

Association of Calcium-Sensing Receptor (CASR rs 1801725) with Colorectal Cancer

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Article information	Abstract
<p>Article history: Received: 20 Aug 2011 Accepted: 10 Oct 2011 Available online: 25 Dec 2011</p> <p>Keywords: Rs 1801725 Colorectal cancer Polymerase chain reaction Restriction fragment length polymorphism Calcium-sensing receptor</p> <p>*Corresponding author at: Gastroenterology and Liver Diseases Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. E-mail: akramsafaei.134@gmail.com</p>	<p>Background: Calcium induces apoptosis in intestinal epithelial cells and subsequently prevents colorectal cancer through ion calcium receptor. Calcium-sensing receptor mutation reduces the expression of this receptor, and subsequently in reduces calcium transportation. Many studies have shown that Calcium-sensing receptor gene polymorphism may increase the risk of colorectal cancer. The purpose of this study is to assess the prevalence of calcium-sensing receptor polymorphisms (rs 1801725) in Iran society and to examine the role of this polymorphism in the increased risk of colorectal cancer (CRC).</p> <p>Materials and Methods: The research was a case-control study. 105 patients with colorectal cancer and 105 controls were randomly studied using polymerase chain reaction and restriction fragment length polymorphism. ² test and software 16- SPSS were used for statistical analysis.</p> <p>Results: In patient samples, the frequency of the genotypes TT, GT, GG in gene CASR rs 1801725 was respectively 64.8, 32.4, and 2.9 and the frequency of this polymorphism in control samples was respectively 51.2, 45.7, and 2.9. Frequency of allele G in patient samples was 0/48 and frequency of allele T was 0.25. In addition, Frequency of allele G in control samples was 0.74 and Frequency of allele T was calculated 0.19.</p> <p>Conclusion: The results show that calcium-sensing receptor variant (1801725 rs) is not associated with increased risk of colorectal cancer.</p> <p>Copyright © 2012 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Colorectal cancer is a disease in which malignant (cancerous) cells will be formed in colon tissue. Several factors are involved in the creation and spread of colorectal cancer, age, diet, family history, smoking, sedentary lifestyle; alcohol consumption and obesity are of factors predisposing colorectal cancer in people [1]. Another cause of colorectal cancer is polyps including a bunch of cells that grow in the colon center. These polyps may become malignant tumors [2]. In addition to all these factors, the role of genetic factors cannot be ignored in the development of colorectal cancer.

Calcium is an elemental that is necessary to maintain the normal structure and activity of all living cells [3]. This element is involved in muscle contraction, heart rate regulation, and production of nerve impulses and increase of intracellular adhesion [4, 5]. Studies have proven that calcium is a protective factor against colorectal cancer [3]. Calcium can prevent the progression of colorectal cancer in two ways: 1- Indirectly; it leads to the deposition of these compounds through binding to fatty acids and bile (factors predisposing colorectal cancer) 2- Directly; it induces apoptosis in colon epithelial cells [6]. Calcium-sensing receptor acts as an informative system in control of calcium homeostasis [7]. This receptor is also responsible for the modulation of parathyroid hormone

secretion and responds to the changes in extracellular calcium levels [8, 9]. The activity of calcium-sensing receptor is GTP-dependent and includes three sections: 1- extracellular section that includes the calcium binding section and has 600 amino acids. 2- Intramembranous section that contains 250 amino acids. 3- Intracellular tail section that contains 220 amino acids.

The position of this gene is on chromosome 3q13.3-21 [10]. Deficiency in the function of this receptor may be involved in carcinogenesis (cancer production) [11]. Protein filamin-A, which is an important molecule for apoptosis, is not able to be connected to calcium-sensing receptor with rs 1801725 polymorphism and genotype TT and this can be the initiation of carcinogenesis. Calcium's role in preventing uncontrolled division of colon epithelial cells is shown [12-15]. The relationship between receptor polymorphisms and colorectal cancer indicate the important role of calcium in preventing tumor initiation [16, 17]. Calcium-sensing receptor establishes a relationship between the serum calcium and a few calcium-dependent intracellular mechanisms [5]. The expression of calcium-sensing receptor (CASR) is high in normal epithelial cells of colon, but the expression of this gene is very low in cancerous tissues of colon [18, 19]. In calcium-sensing receptor (CASR) with rs 1801725 polymorphism, in nine hundred and 86th amino acid of the

receptor, alanine amino acid is replaced by serine and in the 1224th acid nucleoid in exon 7, guanine is replaced by thymidine [20]. In a study conducted in 2008 in Europe on 1600 patients and 1949 normal individuals, CASR polymorphisms were reported to be predisposing factor for colorectal cancer [21]. No report has been so far presented on this polymorphism in Iran society. Therefore, this study was conducted aiming to assess the prevalence of polymorphism of calcium-sensing receptor (rs 1801725) and examine the role of this polymorphism in the increase of the risk of colorectal cancer (CRC) in Iranian population.

Materials and Methods

In this study, 105 patients with colorectal cancer with a mean age of 54.3 ± 13.3 and 105 controls with a mean age of 44.1 ± 17.9 were studied. Male/female ratio was 0.90 in patient group and 0.66 in the control group. Those who referred to Taleghani Hospital in Tehran were studied. Patients included those who had colorectal cancer in terms of pathology and symptoms, and control subjects were selected from those who had colonoscopy and negative pathology results for colorectal cancer. All people were explained on how to use the results and voluntary nature of participation in this study and a written consent form were obtained from patients and control volunteers. The moral written consent form was approved by the Ethics Committee of Research Center for Liver and Digestive of Shahid Beheshti University of Medical Sciences. Five ml of peripheral blood sample was taken to conduct PCR-RFLP genetic tests. Samples used in this study were collected from December 2008 to June 2010, and were used to extract DNA.

Some information including smoking status, age and gender were provided on patients and controls. DNA was extracted through phenol-chloroform method and precipitation was performed with ethanol and PCR-RFLP method was used to determine the genotype. The primers used for amplification of the genetic region of polymorphisms, Were Forward primers (Forward): 5' C TGAGCTTTGATGAGCCTCAGAAGGAC 3' and Reverse primers (Reverse) 5' C CACTGATGACAAGCTCTGTGAACTGGA 3' to make a product at length 269bp. Every PCR reaction contained 10Mm TrisHCL, 50Mm kcl, 0.2Mm dNTP, 5 unite taq polymerase, 200-500 ng/ μ l DNA and the final concentration of 7 picomol/ μ l from each primer (consumables except for primers were prepared from Sinagen Company, primer was prepared from Fermentase Company). For PCR, 35 cycles with 10 min program of 93, 45 seconds of 93, 30 seconds of 74, 45 seconds of 72

and 10 minutes of 72 were used. After PCR, to ensure about amplification of the desired piece, all samples were electrophoresed on 1% agarose gel. Then, the product remaining was put in the vicinity of the enzyme HinII for 3 hours for the enzymatic digestion. After the incubation period, the products were again electrophoresed on agarose 3.5%, and were examined. The observed bands included: three bands 28, 241 and 269bp that represents the heterozygous type, two bands 28 and 241bp which presented homozygous GG and single-band 269bp which represents homozygous TT. Test ² was used to obtain the allele frequency difference between patient and control groups and analyze the classified variables. Using logistic regression analysis, odds ratio (OR) and confidence interval (CI) 95% were calculated and the relationship between polymorphisms and disease was determined. Statistical analyses were conducted via software SPSS-16 and probability of less than 0.05 was considered significant for *p* value.

Results

Most of the studied patients and control subjects were nonsmokers (Table 1). In this study, gender (*p*=0.47) and drug use (*p*=0.88) showed no significant correlation with increased risk of colorectal cancer. In the genotype distribution for healthy individuals, 3 individuals were homozygous TT (2.9%), 48 heterozygous GT (45.7%), 54 homozygous GG (51.4%).

In the genotype distribution for patients, 3 subjects were homozygous TT (2.9%), 34 subjects were heterozygous GT (32.4%), and 68 subjects were homozygous GG (64.8%). Polymorphism genotype distribution of CASR rs 1801725 in two patient and control groups are shown in table 2. Prevalence of alleles T was calculated 25.7% in patients and 19.2% in control group and no significant difference was observed between the two groups in terms of allele frequency (95% confidence interval = 0.42-1.08, odds ratio=0.68, *p*=0.10). The odds ratio were calculated 0.48 (95% Confidence interval=0.08-2.82) for homozygous mutant vs. normal homozygous (GG/TT) and 0.62 (95% Confidence interval=0.34-1.12) for heterozygous vs. normal homozygous (GG/GT).

Table 1. Specifications of the studied patient and control groups

Characteristics	Control (n=105)	Patient (n=105)
Age	44.13 \pm 17.89*	54.25 \pm 13.24*
Male	42(40.0%)	50(47.6%)
Female	63(60.0%)	55(52.4%)
Smoking status		
Never smoking	91(86.7%)	91(86.7%)
Former smoking	2(1.9%)	3(2.9%)
Current smoking	12(11.4%)	11(10.5%)

* Mean \pm SD

The numbers in the parentheses represent percentage

Table 2. Final results from CASR rs 1801725 polymorphism in two patient and control groups with colorectal cancer

Genotype	Patient		Control		OR ^a (CI 95%)	<i>p</i> -Value	OR ^b (CI 95%)	<i>p</i> -Value
	N(%)	Control	N(%)	Control				
GG	68(64.8%)	54(51.4%)	34(32.4%)	48(45.7%)	[1]	—	[1]	—
GT	34(32.4%)	48(45.7%)	3(2.9%)	3(2.9%)	0.56(0.31-0.99)	0.04	0.62(0.34-1.12)	0.11
TT	3(2.9%)	3(2.9%)	154(73.3%)	170(80.9%)	0.79(0.15-4.09)	0.78	0.48(0.08-2.82)	0.41
G	154(73.3%)	170(80.9%)	54(25.7%)	40(19.0%)	0.68(0.42-1.08)	0.1	—	—
T	54(25.7%)	40(19.0%)						

a: inconsistent for age and sex, b consistent for age and sex

Discussion

This study showed that calcium-sensing receptor variant (rs 1801725) is not an important factor for colorectal cancer ($p=0.24$). In addition, the prevalence of allele T in the two populations of patients and controls showed no significant difference. There is some studies reported suggesting that the mutation in CASR is associated with serum calcium level [22, 23]. Pathways during which CASR reduces carcinogenesis are as follows: 1- Improvement of E-cadherin expression level (Glycoprotein that causes cell-to-cell binding) [16]. 2- Suppression of TCF activity (T Cell Factor) which increases the expression level of the factors affecting malignancy such as c-myc [16, 24]. 3- Division control and differentiation of cells through MAPK pathway (Mitogen Activated Protein Kinase) [20]. 4- Inhibition of β -catenin activity (protein that has a significant role in the metastasis of cancerous cells) [16].

Calcium regulates division and differentiation of intestinal epithelial cells through CASR gene [14, 16]. Loss of function of this receptor leads to a malignant development and progression, because on the one hand, it leads to the lack of activity of repressive growth factors that are dependent on calcium ion, and on the other hand, this loss of function increases the production of parathyroid hormone. This hormone is a primary factor in the creation of malignancy and metastasis [5]. Inactivation of the calcium-sensing receptor plays an important role in colorectal cancer, whether because of genetic mutations or gene methylation (which reduces the receptor expression) [25].

In a study conducted on the relationship between calcium-sensing receptor polymorphism and colorectal cancer, Bacs et al. demonstrated that polymorphism CASR 1801725 (A986S) has a significant impact on the development and progression of CRC [20]. In a study conducted by Ulrike pesters et al. on 716 patients and 729 normal individuals, they reported a significant relationship between CASR rs 1801725 polymorphisms and colon adenoma [26]. While, in another study, no significant correlation was observed between this disease and calcium-sensing receptor gene polymorphism [27]. Frequency of allele T in Europe was calculated 17.5 and 13.6 during various studies and in the present study, it was calculated 22.4 (control and patient together) that represent the high incidence of allele T of rs 1801725 calcium-sensing receptor in Iran population.

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associated SNPs near the calcium-sensing receptor (CASR) gene. *PLoS Genet*. 2010; 6(7): 135-9.

There are conflicting studies in these surveys. For example, in the present study, the results were obtained as the lack of relationship between calcium-sensing receptor polymorphisms and colorectal cancer. Achievement of single reasonable result requires more extensive studies in various communities.

The reason of inconsistency in the results of similar studies is that in addition to the calcium-sensing receptor gene polymorphisms (genetic aspect) [19-23]; factors such as diet are effective in increase of the risk of colorectal cancer [28]. Moreover, this inconsistency in various studies can be rooted in racial differences. A factor which is predisposing factor for the particular disease in a specific race or in a region may not be decisive in another race and in a different geographic region. It is recommended to examine more samples in further studies conducted on the relationship between calcium-sensing receptor gene polymorphism (CASR rs 1801725) and colorectal cancer.

Calcium-sensing receptor gene polymorphism (rs 1801725) is not a predisposing factor for colorectal cancer. From a genetic perspective, polymorphism in the calcium-sensing receptor expression controllers such as vitamin D receptor (VDR) is better to be considered along with the study of calcium-sensing receptor gene polymorphism [24]. In addition, it is recommended that further studies to examine the relationship between polymorphisms CASR and colorectal cancer should be conducted with regard to diet, body weight, family history, lifestyle and exposure to environmental carcinogens in the under study individuals.

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Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

No conflict.

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