

# Association of APC and MUTYH Mutations with Colorectal Cancer

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## Research Article

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# Abstract

## Purpose

Colorectal cancer (CRC) is one of the most fatal cancers in the world. Determining if the risk of polymorphism alleles for CRC could contribute to clinical situations suggestive of an increased genetic risk for CRC is of significant importance. The aim of this study was to evaluate the association of genetic polymorphisms in two genes, APC and MUTYH, with CRC susceptibility in Iranian society.

## Methods

In this experimental study, DNA was extracted from 200 blood samples (100 control and 100 patients with CRC). After identifying point mutations in APC and MUTYH genes and designing primers, they were examined by Tetra-arms PCR technique. Chi-square test was used to calculate and analyze the statistical and frequency of SNP in patients and control groups.

## Results

SNPs: rs121913333, rs77542170, rs1801166 and rs869312753 showed significant association with CRC. rs121913333 on 5q22 appeared to have the highest degree of correlation with CRC ( $P=0.0001$ ).

## Conclusion

Our findings indicate that APC and MUTYH mutations are related to the incidence of colorectal cancer. Not only mutant but also heterozygous genotype has a significant role in CRC development.

# Introduction

Colorectal cancer (CRC) is one of the most fatal cancers in the world. The early diagnosis of CRC is of paramount importance as it is one of the most curable cancers if detected early(1). More than 1 million people are diagnosed with CRC and approximately 0.5 million people die from this disease each year worldwide(2). Research data have revealed a significant correlation between DNA mismatch repair genes and gastrointestinal cancer(3). According to previous studies, there is also a relationship between DNA mismatch repair genes and colorectal cancer(4). Reports have shown that mutation in the MUTYH gene is associated with the incidence of gastrointestinal cancers(5). A correlation between MUTYH gene and colorectal polyposis and a high risk of colorectal cancer has been reported in recent years(6). As researches has shown, the effect of Bi-allelic MUTYH mutation carriers in growth of CRC risk are overwhelmingly greater than mono-allelic carriers (7). In most Colorectal cancer, it is the APC which is mutated and play a significant role in constitutive Wnt activation. APC can be dysregulated at both the germ line and somatic level(8). According to a research carried out in 2017, in slightly more than a third CRC cases, loss of heterozygosity of chromosome 5q has been reported and also they observed somatic APC mutations in more than 80% of sporadic colorectal tumors (9, 10). Given Wnt/beta-catenin signaling pathway, there have been number of strong argument in favor of role of APC as a negative regulator and

the destabilization and degradation of beta-catenin are usually observed because of the loss APC function(11). Additionally, the activation of T-cell factor/LEF target gene and initiation of tumorigenesis can arise as the consequence of the nuclear accumulation of beta-catenin (12). Moreover, in 2018, some researchers has pointed out that one of the influential factors which affects in activation of the Wnt/ $\beta$ -catenin pathway and the progression of colorectal tumorigenesis is inactivation of APC(13). Therefore, understanding the predisposing environmental and genetic factors of CRC play significant role to understand the predisposing environmental and genetic factors of CRC in improvement of the prognoses of patients and provide therapies that are more appropriate (14).

Considering the significant prevalence of CRC in the world(15) and in Iran(16) and also serious complications of CRC which impose diverse burden on patients(17), and as well as limitations of already existing routine diagnostic pathways, designing the new diagnostic methods for CRC is needed to be implemented, which certainly will play a significant role in CRC prevention. In this context, we performed the present study to determine the association of APC and MUTYH mutations with CRC in Iranian population.

## **Materials And Methods**

### **Study groups**

We recruited 100 patients (55 males, 45 females) with CRC from stages I, II and III of disease who were admitted to Firoozgar Hospital (Iran, Tehran). To confirm the diagnosis of disease, routine pathological examinations are usually carried out. Location of tumor was colon (49 patients, 49%) and rectum (51 patients, 51%). 100 healthy volunteers (43 males, 57 females) referred to colonoscopy unit of Firoozgar Hospital, with normal results of pathological examinations, were also included as a control group. All study protocols were approved by the Local Ethics Committee of Islamic Azad University, Hamedan Branch and informed consent was obtained from all study participants.

### **Blood sampling**

After a brief explanation of study purpose and obtaining informed consent, 10 ml peripheral venous blood was obtained and collected in sodium EDTA containing tubes, kept on ice, transferred to laboratory and processed within 1 hour after collection.

### **Tetra-primer ARMS-PCR technology**

Combining the advantages of amplification refractory mutation system and tetra-primer PCR, tetra-primer ARMS-PCR is a new technology derived from common PCR and specifically designed to detect SNPs(18). In this technology, Taq DNA polymerase should lack 3'  $\rightarrow$  5' exonuclease activity, as such, the 3' end of the mismatched primer extends slower than that of the matched primer. Due to formation difficulties in a phosphodiester linkage, once the number of mispairing bases was gotten to a certain degree, the 3' end base was not able to extend. This is followed by the termination of reaction and ultimately, the length

amplification bands would not reach to specific level (19). In addition, on both sides of the mutation, there is a pair of outer and specific inner primers(20). These specific inner primers are helpful for screening(21). The outer primers in PCR reaction serves as a positive reference along with amplification of mutant and wild type individual genes with specific inner primers during the mutation occurrence (19). Therefore, it can be seen that employing this strategy leads to reduce the cost and simplify the subsequent optimization process (20, 21). Other distinguishing feature is that primer design plays crucial role in the technical detection sensitivity determination.(22). The 3' end base of primers should be in the position of mutation(23). This base may be fully complementary to the wild-type gene sequence, but mismatched with the mutant type(24). Furthermore, the base can complement with the mutant type, but not with the wild type(25). Based on genetic test, various wild allele, mutation allele, and outer product, such as the APC or MUTYH genes lead to amplification of its heterozygotic SNP locus (26).

Admittedly, the tetra-primer ARMA-PCR method holds several advantages. Firstly, to genotype SNP location, it is strongly advised to employ this technology (18). Another strength of such a method is that SNPs Detection highly efficiently(27). Last but not least, it should be noted that in order to develop SNP markers and assisting mutations detection in diverse gene, the tetra-primer ARMA-PCR method highly utilized (19-21).

## SNP genotyping

DNA was extracted from the samples using FAVORGENE kit. The quality and concentration of DNA were examined using spectrophotometry method. Extracted DNA molecules were kept at -20° C. For genotyping, 8 SNPs associated with colorectal cancer on APC and MUTYH genes were selected from APC mutation database at <http://www.umd.be/APC/>. Variants genotype: rs121913333: C>T (chr5. GRCh38:c.2626 C>T), rs1804197: C>A (chr5.GRCh38:c.86 C>A), rs36053993: G>A (chr1.GRCh38:c.1187 G>A), rs1801166: G>C (chr5.GRCh38: c.3949 G>C), rs77542170: T>C (chr1.GRCh38: c.934-2 T>C), rs869312753: C>T (chr5.GRCh38: c.562 C>T), rs1801155: T>A (chr5. GRCh38: c.3920 T>A) and rs34612342: A>G (chr1. GRCh38: c.536 A>G). Primers were designed and synthesized by Macrogen Company (South Korea). The SNPs were evaluated by Tetra-Arms PCR method and 2x super master mix YTA. The PCR reactions were as follow: 15 minutes at 95 ° C for one cycle, and 32 cycles with 3 steps, the first step 95 ° C for 30 seconds, the annealing temperature of the primer for 30 seconds and the temperature of 72 ° C for a period of 30 seconds. At the end, after completion of 32 cycles, we used 1.5% agarose gel to measure the PCR products. In this study, for the purpose of confirming the PCR results and determining the positive samples, the Sanger sequencing method was used to examine 40 cases.

## Statistical analyses

Chi-square test with p-value <0.05 was used to calculate and analyze the statistical and frequency of SNP in patients and control group and compare them with each other. The risk allele factor (RAF) and odds ratio (OR) and confidence interval (CI) were calculated by X2 test.

# Results

## Mutation in APC gene

According to our findings conducted, it can be seen that the mutation rate of the SNPs: rs121913333, rs1801166 and rs869312753 at APC gene in patients with CRC was overwhelmingly greater than the dcontrol group ( $P=0.0001$ ,  $P=0.003$  and  $P=0.007$ , respectively), indicating that mutation in the SNPs: rs121913333, rs1801166 and rs869312753 at APC gene has significant part in CRC occurrence ( $P=0.007$ ); However, there was no significant difference between mutation rate of the rs1801155 and rs1804197 in the patients with CRC and control group ( $P=0.31$  and  $P=0.61$ , respectively).

There was also significant association between increased mutation rate in rs1801166, rs121913333, rs869312753 with age ( $P=0.0001$ ,  $P=0.0024$ ,  $P=0.0001$ , respectively). The percentage of mutant genotype of rs1801166 was significantly higher in male than female patients with CRC ( $P=0.00013$ ), demonstrating that males tend to be more at risk for CRC than females; However, there was not significant gender difference for rs121913333 and rs869312753 in patients with CRC ( $P=0.052$ ,  $P=0.01$ , respectively).

## Mutation in MUTYH Gene

The results of present study showed that the mutation rate of the SNP rs77542170 at MUTYH gene was significantly higher in the patients with CRC than control group ( $P=0.001$ ), indicating that mutation in the rs77542170 at MUTYH gene has a significant part in CRC occurrence; However, there was not significant difference between mutation rate of the rs34612342 and rs36053993 in the patients with CRC and control group ( $P=0.37$  and  $P=0.60$ , respectively). Among the SNPs, only it was increased mutation rate in the SNP rs77542170 at MUTYH gene to show a significant association with age ( $P=0.0001$ ).

# Discussion

Genetic instability plays a significant role in the development of human cancer. Furthermore, Colorectal cancer (CRC) is increasingly recognized as a serious cause of mortality due to cancer. In the current research, for the first time, a remarkable association of mutation in the SNPs: rs121913333, rs1801166 and rs869312753 at APC with CRC occurrence in Iranian population have been investigated.

The process of neoplastic transformation, which leads to determination of changes in the amino acid sequence of protein or effect the binding locus of transcriptive factors, has been considerably influenced by single nucleotide polymorphisms. These polymorphisms may cause growth the risk for tumor, or bias the effectiveness of applied therapy, then this has provided the significant effect on the pharmacogenetics of anti-cancer medicinal agents, including their transport in the body, metabolism, tissue distribution and excretion(19, 23).

Mutation in APC gene, can disturb numerous processes in which a multifunctional protein participates, including cell adhesion and migration, signal transduction, microtubule assembly and chromosome

segregation(28-30), the consequence of this disturbance may increase the risk of cancer development.

Furthermore, MUTYH, a human gene, which encodes a DNA glycosylase(31), play a significant role in the base excision repair pathway(31), and collaborates in oxidative DNA damage repair. Sites where adenine is inappropriately paired with guanine cytosine, or 8-oxo-7, 8-dihydroguanine, a common form of oxidative DNA damage, are the enzyme's target to excise adenine bases from DNA backbone(32) and numerous studies have highlighted that the mutation in this gene has root in the heritable predisposition to colon and stomach cancer(33).

In the case of MUTYH, the current study confirms that there is a significant correlation between the rs77542170 at MUTYH gene and with CRC occurrence in Iranian population, which have remarkable correlation with recent studies related to this SNP in other societies, namely Hungary, Japan and Korea(34-36).

In recent years there has been a considerable number of researches which reported the APC gene mutations are one of the fundamental factors in colorectal carcinogenesis, namely an investigation, which mentioned c. 2626C>T (rs121913333) as one of frequent mutations in Argentinean patients affected by severe FAP with more than 1000 polyps(37). This was followed by a study which has been carried out by Xiaorong Liu et al in 2007. They have conducted in-depth mutation analysis with systematic analysis techniques in 43 Chinese sporadic CRC patients. 29 somatic mutations have been identified (in 17 different types) in the tumors of 18/43 (42%) patients with sporadic CRC, including c.2626C>T (rs121913333)(38). Furthermore, in 2005, six Italian families with alpha-mannosidosis were analyzed by Michele Sbaragli et al.(39). The aim of this investigation was to identify and characterize the mutations in the selected patients and they have found overall 7 mutations which 5 of them were new, including c..562C>T (rs869312753).

Moreover, in 2014, Djansugurova et al have investigated and screened the APC, MLH1, MSH2 and TP53 Mutations in Patients in Kazakhstan with Early Onset of Colorectal Cancer. They have found that rs1801166 (APC c.3949G>A) which manipulate the functional role of the APC protein, can increase the risk of developing polyposis and significantly contribute with colorectal adenomas and carcinomas(40).

Furthermore, the factor contributing to incorrect localization of the encoded protein, the rs77542170 (c.934-2A>G) variant in MUTYH which is mainly observed in subjects of East Asian descent, and is attributed to main cause of MAP in patients of Asian descent (41).

Moreover, it has been presented that having germline mutations in both copies of the MUTYH gene, increases the risk to develop multiple adenomatous polyps and, hence, colorectal cancer(42). although many studies have attempting to assess the effect of monoallelic MUTYH mutations in increase cancer risk, there is always a degree of ambiguity surrounding this concept.(43). It is conceivable that germline monoallelic MUTYH mutations combined with germline mutations in other genes more strongly increase cancer risk(6).

In conclusion, the findings of this study reveal that not only the occurrence of mutant but also of heterozygous genotype has a significant role in CRC development in Iranian population. Furthermore, for the first time we have shown that the SNPs: rs121913333, rs77542170, rs1801166 and rs869312753 have a significant association with CRC. Another remarkable result of this study was the mutations found in the control group. We have also shown a high unexpected percent of heterozygous genotype in all 8 SNPs in the control subjects showing a high CRC susceptibility in the population. However, further researches are required to evaluate the prevalence of both APC and MUTYH mutations with CRC in Iranian society using large population study.

## **Declarations**

### ***Conflict of interest statement***

*The authors declare that there are no conflicts of interest regarding the publication of this article.*

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