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Cyclooxygenase-2 polymorphisms and the risk of esophageal adeno- or squamous cell carcinoma

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Abstract

AIM: To determine whether *-1195 A→G* and/or *-765 G→C* polymorphisms in *Cyclooxygenase-2* (*COX-2*) may have a risk modifying effect on the development of esophageal carcinoma in a Dutch Caucasian population.

METHODS: Two study groups were recruited, 252 patients with esophageal carcinoma and 240 healthy controls, matched for race, age, gender and recruiting area. DNA was isolated from whole blood and used for genotyping. PCR products were digested with restriction enzymes and products were analyzed by agarose gel electrophoresis. Odds ratios (OR) and 95% confidence intervals (CI) were estimated.

RESULTS: The distribution of the *-1195 A→G* polymorphism was significantly different in esophageal cancer patients compared to controls. The *-1195 GG* genotype resulted in a higher risk of developing esophageal adenocarcinoma (OR = 3.85, 95% CI: 1.45-10.3) compared with the *-1195 AA* genotype as a reference. The *-765 G→C* genotype distribution was not different between the two groups. The *GG/GG* haplotype was present more often in esophageal adenocarcinoma patients than in controls (OR = 3.45, 95% CI: 1.24-9.58; with *AG/AG* as a reference). The same trends were observed in patients with squamous cell carcinomas, however, the results did not reach statistical significance.

CONCLUSION: Presence of the *COX-2 -1195 GG* genotype and of the *GG/GG* haplotype may result in a higher risk of developing esophageal carcinoma.

Key words: Adenocarcinoma; Cyclooxygenase-2; Esophagus; Genetic polymorphism; Squamous cell carcinoma

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INTRODUCTION

During the last few decades, the incidence of esophageal carcinoma has sharply increased in Western-lifestyle countries. Two main types of esophageal carcinoma exist, adenocarcinoma and squamous cell carcinoma. The main difference between adenocarcinoma and squamous cell carcinoma is the cell type from which the tumor originates; glandular or squamous epithelial cells, respectively.

Adenocarcinoma of the esophagus predominantly occurs in Western societies. There is a strong and probably

causal relation between gastro-esophageal reflux and the development of esophageal adenocarcinoma[1]. Gastro-esophageal reflux may cause damage to the esophageal tissue due to the high concentrations of acid and bile salts, which may induce metaplasia and cell proliferation, thereby increasing the risk of mutations. This can lead to Barrett's esophagus with high grade dysplasia and ultimately to adenocarcinoma of the esophagus[1,2].

In contrast to adenocarcinoma, squamous cell carcinoma of the esophagus is thought to be caused predominantly by specific lifestyle or environmental factors such as heavy smoking in combination with alcohol use, chewing of tobacco or consumption of spicy foods and hot beverages[3]. In certain developing countries such as China, India or Iran, squamous cell carcinoma of the esophagus is very common, probably due to particular lifestyle habits[3]. As a result, damage to esophageal tissue may occur and tissue renewal may increase. This increased cell proliferation can lead to mutations, dysplasia and carcinoma.

Cell proliferation may play a key role in tumor genesis and cyclooxygenases (COXs) are important regulatory enzymes in this process. COXs are enzymes that catalyze the conversion of free arachidonic acid into prostaglandin H_2, which is the precursor of prostaglandins, prostacyclin and thromboxanes. These regulatory compounds play a role in many biological processes such as cell proliferation, angiogenesis, immune function and inflammation, which are all crucial in the development and progression of neoplasms[4]. The human COX family consists of three members, COX-1-3[4,5]. COX-1 is found in most tissues and plays a role in homeostasis of many physiologic processes. COX-3 is an alternative splice product of COX-1 and is believed to be involved in the regulation of pain and fever. COX-2 is probably very important in the development and progression of neoplasms. COX-2 is an inducible enzyme whose expression can be induced by pro-inflammatory and mitogenic stimuli like cytokines and growth factors. COX-2 plays an important role in the development of otherwise healthy tissue into metaplastic and dysplastic tissue, as well as in the development and progression of a tumor, by taking part in the regulation of cell proliferation, cell transformation, tumor growth, metastasis and invasion. COX-2 is often found over-expressed in gastrointestinal tumors, including those of the esophagus[6-10]. Tumors which exhibit a high level of COX-2 seem to be more aggressive[6] and patients bearing those tumors showed a significantly reduced survival[10]. In addition, when COX-2 expression in laboratory animals was suppressed with medication, fewer animals developed esophageal adenocarcinoma[11]. Therefore, the role of COX-2 in the development of normal or metaplastic tissue into neoplasms seems evident.

Recently, several functional Single Nucleotide Polymorphisms in the *COX-2* gene have been discovered which may contribute to the variance in inter-individual COX-2 expression. The *-1195 A→G* substitution in the COX-2 promotor was found to be associated with a lower expression of COX-2 in a Chinese population[12].

Another SNP, *-765 G→C* was first described in a UK population[13]. This polymorphism was shown to result in a lower promoter activity, which could subsequently lead to a lower expression of COX-2.

The purpose of this study is to determine the possible modulating effect of the *COX-2* polymorphisms *-1195 A→G* and *-765 G→C* on the risk for developing esophageal cancer in a Dutch Caucasian population.

MATERIALS AND METHODS

Patients and controls

A group of 252 patients with esophageal carcinoma was recruited during the period October 2002 to January 2008, in four hospitals all localized in the South-East area of The Netherlands. These hospitals were: (1) Radboud University Nijmegen Medical Center, (2) Canisius Wilhelmina Hospital, Nijmegen, (3) Hospital Gelderse Vallei, Ede and (4) Rijnstate Hospital, Arnhem. Only patients with a diagnosis of esophageal carcinoma as confirmed by a pathologist were included in the study.

Following an advertisement in local papers, a group of 240 healthy controls was recruited from the same geographical area of The Netherlands. Controls were matched with the esophageal carcinoma patients for age, ethnicity and gender.

The study was approved in 2002 by the Medical Ethical Review Committee, region Arnhem-Nijmegen (CMO 2002/114). EDTA blood was collected from patients and controls. The whole blood samples were stored at -22℃ until use. DNA was extracted from whole blood by using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, USA) according to the manufacturer's instructions. The extracted DNA was stored at 4℃ until use.

The extracted DNA was used for determination of the *-1195 A→G* and *-765 G→C* polymorphisms in the *COX-2* promoter by polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP), exactly as described by Zhang *et al*[12].

Statistical analysis

The differences between characteristics of patients with esophageal carcinoma and controls were analysed with the Student's *t*-test. All genotypes of controls and patients were tested to determine whether they were distributed according to the Hardy-Weinberg equilibrium. The chi-square test was used to test for differences in distribution of genotypes between the two groups, or to estimate differences in allele frequencies. Odds ratios (OR) with 95% confidence interval (95% CI) were calculated for genotypes associated with predicted normal versus predicted altered enzyme activities (variant genotypes). *COX-2* haplotypes were studied using the PL-EM software as described by Qin *et al*[14]. $P < 0.05$ was considered to be statistically significant. All data were processed using SPSS software for Windows version 16.0 (SPSS Inc, Chicago Illinois, USA).

RESULTS

Patients with esophageal carcinoma and controls were

Table 1 Characteristics of patients with oesophageal carcinoma and controls *n* (%)

Characteristics	Patients with oesophageal carcinoma				Controls
	Total	Adeno carcinoma	Squamous cell carcinoma	Mixed	
n	252	174 (69.0)	70 (27.8)	8 (3.2)	240
Age (yr; mean ± SD)	64.3 ± 10.8	64.7 ± 11.0	62.7 ± 10.2	69.9 ± 8.0	64.6 ± 10.9
Gender					
Female	51 (20.2)	24 (13.8)	26 (37.1)	1 (12.5)	51 (21.2)
Male	201 (79.8)	150 (86.2)	44 (62.9)	7 (87.5)	189 (78.8)

Table 2 Distribution of the *COX-2 -1195A→G* and *-765 G→C* genotypes and corresponding ORs in patients with oesophageal adenocarcinoma or squamous cell carcinoma *versus* controls

Genotype *COX-2*	Adenocarcinoma		Squamous cell carcinoma		Controls *n* (%)
	n (%)	OR (95% CI)	*n* (%)	OR (95% CI)	
-1195A→G					
-1195A/-1195A	100 (58)	Reference	39 (56)	Reference	154 (64)
-1195G/-1195A	59 (34)	1.13 (0.75-1.73)	26 (37)	1.28 (0.73-2.26)	80 (33)
-1195G/-1195G	15 (9)	3.85 (1.45-10.3)	5 (7)	3.29 (0.95-11.4)	6 (3)
Total	174		70		240
-765 G→C[1]					
-765G/-765G	112 (69)	Reference	41 (69)	Reference	157 (66)
-765G/-765C	46 (28)	0.88 (0.57-1.37)	16 (27)	0.84 (0.44-1.60)	73 (31)
-765C/-765C	5 (3)	1.17 (0.35-3.92)	2 (3)	1.28 (0.25-6.56)	6 (3)
Total	163		59		236

[1]In both the cases and control group, there are some missing data because of unsuccessful PCR; OR: Odds ratio; CI: Confidence interval.

Table 3 *COX-2* haplotype distribution and corresponding ORs in patients with oesophageal adenocarcinoma or squamous cell carcinoma carcinoma *versus* controls

Haplotype *COX-2*	Adenocarcinoma		Squamous cell carcinoma		Controls *n* = 236 (%)
	n = 163 (%)	OR (95% CI)	*n* = 59 (%)	OR (95% CI)	
AG/AG	59 (36.2)	Reference	18 (30.5)	Reference	94 (39.8)
AG/AC	29 (17.8)	0.87 (0.50-1.52)	12 (20.3)	1.18 (0.53-2.64)	53 (22.4)
AC/AC	5 (3.1)	1.33 (0.39-4.55)	2 (3.4)	1.74 (0.33-9.32)	6 (2.5)
GC/AC	0 (0)	-	0 (0)	-	0 (0)
GG/AC	17 (10.4)	1.29 (0.63-2.64)	4 (6.8)	0.99 (0.31-3.24)	21 (8.9)
GG/AG	40 (24.5)	1.14 (0.68-1.91)	19 (32.2)	1.77 (0.86-3.66)	56 (23.7)
GG/GG	13 (8.0)	3.45 (1.24-9.58)	4 (6.8)	3.48 (0.89-13.6)	6 (2.5)

matched for race, age, gender and recruiting area. Table 1 shows the characteristics of the patients and controls. The *COX-2* genotype distributions in patients and controls are summarized in Table 2. The polymorphisms tested here were distributed according to the Hardy-Weinberg criteria, *P*-values in patients and controls were 0.98 and 0.47 for the *-765 G→C* polymorphism and 0.21 and 0.24 for the *-1195 A→G* polymorphism, respectively.

No significant differences in the distribution of the *-765 G→C* polymorphism between patients with esophageal carcinoma and controls were observed ($P = 0.80$; χ^2 test). However, a significant difference in the distribution of the *-1195 A→G* polymorphism between patients and controls was observed ($P = 0.02$; chi square test). The *-1195 G/-1195 G* genotype was present more often in patients with esophageal carcinoma (whole group) as compared to the *-1195 A/-1195 A* genotype in controls (OR 3.57, 95% CI 1.39-9.13, $P = 0.005$). When analyzed according to the type of tumor, ORs were 3.85 (95% CI 1.45-10.3) for patients with adenocarcinoma and 3.29 (0.95-11.4) for patients with squamous cell carcinoma (Table 2). Allele frequencies in all patients with esophageal cancer (*-1195 A vs -1195 G*) also differed significantly from those in controls ($P = 0.02$).

When comparing the squamous cell carcinoma group ($n = 70$) with the adenocarcinoma group ($n = 174$), there were no significant differences with respect to the *-1195* genotype distribution: *-1195 AA*, 55.7% *vs* 57.5%; *-1195 AG*, 37.1% *vs* 33.9% and *-1195 GG*, 7.2% *vs* 8.6% ($P = 0.97$). For the *-765* genotypes no differences in distribution between the squamous cell carcinoma and adenocarcinoma groups were found: *-765 GG*, 69.5% *vs* 68.7%; *-765 GC*, 27.1% *vs* 28.2% and *-765 CC*, 3.4% *vs* 3.1% ($P = 0.95$).

Table 3 shows the results of a comparison of the distribution of the *COX-2 -765* and *-1195* haplotypes, according to the type of tumor. Only one significant difference was found, the *GG/GG* haplotype was present more often in the esophageal adenocarcinoma group than in the control group (OR = 3.45, 95% CI = 1.24-9.58). However, the number of individuals

present in these subgroups was very small (n = 13 *vs* n = 6, respectively). The same trend was observed in the squamous cell carcinoma group, however, statistical significance was not reached.

DISCUSSION

The *-1195 GG* genotype was present more often in patients with esophageal carcinoma than in controls. This is in contrast to the findings of Zhang *et al*[12] who identified the *-1195 AA* genotype as a risk factor for esophageal carcinoma. It is commonly reported that COX-2 expression is higher in cancerous tissue, because high COX-2 expression contributes to and sustains inflammatory and pre-cancerous processes[4,6]. Zhang *et al*[12] also concluded that COX-2 mRNA expression in *-1195 AA* genotypes was much higher than the mRNA expression in tissues of patients with the *-1195 GG* genotype. Our findings now suggest that the *COX-2 -1195* polymorphism has the opposite effect on esophageal carcinoma risk in Caucasians, as compared to Chinese patients. However, two limitations must be noted: firstly, we did not measure whether the COX-2 mRNA expression in *-1195 AA* genotypes was highest in our group of Caucasian patients, similar to the findings of Zhang *et al*[12] in Chinese patients. Secondly, there is a difference between our study population and that of Zhang *et al*[12]; the majority of our patients had adenocarcinoma (69%) and the minority suffered from squamous cell carcinoma (28%), whereas the Chinese patients in the study by Zhang *et al*[12] all had squamous cell carcinoma. In China, esophageal squamous cell carcinoma is significantly more common than adenocarcinoma, as it is mainly caused by lifestyle factors such as drinking hot beverages and eating spicy foods, whereas adenocarcinoma is associated with acid reflux as a result of the Western lifestyle[1]. In our patient group, we found no differences in the distribution of both *COX-2* polymorphisms between patients with adenocarcinoma and squamous cell carcinoma, which suggests that the differences found when compared to the results of Zhang *et al*[12] could be assigned merely to racial differences rather than to differences in the type of tumor.

Another indication that racial differences in the study populations may explain the apparent contradictory results is obtained by comparing the distribution of the *COX-2* polymorphisms in the Chinese and Dutch control populations. The genotype frequencies found in our Dutch controls for the *-765 G→C* and *-1195 A →G* polymorphisms were: 66.5% *GG*, 30.9% *GC*, 2.9% *CC* and 64.2% *AA*, 33.3% *GA*, 2.5% *GG*, respectively. Zhang *et al*[12] in a Chinese population reported genotype frequencies of 95.7% *GG*, 4.3% *GC*, 0% *CC* and 24.1% *AA*, 53.4% *GA* and 22.5% *GG*, respectively. Tan *et al* in Chinese controls more recently reported approximately the same genotype frequencies as Zhang *et al*: 95.2% *GG*, 4.8% *GC*, 0% *CC* and 23.7% *AA*, 53.2% *GA* and 23.1% *GG*, respectively[15].

On the other hand, our control group data on the *COX-2 -765* genotype were in good agreement with other European control data recently reported from Denmark, being 73.2%, 24.8% and 2.0% for -765 *GG*, *GC* and *CC* genotypes, respectively[16]. In addition, the *COX-2* polymorphism data in our patients are very similar to the recently reported *COX-2 -765* and *-1195* genotype distributions in Dutch esophageal adenocarcinoma patients by Moons *et al*[17], except for the *-1195 GG* genotype, which was present in 8.0% of our patients *vs* only 2.0% in the patients in the study by Moons *et al*[17].

The distribution of the *-765* genotypes in the control group was not found to be significantly different when compared to the esophageal carcinoma group, whereas Moons *et al*[17] reported a significantly different *-765 CC* genotype distribution between a Dutch esophageal carcinoma group (n = 140) and a Barrett's esophagus (n = 255) or reflux esophagitis (n = 240) patient group. It should be noted, however, that the number of *-765 CC* genotype individuals in these patient groups was very low, being seven, four and zero individuals, respectively[17]. Two main reasons for the difference in results between the two Dutch studies are as follows: firstly, our study was performed on a larger patient population than the study by Moons *et al*[17] (252 *vs* 140 patients), and secondly in our study, similar to the study by Zhang *et al*[12], a comparison between patients with esophageal cancer and healthy controls was made, in contrast to the study by Moons *et al*[17] where patients with Barrett's esophagus or reflux esophagitis, both of which are at risk for esophagus carcinoma, were used for comparison.

Analyzing the *COX-2* haplotypes showed that the *GG/GG* haplotype was present more often in the esophageal carcinoma group, which again is not in accordance with the results of Zhang *et al*[12] and Moons *et al*[17], who both found that the *CA* containing haplotypes carried the highest risk. Since the results of Zhang *et al*[12] and Moons *et al*[17] on different types of tumors (squamous cell carcinoma *vs* adenocarcinoma, respectively) are very similar, and more or less contradict our results, it was of interest to compare the haplotype distribution between our patients with squamous cell carcinoma *vs* adenocarcinoma. However, no significant differences were found.

In conclusion, the presence of the *COX-2 -1195 GG* genotype and of the *GG/GG* haplotype may result in a higher risk of developing esophageal adenocarcinoma and possibly also squamous cell carcinoma.

COMMENTS

Background

Cyclooxygenase-2 (COX-2) is claimed to be a key enzyme in the development and progression of neoplasms. COX-2 is often found over-expressed in gastrointestinal tumors, including those of the esophagus. The corresponding *COX-2* gene is polymorphic and two single nucleotide polymorphisms; *-1195 A →G* and *-765 G→C* were demonstrated to influence the expression of COX-2. Therefore, these polymorphisms might modulate the risk for gastrointestinal cancers, including cancer of the esophagus.

Research frontiers

In this study, the *COX-2 -1195 GG* genotype was found to be present more often in Caucasian patients with esophageal carcinoma than in controls. This is in contrast to earlier findings in a Chinese population, where the *-1195 AA* genotype was revealed as a risk factor for esophageal carcinoma.

Innovations and breakthroughs

Presence of the *COX-2 -1195 GG* genotype and of the *GG/GG* haplotype may result in a higher risk of developing esophageal carcinoma.

Applications

Screening for the *COX-2 -1195 GG* genotype in a population at risk for esophageal cancer may be valuable in the future in order to select high risk patients. Information and prevention programs can than be focused on these patients.

Terminology

COX-2 is an enzyme that catalyzes the conversion of arachidonic acid in prostaglandin H_2, the precursor of other prostaglandins, prostacyclin and thromboxanes. These regulatory compounds play a role in many biological processes such as cell proliferation, angiogenesis, immune function and inflammation, which are all crucial in the development and progression of neoplasms.

Peer review

This study offered a controversial view of COX-2 polymorphisms in the esophageal carcinomas, compared with existing studies in Europe and China. The authors thoroughly discussed various possibilities that may lead to the different findings among studies. This manuscript is well written. Although the finding is controversial, the authors discussed this issue very well.

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