RESEARCH ARTICLE

Association of 8q24.21 rs10505477-rs6983267 Haplotype and Age at Diagnosis of Colorectal Cancer

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Abstract

Background: Colorectal cancer (CRC) is the fourth most common cause of cancer death in the world. Genetic variants in 8q24.21 including rs10505477 and rs6983267 have been hypothesized to be involved in susceptibility to CRC. This study aims to investigate the possible association between these loci and their haplotypes with CRC risk in Iranian population. Materials and Methods: Subjects were recruited from two hospitals in Tehran. The rs10505477 and rs6983267 polymorphisms were genotyped by TaqMan real time PCR using subject genomic DNA, extracted either from formalin-fixed, paraffin-embedded tissue of patients or from blood of the controls by standard methods. Results: A total of 715 subjects (380 CRC patients and 335 matched controls) were genotyped in this study. Allele and genotype analysis of the rs10505477 and rs6983267 polymorphisms by gender, age at diagnosis, tumor location, tumor grade, and tumor node metastasis (TNM) showed no significant association with CRC risk. There was a significant relationship between GG haplotype and susceptibility to age at diagnosis for both <60 and ≥60 (p=0.0005 and p=0.000004, respectively) and between GT and CRC in the age at diagnosis ≥ 60 (Table 3: p=0.031). The GG haplotype was less frequent in CRC patients with the age at diagnosis <60, but was more common in subjects with the age at diagnosis ≥ 60. Conclusions: Results of this study suggests that the rs6983267 and rs10505477 polymorphisms alone may not be relevant to CRC risk, but their GG haplotype plays a notable role in age at diagnosis of CRC in the Iranian population.

Keywords: Colorectal cancer - 8q24.21 - polymorphism - age at diagnosis - Iran

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Introduction

Colorectal cancer (CRC) is a major public health problem in the world wide and is considered as the second most common cause of cancer death in the western. This disease is the second most common cancer in female and the third in male in the world. A significantly increased risk of CRC has been reported by epidemiological studies (Siegel et al., 2012). In Iran, the incidence rate of CRC in males and females is about 40 and 36 per 100,000 people, respectively, which is relatively higher than other countries (Kolahdoozan et al., 2010; Safaee et al., 2012). Approximately, 5000 new diagnosed Iranian CRC patients have been reported every year, of them 43% were younger than aged 50 years, a decade younger than the range in developed countries. The majority of CRC tumors were detected in the colon. Since CRC impacts on morbidity and mortality of the patients, early diagnosis of this disease will reduce the number of cases (Foroutan et al., 2008; Safaee et al., 2010).

CRC is a complex disease contributed by environmental and genetic risk factors. Twin studies indicated that approximately 35% of CRC aetiology is contributed by genetic effect, of which 30% is related to low penetrance variants (Lichtenstein et al., 2000; Goel et al., 2010). Genome-wide association studies (GWAS) have reported numerous CRC risk variants. Among these variants, the rs6983267 and rs10505477 located at 8q24, have been of extensive interest in the development of different types of cancer, including CRC (Tomlinson et al., 2007; Zanke et al., 2007; Tenesa et al., 2008). Recently, meta-analyses of the pooled related data verified the association of rs6983267 and rs10505477 polymorphisms with susceptibility to CRC (Haerian et al., 2011; Theodoratou et al., 2012). A study has verified this finding for rs6983267 polymorphism in Iranian population with CRC (Daraei et al., 2012). However, there is no report of relevance of the rs10505477 polymorphism and its combination with rs6983267 to CRC in Iranians. Hence, we performed a case control study to examine the possible link between

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the rs10505477 polymorphism and susceptibility to CRC in a sample of this population.

Materials and Methods

This study is a multicenter collaboration between the Research Center of Gastroenterology and Liver Disease (RCGLD) of Shahid Beheshti University of Medical Sciences (SBUMS), the Iran Social Security Organization (ISSO), and the Tehran University of Medical Sciences (TUMS). The study protocol was approved by the ethics committee of three participating centers. The unrelated CRC patients were recruited from gastroenterology clinics in Milad and Sina hospitals, branches of ISSO, and TUMS, respectively. Patients were diagnosed by clinicians and pathologists who were blinded to the genotype data. Colorectal tumors were classified according to the WHO Classification of Tumors of the Digestive System. The pathologic stage of colorectal tumors were classified into stage 0 (TisN0M0), stage I (T1-2, N0, M0), stage II (T3-4, N0, M0), stage III (any T, any N, M0), and stage IV (any T, any N, M1). According to the WHO histological classification, tumor grade was categorized into high (poorly differentiated), intermediate (moderately differentiated), and low (well differentiated) (Jass et al., 1989; WHO, 1977).

Patients with Iranian origin undergone surgery for a primary invasive colorectal tumor and pathologically diagnosed as CRC adenocarcinoma were included in this study. Any patient with CRC recurrences, history of CRC surgery, familial adenomatous polyposis (FAP), hereditary nonpolyposis colorectal cancer (HNPCC), history of other cancer(s), inflammatory bowel disease, and ulcerative colitis was excluded from the study. A standardized extraction template was administered to extract demographic and clinical data from the patient's folder. Controls were cancer free people recruited from blood transfusion center of SBUMS at Taleghani hospital. Genomic DNA was extracted either from formalin-fixed, paraffin-embedded tissue of patients or from blood of the controls by standard methods. The rs6983267 and rs10505477 polymorphisms were genotyped by using TaqMan real time PCR, 7500 real time PCR system (Applied Biosystems) at the RCGLD. To control the quality of the genotype data, about 5% of the real time PCR products were tested by direct sequencing.

All values were presented either as the mean±SD for continuous data or as frequency for categorical data. Differences in categorized data (Age, gender, tumor site, grade and stage) were calculated by chi-2 test. Odds ratios (ORs) with 95% confidence interval (CI), adjusted for covariates (gender and age at recruitment), were obtained

Table 1. Demographic Characteristics of the Subjects

Characteristics		Total (n=715)		
		CRC (380)	C (335)	p
Sex, N (%)	Female	165 (43)	151 (45)	0.706
	Male	215 (57)	184 (55)	
	Age (years), mean (SD)	58 (14)	57 (15)	0.442
Age at diagnosis, N (%)	<60	210 (55)	187 (56)	0.940
	≥60	170 (45)	148 (44)	
Tumor location, N (%)	Colon	275 (72)		
	Rectum	105 (28)		
Grade, N (%)	Poor	47 (12)		
	Moderate	189 (50)		
	Well	144 (38)		
TNM, N (%)	I	39 (10)		
	II	156 (41)		
	III	164 (43)		
	IV	21 (6)		

*CRC, colorectal cancer; C, controls; TNM, Tumor Node Metastasis

Table 2. The Relationship between of rs10505477 and rs6983267 Polymorphisms and Susceptibility to CRC Based on the Gender

Allele/genotype/haplotype		Female (n=316)				Male (n=399)				Total (n=715)			
		CRC (%)	C (%)	p	OR (CI 95%)	CRC (%)	C (%)	p	OR (CI 95%)	CRC (%)	C (%)	p	OR (CI 95%)
rs10505477	A	164 (50)	144 (48)	-	Referent	194 (45)	154 (42)	-	Referent	358 (47)	298 (45)	-	Referent
	G	166 (50)	158 (52)	0.613	1.08 (0.79-1.48)	236 (55)	214 (58)	0.353	1.14 (0.86-1.51)	402 (53)	372 (56)	0.320	1.11 (0.90-1.37)
	A/A	46 (28)	33 (22)	-	Referent	42 (20)	25 (14)	-	Referent	88 (23)	58 (17)	-	Referent
	A/G	72 (44)	78 (52)	0.142	1.51 (0.87-2.62)	110 (51)	104 (56)	0.107	1.59 (0.91-2.79)	182 (48)	182 (54)	0.036	1.52 (1.03-2.24)
	G/G	47 (28)	40 (26)	0.586	1.19 (0.64-2.19)	63 (29)	55 (30)	0.221	1.47 (0.79-2.71)	110 (29)	95 (28)	0.218	1.31 (0.85-2.02)
rs6983267	G	196 (59)	183 (61)	-	Referent	268 (62)	219 (60)	-	Referent	464 (61)	402 (60)	-	Referent
	T	134 (41)	119 (39)	0.758	0.95 (0.69-1.31)	162 (38)	149 (40)	0.416	1.13 (0.85-1.50)	296 (39)	268 (40)	0.684	1.05 (0.85-1.29)
	G/G	59 (36)	59 (39)	-	Referent	78 (36)	64 (35)	-	Referent	137 (36)	123 (37)	-	Referent
	G/T	78 (47)	65 (43)	0.464	0.83 (0.51-1.36)	112 (52)	91 (49)	0.964	0.99 (0.64-1.52)	190 (50)	156 (47)	0.587	0.92 (0.66-1.26)
	T/T	28 (17)	27 (18)	0.911	0.94 (0.51-1.83)	25 (12)	29 (16)	0.280	1.41 (0.75-2.65)	53 (14)	56 (17)	0.476	1.18 (0.75-1.84)
rs10505477-rs6983267	AG	164 (50)	144 (48)	0.613	1.08 (0.79-1.48)	194 (45)	154 (42)	0.353	1.14 (0.86-1.51)	358 (47)	298 (44)	0.320	1.11 (0.90-1.37)
	GG	32 (10)	39 (13)	0.201	0.72 (0.44-1.19)	74 (17)	65 (18)	0.866	0.97 (0.67-1.40)	106 (14)	104 (16)	0.401	0.88 (0.66-1.18)
	GT	134 (40)	119 (39)	0.758	1.05 (0.76-1.45)	162 (38)	149 (40)	0.416	0.89 (0.67-1.18)	296 (39)	268 (40)	0.685	0.96 (0.77-1.18)

*CRC, colorectal cancer; C, controls

through binomial logistic regression analysis. Haplotype and linkage disequilibrium (LD) analysis for the two loci was performed with the Haploview 4.2 program (Broad Institute. Haploview.www.broad.mit.edu/mpg/haploview) and corrected for multiple testing by using 100,000 permutations for individual locus and haplotypes. The Bonferroni procedure was used for the correction of multiple comparisons. Two sided tests of statistical significance were used to determine statistically significant p values ($p<0.05$) with the SPSS software package (ver. 15.0; SPSS, Chicago, IL, USA).

Results

Demographic characteristics

Table 1 is a summary of demographic data of 715 subjects (380 CRC patients and 335 matched controls for age and gender). CRC was more common in males than in females ($p<0.01$, 57% and 43%, respectively). The diagnosed of CRC in the patients was more carried out at age of <60. Mean age of females with CRC was significantly more than males with CRC ($p=0.001$, respectively). Colon cancer in the patients was more frequent than rectal cancer (72% and 28%, respectively). In histological differentiation 12%, 50%, and 38% of patients were classified as poor, moderate, or well grade, respectively. As regards tumor node metastasis (TNM) at the time of diagnosis, 10%, 41%, 43%, and 6% of patients were classified from I to IV, respectively.

Association study

Tables 2 to 6 list alleles and genotypes frequencies data of the rs10505477 and rs6983267 polymorphisms and their haplotypes, analyzed by gender, age at diagnosis, location of tumor, tumor grade, and TNM in 380 CRC patients and 335 controls. Significant allele association was observed between the rs6983267 susceptibility to CRC in patients with the age at diagnosis ≥60 ($p=0.031$). The G allele carriers were more prone to CRC than T allele carriers (58% and 42%, respectively). However, after adjustment for covariates and Bonferroni correction, this association was lost. Genotype distribution of the rs6983267and rs10505477 was consistent with HWE in patients and in controls. There was significant association of rs10505477 genotype with susceptibility to CRC observed in overall (Table 2: A/G versus A/A, $p=0.036$), in the age at diagnosis <60 (Table 3: A/G versus A/A, $p=0.021$ and GG versus A/A, $p=0.052$), in colon location (Table 4: A/G versus A/A, $p=0.029$), and tumor grade at poor and moderate stages (Table 5: A/G versus A/A, $p=0.020$ and GG versus A/A, $p=0.009$, respectively). Significant association between rs6983267 and susceptibility to CRC was observed in the age at diagnosis ≥60 as well (Table 3: T/T versus G/G, $p=0.026$). After adjustment for covariates and Bonferroni

Table 3. The Relationship between of rs10505477 and rs6983267 Polymorphisms and Susceptibility to CRC Based on the Age at Diagnosis

Allele/genotype/haplotype		Age at diagnosis <60				Age at diagnosis ≥60			
		CRC (%)	C (%)	p	OR (CI 95%)	CRC (%)	C (%)	p	OR (CI 95%)
rs10505477	A	208 (49)	161 (43)	-	Referent	150 (44)	137 (46)	-	Referent
	G	212 (51)	213 (57)	0.068	1.30 (0.98-1.72)	190 (56)	159 (54)	0.584	0.92 (0.67-1.25)
	A/A	57 (27)	32 (17)	-	Referent	31 (18)	26 (18)	-	Referent
	A/G	94 (45)	97 (52)	0.021	1.84 (1.10-3.08)	88 (52)	85 (57)	0.645	1.15 (0.63-2.10)
	G/G	59 (28)	58 (31)	0.052	1.75 (1.00-3.08)	51 (30)	37 (25)	0.672	0.87 (0.44-1.69)
rs6983267	G	265 (63)	254 (68)	-	Referent	199 (58)	148 (50)	-	Referent
	T	155 (37)	120 (32)	0.155	0.81 (0.60-1.08)	141 (42)	148 (50)	0.031	1.41 (1.03-1.93)
	G/G	83 (40)	90 (48)	-	Referent	54 (32)	33 (22)	-	Referent
	G/T	99 (47)	74 (40)	0.085	0.69 (0.45-1.05)	91 (53)	82 (56)	0.148	1.48 (0.87-2.49)
	T/T	28 (13)	23 (12)	0.385	0.76 (0.41-1.42)	25 (15)	33 (22)	0.026	2.16 (1.10-4.25)
rs10505477-rs6983267	AG	208 (50)	161 (43)	0.068	1.30 (0.98-1.72)	150 (44)	137 (46)	0.584	0.92 (0.67-1.25)
	GG	57 (13)	93 (25)	0.0005	0.47 (0.33-0.68)	49 (14)	11 (4)	0.000004	4.36 (2.22-8.56)
	GT	155 (37)	120 (32)	0.154	1.24 (0.92-1.66)	141 (42)	148 (50)	0.031	0.71 (0.52-0.97)

*CRC, colorectal cancer; C, controls

Table 4. The Relationship between of rs10505477 and rs6983267 Polymorphisms and Susceptibility to CRC Based on the Location of Tumor

Allele/genotype/haplotype		Colon				Rectum			
		CRC (%)	C (%)	p	OR (CI 95%)	CRC (%)	C (%)	p	OR (CI 95%)
rs10505477	A	262 (48)	298 (45)	-	Referent	96 (46)	298 (45)	-	Referent
	G	288 (52)	372 (55)	0.271	1.14 (0.91-1.42)	114 (54)	372 (55)	0.753	1.05 (0.77-1.44)
	A/A	66 (24)	58 (17)	-	Referent	22 (21)	58 (17)	-	Referent
	A/G	130 (47)	182 (54)	0.029	1.59 (1.05-2.42)	52 (50)	182 (54)	0.338	1.33 (0.74-2.37)
	G/G	79 (29)	95 (29)	0.183	1.37 (0.86-2.17)	31 (29)	95 (29)	0.643	1.16 (0.62-2.20)
rs6983267	G	340 (62)	402 (60)	-	Referent	124 (59)	402 (60)	-	Referent
	T	210 (38)	268 (40)	0.517	1.08 (0.86-1.36)	86 (41)	268 (40)	0.806	0.96 (0.70-1.32)
	G/G	104 (38)	123 (37)	-	Referent	33 (32)	123 (37)	-	Referent
	G/T	132 (48)	156 (46)	0.997	1.00 (0.71-1.42)	58 (55)	156 (46)	0.190	0.72 (0.44-1.18)
	T/T	39 (14)	56 (17)	0.433	1.21 (0.75-1.97)	14 (13)	56 (17)	0.843	1.07 (0.53-2.16)
rs10505477-rs6983267	AG	262 (48)	298 (44)	0.271	1.14 (0.91-1.42)	96 (46)	298 (44)	0.753	1.05 (0.77-1.44)
	GG	78 (14)	104 (16)	0.513	0.90 (0.65-1.24)	28 (13)	104 (16)	0.438	0.84 (0.53-1.31)
	GT	210 (38)	268 (40)	0.518	0.93 (0.74-1.17)	86 (41)	268 (40)	0.806	1.04 (0.76-1.43)

*CRC, colorectal cancer; C, controls

Table 5. The Relationship between of rs10505477 and rs6983267 Polymorphisms and Susceptibility to CRC Based on the Tumor Grade

Allele/genotype/haplotype		Poor				Moderate				Well			
		CRC (%)	C (335)	p	OR (CI 95%)	CRC (%)	C (335)	p	OR (CI 95%)	CRC (%)	C (335)	p	OR (CI 95%)
rs10505477	A	42 (45)	298 (45)	-	Referent	181 (48)	298 (45)	-	Referent	135 (47)	298 (45)	-	Referent
	G	52 (55)	372 (55)	0.970	1.01 (0.65-1.56)	197 (52)	372 (55)	0.288	1.15 (0.89-1.48)	153 (53)	372 (55)	0.494	1.10 (0.84-1.45)
	A/A	10 (17)	58 (17)	-	Referent	49 (25)	58 (17)	-	Referent	29 (23)	58 (17)	-	Referent
	A/G	24 (41)	182 (54)	0.020	2.55 (1.16-5.61)	83 (43)	182 (54)	0.009	1.85 (1.17-2.94)	75 (59)	182 (54)	0.949	1.22 (0.58-1.67)
	G/G	24 (41)	95 (29)	0.675	1.18 (0.54-2.59)	62 (32)	95 (29)	0.182	1.41 (0.85-2.33)	24 (19)	95 (29)	0.524	2.23 (0.67-2.23)
rs6983267	G	74 (64)	402 (60)	-	Referent	251 (65)	402 (60)	-	Referent	139 (54)	402 (60)	-	Referent
	T	42 (36)	268 (40)	0.441	1.18 (0.78-1.77)	137 (35)	268 (40)	0.131	1.22 (0.94-1.58)	117 (46)	268 (40)	0.116	0.79 (0.59-1.06)
	G/G	25 (43)	123 (37)	-	Referent	77 (40)	123 (37)	-	Referent	35 (27)	123 (37)	-	Referent
	G/T	24 (41)	156 (46)	0.369	1.32 (0.32-2.43)	97 (50)	156 (46)	0.972	1.01 (0.69-1.47)	69 (54)	156 (46)	0.327	0.82 (0.55-1.22)
	T/T	9 (16)	56 (17)	0.577	1.27 (0.55-2.89)	20 (10)	56 (17)	0.060	1.75 (0.98-3.15)	24 (19)	56 (17)	0.484	0.83 (0.49-1.41)
rs10505477-rs6983267	AG	44 (38)	298 (45)	0.189	0.76 (0.51-1.14)	181 (47)	298 (45)	0.494	1.09 (0.85-1.40)	136 (53)	298 (45)	0.041	1.35 (1.01-1.80)
	GG	30 (26)	104 (14)	0.006	1.90 (1.19-3.02)	70 (18)	104 (14)	0.287	1.20 (0.86-1.67)	3 (1)	104 (14)	-	-
	GT	42 (36)	268 (40)	0.440	0.85 (0.57-1.28)	137 (35)	268 (40)	0.130	0.82 (0.63-1.06)	117 (46)	268 (40)	0.115	1.26 (0.94-1.69)

*CRC, colorectal cancer; C, controls

correction, these associations did not remain.

Haplotype analysis showed significant association between GG and CRC susceptibility in both ages at diagnosis <60 and ≥60 (Table 3: p=0.0005 and p=0.000004) and between GT and CRC in the age at diagnosis ≥60 (Table 3: p=0.031). Moreover, significant association of GG and AG haplotypes was observed with tumor grade at poor or well stages (Table 5: p=0.006 and p=0.041). After 100 000 permutation test, only association of GG haplotype and CRC susceptibility in both ages at diagnosis <60 and ≥60 was remained. The GG haplotype was less and more frequent in patients with the age at diagnosis <60 and at diagnosis ≥60, respectively. A strong LD was obtained between these two loci in overall subjects or in subsidiary analysis by gender, age at diagnosis, location of tumor, tumor grade, and TNM subgroup analysis (D'=85-96). Altogether, GG carriers were more sensitive to late onset of CRC but less sensitive to early onset of CRC.

Discussion

Evidence identified a strong association between rs6983267 and rs10505477 genetic markers in chromosome 8q24.21 and risk of some types of cancers such as CRC. The rs10505477 locus lies in a hotspot within a 1.5-Mb gene-free region (gene desert) with a distance of 5.90 kbp from rs6983267 polymorphism located in the putative MYC regulatory element which has been suggested as a causal variant for CRC (Tomlinson et al., 2007; Zanke et al., 2007; Tenesa et al., 2008; Haerian et al., 2011). Experiment in mouse model lacking a MYC enhancer that includes human rs6983267 model showed resistance to intestinal tumours in these animals (Sur et al., 2012; Takatsuno et al., 2013). Our meta-analysis also supported the effect of these loci in CRC, however small sample size in Asian studies was one of the limitation in our study (Gruber et al., 2007; Matsuo et al., 2009; Xiong 2010; Cui et al., 2011; Haerian et al., 2011; Li et al., 2011; Thean et al., 2012). To date, seven studies have reported the association of rs6983267 or rs10505477 with the risk of CRC in Asia. One study was done for rs10505477 in Jewish population and six studies were performed for rs6983267 in Chinese from China and Singapore, Iran and Japan (Table 7). In the current study, we examined this association for both loci and their haplotype with larger sample size from Iran. Our results showed a relationship between rs6983267 -rs10505477 GG haplotype and onset age of CRC. Patients with GG haplotype at age of 60 or more were more prone to CRC but less sensitive to early onset of CRC.

Results of the current study were inconsistent with one previous report for the rs10505477 from Jewish population. The rs10505477 locus was risk factor in about 14% of CRC in this population in autosomal codominant (p=0.03) and dominant (p=0.008) genetic models. However, we did not observe this association for these two genotype models in Iranian population which may be influenced by difference of ethnicity or smaller sample size. Ethnicity is an important factor that may affect the results. Variation of genetic or environmental condition between different ethnic groups influences on their susceptibility to disease or treatment. Interplay between genetic difference and environmental factors such as regional climate, culture, and pathogens may lead to a variety of adaptations of populations or individuals and thereby modification of risk of disease in populations of different ancestry. Stratified population is another cause of different results (Thomas et al., 2002; Spencer et al., 2009). The previous report was performed in a heterogenous Jewish population composed of Ashkenazi Jewish, Sephardi Jewish, non-Jewish ethnicity (Gruber et al., 2007). Concerns about population stratification or mixture of individuals from heterogeneous genetic backgrounds in association studies investigating the effect of a genetic factor on risk of disease

Table 6. The Relationship between of rs10505477 and rs6983267 Polymorphisms and Susceptibility to CRC Based on the Tumor Stage (TNM)

Allele/genotype/haplotype		I-II				III-IV			
		CRC (%)	C (%)	p	OR (CI 95%)	CRC (%)	C (%)	p	OR (CI 95%)
rs10505477	A	182 (47)	298 (45)	-	Referent	176 (48)	298 (45)	-	Referent
	G	208 (53)	372 (55)	0.490	1.09 (0.85-1.40)	194 (52)	372 (55)	0.338	1.13 (0.88-1.46)
	A/A	44 (23)	58 (17)	-	Referent	44 (24)	58 (17)	-	Referent
	A/G	94 (48)	182 (54)	0.105	1.47 (0.92-2.34)	88 (48)	182 (54)	0.059	1.57 (0.98-2.50)
	G/G	57 (29)	95 (29)	0.368	1.26 (0.76-2.11)	53 (29)	95 (29)	0.243	1.36 (0.81-2.28)
rs6983267	G	233 (60)	402 (60)	-	Referent	231 (62)	402 (60)	-	Referent
	T	157 (40)	268 (40)	0.935	1.00 (0.77-1.28)	139 (38)	268 (40)	0.442	1.11 (0.85-1.44)
	G/G	65 (33)	123 (37)	-	Referent	72 (39)	123 (37)	-	Referent
	G/T	103 (53)	156 (46)	0.263	0.80 (0.54-1.18)	87 (47)	156 (46)	0.808	1.05 (0.71-1.55)
	T/T	27 (14)	56 (17)	0.743	1.10 (0.63-1.90)	26 (14)	56 (17)	0.408	1.26 (0.73-2.18)
rs10505477-rs6983267	AG	182 (47)	298 (44)	0.490	1.09 (0.85-1.40)	176 (48)	298 (44)	0.338	1.13 (0.88-1.46)
	GG	51 (13)	104 (16)	0.277	0.82 (0.57-1.18)	55 (14)	104 (16)	0.778	0.95 (0.67-1.36)
	GT	157 (40)	268 (40)	0.934	1.01 (0.78-1.30)	139 (38)	268 (40)	0.441	0.90 (0.70-1.17)

*CRC, colorectal cancer; C, controls

Table 7. Studies of the Relationship between rs10505477 and rs6983267 Polymorphisms and Susceptibility to CRC

No.	Author, Year	Origin (sub-population)	Polymorphisms		Subjects (N)		Association	Ref.
			rs10505477	rs6983267	CRC	C		
1	Gruber et al. 2007	Israel*	+	-	1,861	1,937	Yes	18
2	Matsuo et al. 2009	Japan	-	+	481	962	Yes	25
3	Xiong et al. 2010	China	-	+	2,124	2,124	Yes	26
4	Cui et al. 2011	Japan	-	+	6,167	4,494	Yes	28
5	Daraei et al. 2011	Iran (Isfahan)	-	+	115	120	Yes	13
6	Li et al. 2011	China	-	+	435	788	Yes	27
7	Thean et al. 2012	Singapore, Chinese	-	+	991	993	Yes	29
8	This study 2013	Iran (Tehran)	+	+	380	335	GG haplotype with onset age of CRC	-

*Ashkenazi Jewish, Sephardi Jewish, non-Jewish; **CRC, colorectal cancer; C, control

have been raised doubts about the reliability of reported findings (Altshuler et al., 1998). Future studies with more homogeneous Asian population are suggested to find the reason of this discrepancy. Further studies with larger sample size from Iranian population are suggested to focus more on this inconsistency.

Unlike last reports from Asia including Iran, we also did not identify any association between the rs6983267 and CRC risk in Iranians. This controversy with the previous report from Iran may be related to larger sample size in this study (N=715) as compared with previous one from Iran (N=235) and difference of ethnicity with studies performed in Chinese and Japanese populations. Small sample size may results in false positive and underpowered results. We also corrected our results by applying Bonferroni procedure for multiple comparisons effects, whereas the previous study from Iran did not correct the final results. However, we found a strong association between GG haplotype of rs10505477 and late onset of CRC in Iranian subjects. A strong relationship between the rs10505477 and rs6983267 loci is reported in CRC patients [Tomlinson et al., 2007]. Evidence suggests that the rs6983267 locus is a factor of the cis-regulatory enhancer element for the MYC gene, with a long-range physical interaction with this gene in CRC. This locus can promote CRC occurrence via alterative binding of G or T alleles to transcription factor 7- like 2 (TCF7L2) of Wnt signalling pathway (Cicek et al., 2009; Curtin et al., 2009). Although, another study suggested that MYC expression in colon tissues might be regulated by some splicing forms of TCF7L2, not through the direct differential interaction of rs6983267 alleles (Matsuo et al., 2009). Therefore, rs6983267 may not be effective alone in CRC risk, but can play a remarkable role in susceptibility to this disease in combination with rs10505477 locus.

Some limitations of the present study should be acknowledged. First, small sample size in the Iranian case-control study. Second, incomplete data in the patient folder resulted in excluding some patients. In conclusion, this study suggests that the rs6983267 and rs10505477 polymorphisms alone may not be relevance to CRC risk, but their GG haplotype plays a remarkable role in onset age of CRC in Iranian population.

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