RESEARCH ARTICLE

Is there an Association between Variants in Candidate Insulin Pathway Genes IGF-I, IGFBP-3, INSR, and IRS2 and Risk of Colorectal Cancer in the Iranian Population?

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Abstract

Background: Several epidemiological studies have shown associations between colorectal cancer (CRC) risk and type 2 diabetes and obesity. Any effects would be expected to be mediated through the insulin pathway. Therefore it is possible that variants of genes encoding components of the insulin pathway play roles in CRC susceptibility. In this study, we hypothesized that polymorphisms in the genes involving the insulin pathway are associated with risk of CRC. Materials and Methods: The associations of four single nucleotide polymorphisms (SNPs) in IGF-I (rs6214), IGFBP-3 (rs3110697), INSR (rs1052371), and IRS2 (rs2289046) genes with the risk of CRC were evaluated using a case–control design with 167 CRC cases and 277 controls by the PCR–RFLP method. Results: Overall, we observed no significant difference in genotype and allele frequencies between the cases and controls for the IGF-I, IGFBP-3, INSR, IRS2 gene variants and CRC before or after adjusting for confounders (age, BMI, sex, and smoking status). However, we observed that the IRS2 (rs2289046) GG genotype compared with AA+AG genotypes has a protective effect for CRC in normal weight subjects (p=0.035, OR=0.259, 95%CI= 0.074-0.907). Conclusions: These findings do not support plausible associations between polymorphic variations in IGF-I, IGFBP-3, INSR, IRS2 genes and risk of CRC. However, the evidence for a link between the IRS2 (rs2289046) variant and risk of CRC dependent on the BMI of the subjects, requires confirmation in subsequent studies with greater sample size.

Keywords: Colorectal cancer - insulin pathway genes - polymorphism - PCR-RFLP

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Introduction

Colorectal cancer (CRC) is the most frequently causes of cancer morbidity and mortality throughout the world, accounts for 608,000 cancer deaths worldwide (Ferlay et al., 2010). The previous investigations showed a great share of obesity and lifestyle factors such as diet, cigarette smoking, alcohol consumption, and physical inactivity, in individual's susceptibility to CRC (Giovannucci, 2001; Chapelle, 2004). The effect of these etiological factors on the risk of CRC mediated through the insulin (INS) pathway (Giovannucci, 1995; Lee et al., 2013). Various studies have linked diabetes and CRC risk (Yang et al., 2004; Nagel et al., 2006; Chung et al., 2008). Thus, genetic variants in the genes involve in the insulin pathway may affect the CRC predisposition.

Insulin and its structural homologue, insulin-like growth factors-I (IGF-I) implicate in inducing cell proliferation and inhibiting apoptosis (Giovannucci, 2001; Pollak et al., 2004; Larsson et al., 2005). IGF-I has a strong mitogenic activity and insulin may implicate in colorectal carcinogenesis indirectly by upregulating IGF-I biosynthesis, increasing IGF-I bioavailability and inhibiting the production of IGF-binding protein-3 (IGFBP-3) (Khandwala et al., 2000; Kaaks et al., 2001; Moschos et al., 2002). IGFBP-3 makes a complex with IGF-I and therefore influence on IGF-I bioavailability. Independent of IGF-I, IGFBP-3 inhibits growth and promotes apoptosis (Renehan et al., 2004; Davies et al., 2006). Insulin binds to its cell surface receptor (INSR), which regulates its metabolic action through initiating a series of tyrosine residues phosphorylation (Kohanski, 1993; White, 1997; Zhang et al., 2010). Insulin receptor substrate-2 (IRS2) is one of the major substrate of INSR,

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given its key role as an adaptor to mediate insulin signals to the downstream molecules by binding to the p-85 subunit of phosphatidylinositol (PI)-3 kinase and activating the serine kinase PKB/Akt pathway (Biddinger et al., 2006; White, 2006). Additionally there are some inconsistent results about association between IRS2 haplotypes and obesity risk (Lautier et al., 2003).

Some previous studies conducted to investigate the effect of IGF-I, INSR and IRS2 gene polymorphisms on insulin resistant and type 2 diabetes mellitus (Flores-Martínez et al., 2004; Bodhini et al., 2007; Vella et al., 2008; Baroudi Ouederni et al., 2009; Malodobra et al., 2011). So far, the associations of insulin pathway gene polymorphisms with prostate and breast cancer have been studied intensively but their results were inconsistent (Ho et al., 2003; Neuhausen et al., 2005; Deming et al., 2007; Deming et al., 2008; Sarma et al., 2008; Xuefen et al., 2010). Only a few studies have focused on variants in genes along the insulin pathway regarded to their effect on the risk of CRC (Slattery et al., 2005; Pechlivanis et al., 2007). Additionally one study has evaluated the effect of insulin pathway gene polymorphisms on advanced colorectal adenoma (Gunter et al., 2007).

The polymorphisms that were located in protein coding region can induce amino acid changes, resulting in functional changes in the protein. Several groups have studied the relationship between breast and colorectal cancers and the rs6214 polymorphism located in exon4 of the IGF-I gene with inconsistent results (Deming et al., 2008; Feik et al., 2010). The rs3110697 polymorphism in intron3 region of IGFBP-3 gene has been associated with breast cancer in one study and may be related to its affect on splicing or expression of the gene (Deming et al., 2007; Xuefen et al., 2010). 3' untranslated region (3'UTR) in human genes plays a crucial role in regulating gene expression at post-transcriptional level. Mutations in the 3'UTR region have been connected with some disorders and diseases, especially tumors (Conne et al., 2000). The SNP located in 3'UTR of the INSR gene showed an association with insulin resistance (Malodobra et al., 2011). Actually despite the biological plausibility the rs2289046 polymorphism at IRS2 gene in this regulatory region demonstrated no relation with advanced left-sided colorectal adenoma (Gunter et al., 2007).

To obtain a better understanding of the association between SNPs in several insulin pathway-related genes and the CRC risk, we selected variants of the IGF-I rs6214 in exon4, IGFBP-3 rs3110697 in intron3, INSR rs1052371 in 3'UTR, and IRS2 rs2289046 in 3'UTR region of the genes. These polymorphisms were selected according to their position in the gene, degree of heterozygosity and their use in previous genetic investigations.

Materials and Methods

Participants

Total of 444 recruited subjects, including 167 CRC patients (age range 23-85) as cases and 277 controls (age range 13-78), were evaluated in a case-control study. The patients who were undergoing colonoscopy for various gastrointestinal complain were recruited by Gastroenterology and Liver Diseases Research Center. Cases were defined as the patients with positive pathological reports for CRC and control participants had negative colonoscopy reports for malignancy or polyps (including adenomatous and other polyps). Some of the subjects in cases and control groups presented positive family history (first-degree relatives, including parents, siblings, and children) for CRC (Table 1). At colonoscopy, anthropometric measurements, smoking habits, and family history for CRC were recorded. The recruitment of the participants was between September 2011 and February 2012 (all of them were Iranian and genetically unrelated). Informed consent was provided from all the subjects at recruitment and the Ethical Review Boards of the Institution approved the study protocol. The body mass index (BMI) of each subject was calculated by weight (kg)/height (m^2) formula. Subjects were divided into subgroups, on the basis of their BMI values denoted as following: normal-weight (BMI<25kg/m^2) cases (n=84), overweight/obese (BMI≥25kg/m^2) cases (n=83), normal-weight (BMI<25kg/m^2) control (n=154) and overweight/obese (BMI≥25 kg/m^2) control (n=123).

Genotype analysis

Genomic DNA was extracted from 5ml EDTA-anticoagulated whole blood by using the standard "phenol chloroform" method.

The IGF-I G>A (rs6214) polymorphism was examined by means of PCR using forward primer: 5'-TTCTGTGGAATAAGATACTGGAC -3' and rivers primer: 5'- TGAAGGAAATAAGTCATAGACACT -3'. Cycling was at 94°C for 5 min and 30 cycles of 94°C for 45s, 57°C for 40s, and 72°C for 45s followed by 72°C for 5 min. The resulting PCR products were electrophoresis on 2% agarose gels and digested overnight with restriction enzyme Hin1II at 37°C and then electrophoresed on 3% agarose gels. Genotyping of the subjects were evaluated according to the alleles digestion pattern of the Hin1II restriction site (absence of ("A") or presence of ("A") allele). Hin1II digestion showed genotypes as follow GG (190 bp), GA (190, 133 and 57 bp), or AA (133 and 57 bp).

The IGFBP-3 A>G (rs3110697) polymorphism was amplified using primer forward 5'-

Table 1. Clinical Data Analysis (IGF-I (Hin1II)/ IGFBP-3 (BtsI)/INSR (LweI)/IRS2 (PvuII)

Variables		Controls (n=277)		Cases (n=167)		p value
Age (years)		42.38 (15.40)		53.88 (13.45)		<0.001
BMI (kg/m^2)		24.94	(3.62)	25.27	(4.94)	0.451
Gender	Men	128	(46.2)	91	(54.5)	
	Women	149	(53.8)	76	(45.5)	0.091
Smoking history	No	233	(84.1)	132	(79.0)	
	Former	31	(11.2)	21	(12.6)	
	Current	13	(4.7)	14	(8.4)	0.243
Family history of colorectal cancer						
	No	252	(91.0)	145	(86.8)	
	Yes	25	(9.0)	22	(13.2)	0.169
Tumor site	Colon		-	104	(62.3)	
	Rectal			63	(37.7)	
Metastasis	No			149	(89.2)	
	Yes			18	(10.8)	

*Variables presented as mean (SD) or number (%)

CTCCGACTCACTGGCATTTC -3' and rivers 5'-ACCAGCCCTTGTAGAACCTC -3'. PCR conditions consisted of a 5 min denaturation at 94°C followed by 30 cycles of 94°C for 45 sec, 66°C for 40 sec, and 72°C for 45 sec and final extension at 72°C for 5 min. PCR products were confirmed by electrophoresis on 2% agarose gel and digested 3 hour with restriction enzyme BtsI at 55°C and then electrophoresed on 3% agarose gels. Each subject was genotyped according to BtsI restericition site (absence of (''G'') or presence of (''G'') allele). BtsI digestion demonstrated genotypes as AA (616 bp), AG (616, 397 and 219 bp), or GG (397 and 219 bp).

The INSR T>C (rs1052371) polymorphism was detected using PCR amplification with 5'-CTAGTCAAGGTCCAGAACC -3' as the forward primer and 5'- AGGCACACAAAGGGACGAG -3' as the reverse primer. PCR cycling consisted of an initial denaturation at 94°C for 5 min and then 30 cycles of 94°C, 57°C and 72°C for respectively 45, 40 and 45 sec. PCR products were confirmed by electrophoresis on 2% agarose gel and digested 3 hour with restriction enzyme LweI at 37°C and then electrophoresed on 3% agarose gels. Genotypes were scored according to the absence of (''C'') or presence of (''C'') allele in the LweI site. Enzyme digestion reveals genotypes denoted TT (224 bp), TC (224, 154 and 70 bp), or CC (154 and 70 bp).

The IRS2 A>G (rs2289046) polymorphism was evaluated using PCR amplification with 5'-TTGGACTTTGAAGACGGATTAC-3' as the forward primer and 5'-TTCCATCAATAACATAGGGGCT-3' as the reverse primer. The PCR reaction was started with an initial denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 45 s, 61°C for 40 s, 72°C for 45 s, and final extension at 72°C for 5 min. PCR products were electrophoresis on 2% agarose gel and digested overnight with restriction enzyme PvuII at 37°C and then electrophoresed on 3% agarose gels. Genotypes of each subject were denoted according to the alleles digestion pattern of the PvuII restericition site [absence of (''G'') or presence of (''G'') allele]. PvuII digestion reveals genotypes noted as AA (471 bp), AG (471, 399 and 72 bp), or GG (399 and 72 bp).

The concordance of genotyping was confirmed by duplicate analysis on 10% of the total samples and the results were 100% accurate.

Statistical methods

The relative association between patients and controls for genotype and allele frequencies was performed by χ^2 test. Departures from Hardy–Weinberg equilibrium were assessed using χ^2 test among cases and controls, separately. To adjust confounding factors including age, BMI, sex and smoking statues, logistic regression analysis was used. Odds ratios (OR) were given with the respective 95% confidence intervals (95%CI) to qualified the roll of distinctive genotypes in prevalence of CRC. The t-test was used to evaluate the variations in demographic factors. Statistical tests were performed by SPSS software (version 15.0; SPSS, Chicago, IL, USA). Significance was assumed for $p<0.05$.

Results

Characteristics of cases and control groups are provided in Table 1. As can be seen from the table CRC cases were significantly older than controls ($p<0.001$). Furthermore, no significant differences were found between the cases and control subjects according to their BMI, gender and smoking history. Additionally there was no statistically significant difference between two groups regarded to their family history. However in the case group 62.3% of tumors were diagnosed in the colon and just 10.8% of them had metastasis to the other parts of the body.

The genotype and allele frequencies for IGF-I rs6214, IGFBP-3 rs3110697, INSR rs1052371 and IRS2 rs2289046 gene polymorphisms loci are presented in Table 2 (Figure 1). It should be noted that there was some missing genotype in INSR and IRS2 polymorphism loci and 7 samples of controls and 9 samples of cases remained untyped.

Among both case and control population, the genotyped polymorphisms were distributed in compliance to Hardy-Weinberg equilibrium ($p>0.05$) except in case group of rs3110697 in IGFBP-3 gene ($p<0.05$). No significant differences were observed in the IGF-I, IGFBP-3, INSR and IRS2 gene polymorphisms neither for genotypes nor for allele frequencies between the patients and the controls. Although adjustment for covariates like age, BMI, gender, and smoking history did not significantly alter the association between these four SNPs and the risk of CRC. Categorizing the analyses by gender and tumor site (data not shown), resulted no statistically significant differences in IGF-I, IGFBP-3, INSR and IRS2 polymorphisms between the cases and the controls. We also assessed the risk of CRC in relation to IGF-I, IGFBP-3, INSR and IRS2 polymorphisms between the cases with CRC and controls regarding to their BMI, and found IRS2 AG genotype in normal weight subjects increase risk of CRC (p=0.040, OR=1.986, 95%CI=1.031-

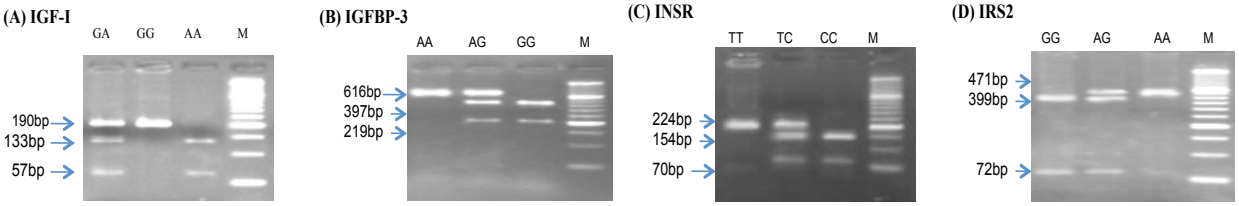

Figure 1. PCR-RFLP Analysis of Genetic Polymorphism for Several Insulin Pathway-Related Genes. Genomic DNA was amplified with appropriate primers followed by an enzyme digestion, and resulting products were analyzed on 3% agarose gels for **(A)** IGF-I; **(B)** IGFBP-3; **(C)** INSR; and **(D)** IRS2. M: DNA marker 50bp

Table 2. The Genotype and Allele Frequencies of IGF-I, IGFBP-3, Insr and Irs2 Gene Polymorphisms in Cases with Colorectal Cancer and in Controls

Variant		Controls	Cases	Crude		Adjusted	
				OR (95% CI)	p value	OR (95% CI)	p value
IGF-I/ Hin1II/rs6214 G>A		(n=277)	(n=167)				
Genotype-wise comparison	GG	120 (43.3)	78 (46.7)	1.0 (reference)		1.0 (reference)	
	AA	38 (13.7)	22 (13.2)	0.891 (0.490-1.619)	0.704	0.749 (0.395-1.423)	0.378
	GA	119 (43.0)	67 (40.1)	0.866 (0.573-1.310)	0.496	0.846 (0.542-1.321)	0.463
	AA and GA	157 (56.7)	89 (53.3)	0.872 (0.593-1.283)	0.487	0.821 (0.542-1.243)	0.351
	AA versus others	38 (13.7)	22 (13.2)	0.954 (0.543-1.678)	0.871	0.811 (0.442-1.487)	0.498
Allele-wise comparison	G	359 (64.8)	223 (66.8)	1.0 (reference)		-	
	A	195 (35.2)	111 (33.2)	0.916 (0.688-1.221)	0.551	-	
IGFBP-3/BtsI/rs3110697 A>G		(n=277)	(n=167)				
Genotype-wise comparison	AA	99 (35.7)	47 (28.1)	1.0 (reference)		1.0 (reference)	
	GG	55 (19.9)	34 (20.4)	1.302 (0.751-2.259)	0.348	0.982 (0.538-1.790)	0.952
	AG	123 (44.4)	86 (51.5)	1.473 (0.945-2.294)	0.087	1.467 (0.913-2.355)	0.113
	GG and GA	178 (64.3)	120 (71.9)	1.420 (0.936-2.155)	0.100	1.306 (0.837-2.039)	0.240
	GG versus others	55 (19.9)	34 (20.4)	1.032 (0.639-1.665)	0.898	0.780 (0.460-1.320)	0.354
Allele-wise comparison	A	321 (57.9)	180 (53.9)	1.0 (reference)		-	
	G	233 (42.19)	154 (46.1)	1.179 (0.897-1.549)	0.238	-	
INSR/LweI rs1052371 T>C		(n=270)	(n=158)				
Genotype-wise comparison	TT	171 (63.3)	99 (62.6)	1.0 (reference)		1.0 (reference)	
	CC	12 (4.5)	5 (3.2)	0.720 (0.246-2.103)	0.548	0.720 (0.246-2.103)	0.548
	TC	87 (32.2)	54 (34.2)	1.120 (0.713-1.760)	0.622	1.120 (0.713-1.760)	0.622
	CC and TC	99 (36.7)	59 (37.4)	1.029 (0.686-1.546)	0.889	1.029 (0.686-1.546)	0.889
	CC versus others	12 (4.4)	5 (3.2)	0.703 (0.243-2.033)	0.515	0.703 (0.243-2.033)	0.515
Allele-wise comparison	T	429 (79.4)	252 (79.7)	-		1.0 (reference)	
	C	111 (20.6)	64 (20.3)	0.982 (0.695-1.386)	0.916		
IRS2/ PvuII rs2289046 A>G		(n=270)	(n=158)				
Genotype-wise comparison	AA	117 (43.3)	66 (41.8)	1.0 (reference)		1.0 (reference)	
	GG	34 (12.6)	13 (8.2)	0.678 (0.334-1.374)	0.281	0.678 (0.334-1.374)	0.281
	AG	119 (44.1)	79 (50.0)	1.177 (0.777-1.782)	0.441	1.177 (0.777-1.782)	0.441
	GG and GA	153 (56.7)	92 (58.2)	1.066 (0.716-1.586)	0.753	1.066 (0.716-1.586)	0.753
	GG versus others	34 (12.6)	13 (8.2)	0.622 (0.318-1.218)	0.166	0.622 (0.318-1.218)	0.166
Allele-wise comparison	A	353 (65.4)	211 (66.8)			1.0 (reference)	
	G	187 (34.6)	105 (33.2)	0.939 (0.700-1.260)	0.676		

*Variables presented as number (%); **Adjusted for age, BMI, sex, smoking status, and family history

3.826), also GG genotype compared with AA+AG genotypes has protective effect for CRC in normal weight subjects (p=0.035, OR=0.259, 95%CI=0.074-0.907).

In addition cases and control subjects separately were further divided into two groups according to their BMI (normal weight and overweight/obese), and their obesity risks were evaluated in regarding to IGF-I, IGFBP-3, INSR and IRS2 polymorphisms allele and genotype frequencies. Only AG (p=0.000, OR=2.632, 95%CI=1.556-4.454) and GG+GA versus AA (p=0.002, OR=2.186, 95%CI=1.338-3.571) genotypes in control group of IRS2 polymorphism were found to have significant association with obesity risk.

Discussion

We have performed a case-control study to assess the role of variants in four genes along the INS pathway on the risk of CRC. Results from this evaluation did not provide any evidence for association between polymorphisms of IGF-I rs6214, IGFBP-3 rs3110697, INSR rs1052371, and IRS2 rs2289046 genes and the disease risk. However, we observed that IRS2 (rs2289046) GG genotype compared with AA+AG genotypes has a protective effect for CRC in normal weight subjects.

Previously, the links between insulin resistance, obesity, and CRC have been noted, and therefore the important roles of insulin pathway in the etiology of CRC have been clarified (Yam et al., 1996; Tamakoshi et al., 2004). The potential associations between variants of genes encoding components of the insulin pathway and risk of CRC have been investigated in limited studies. In the investigation that was conducted by Slattery et al. (2005) IGF-I, IGFBP-3, and IRS2 SNPs have been evaluated regarded to their effect on the CRC risk. Pechlivanis et al. (2007) also have examined the association between INSR and IRS2 variants and the risk of CRC.

Additionally, the associations between INSR and IRS2 SNPs have been assessed by Gunter et al (2007) regarded to their effect on advanced left-sided colorectal adenoma.

Although most of prior studies have assessed the association between (CA)n repeat polymorphism in the IGF-I promoter region and CRC risk (Slattery et al., 2005; Wong et al., 2005; 2008; Pechlivanis et al., 2007), only one study (Feik et al., 2010), has evaluated the association between rs6214 polymorphism at exon4 of the gene and risk of colorectal polyps and CRC. However significant association was reported by Feik et al. (2010), in our investigation no association was found between the IGF-I rs6214 gene polymorphism and risk of the disease. In agreement with our results Deming et al. (2008), found no association between this polymorphism and breast cancer risk. In addition, Vella et al. (2008) reported rs6214 influence on IGF-I concentrations, since no association was showed between polymorphism and growth, glucose metabolism or type 1 diabetes. The SNP is one of the

non-synonymous SNP in this region but functional consequence of this polymorphism, is yet unknown. These conflict findings may contribute to small sample size, false positive results, difference in subject's definitions, genotyped markers and statistical methods.

This is the first attempt to evaluate the association between rs3110697 of IGFBP-3 gene and risk of CRC. The polymorphism is located in intron3 region of the gene and may play its role by involving in gene splicing or expression. Breast cancer was studied extensively considering this polymorphism, with inconsistent results. Xuefen et al. (2010) demonstrated that A allele of the SNP was inversely associated with benign breast disease. Nevertheless, Deming et al. (2007) found significant association between rs3110697 and breast cancer risk, Patel et al. (2008) reported no association between the SNP and breast cancer susceptibility. Interestingly, in both studies were demonstrated the rs3110697 is significantly influence circulating levels of IGFBP-3. Furthermore, Sarma et al. (2008) observed no significant associations between the SNP and prostate cancer risk. However, we did not find any association between IGFBP-3 rs3110697 genotypes and alleles and CRC risk. The best explanation for the result is our sample size that was not large enough to demonstrate the differences in genotypes and alleles frequencies between cases and control groups. To confirm our data this evaluation should be replicated in other population with larger sample size. In present study, the distribution of the rs3110697 genotypes deviated significantly from HWE in cases. Most probably it is a function of sample size. However genotyping error, population stratification, and inbreeding are the other factors that could influence on deviations from HWE.

There are rare studies about association between INSR gene polymorphism and CRC. Pechlivanis et al. (2007) showed significant association between the SNP in promoter region of INSR gene and the risk of CRC. Since Gunter et al. (2007) found no association between several INSR gene variants and advanced colorectal adenoma. To our knowledge this is the first investigation into association of the INSR rs1052371 and CRC. The polymorphism located in 3'end of the INSR gene. 3' untranslated region (3'UTR) involved in gene expression, through regulation of mRNA stability. However we did not find any association between INSR rs1052371 genotypes and alleles and CRC risk. Malodobra et al. (2011) also reported no association between the SNP and the insulin resistant. Obtained results need to be verified on larger number of subject and replicated within distinct population.

At least one previous study by Gunter et al. (2007), has examined whether rs2289046 in 3'UTR region of IRS2 gene is associated with CRC susceptibility, but has not found any association. Concordant to their results we have investigated no significant association between the SNP and the disease risk. Feigelson et al. (2008) indicated that the G allele of rs2289046 was associated with breast cancer risk. Furthermore, we found that among the normal weight subjects, the GG genotype act as a protective factor for CRC susceptibility when compared to the AA+AG genotypes. For concluding this result small sample size emerges as a major issue of our study, therefore, it should be replicated in other studies with larger sample size. Additionally we observed association between IRS2 GG+AG comparing to AA genotype to increase obesity risk among our controls. Because the association is not seen among the cases, it is not reliable and we suppose that the result is due to chance.

Several limitations of this study merit to be considered. The primary limitation of this study includes relatively small sample size that prevents representing strong conclusion. The other limitation in our investigation is that we examined only four polymorphisms in four genes along the insulin signaling pathway, and thus coverage of each gene remains to be determined. Another limitation was the lake of information about the effect of these polymorphisms on serum level of the IGF-I, IGFBP-3 and IRS2 that might influence on our conclusion. Further limitation is a potential information bias from the case-control study design. Accordingly, we could not completely rule out the possibility of chance findings. Nevertheless, the possibility of true finding should not be excluded.

In summary, the findings presented here did not provide any support for a putative role of genetic variants in insulin pathway in relation to CRC risk. However, we present evidence for the interaction between the rs2289046 variant of IRS2 gene and BMI in risk of CRC. These results should be confirmed in additional investigations with increased numbers of subjects to further evaluate the potential association between these polymorphism and risk of CRC.

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