IRS-2 G1057D polymorphism in Turkish patients with colorectal cancer

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Abstract

Introduction: Gene polymorphisms have a broad range of analysis, but are of particular use in molecular medicine due to their potential in revealing the genetic tendency in diseases such as cancer, heart attack etc. These studies basically depend on mutations that can be detected by proper techniques. The genes coding the insulin receptor substrate (IRS) proteins are among the most widely analysed polymorphisms in various cancer types, in which a G1057D mutation is seen.

Aim: To determine the risk of colon cancer by analysing the *IRS-2* gene polymorphism in Turkish patients.

Material and methods: A total of 161 newly diagnosed colorectal cancer patients were analysed and compared to 197 unrelated healthy controls. A polymerase chain reaction-based restriction fragment length polymorphism method was carried out.

Results: No differences were observed between the patient and control groups for both allele and genotype frequencies of the *IRS-2* G1057D gene.

Conclusions: Our results demonstrated that *IRS-2* G1057D polymorphism is not associated with colorectal cancer in the Turkish population. This research is a preliminary and original study in Turkish patients with colorectal cancer. It also provides population-level genetic data on *IRS-2* in the Turkish population. Further studies should be performed on larger number of patients and controls for more reliable results about the genetic tendency in colorectal cancer in Turkey. The study is a collaborative work of different universities and scientists.

Introduction

Cancer is the primary cause of death in the world. Among the different cancer types, colon cancer is the fourth most frequently seen and the fourth most frequent cause of death. Every year 677,000 people die because of colon cancer [1]. Colorectal cancer or colon cancer is described as the cancer type occurring in colon tissues, which form the longest part of the large intestine. Most colon cancers are adenocarcinomas that begin at the cells producing and secreting mucus and other fluids [2]. Colorectal adenomas or adenocarcinomas are the first phase of the pathological transformation of normal colon epithelial cells to colorectal cancer cells [3].

About 95% of colorectal cancers occur at irregular intervals, which infers that the genes are damaged by chance. The rest (about 5%) are transmitted genetically and are less common [2]. Although the factors that can influence the risk of cancer are not precisely known, some environmental and genetic factors are considered to be effective. Research is ongoing on this issue. Diet,

anti-nonsteroidal anti-inflammatory drugs (NSAIDs), smoking and physical activity are some of the environmental agents being studied [2]. There are several studies revealing that physiological differences such as hormone levels are also determinants of breast, prostate, colorectal and lung cancers [3–5]. For example, the interruption of the connection of insulin secretion and function results in hyperinsulinaemia, hyperglycaemia and type 2 diabetes. Hyperinsulinaemia and type 2 diabetes increase the risk of colon cancer [6]. Insulin-like growth factor (IGF), insulin-like growth factor binding proteins (IGFBPs), insulin and insulin receptor substrate (IRS) play significant roles in the initiation of cell growth and colorectal cancer proliferation [4, 7, 8]. Studies linking insulin resistance, obesity and colorectal cancer suggest that the insulin pathwaymay play an important role in the aetiology of colorectal cancer [9–11]. IRS proteins are *IRS-1* and *IRS-2*, which are very common, *IRS-3* found mostly in adipose tissues, and *IRS-4* in the thymus, brain and kidney [12].

The genes expressing the proteins have recently been studied to analyse the gene polymorphisms in different populations, due to their clinical significance as some of the polymorphic genes will produce polymorphic proteins, whose functions in the body might be affected and clinical diseases might occur. Therefore scientists are researching the gene polymorphisms and the clinical outcomes in order that the genetic tendency of the medical conditions could be specified and diagnostic-prognostic markers could be represented [13].

The gene encoding the insulin receptor substrate 2, *IRS-2* gene, will only be discussed here due to the concept of the paper. Several polymorphisms have been identified in the *IRS-2* gene, but in humans the prevalent polymorphism is Gly1057Asp, which is also known as G1057D single nucleotide polymorphism (SNP) (rs1805097). The genotypes of this SNP (GG, GD and DD) are analysed by means of their frequency in various patients and control groups in order to specify the association between *IRS-2* G1057D polymorphism and clinical diseases. For instance, Slattery *et al*. [14] showed that *IRS-1* (G972D) and *IRS-2* (G1057D) gene polymorphisms have coherent and independent effects in colon cancer. Studies on gene polymorphisms in colorectal cancer, which have been conducted around the world, have to be initiated also in Turkey; this would allow analysis of the genetic tendency in colorectal cancer.

Aim

This study aims to investigate whether *IRS-2* G1057D gene polymorphism is associated with colorectal cancer in Turkish patients.

Material and methods

Subjects

This study includes all cases of colorectal cancer consecutively hospitalised in the Surgery Departments of Mersin and Kocaeli University Hospitals, including patients initially seen as outpatients, from September 2001 through April 2004. The 161 newly diagnosed colorectal cancer patients and 197 unrelated healthy controls were recruited from two hospitals in Turkey. The study was approved by the ethics committees of the institutions involved in this research.

IRS-2 genotyping

Genomic DNA was extracted from 200 µl of peripheral blood by High Pure DNA isolation Kit (Qiagen, Inc., Chatsworth, CA) following then manufacturer's instructions. A polymerase chain reaction (PCR)-based restriction fragment-length polymorphism (RFLP) method was used for genotype *IRS-2* G1057D polymorphism, which removes the Hae-II restriction enzyme site. The PCR was performed in a 25 µl volume containing 20 ng genomic DNA, 10×PCR buffer with 1.5 mM $MgCl_2$, 0.25 mM dNTPs, 10% dimethylsulphoxide, 0.5 units of Taq polymerase (Fermantas, MBI) and 5 pmol of each primer, *IRS-2*F (5' GCT CCC CCA AGT CTC CTA A 3') and *IRS-2*R (5' CTC AGC CTC TTC ACG CCC 3'). The PCR thermal cycling conditions were an initial melting period at 95°C for 2 min; then 35 amplification cycles of 95°C for 45 s, 62°C for 45 s and 72°C for 45 s; and a 7-minute extension step at 72°C. The PCR products were checked on 1.5% agarose gel for the assay completion, and then the PCR products of 375 base pairs (bp) were digested with restriction enzyme Hae-II by overnight incubation at 37°C. The digestion products were electrophoresed on 3% agarose gel and visualised by staining with ethidium bromide and evaluated using the gel documentation system (Vilber-Lourmat, Cedex, France).

Statistical analysis

A case-control study was performed and allelic frequency of the polymorphism was calculated both in cases and controls. The χ^2 test was used to compare the genotype frequency of the *IRS-2* gene polymorphism between colorectal cancer patients and controls. The association between *IRS-2* polymorphisms and colorectal cancer was modelled through binary logistic regression analysis. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated to compare colorectal cancer risk for the genotypes. Values of $p \leq 0.05$ was considered as the significance level. The software used for the calculation was SPSS version 11.5 (SPSS Inc., Chicago, IL).

Results

A total of 161 patients with colorectal cancer (male : female = 94 : 67; mean age: 59.91 ±13.26 years) and 197 control subjects (male : female = 95 : 102; mean age: 57.11 ±14.60 years) participated in this study. Age and gender data of both groups are shown in Table I.

As shown in Table II, the frequencies of GG, GD and DD were 49.1%, 36.0% and 14.9% in cases and 44.7%, 43.1% and 12.2%, respectively, in controls for *IRS-2* G1057D polymorphism. No difference was found between the genotypes in groups ($p > 0.05$; Table II). The frequency of G allele was 0.671 for cases and 0.662 for controls, whereas the frequency of D allele was 0.329 for cases and 0.338 for controls. There was also no difference between cases and controls for the frequency of G and D alleles ($p > 0.05$; Table II).

Discussion

Insulin, the hormone that controls the energy homeostasis by functioning on target tissues such as liver, muscle and adipose, acts as if being attached to its receptors on the cell membrane. The insulin receptors phosphorilate the polymorphic substrate proteins, called insulin receptor substrates. *IRS* proteins, particularly *IRS-1* and *IRS-2*, are present in almost all cells and tissues. *IRS-1* controls body growth and peripheral insulin action, whereas *IRS-2* regulates body weight control and glucose homeostasis [14].

The levels of *IRS* proteins were examined in pancreatic cancer [3] and breast cancer [5, 7], whereas Keku *et al*. [6] studied insulin, glucose, IGF and IGFBP levels, and Wei *et al*. [15] studied C-peptide, *IGFBP-1* and haemoglobin levels in colorectal adenoma cases.

There are several pieces of evidence indicating that insulinis associated with the risk of colorectal cancer [9, 16]. In rats, insulin enhances the growth of aberrant crypt foci and colorectal cancer precursor lesions, and increases the number and the size of the tumours [9].

Several studies have also found a link between insulin, type 2 diabetes, obesity and body size and the risk of colorectal cancer [9–11, 17]. There isalso evidence for the association between elevated levels of insulin and increased risk of colorectal cancer in humans [17, 18].

The gene polymorphisms coding the *IRS* proteins were comprehensively studied by means of diseases such as polycystic ovary syndrome [19], obesity [20], type 2 diabetes [21], prostate cancer [22], breast cancer [8], gastric cancer [23], ovarian cancer [24] and colon cancer [14, 25, 26]. Tumour size, lymph nodes and metastasis are widely examined in cancer research studies; however, only specific genes that are known to play a role in tumour initiation, progression and metastasis are analysed to identify the relevance of tumour characteristics. For instance, Röcken *et al*. [27] showed that an enzyme that participates locally in the pathology of carcinoma, angiotensin I–converting enzyme (ACE), gene polymorphism was associated with gender-specific differences in primary tumour size. However, the IRS gene polymorphisms have not yet been studied regarding tumour size, lymph nodes and even metastasis.

Most of the research regarding *IRS-2* G1057D polymorphism has been detected using PCR and certain

Table I. The distribution of patients and controls by age and gender

Parameter	Patients	Controls	Value of *p*
Total number	161	197	
Age, mean ±SD [years]	59.91 ±13.26	57.11 ±14.60	0.06
Gender:			0.06
Female, *n* (%)	67 (41.6)	102 (51.8)	
Male, *n* (%)	94 (58.4)	95 (48.2)	

n – number of sample

Table II. *IRS-2* genotype and allele frequencies of patients and controls

Variable	Patients (*N* = 161)	Controls (*N* = 197)		
	n (%)	*n* (%)	Odds ratio	95% CI
Genotype:				
GG	79 (49.1)	88 (44.7)	1 (reference)*	
GD	58 (36.0)	85 (43.1)	0.76	0.48–1.19
DD	24 (14.9)	24 (12.2)	1.11	0.58–2.11
Allele:				
G	216 (67.1)	261 (66.2)	1 (reference)*	
D	106 (32.9)	133 (33.8)	1.50	0.99–2.29

*n – number of sample, 95% CI (95% confidence interval) from conditional logistic regression. *Carriers of at least one intact allele are used as reference*

subsequent enzyme digestions; Gunter *et al*. [26] used SNP selection to analyse this polymorphism. It was detected that none of the *IRS-2* polymorphisms were related to colorectal adenoma.

IRS-2 G1057D polymorphism also lies close to two putative phosphorylation tyrosine sites; however, no effect of its binding ability to the p85 subunit of PI-3 kinase has been observed [28]. Similar to *IRS-1*, several studies showing inconsistent effects of *IRS-2* SNP have been observed in insulin resistance-related diseases [29]. No association has been detected in studies regarding prostate cancer [22], breast cancer [28], type 2 diabetes and obesity [30]. In the study of Slattery *et al*. [14], heterozygotes, but not the homozygotes, for the D allele were shown to be at reduced risk of colorectal cancer. Pechlivanis *et al*. [31] showed that *IRS-2* G1057D polymorphism was not associated with the risk of colorectal cancer.

Colon cancer risk also affects specific types of mutations. Samowitz *et al*. [25] analysed 1788 cases and 1981 controls for *IRS-1*, *IRS-2*, *IGF-1* and *IGFBP-3* as well as tumour mutations and microsatellite instability. Only the heterozygous genotype of *IRS-2* GD was determined to be related to a decreased risk of microsatellite instability.

So far, there have been various studies and data regarding *IRS-2* G1057D polymorphism in the world. No information is available for this polymorphism among Turkish colon cancer patients. We report herein the genotype and allele frequencies of this polymorphism and the role of *IRS-2* G1057D polymorphism in colorectal cancer susceptibility in patients and healthy subjects representing the Turkish population. For *IRS-2* G1057D polymorphism, the frequencies of GG, GD and DD genotypes were 49.1%, 36.0% and 14.9% in cases and 44.7%, 43.1% and 12.2% in controls, respectively. Slattery *et al*. [14] studied 1181 colon cancer cases and 1194 controls. The frequencies of GG, GD and DD genotypes were 46.5%, 40.7% and 12.8% in cases and 41.2%, 47.3% and 11.5% in controls, respectively. Slattery *et al*. [14] detected that the GD genotype is related to a reduced risk in colon cancer. Samowitz *et al*. [25] studied 1788 colon cancer cases and 1981 controls. They observed GG genotype as 45.7% in cases (42.2% in controls), GD was 41.8% in cases (46.1% in controls) and DD was 12.5% in cases (11.7% in controls). When compared to our study, there is not a significant difference in the frequencies of the genotypes analysed. The increase of heterozygote individuals in both studies of Slattery [14] and Samowitz [25] might be due to the large sample group size. Recently, Cayan *et al*. [24] examined the association of *IRS-2* gene polymorphism with ovarian cancer in 185 Turkish women and found the GG genotype to be 53.6%; GD 38.6% and DD 7.9% in the control group, whereas our control results were 44.7%, 43.1% and 12.2%, respectively.

The frequency of G allele was 0.671 for cases and 0.662 for controls in our study. The frequency of D allele was 0.329 for cases and 0.338 for controls. Slattery *et al*. [14] determined that the *IRS-2* G allele frequencies were 0.568 and 0.658, and D allele frequencies were 0.281 and 0.342 for cases and controls, respectively. Samowitz *et al*. [25] observed that the *IRS-2* G allele frequencies were 0.585 and 0.476, and D allele frequencies were 0.294 and 0.515 for cases and controls, respectively. The decrease in the allele frequencies of *IRS-2* G1057D polymorphism when compared to our study might also be due to the size of the groups. For healthy control groups, Cayan *et al*. [24] specified that the *IRS-2* G allele frequency was 0.729, and D allele frequency was 0.271 in a Turkish population, whereas our control results were 0.662 and 0.338, respectively.

Conclusions

The results obtained from the current study demonstrate that *IRS-2* G1057D polymorphism is not a susceptible factor for colorectal cancer in Turkish patients. This research is a preliminary study in Turkish patients with colorectal cancer. It also provides population-level genetic data on *IRS-2* in the Turkish population. Further studies should be performed on a larger number of patients and controls for more reliable results regarding the genetic tendency in colorectal cancer in Turkey.

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