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A Common Promoter Polymorphism (-23HphI) in Insulin Gene and Susceptibility to Colorectal Cancer

Hossein Nobakht,[1] Touraj Mahmoudi,[2,*] Mojgan Mirakhorli,[3] Reza Dabiri,[1] and Mohammad Reza Zali[2]

[1]Internal Medicine Department, Semnan University of Medical Sciences, Semnan, Iran
[2]Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[3]Colorectal Research Center, Rasoul Akram Hospital, Iran University of Medical Sciences, Tehran, Iran

[*]*Corresponding author*: Touraj Mahmoudi, MSc, Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Velenjak, Shahid Chamran Highway, 1985711151, Tehran, Iran. Tel: +98-9124148851, Fax: +98-2122432517, E-mail: mahmouditouraj@gmail.com

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Abstract

Background: With regard to the major role of insulin resistance in colorectal cancer (CRC), this study investigated whether insulin (*INS*) gene -23HphI variant was associated with susceptibility to CRC risk.
Methods: Our study was conducted as a case-control study and 312 cases with CRC and 438 controls were enrolled. All 750 subjects were genotyped for *INS* gene -23HphI variant using PCR-RFLP method.
Results: There was no significant difference for the -23HphI variant of *INS* gene in either genotype or allele frequencies between the cases and the controls and this lack of difference remained non-significant even after adjustment for age, BMI, sex, smoking status, regular NSAID use, and family history of CRC. No evidence for the effect modification of the association -23HphI variant and CRC by BMI, sex, or tumor site was also observed. Moreover, the risk of obesity in relation to the -23HphI variant in the controls and the cases was separately analyzed and we observed no significant difference between normal weight (BMI < 25 kg/m^2) and overweight/obese (BMI ≥ 25 kg/m^2) subjects.
Conclusions: These findings do not support the plausible effect of the *INS* gene -23HphI variant on CRC risk; nonetheless, our finding requires confirmation and the role of the gene variant in carcinogenesis needs to be further evaluated.

Keywords: Colorectal Cancer, Insulin Gene, PCR-RFLP, Variant

1. Background

Colorectal cancer (CRC), a complex metabolic disease, is a major health problem throughout the world and is the second leading cause of cancer-related mortality (1, 2). Previous epidemiological studies have shown that CRC was associated with insulin resistance and obesity as well as CRC and obesity are related to each other through hyperinsulinemia (3-5). Moreover, an increased susceptibility to CRC in people with type 2 diabetes mellitus has been reported in a large number of previous reports and as we know increased insulin secretion and insulin resistance are involved in the etiology of type 2 diabetes mellitus (6). And lastly, Insulin is implicated in inducing cell proliferation and inhibiting apoptosis (4, 6). Furthermore, insulin can increase the bioavalibilty and expression of insulin like growth factor 1 (IGF1), which in turn, can regulate cell differentiation and increase metastasis (4, 7, 8).

The association between the gene polymorphisms of insulin signaling pathway and CRC risk has almost widely investigated, however, to our knowledge, just two previous studies (9, 10) explored the implication of the *INS* gene -23HphI variant with CRC risk, and the role of this gene in the etiology of CRC is still equivocal.

Therefore, these observations led we look for the possible association of the -23HphI variant located in the promoter of *INS* gene with CRC risk. This polymorphism was selected based on its commonly use in previous studies, position in the gene, degree of heterozygosity, and somewhat functional importance.

2. Methods

2.1. Participants

The study population consisted of 750 subjects including 312 cases with CRC and 438 controls reporting to the research institute for gastroenterology and liver diseases (RCGLD), Shahid Beheshti University of Medical Sciences. This study was conducted as a case-control study and all the subjects were recruited from patients who were under going colonoscopy either because of various gastrointestinal symptoms or because of they were considered high risk for CRC. Cases were the patient with positive pathologic report for CRC, and eligibility criteria for control subjects in-

cluded no individual history of colorectal malignancy or polyps (including adenomatous and other polyps). All patients and controls were Iranian and genetically unrelated. Before subject's colonoscopy, self administration had been used to collect the information about their demographic, anthropometric, and clinical characteristics. The present study was approved by the ethical committee of the institute and all study participants were informed about the aims of the study and gave written consents.

2.2. Genotype Analysis

Blood samples from all the subjects were collected in tubes containing EDTA as an anticoagulant and store at 4C. Genomic DNA was purified from peripheral blood leucocytes using standard "phenol chloroform" method. In this study, genotyping was done by using PCR-RFLP method. Also, genotyping of the *INS* gene was performed by investigators who were blinded to the participants' clinical data. The -23HphI variant was evaluated by a PCR that amplified a 441 bp fragment using a forward (5'-TCCAGGACAGGCTGCATCAG-3') and a reverse (5'-AGCAATGGGCGGTTGGCTCA-3') primer. The PCR reaction was run at 93°C for 10 min followed by 35 cycles of 93°C for 45 seconds, 57°C for 30 seconds, 72°C for 45 seconds, and final extension at 72°C for 10 minutes. The PCR products were digested overnight with restriction enzyme Alw26I (Fermentas, Leon-Rot, Germany) at 37°C and the RFLP products were run on 3.5% agarose gel, and stained with ethidium bromide for visualization under UV light. *INS* genotypes of each subject were identified according to the digestion pattern and alleles according to the absence ("A") or presence ("T") of the Alw26I site. Alw26I digestion reveals genotypes denoted AA (441bp), AT (441, 230 bp and 211 bp), or TT (230 bp and 211 bp). The concordance of genotyping was confirmed by duplicate analysis of approximately 15% of the samples and DNA sequencing of approximately 2% of the samples that all of them were selected randomly (all results were accurate).

2.3. Statistical Methods

We calculated differences in demographic or anthropometric factors using t-test or χ^2 test when appropriate. Testing Hardy-Weinberg equilibrium for the *INS* gene -23HphI variant among cases and controls, separately, and comparisons of the distribution of the allele frequencies between the groups were performed using the χ^2 test. Comparisons of the distribution of the genotype frequencies between the different groups were performed using the logistic regression. Logistic regression analysis was also used to adjust for confounders such as for age, BMI, sex, smoking status, regular NSAID use, and family history of CRC. The odds ratios (OR) are given with the respective 95% confidence intervals (95% CI). SPSS statistical package (version 15.0; SPSS Inc. Chicago, IL, USA) was used to analyze the data. In all statistical tests, a P value of less than 0.05 was considered to indicate statistical significance.

3. Results

3.1. Clinicopathological Analysis

Selected characteristics of the study population and their statistical significance are summarized in Table 1. On average, cases with CRC were older ($P < 0.001$) and less likely to use NSAIDs ($P < 0.001$) when compared with their control counterparts. However, there were no significant differences between the cases with CRC and the controls in terms of BMI, sex, smoking status, and family history of colorectal cancer.

3.2. INS gene -23HphI Variant Analysis

The distribution of genotypes and alleles of the *INS* gene -23HphI variant in cases with CRC and controls are provided in Table 2. As shown in Table 2, no significant difference was observed in genotype and allele frequencies between the cases with CRC and the controls for the -23HphI polymorphism. Furthermore, after adjustment for age, BMI, sex, smoking status, regular NSAID use, and family history of CRC, no significant association between the polymorphism and the risk of CRC was found.

Additionally, when we stratified the analyses by tumor site (Table 3) or sex (Table 4), we found no statistically significant differences in the *INS* gene -23HphI variant either before or after adjustment for confounding factors. We also conducted a breakdown comparison between cases and controls within different BMI categories (Table 5). In the comparison between normal weight controls and normal weight cases with CRC, as well as in the comparison between overweight/obese controls and overweight/obese cases with CRC, we found no differences between these groups with respect to allele and genotype frequencies of the -23HphI variant either before or after adjustment for age, sex, smoking status, and family history of CRC.

Finally, in this study the risk of obesity in relation to the *INS* gene -23HphI variant was also examined (data not shown). We observed no significant difference in genotype and allele frequencies between the normal weight cases with CRC and overweight/obese cases with CRC and between normal weight controls and overweight/obese controls for the -23HphI variant.

Table 1. Selected Characteristics of the Study Subjects[a]

Variables	Controls (N = 438)	Cases (N = 312)	P Value
Age, y	44.2 (16.2)	55.8 (12.9)	< 0.001
BMI, kg/m^2	25.2 (3.9)	25.8 (5.4)	0.092
Gender			0.151
Men	221 (50.5)	174 (55.8)	
Women	217 (49.5)	138 (44.2)	
Smoking status			0.294
Never smoker	369 (84.2)	260 (83.3)	
Former smoker	56 (12.8)	36 (11.6)	
Current smoker	13 (3.0)	16 (5.1)	
Regular NSAID use			< 0.001
No	357 (81.5)	302 (96.8)	
Yes	81 (18.5)	10 (3.2)	
Family history of colorectal cancer			0.473
No	392 (89.5)	274 (87.8)	
Yes	46 (10.5)	38 (12.2)	
Tumor location			-
Colon	-	203 (65.1)	
Rectum	-	109 (34.9)	
Metastasis			-
No	-	286 (91.7)	
Yes	-	26 (8.3)	
HNPCC			-
No	-	288 (92.3)	
Yes	-	24 (7.7)	

[a]Variables presented as mean (SD) or number (%).

4. Discussion

We conducted a case-control study to explore the possible association between the *INS* gene -23HphI variant and CRC risk. In the present study, no statistically significant difference was found for this polymorphism in either genotype or allele frequencies between the cases with CRC and the controls and this lack of difference remained non-significant even after adjustment for age, BMI, sex, smoking status, regular NSAID use, and family history of CRC. Furthermore, no evidence for effect modification of the association -23HphI variant and CRC by BMI, sex, or tumor site was observed. In addition, the -23HphI variant was not associated with the risk of obesity in controls and cases with CRC.

4.1. INS Gene -23HphI Variant

At the present time, CRC is considered as a multifactorial disease that might result from the interaction between genetic as well as environmental factors. Nevertheless, the number and nature of genes that influence susceptibility to CRC are mostly unknown. The links between insulin resistance, obesity and CRC have previously been noted, and therefore the major role of insulin pathway signaling in the etiology of CRC have been clarified. Furthermore, insulin signaling pathway related genes are likely to affect the pathogenesis of CRC due to the significant role of insulin resistance and obesity in this cancer. Having said that, however, the association studies of the effects of insulin pathway gene variants on CRC risk have been inconclusive, and unfortunately, such inconsistencies are common in genetic association studies (11, 12). These discrepancies might be owing to differences in the genetic and/or environmental factors triggering the development of CRC, the exact definition of the disease, small sample size, differences in the statistical methods, and finally possible linkage disequilibrium with the other unknown variants.

To date, just two epidemiological studies (9, 10) have evaluated the association between the *INS* gene -23HphI variant and the risk of CRC. Both of these studies could not find any significant association. Moreover, there is another study which investigated the association between the *INS* gene variant and advanced colorectal adenoma and again no association was found (13). Interestingly enough, our study found no significant association between the *INS* gene -23HphI variant and CRC risk too. Previous studies have demonstrated higher serum level of insulin in CRC patients than controls (5) and insulin therapy may boost the CRC risk (14). The -23HphI polymorphism is situated in the promoter region and hence its sequence alterations can affect the expression of INS. It has also been reported that the -23HphI variant is in complete linkage disequilibrium with the VNTR classes. The short class I VNTR alleles which are in linkage disequilibrium with 'A' allele of the -23HphI polymorphism are associated with the increased expression level of *INS* gene, while the long class III alleles are associated with the 'T' allele of the -23HphI variant (15). However, in spite of the biological plausibility, this study did not recommend that the -23HphI polymorphism of *INS* gene might play a role in CRC pathogenesis in the Iranian population. Nonetheless, further studies with increased numbers of CRC patients in other populations are required to confirm these findings.

4.2. Study Limitations

A number of limitations of this study merit to be considered. The first limitation is the relatively small sam-

Table 2. The Genotype and Allele Frequencies of Insulin (*INS*) Gene -23HphI Variant in Cases with Colorectal Cancer and Controls[a]

Variant	Controls (N = 438)	Cases (N = 312)	Crude		Adjusted[b]	
			OR (95% CI)	P value	OR (95% CI)	P value
- 23HphI T > A						
Genotype - wise comparison						
AA	277 (63.2)	196 (62.8)	1.0 (reference)		1.0 (reference)	
AT	94 (21.5)	79 (25.3)	1.18 (0.83 - 1.68)	0.336	1.16 (0.78 - 1.73)	0.454
TT	67 (15.3)	37 (11.9)	0.78 (0.50 - 1.21)	0.271	0.72 (0.44 - 1.17)	0.190
TT and AT	161 (36.8)	116 (37.2)	1.01 (0.75 - 1.37)	0.906	0.97 (0.69 - 1.36)	0.864
TT versus others	67 (15.3)	37 (11.9)	0.75 (0.48 - 1.15)	0.180	0.69 (0.43 - 1.11)	0.132
Allele - wise comparison						
A	648 (74.0)	471 (75.5)	1.0 (reference)		-	-
T	228 (26.0)	153 (24.5)	0.92 (0.72 - 1.17)	0.508	-	-

[a]Variables presented as number (%).
[b]Adjusted for age, BMI, sex, smoking status, regular NSAID use, and family history.

Table 3. The Association Between Insulin (INS) Gene -23HphI Variant and Risk of Colon and Rectal Cancers After Adjustment for Age, BMI, Sex, Smoking Status, Regular NSAID use, and Family History[a]

Variant	Control (N = 438)	Colon (N = 203)	OR (95%CI)	P Value	Control (N = 438)	Rectal (N = 109)	OR (95%CI)	P Value
-23HphI T>A								
Genotype-wise comparison								
AA	277 (63.2)	128 (63.1)	1.0 (reference)		277 (63.2)	68 (62.4)	1.0 (reference)	
AT	94 (21.5)	50 (24.6)	1.11 (0.70 - 1.72)	0.674	94 (21.5)	29 (26.6)	1.29 (0.73 - 2.30)	0.369
TT	67 (15.3)	25 (12.3)	0.74 (0.43 - 1.29)	0.297	67 (15.3)	12 (11.0)	0.73 (0.35 - 1.49)	0.395
TT and AT	161 (36.8)	75 (36.9)	0.94 (0.64 - 1.38)	0.786	161 (36.8)	41 (37.6)	1.03 (0.63 - 1.68)	0.885
TT versus others	67 (15.3)	25 (12.3)	0.72 (0.42 - 1.25)	0.246	67 (15.3)	12 (11.0)	0.68 (0.34 - 1.38)	0.294
Allele-wise comparison								
A	648 (74.0)	306 (75.4)	1.0 (reference)		648 (74.0)	165 (75.7)	1.0 (reference)	
T	228 (26.0)	100 (24.6)	0.92 (0.70 - 1.21)	0.594	228 (26.0)	53 (24.3)	0.90 (0.68 - 1.15)	0.584

[a]Variables presented as number (%).

ple size and hence the genotype differences may be attributable strictly to chance. The second is that we examined only one polymorphism in *INS* gene. For this reason the coverage of the gene remains to be determined. The third is lacking of data on serum glucose and insulin levels as well as insulin resistance index (HOMA-IR), which in turn, could change the results of this study. The fourth is that our study was a hospital-based study and the population may not be representative of the general population and thus selection bias may have existed. Accordingly, we could not totally rule out the possibility of chance findings. Nonetheless, in spite of these limitations, our study protocol was well designed and the possibility of true findings should not be excluded too.

To put it briefly, all the observed findings lead us to the conclusion that in this case-control study, the -23HphI variant located in the promoter of *INS* gene does not seem to affect the development of CRC in the Iranian population. Further large-scale studies in other populations, however, are warranted to confirm our findings.

Table 4. The Association Between Genotypes and Alleles of Insulin (INS) Gene -23HphI Variant and Colorectal Cancer Risk According to Sex Category After Adjustment for Age, BMI, Smoking Status, Regular NSAID use, and Family History[a]

Variant	Male				Female			
	Control (n= 221)	Case (n= 174)	OR (95%CI)	P value	Control (n= 217)	Case (n= 138)	OR (95%CI)	P value
-23HphI T > A								
Genotype-wise comparison								
AA	143 (64.7)	114 (65.5)	1.0 (reference)		134 (61.8)	82 (59.4)	1.0 (reference)	
AT	46 (20.8)	41 (23.6)	1.16 (0.67 - 2.02)	0.597	48 (22.1)	38 (27.5)	1.16 (0.65 - 2.05)	0.609
TT	32 (14.5)	19 (10.9)	0.73 (0.37 - 1.47)	0.381	35 (16.1)	18 (13.1)	0.72 (0.36 - 1.44)	0.362
TT and AT	78 (35.3)	60 (34.5)	0.98 (0.61 - 1.56)	0.932	83 (38.2)	56 (40.6)	0.96 (0.59 - 1.57)	0.897
TT versus others	32 (14.5)	19 (10.9)	0.70 (0.35 - 1.39)	0.319	35 (16.1)	18 (13.1)	0.69 (0.35 - 1.36)	0.288
Allele-wise comparison								
A	332 (75.1)	269 (77.3)	1.0 (reference)		316 (72.8)	202 (73.2)	1.0 (reference)	
T	110 (24.9)	79 (22.7)	0.88 (0.63 - 1.23)	0.475	118 (27.2)	74 (26.8)	0.98 (0.69 - 1.37)	0.912

[a]Variables presented as number (%).

Table 5. The Association Between Insulin (*INS*) Gene -23HphI Variant and Colorectal Cancer Risk According to BMI Category After Adjustment for Age, Sex, Smoking Status, Regular NSAID Use, and Family History[a]

Variant	Normal weight, BMI < 25 kg/m^2				Overweight/obese, BMI ≥ 25 kg/m^2			
	Control (n = 211)	Case (n = 139)	OR (95%CI)	P value	Control (n = 227)	Case (n = 173)	OR (95%CI)	P value
-23HphI T > A								
Genotype-wise comparison								
AA	130 (61.6)	90 (64.7)	1.0 (reference)		147 (64.7)	106 (61.3)	1.0 (reference)	
AT	45 (21.3)	35 (25.2)	1.07 (0.60 - 1.92)	0.806	49 (21.6)	44 (25.4)	1.21 (0.70 - 2.10)	0.486
TT	36 (17.1)	14 (10.1)	0.52 (0.25 - 1.10)	0.090	31 (13.7)	23 (13.3)	0.91 (0.49 - 1.78)	0.790
TT and AT	81 (38.4)	49 (35.3)	0.82 (0.50 - 1.36)	0.453	80 (35.3)	67 (38.7)	1.09 (0.68 - 1.74)	0.717
TT versus others	36 (17.1)	14 (10.1)	0.51 (0.25 - 1.07)	0.074	31 (13.7)	23 (13.3)	0.86 (0.45 - 1.65)	0.666
Allele - wise comparison								
A	305 (72.3)	215 (77.3)	1.0 (reference)		343 (75.6)	256 (74.0)	1.0 (reference)	
T	117 (27.7)	63 (22.7)	0.76 (0.53 - 1.08)	0.134	111 (24.4)	90 (26.0)	1.24 (0.89 - 1.54)	0.192

[a]Variables presented as number (%).

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Footnotes

Authors' Contribution: None declared.

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