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Investigation of the Association between the *CYP7A1 A-203C* Polymorphism and Barrett's Esophagus in British Population

Zuhal UCKUN[1]* Lizzy MCADAM[2] Halit Sinan SUZEN[3] Gareth J.S. JENKINS[2]
[1]Department of Pharmaceutical Toxicology, Faculty of Pharmacy, Mersin University, Yenisehir, Mersin, Turkey
[2]Swansea School of Medicine, University of Swansea, SA28PP, UK
[3]Department of Toxicology, Faculty of Pharmacy, Ankara University, Tandogan, Ankara, Turkey

*Corresponding author:
E-mail: uckunzuhal@gmail.com

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Abstract

Cholesterol 7α -hydroxylase (CYP7A1, EC 1.14.13.17) encoded by *CYP7A1* gene is the rate-limiting enzyme in synthesis of bile acids from cholesterol in the liver. Polymorphisms in *CYP7A1*gene can affect CYP7A1 activity, and thus affect cholesterol metabolism and bile acid production. Bile acids have an important role in development of Barrett's esophagus (BE) which is a condition in which changing of the cells lining the esophagus occurs. They are known extremely toxic substances at high doses. Thus, bile acids and thereby BE, might contribute to cancer. The aim of this pilot study was to investigate the association between the *CYP7A1 A-203C* polymorphism and BE. In the study, 55 samples from Barrett's esophagus and 104 samples from control group were analyzed by using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. No statistical differences between cases and controls were found in the distribution of genotype or allele frequencies. However, the *AC* genotype was less frequent in the case group (38%) versus controls (51%). The results also showed that crude odds ratio of individuals with the *CYP7A1* heterozygote (*AC*) genotype and Barrett's esophagus for the *CC* genotypes versus *AA* genotypes was 0.528 (95% CI 0.261-1.070; p= 0.077) and 0.622 (95% CI 0.229-1.704; p= 0.363), respectively. The variant *C* allele may have protective effect with regard to risk of BE. This study is the first to demonstrate the relationship between *CYP7A1*gene and BE. However, these results need further investigation and confirmation.

Keywords: *CYP7A1*, Barrett's esophagus, bile acids, polymorphism

INTRODUCTION

Barrett's esophagus (BE) is a condition in which normal squamous mucosa of the distal esophagus is replaced by a specialized intestinal metaplasia. This premalignant lesion can give rise to esophageal adenocarcinoma (EAC) which is a very aggressive type of cancer, and BE is associated with a nearly 40-fold increased risk of EAC [1]. The incidence of EAC has increased at a rate that is among the highest of all cancers [2]. Bile acids have long been implicated in the etiology of BE. Primary bile acids such as cholic acid and chenodeoxycholic acid are excreted in the liver, and are converted to secondary bile acids, primarily deoxycholic and lithocholic acids, by anaerobic bacteria in the intestinal lumen. Bile acids are known to induce oxidative stress, DNA damage, and mitochondrial damage [3]. At high doses, bile acids are overly toxic substances, probably through damaging cell membranes, mitochondrial membranes or disrupting cellular function [4]. Therefore, this cytotoxicity may be involved in stimulating proliferation and thus contribute to cancer [4,5]. On the other hand, at lower doses, bile acids stimulate cell signalling effects involving, protein kinase C, c-myc, COX-2 and NF-kappaB [4]. As a result, bile acids may be important in carcinogenesis. Bile acids synthesize from cholesterol in the liver through cholesterol 7α-hydroxylase enzyme (CYP7A1, EC 1.14.13.17) [6]. CYP7A1 encoded by *CYP7A1* gene is the rate-limiting enzyme for cholesterol catabolism and bile acid synthesis. Therefore, polymorphisms in *CYP7A1* gene can affect CYP7A1 activity, and thereupon affect cholesterol metabolism and bile acid synthesis [7]. A common single-nucleotide polymorphism *c.203 A>C* in the promoter region of *CYP7A1* has been described. The objective of this pilot study was to investigate the association between the *CYP7A1* A-203C polymorphism and BE in British population.

MATERIALS AND METHODS

Study subject

A case-control study was carried out using DNA samples from Barrett's biopsies which was collected in Swansea along with DNA samples from a control population bought in from ECACC (European Collection of Cell Cultures), England. The study consisted of 55 samples for cases and 104 samples for control. Barrett's biopsies were recruited between pre-2006/2006 and 2008. Control samples were bought from ECACC in 2008. Pre approval from ethics committees and written consent were obtained before the commencement of the study.

Procedures

CYP7A1 A-203C polymorphism was determined according to method described by Han et al [6] with minor modifications. Briefly, PCR amplification of *CYP7A1* was done using the forwad and reverse primers: 5'-AATGTTTTTCCCAGTTCTCTTTC-3' and 5'-AATTAGCC ATTTGTTCATTCTATTAG-3'. PCR was performed in a 20-µl reaction mixture containig 300-500 ng of genomic DNA, 10 pmol of each primer, 0.2 mM each

deoxynucleotide triphosphate, 10 x PCR buffer, 1.5 mM MgCl2, and 1.25 unit of Taq polymerase (Fermentase) on the MBS Satellite Thermal Cycler (Thermo, UK). After initial denaturation for 4 min at 94 ˚C, the PCR was performed for 35 cycles of 30 s at 94 ˚C, 30 s at 53 ˚C, 30 s at 72 ˚C with a final step of 72 ˚C for 5 min for elongation. Negative control reactions with no added DNA were included in each PCR analysis to ensure the reagents used that contained no contaminating DNA. The PCR product (393 bp) was analyzed electrophoretically on a 2 % agarose gel stained with ethidium bromide (500 ng/ml). 10 µl of the PCR product was digested in 50°C overnight with 10 U of Bsa I with the appropriate buffer in total volume of 20 µl. The digestion resulted in fragments of 300 and 93 bp for the *A* allele, and fragments of 261, 93 and 39 bp for the *C* allele. The digested fragments were electrophoresed on a 2 % agarose gel and visualized using ethidium bromide.

Statistical analysis

The genotype and allele frequencies of *CYP7A1* were calculated by genotype counting method. The chi-square test was used to compare the genotype and allele frequencies between cases and control groups and the association of these genotype frequencies with the risk of BE was examined in terms of odds ratio (OR) and 95% confidence intervals (CI). ORs and 95% CI were calculated using SPSS for Windows computer Software version 12. A *P* value <0.05 was considered statistically significant.

RESULTS

In this study, we analyzed 55 samples from Barrett's esophagus and 104 samples from control group to identify the *A-203C* polymorphism in the promoter region of *CYP7A1* gene. The frequencies of the *AA*, *AC*, and *CC* genotypes in Barrett's esophagus samples were 49%, 38% and 13%, respectively. According to these results, the frequencies of *A* and *C* alleles were 0.682 and 0.318, respectively. On the other hand, the frequencies of the *AA*, *AC*, and *CC* genotypes for control group were 35%, 51% and 14%, respectively. The frequencies of *A* and *C* alleles were 0.600 and 0.400, respectively (Table 1).

Table 1. Distribution of genotype and allele frequencies of CYP7A1 gene A-203C polymorphism in cases and controls

	Genotype frequencies n (%)			Allele frequencies			
	AA	*AC*	*CC*	*A*	*C*	X^2	*p*
Cases	27(49)	21 (38)	7 (13)	0.682	0.318	2.016	0.156
Control	36(35)	53 (51)	15 (14)	0.600	0.400		

The results of the control group were in good accordance with the expected genotype distributions, calculated using the Hardy–Weinberg equation (χ2: 0.406; p= 0.52). No statistical differences between cases and controls were found in the distribution of genotypes and allele frequencies. In spite of statistical insignificancy, the *AC* genotype was less frequent in the case group (38%) compared to control group (51%). Furthermore, the results showed that crude odds ratios of individuals with the *AC* genotype and *CC* genotype versus the *AA* genotypes were 0.528 (95% CI 0.261-1.070; p= 0.077) and 0.622 (95% CI 0.229-1.704; p= 0.363), respectively (Table 2).

Table 2. Crude ORs and 95% CI Barrett's esophagus according to CYP7A1 A-203C polymorphisms

Genotype	Cases n (%)	Control n (%)	Crude OR (95% CI)	*p-value*
AA	27 (49)	36 (35)	1.00 (referent)	
AC	21 (38)	53 (51)	0.528 (0.261-1.070)	0.077
CC	7 (13)	15 (14)	0.622 (0.229-1.704)	0.363

DISCUSSIONS

There are no reported previous studies to compare with the results of this study. The study is the first to demonstrate the relationship between *CYP7A1* gene and BE. The results tend to show a decreased risk of BE in individuals with the *AC* and *CC* genotypes. In spite of statistical insignificant, these genotypes may probably be associated with lowered capability of synthesizing bile acids [8-10].

Bile acids have been identified as endogenous etiologic agents for gastrointestinal tract cancers including esophagus, stomach, small intestine, liver, pancreas, biliary tract, colon/rectum cancer [11-14].

As shown in Figure 1, at high physiologic concentrations, bile acids can cause generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Increased production of ROS/RNS can lead to increased DNA damage, and then increased mutation and apoptosis. Moreover, often repeated and elongated exposure of tissues to high physiological levels of bile acids can lead to generation of genomic instability, development of reduced apoptosis capability and, finally, GI cancer [14]. Thereupon, the factors changing levels of bile acids are important.

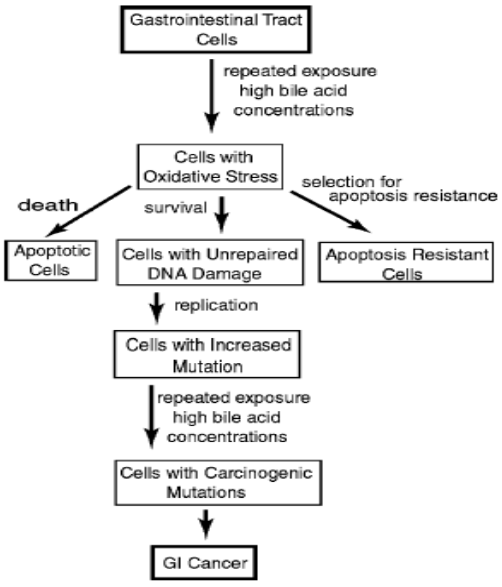

Figure 1. GI cancer pathway caused by the role of bile acid - induced DNA damage [14]

CYP7A1 is the rate-limiting enzyme producing bile acids from cholesterol. Spady et al [15] reported that

overexpression of CYP7A1 activity in hamsters resulted in a dose-dependent decrease in plasma cholesterol concentrations. Schwarz et al [16] demonstrated that in mice deficient in *CYP7A1*, fecal excretion of bile acids as well as the bile acid pool was decreased. Furthermore, in humans, *CYP7A1 A-203C* polymorphism is associated with plasma concentrations of total or low density lipoprotein (LDL) cholesterol, suggesting lower enzyme activities in those with the -203*CC* genotype [9,10]. The *CYP7A1 A-203C* polymorphism probably renders lower activity of the enzyme expressing bile acids.

The results of this study are in agreement with Hagiwara et al [8] examined the association between the *CYP7A1 A-203C* polymorphisms and colorectal cancer in 685 colorectal cancer cases and 778 controls in Japanese population. In the case group, the *CC* genotype was slightly less frequent compared to control group, and the adjusted odds ratio for the *CC* versus *AA* genotype was 0.88 (95% confidence interval, 0.65-1.20). In proximal colon cancer group, the adjusted odds ratios (95% confidence intervals) of the *CC* genotype compared with the *AA* genotype and the *AA* and *AC* genotypes combined were 0.63 (0.36-1.10) and 0.59 (0.37-0.96), respectively. As a result, a decreased risk associated with the *CYP7A1 CC* genotype was observed for proximal colon cancer. The results of the study by Tabata et al [17] were consistent with those of the study by Hagiwara et al [8]. Tabata et al [17] reported that the *CC* genotype was associated with a decreased risk of proximal colon adenomas. Adjusted odds ratios of proximal colon adenomas (95% confidence intervals) for the *AC* and *CC* genotype were 0.82 (0.54–1.24) and 0.56 (0.34–0.95) compared with *AA* genotype, respectively. Furthermore, Srivastava et al [7] also examined the association between the *CYP7A1 A-203C* polymorphisms and gallbladder cancer (GBC) and gallstone disease in 141 GBC, 185 gallstone patients and 200 healthy controls in Indian populations. The *CC* genotype of *CYP7A1* is an independent genetic risk factor for GBC but plays a modest role in susceptibility to gallstone disease.

As a result, the *AC* and/or *CC* genotype may have a decreased risk of GI cancer compared to the *AA* genotype.

CONCLUSION

In conclusion, the *AC* and/or *CC* genotype may probably be associated with lowered capability of synthesizing bile acids. The variant *C* allele may have protective effect with regard to risk of BE. However, the findings provide further evidence on the role of bile acids in BE.

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