

## RESEARCH ARTICLE

# The Prostaglandin Synthase 2/cyclooxygenase 2 (PTGS2/COX2) rs5277 Polymorphism Does not Influence Risk of Colorectal Cancer in an Iranian Population

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## Abstract

**Background:** The prostaglandin-endoperoxide synthase 2 [PTGS2, commonly known as cyclooxygenase-2 (COX-2)] is an enzyme induced by proinflammatory stimuli that is often overexpressed in malignant tissue and involved in the synthesis of prostaglandins and thromboxanes, regulators of processes such as inflammation, cell proliferation, and angiogenesis, all relevant for cancer development. We investigated whether a functional genetic polymorphism, rs5277, in COX-2 may have a risk-modifying effect on sporadic colorectal cancer in an Iranian population. **Materials and Methods:** We conducted a case-control study on 167 patients with colorectal cancer and 197 cancer-free controls in Taleghani Hospital in Tehran, Iran, between 2007 and 2011. Peripheral blood samples of both groups were processed for DNA extraction and genotyping of the COX-2 gene polymorphism (rs5277) using PCR-RFLP. RFLP results were confirmed by direct sequencing. Logistic regression analysis was performed to calculate the adjusted odds ratio (OR) and 95% confidence interval (95% CI). **Results:** There was no significant difference in the distribution of COX-2 gene rs5277 polymorphism genotype and the allelic form, among CRC patients compared with the healthy control group ( $p = 0.867$ ). **Conclusions:** Our results suggest that rs5277 polymorphism in COX2 could not be a good prognostic indicator for patients with CRC.

**Keywords:** Colorectal cancer - cyclooxygenase-2 - genetic polymorphism - Iran

*Asian Pac J Cancer Prev*, 15 (8), 3507-3511

## Introduction

Inflammation plays a key role in the development of colorectal cancer. Several studies revealed that inflammatory disease of the colon, such as Crohn's disease and Ulcerative colitis, substantially increase the risk of colorectal cancer (Lennard-Jones et al., 1977; Lashner et al., 1989; Rhodes and Campbell, 2002).

Prostaglandins are molecules of particular interest in the inflammatory response. The cyclooxygenase (COX) enzymes, also known as prostaglandin H<sub>2</sub> synthase (PGHS) or prostaglandin-endoperoxide synthase (PTGS), are key enzymes in the production of prostaglandins. Currently, two isoenzymes of COX have been identified; COX1 and COX2. COX-1 (PTGS1) which is considered to be a constitutive enzyme expressed in most cell types and normal tissues (Dubois et al., 1998; Vane et al., 1998). Conversely, the COX2 (PTGS2) isoform, undetectable under normal physiological conditions, is

an inducible enzyme therefore is induced in response to cytokines, mitogens, growth factors, tumor promoters, proinflammatory stimuli and tumor development (Dubois et al., 1998; Bakhle, 2001; Cao and Prescott, 2002). Recently, researchers have demonstrated that COX-2 could have had a substantial role in the etiology of colorectal and other cancers (Brown and DuBois, 2005; Eisinger et al., 2007).

It has been indicated that overexpression of COX-2 is correlated with tumor recurrence, shorter survival, lymphatic metastases, later stage and larger tumor size and particularly with hematogenous spread of the colorectal cancer (Sheehan et al., 1999; Tomozawa et al., 2000; Zhang and Sun, 2002; Soumaoro et al., 2004). Furthermore, expression of COX-2 is thought to accommodate tumor promotion and carcinogenesis through stimulation of cell proliferation, inhibition of apoptosis, and of angiogenesis and invasiveness (Cao and Prescott, 2002; Kanaoka et al., 2007).

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Associations between COX-2 polymorphisms and risk of colorectal cancer have recently been studied; some of these polymorphisms are rs20417, rs20432, rs5275 and rs4648310 (Pan1 et al., 1941; Hamajima et al., 2001; Lin et al., 2002; Goodman et al., 2004; Koh et al., 2004; Siezen et al., 2006; Poole et al., 2007; Tan et al., 2007).

We have selected gene polymorphism rs5277 (3050) located in the coding region of exon 3 on the basis of reported functional and biological relevance. Previous studies have shown that the selected polymorphism are the more prevalent and has biological relevance in colorectal cancer (Barry et al., 2009; Pereira et al., 2009). Besides the COX2.3050 polymorphism is one of the most prevalent in the Caucasian population and it has been studied in several pathologic disorders, and associations with disease have been found (Campa et al., 2004; Cheng et al., 2007; Cox et al., 2007; Lee et al., 2007), however, the functional impact of this polymorphism on COX-2 activity still remains unknown. There is one hypothesis that this polymorphism itself or with another linked marker may have biological effects on COX-2 activity (Xaubet et al., 2010).

The aim of this study was to assess the association between single nucleotide polymorphism rs5277 of COX-2 gene involved in the Prostaglandins pathway and the occurrence of colorectal cancer in an Iranian population.

## Materials and Methods

### Study population and sample collection

This case-control study was conducted to assess risk factors of colorectal cancer. Cases were 167 patients with a histologically confirmed diagnosis of colorectal cancer attending the Taleghani Hospital in Tehran, Iran, between 2007 and 2011. 197 subjects without any colorectal tumors or other tumors were evaluated as control group. This study was confirmed by the Ethics Committee of the Research Center for Gastroenterology and Liver diseases, Shahid Beheshti University of Medical Sciences.

### DNA extraction

Peripheral blood samples of the both groups (cases and controls) were processed for the DNA extraction, immediately after their collection. DNA was isolated by standard methods using proteinase K digestion, phenol chloroform extraction and ethanol precipitation and stored at 4°C. DNA concentration was determined by using Nanodrop (Thermo Scientific NanoDrop 1000 Spectrophotometer/USA).

### PTGS2 rs5277 polymorphism genotyping

Polymerase chain reaction (PCR) amplification of a 511 bp region of COX-2 was performed in a 25µl reaction containing 10mM Tris-HCl (pH 8.3), 50mM KCl, 2mM MgCl<sub>2</sub>, 0.2 mM each dNTP, 0.5 mM each primer (forward 5' CACTACATACTTACCCACTTC 3') and reverse 5' CCATCTGCTCACTATCCAAG 3'), 1 unit Taq polymerase, and 100ng of genomic DNA.

The PCR profile consisted of an initial denaturation of 95°C for 5 min, followed by 30 cycles of 95°C for 45 s, 60.5°C for 40 s, and 72°C for 40 s with final extension of 72°C for 5 min. PCR products (10µl) were incubated

with 10U of restriction enzyme HincII at 37°C overnight and analyzed by 2.5% agarose gel electrophoresis, and visualized by ethidium bromide staining. The 306G>C genotypes that could be detected were: 306CC (511 bp fragment), 306GC (511+424+87 bp fragments), and 306GG (424 + 87 bp fragments). The results of the RFLP (restriction fragment length polymorphism) analysis were confirmed by randomly selected samples for direct sequencing (Figure 1).

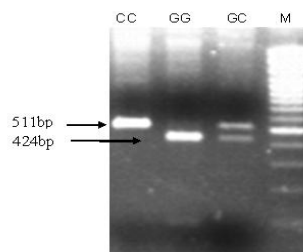
### Statistical analysis

Data were analyzed using SPSS 13. The X<sup>2</sup> or Fisher's exact tests were applied to determine differences in genotype/allele frequencies. The confounding effect of age and gender was adjusted using conditional logistic regression. The p value <0.05 was considered as significant. Data were expressed as mean±SD or frequency (%). Logistic regression was used to calculate odds ratio (OR) and 95% confidence interval (CI) for the association between genotypes and sporadic CRC. The observed genotype frequencies were tested by X<sup>2</sup> test for deviation from the Hardy-Weinberg equilibrium.

## Results

Selected characteristics of cases and controls in the studied population are displayed in Table 1.

One hundred and sixty seven patients with the diagnosis of CRC with mean age of 57.22±13.195 years, including 94 males (56.3%) and 73 females (43.7%) were studied, the control group consisted of 197 non-cancer subjects with mean age of 44.43±15.639 years, including 84 males (42.6%) and 113 females (57.4%).



**Figure 1. Results of rs5277 Polymorphism.** Band pattern corresponding to the three genotypes. Restriction fragments of 511 bp(CC), 424/87 bp(GG), 511/424/87 bp(GC) can be observed

**Table 1. Characteristics of Cases and Controls in Population Study**

Variables	Cases (N=167)	Control (N=197)
Age (mean±SD)	57.22±13.195	44.43±15.639
BMI (mean±SD) (kg/m <sup>2</sup> )	25.49±4.24	25.05±3.76
Gender	Male	84 (42.6%)
	Female	113 (57.4%)
Smoking	Ever	22 (11.2%)
	Never	175 (88.8%)
NSAID use	Regular	27 (13.7%)
	Irregular	170 (86.3%)
Localization of tumor	Colon	-
	Rectum	-
Metastasis	No	-
	Yes	-

**Table 2. Allele and Genotype Distribution of Studied SNP among CRC Patients and Healthy**

SNP Variable	Cases (N=167) n (%)	Control (N=197) n (%)	OR* (95% C.I.)	p value
<b>Rs5277</b>				
Genotypes				0.867
GG	137 (82.0%)	162 (82.2%)	1.00 (Reference)	
GC	28 (16.8%)	32 (16.2%)	1.201 (0.614-2.348)	
CC	2 (1.2%)	3 (1.5%)	1.029 (0.145-7.310)	
Alleles				0.977
G	302 (90.4%)	356 (90.4%)	1.00 (Reference)	
C	32 (9.6%)	38 (9.6%)	0.993 (0.605-1.628)	

\*Adjusted for confounder variables

Of the patients, 114 had colon cancer and 53 had rectal cancer. BMI was 25.49±4.24 for cases and 25.05±3.76 for controls. Smoking status was not statistically different (p=0.281) between the two group. Approximately more NSAID consumers (13.7% vs 4.2%) were found among controls compared to sporadic colorectal cancer cases.

To remove the effect of confounder variables, the logistic regression method was used for adjustment of data. There was no significant deviation in the genotype frequency of rs5277 polymorphism from the Hardy-Weinberg equilibrium (p>0.05).

Regarding the rs5277 polymorphism, genotypes GG, GC and CC (Figure 1) were detected in 137 (82.0%), 28 (16.8%) and 2 (1.2%) of patients (n=167) and in 162 (82.2%), 32 (16.2%) and 3 (1.5%) of healthy control samples (n=197) respectively. The results obtained from the analysis of genotyping are presented in Table 2.

The frequency of variant allele G was 302 (90.4%) and allele C was 32 (9.6%) in the cases, and also 356 (90.4%) and 38 (9.6%) in the controls, respectively.

There was no significant difference in the distribution of COX-2 gene rs5277 polymorphism genotype and the allelic form, when CRC patients were compared with the healthy control group (p: 0.867). The distribution of genotype and allele frequency among the healthy controls and the patients are revealed in Table 2. In the overall studied samples, the rs5277 polymorphism was not significant associated with a CRC risk.

The potential association of genotype distribution of the COX-2 polymorphism with tumor localization and metastasis was studied. No association between the rs5277.

## Discussion

Influence of genetic alterations regarding the increase or decrease of the risk of cancer development has been demonstrated by various investigations (Loktionov, 2004; Park et al., 2006; Mahmoudi et al., 2010; Lubbe et al., 2011). Recent studies has been reported a number of associations between a variety of SNPs in COX-2 and colorectal cancer (Lin et al., 2002; Cox et al., 2004; Siezen et al., 2005; Ulrich et al., 2005). Our previous study has reported association between rs20417 polymorphism in Cox-2 and sporadic colorectal cancer in Iranian population (Khorshidi et al., 2013). Accordingly, These results accentuate the significance of this gene and the

pro-inflammatory AA-pathway in the development of colorectal cancer (Siezen et al., 2006).

The present study was designed to determine the probable association between one of the polymorphisms of COX-2 gene and the risk of CRC among Iranian population.

To our knowledge, this is the first study on association between rs5277 polymorphism in COX-2 and sporadic colorectal cancer in Iranian population. The frequencies of the genotypes in our patients with colorectal cancer were 82.0% GG, 16.8% GC and 1.2% CC, respectively. No significant correlation is established between both allele and genotype frequencies of PTGS2 rs5277 polymorphism and sporadic CRC risk in population under study (p value=0.867). However, the findings of the current study do not support some of the previous research which was declared a significant association between this polymorphism and CRC. For instance, in one study Barry et al. (2009) reported a statistically significant increased risk for adenoma recurrence of 49% for the rs5277 CC genotype, based on this study demonstrated the pattern of rs5277 was indicative of a recessive inheritance mode because heterozygotes did not have an increased risk (Barry et al., 2009). In the pooled analysis showed V102V COX-2 polymorphism influenced the development of colorectal cancers in early stages of carcinogenesis (Pereira et al., 2009) furthermore there was a non- significant trends toward a reduced adenoma risk based on two previous studies (Gunter et al., 2006; Siezen et al., 2006). Yu et al. (2010) in a report related that rs5277 attained a marginal significant in breast cancer (Yu et al., 2010). GC genotype of SNP V102V has been shown to be inversely associated with risk of colorectal cancer (Siezen et al., 2006). Besides, in an aspirin trial of colorectal cancer, rs5277 polymorphism was associated with increased cancer recurrence (Menter et al., 2010). Nonetheless, in our study, genotypes stratification by aspirin or NSAID intake and smoking status did not show any altered effect of rs5277 polymorphism on the development of sporadic colorectal cancer. Therefore, in our population this polymorphism is not influenced by aspirin or NSAID use and smoking. Moreover, one study was reported that no significant association was found between rs5277 polymorphism and cervical cancer risk in the Korean population (Lee et al., 2007). Also, our finding is supported by a study in United State which is reported no statistically significant association between rs5277 and colon cancer risk (Thompson et al., 2009). As a final point, some of the previous studies have shown rs5277 polymorphism is not associated with risks of Gastric cancer and (Hou et al., 2007), Prostate cancer (Danforth et al., 2008). Apparently, further investigations are required to clarify these conflicting findings.

In conclusion, this preparatory study was the first report of rs5277 COX-2 gene polymorphism among colorectal cancer patients from Iran. Overall, there was no association between COX-2 rs5277 polymorphism and the risk of sporadic CRC in Iranian population. Further studies to replicate this finding in different populations are needed to validate this result.

## Acknowledgements

The present project was supported by the Research Center for Gastroenterology and Liver Diseases (RCGLD), Shahid Beheshti University of Medical sciences, Tehran, Iran. The authors gratefully acknowledge the suggestions and advice of Dr. Hamid Mohaghegh Shalmani.

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