of CDKN1A Gene and Risk of Colorectal Cancer

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Authors' contributions

This work was carried out in collaboration between all authors. Author NZ wrote the proposal, Lab working and wrote the first draft of the manuscript. Authors MH and MMH designed the study, managed the analyses of the study. Authors VC and SS Lab working and wrote the protocol. Author MV performed the statistical analysis. Authors MH, MRZ, HAA and ENM managed the literature searches, managed the analyses of the study and wrote the final draft of the manuscript.

Original Research Article

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ABSTRACT

Aims: Progressive loss of cell cycle control is an important feature on the colorectal cancer. CDKN1A gene encoded p21 protein that's one of the cyclin-dependent kinase inhibitors, plays a key role in regulating the cell cycle. The aim of this study was to

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investigate associations of the CDKN1A gene polymorphism rs762624 and rs3176336 with risk of colorectal cancer in an Iranian population.
Methods: A case-controls study was conducted to investigate the association of polymorphism rs3176336 and rs762624, with colorectal cancer risk in Iranian population. In this study 150 cases of sporadic CRC and 150 healthy controls were recruited, genomic DNA were extracted from peripheral blood, the genotypes were determined using the polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method and the result was validated by direct sequencing.
Results: The rs762624 frequencies of the AA, AC, and CC genotypes among cases were 9.3%, 74.7%, and 16%, respectively, while in controls genotype frequencies were 10%, 74%, and 16%, respectively. The rs3176336 frequencies of the AA, AC, and CC genotypes among cases were 29.3%, 18% and 52.7%, respectively, while in controls genotype frequencies were18%, 20%, and 62%, respectively. No association was found for the CDKN1A rs3176336 AT/AA genotype (Adjusted odds ratio (OR), 0.726, 95% confidence interval (CI), 0.365–1.443 for AT genotype; OR, 1.67, 95% CI, 0.754–3.702 for AA genotype) with risk of colorectal cancer, compared with the TT genotype. In our research, we could not found significant relation between stage of colorectal cancer and genotypes of rs762624 and rs3176336 polymorphisms (p=0.081, p=0.988).
Conclusion: Present data do not confirm association of rs3176336 and rs762624 polymorphisms with susceptibility of Iranian to colorectal cancer.

Keywords: Single nucleotide polymorphism; colorectal cancer; CDKN1A; rs762624; rs3176336.

1. INTRODUCTION

Colorectal cancer (CRC) is the third widespread cancer in the world, and one of the main causes of death arising out of cancer in all societies [1]. Studies showed that CRC is rising in Asian countries such as Japan, China, Singapore and South Korea [2-5]. CRC is the fifth most common cancer among Iranian men and third among women [5].

CRC is the result of uncontrolled growth of cancer cells in large intestine [6]. The pathogenesis of colorectal carcinoma is multifactorial. The genetic heterogeneity of CRC is now well established [7]. Several genes and pathway are involved in the initiation and development of CRC [8]. Although environmental factors are effective in the occurrence of CRC, genetic factors play the main role in this type of cancer [9,10].

One of the genes that have role in the cancer is CDKN1A (P21/CIP1/WAF1), p53 activates the transcription of CDKN1A, and there is a direct link between p53 and cell cycle regulation [11,12]. CDKN1A gene is located on 6p21 chromosome, whose sequence, structure, and activities are protected by p53 [13]. Cell cycle regulation is required for the growth and differentiation of cells, and disruption of cell cycle leads to the growth of tumor [14]. P53 can stop cell cycle by stimulating CDKN1A gene that encodes cyclin-dependent kinase inhibitor 1 (p21) protein. p21 play a great role in the inhibit of cell cycle after damaging DNA [15]. p21 inhibits CDKs, which are the main enzymes for the regulation of cell cycle [11]. p21 prevents apoptosis by the cutting induction of proapoptotic protein such as procaspase 3 and procaspase 8 [15]. In the most reproducing cells, p21 exists in low amounts [16].

Despite curative surgical resection of the primary tumor and adjuvant chemotherapy, CRC continues to be a major healthcare concern [17,18]. Although, the primary diagnosis and therapy is colonoscopy and surgical method respectively, on the other hand the predictive and prognostic value of CRC cancer is still poor; hence, finding new biomarker is necessary [19,20].

Single nucleotide polymorphism (SNP) is the important source of variation in the genome, which could lead to increased susceptibility to cancer. Various frequencies of SNP genotypes in different population have been observed [21]. In our previously study we found no significant association between EGF rs4444903 polymorphism and CRC in an Iranian population [22]. Although, according to meta-analysis report from the Ying Piao et al. EGF rs4444903 polymorphism might increase the risk of esophageal and CRC [23]. According of our knowledge, no study reported that CDKN1A rs762624 and rs3176336 polymorphisms are associated with susceptibility to CRC in Iranian individuals. Therefore, we hypothesized that CDKN1A gene polymorphisms may modulate the susceptibility to CRC. To test this hypothesis, we conducted a case control study to evaluate the potential association between CDKN1A rs762624 and rs3176336 polymorphisms and the risk of CRC in an Iranian population.

2. MATERIALS AND METHODS

2.1 Study Population

In this case-control study, a total of 300 subjects were enrolled (150 sporadic colorectal cancer, 150 healthy controls). All patients were afflicted with sporadic colorectal cancer and were selected from the individuals referred to Taleghani Hospital Tehran during the period 2010-2012 for diagnosis and treatment. A colonoscopy was performed on all participants in the patient and control groups. The control group consists of the persons, who referred to this center for screening purposes and whose negative colonoscopy test confirmed that they are not suffering from CRC. Written informed consent was obtained from all the subjects. This study was conducted under the approval of the ethics committee (protocol number: 4035) of the Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences (Tehran, Iran).

2.2 Genotyping

Genomic DNA was extracted from peripheral blood using salting out standard method [24]. The genotypes of the both polymorphisms were analyzed by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP). PCR amplification of genomic DNA was performed with specific primers. To confirm the PCR products were subjected to 1% agarose gel, the PCR products were digested by the restriction enzymes (New England Biolabs). The digested PCR products were determined on a 3% agarose gel and stained with ethidium bromide for visualization under UV light. SNPs analysis information is shown in the (Table 1).

2.3 Sequencing

To confirm the RFLP procedure, 10% of the PCR products were sequenced using the ABI PRISM 3130xL Genetic Analyzer (Applied Biosystems®, Invitrogen Life Technologies, Carlsbad, CA, USA) and the chain termination method (Fig. 1).

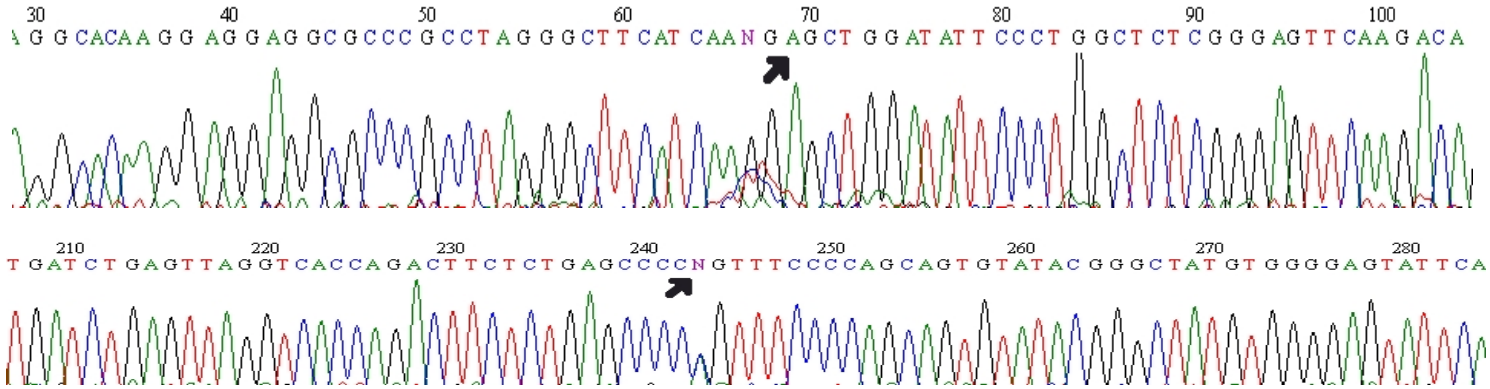

Fig. 1. Direct DNA sequencing results for the CDKN1A rs3176336 and rs762624 gene polymorphisms

Table 1. SNPs analysis information

SNPs (reference sequence)	Location (base change)	Primer sequence	PCR product size (bp)	Restriction enzyme	RFLP fragments size (bp)
rs762624	Promoter -899 (A/C)	Forward prime 5′GTGTGAGGTAGATGGGAG3′ Reverse primer: 5′GAAGGGGAGGATTTGACG3′	350	BmrI	C: 350 A: 264+86
rs3176336	Intron+2330 (A/T)	Forward primer: 5′GTTTCTGAGTTTTCTTTG3′ Reverse primer: 5′AGCGGAGACACACTGGTA3′	268	AluI	A: 268 T: 172+96

2.4 Statistical Analysis

The cases and controls were compared using a Student's t-test for the continuous variables and a χ2 test for the categorical variables. Hardy–Weinberg equilibrium (HWE) was tested using a goodness-of-fit χ2 test. Unconditional logistic regression analysis was used to calculate the odds ratios (ORs) and 95% confidence intervals (CIs), with an adjustment for possible confounders (gender, age and smoking). The statistical significance level consider lower than 0.05 (P<0.05). The data were analyzed using of SPSS software (version 13).

3. RESULTS

In the present study, we evaluated effect of polymorphisms rs762624 and rs3176336 at tumor suppressor gene CDKN1A with risk of CRC in the total of 150 patients and 150 cancer-free controls in Iranian population. Demographic characteristics of case patients and control subjects are summarized in (Table 2). There were no significant differences between cases and controls according to gender and smoking status (P=0.105 and P=0.650, respectively). Although we observed the patients had significantly higher average age and BMI than the controls individual (P<0.001 and P=0.035, respectively). However, all of these variables were adjusted further for any residual confounding effect in later multivariate logistic regression analyses.

The genotype and allele frequencies of CDKN1A polymorphisms rs3176336 and rs762624 for the controls and the cases are shown in (Table 3).The rs3176336 frequencies of the TT, AT, and AA genotypes were 18%, 52.7%, and 29.3%, respectively, among the cases, and 20%, 62%, and 18%, respectively, among the controls. The rs762624 frequencies of the AA, AC, and CC genotypes were 9.3%, 74.7%, and 16%, respectively among the cases, and 10%, 74%, and 16%, respectively among the controls. Genotype distributions in controls and cases were consistent with the Hardy–Weinberg equilibrium (HWE). In these SNPs analyzed, the P value is larger than 0.05. There was no apparent association between the CDKN1A geners762624 and rs3176336 polymorphisms and stage of CRC (p=0.081, p=0.988) (Table 4).

No significant association was found between rs762624 and rs3176336 of CDKN1Agene and risk of CRC. Nevertheless the statistical significance level consider lower than 10%, rs3176336 polymorphism can be described as significant result.

Table 2. Demographic characteristics of the colorectal cancer case control study participants

Variables	Controls	Cases	*p*
Age (mean±S.D.)	45.21±15.810	58.35±12.488	*P*<0.001
BMI	28.82±3.19	24.39±3.11	0.035
Gender, n (%)			0.105
Male	65(43.3%)	83(55.3%)	
Female	85(56.7%)	67(44.7%)	
Smoking			0 .650
Smoker	12(8%)	13(8.7%)	
Non-smoker	138(92%)	137(91%)	

Table 3. Allele and genotype distribution of two studied SNPs among CRC patients and healthy control subjects

SNP	Variable	Controls	Cases	Unadjusted OR (95%CI)	†Adjusted OR (95%CI)	*p*
rs3176336						
	Genotypes					
	TT	30(20%)	27(18%)	1.00 (Reference)	1.00(Reference)	
	AT	93(62%)	79(52.7%)	0.944(0.518-1.720)	0.726(0.365-1.443)	0.850
	AA	27(18%)	42(29.3%)	1.811(0.893-3.672)	1.67(0.754-3.702)	0.100
	Alleles					
	T	153(51%)	133(44.3%)	1.00 (Reference)		
	A	147(49%)	167(55.7%)	1.37(0.948-1.82)		0.102
rs762624						
	Genotypes					
	AA	15(10%)	14(9.3%)	1.00(Reference)	1.00(Reference)	
	AC	111(74%)	112(74.7)	1.081(0.498-2.345)	0.860(0.364-2.034)	0.844
	CC	24(16%)	24(16%)	1.071(0.426-2.695)	1.399(0.828-2.366)	0.883
	Alleles					
	A	141(47%)	140(46.7%)	1.00(Reference)		
	C	159(53%)	160(53.3%)	1.013(.735-1.397)		0.935

† *Adjusted for confounder variables such as age and gender. CI, confidence interval; OR, odds ratio*

Table 4. Shows the tumor-stage specific distribution of CDKN1A rs3176336 and rs762624 genotypes among colorectal cancer patients

SNP	Genotype	Stage 0	Stage I	Stage II	Stage III	Stage IV	*P* value
rs3176336							0.081
	TT	0(0%)	0(0%)	3(7.0%)	15(27%)	2(16.7%)	
	AT	0(0%)	4(66.7%)	24(55.8%)	25(45.5%)	9(75%)	
	AA	1(100%)	2(33%)	16(37.2%)	15(27%)	1(803%)	
rs762624							0.988
	AA	0(0%)	0(0%)	3(8.8%)	4(8.9%)	1(4.3%)	
	AC	2(100%)	4(80%)	26(76.5%)	33(73.3%)	18(78.3%)	
	CC	0(0%	1(20%)	5(14.7%)	8(17.8)	4(17.4%)	

4. DISCUSSION

CDKN1A gene is a well-studied gene encoding a protein that controls the cell cycle arrest under multiple stimulations. p21 is involved in DNA repair, such as nucleotide excision and base excision repair [25-27]. Previous meta-analysis by Ma et al. showed that polymorphism within exonic region (Ser31 Arg) of CDKN1A gene were associated with the risk of cancer especially CRC [28]. In our knowledge polymorphisms in other region (intronic and promotor) of CDKN1A gene have been studied less in colorectal cancer population extensively. In this studied, we examining SNPs of CDKN1A gene rs3176336 which is located in the intronic region, and rs762624 which is located at promoter position in Iranian CRC population. In our study, we could not find significant association between two SNPs and risk CRC. Other studies showed that inconsistent relationships of these SNPs and cancer such as Mcinerney et al. in 2009 reported that no evidence of an association between rs3176336 and breast cancer [29]. Driver et al. [30] performed a research on the effects of rs3176336 on breast cancer in the patients of the British population, and found that there was a significant relation between this polymorphism and breast cancer (p=0.0026).

In a study of K. Kim and colleagues conducted on the relation between rs762624 and systematic lupus erythematosus (SLE), the researchers provided a hypothesis stating that the SNP rs762624 is located in the binding site of transcriptional c-Myb in CDKN1A promoter. This SNP converts allele C to allele A, and changes the binding site of c-Myb, leading to the decrease in gene expression in intercellular conditions. They also found that the minor allele A of rs762624 is related to increase the susceptibility to systemic lupus erythematosus (SLE) and lupus nephritis [31]. According to the Song et al. study, no significant relation was reported between rs762624 polymorphism and ovarian cancer survival [32]. Also one year later, L Goode et al. examined the relation between 11 genes involved in cell cycle and ovarian cancer in American people. They found no significant relation between rs3176336, rs762624, and ovarian cancer [33]. We presume that these polymorphisms are in different levels of correlation with different cancers in different populations. Allelic frequency difference or imbalance in correlation may be the causes of allelic heterogeneity.

We considered the tumor stage as a possible effect on CRC progression; however, no significant relation between these data and CRC was founded.

Although no significant association between the studied polymorphisms and CRC is reported, the relation found is considered to some extent significant. Moreover, it is suggested that the relation of the expression of the different transcriptions of rs762624 polymorphism with CRC be studied.

5. CONCLUSION

Present data provide the first evidence that these polymorphisms (rs3176336 and rs762624) are not a potential contributor to the risk of colorectal cancer and clinicopathological features in an Iranian population, and suggests the need of a large-scale case-control study to validate our results.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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