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N-Acetyltransferase 2 gene polymorphism in patients with colorectal carcinoma

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The acetylation polymorphism is a common inherited variation in human drug and carcinogen metabolism. Because N-acetyltransferase (NAT2) is important for the detoxification and/or bioactivation of drugs and carcinogens, polymorphisms of this gene have important implications in therapeutics of and susceptibility to cancer. In this study, NAT2 genotype (NAT2*5A ($C^{481}T$), NAT2*6A ($G^{590}A$), NAT2*7A/B ($G^{857}A$)) and NAT2*14A ($G^{191}A$) and phenotype were determined in 125 patients with colorectal carcinoma and 82 healthy control in Mersin, a city located in the southern region of Turkey. Isolation of the subjects' DNA was performed by using a highly purified PCR template preparation kit/(Roche Diagnostics cat. no: 1 796 828) and the NAT2 polymorphism was detected using real-time PCR (Roche Diagnostics, GmbH, Mannheim, Germany). According to this study high protein intake is associated with the increased risk for the development of colon cancer (OR = 1.73; 95% CI, 1.10–3.07). Although only NAT2*14A fast type was associated with increased risk in patients with colorectal carcinoma (OR = 3.03; 95% CI, 1.56–5.86), when a high protein diet was considered, NAT2*7A/B fast genotype was also found to be associated with an increased risk (OR = 2.06, 95% CI for NAT2*7A/B, 1.10–3.86; OR = 2.65; 95% CI, 1.29–5.46 for NAT2*14A). Smoking status did not differ between the control and patient groups. Our data suggest that exposure to carcinogens through consumption of a high-protein diet may increase the risk of colorectal carcinoma only in genetically-susceptible individuals. Copyright © 2004 John Wiley & Sons, Ltd.

KEY WORDS — colorectal carcinoma; NAT2 polymorphism

INTRODUCTION

Human arylamine N-acetyltransferases (NAT) are known to exist as two isoenzymes, NAT1 and NAT2, with different, though overlapping, substrate specificity.[1] N-Acetyltransferases are present in the cells of most mammalian species. NAT1 is expressed in the cells of the majority of tissues and organs, whereas NAT2 is expressed only in the liver and intestine.[2] Arylamine N-acetyltransferases (EC 2.3.1.5) in humans catalyses the acetylation of arylamines derived from food to heterocyclic arylamine carcinogens.[3,4] Aromatic amines and hydrazine (N-acetylation) and N-hydroxy-aromatic and heterocyclic amines (O-acetylation) are both examples of acceptor substrates that, in general, are deactivated (N-acetylation) or activated (O-acetylation) by NAT1 and/or NAT2. NAT1 and NAT2 also catalyse the intramolecular N, O-acetyltransfer of N-hydroxy-N-acetyl-aromatic amines.[5] Genetic polymorphism in NAT1 and/or NAT2 may modify the cancer risk related to exposure to these carcinogens.[6,7]

Human epidemiological studies suggest that a rapid acetylator phenotype may be associated with a higher incidence of colorectal cancer, but a decreased risk of bladder cancer.[5,8–12]

Ethnic differences exist in NAT1 and NAT2 genotype frequencies that may be a factor in cancer incidence. Large-scale molecular epidemiological

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studies that investigate the role of NAT1 and NAT2 genotypes and/or phenotypes together with other susceptibility gene polymorphisms and biomarkers of carcinogen exposure are necessary to expand our current understanding of the role of NAT1 and NAT2 acetylation polymorphism in cancer risk.[5]

In this study, NAT2 genotype (NAT2*5A (C481T), NAT2*6A (G590A), NAT2*7A/B (G857A) and NAT2*14A (G191A) and phenotype were determined in 125 patients with colorectal carcinoma and 82 healthy controls in Mersin, a city where the local diet is rich in protein and where individuals generally consume well-cooked meat.

MATERIAL AND METHODS

Subjects

The study subjects comprised 125 patients with colorectal carcinoma attending the clinic of the General Surgery Department, University of Mersin and 82 healthy controls who visited our hospital for an annual check-up. Control subjects were selected from healthy persons without a history of malignancy, atopy, or autoimmune disease. The study was carried out according to the Declaration of Helsinki and approved by the Mersin University, Faculty of Medicine, Investigational Review Board. Informed consent was obtained from all participating patients. All patients with colorectal carcinoma had undergone surgical procedures depending upon the location of their tumours. All of the specimens examined had been diagnosed histopatologically as adenocarcinoma. The distribution of genders, ages, protein diet consumption and smoking status between the groups are given in Tables 1 and 2, respectively. Persons who smoked 20 cigarettes/day for 5 years were considered as smokers. High protein intake was defined as a usual consumption of more than 50 g protein/day.

DNA extraction and genotyping of NAT2

Blood was collected in EDTA-containing tubes and DNA was extracted from the lymphocytes with a highly purified PCR template preparation kit (Roche Diagnostics, GmbH, Mannheim, Germany; cat. no: 1 796 828). NAT2*5A, NAT2*6A, NAT2*7A/B and NAT2*14A polymorphisms of NAT2 were detected using a LightCycler-NAT2 mutation detection kit in real-time PCR (LightCycler instrument, Roche Diagnostics, GmbH, Mannheim, Germany; cat. no: 3113914). The presence of mutations in both alleles of NAT2 was assumed to represent a slow acetylation phenotype. The wild types and heterozygotes were classified as fast acetylators.

Table 1. The mean ± standard deviation of ages of patients and controls

Group	Gender (n) Male *n* (%)	Female *n* (%)	Age (mean ± SD)
Patients	65 (52.0)	60 (48.0)	57.6 ± 11.8
Controls	41 (50.0)	41 (50.0)	53.3 ± 7.4

The results were expressed in terms of arithmetic means (X) ± Standard deviation (SD); *n*, number in sample.

Table 2. Cigarette consumption and high protein diet distribution between patients and controls

	Patients *n* (%)	Control *n* (%)	OR (CI)*
Cigarette consumption	63 (50.4)	44 (46.8)	1.21 (0.69–2.12)
High protein intake	69 (55.2)	43 (45.7)	1.73 (1.10–3.07)

*ORs (odds ratio), CI (confidence interval) from conditional logistic regression.
n, number in sample.

Statistical analysis

The statistical program SPSS 11.5 for Windows was used. Patients and control group ages are presented as mean and standard deviation (SD). The association between NAT2 polymorphisms, cigarette consumption, protein diet and the development of colorectal cancer was examined by use of multiple logistic regression analysis to calculate the odds ratios (OR) and 95% confidence intervals (CI). The NAT2 phenotypes fall into two distinct categories: those with slow or fast phenotypes. The risk group consisted of individuals who smoked cigarettes (20 cigarettes/day), had a high protein intake (a usual consumption of more than 50 g protein/day) and had high-risk genotypes (the fast acetylator of NAT2).

RESULTS

We determined the mutations of NAT2 enzymes in 125 patients with colorectal carcinoma and 82 healthy controls aged between 40 and 70 years. Four of the mutation combinations of the NAT2 gene were determined: NAT2*5A (C481T), NAT2*6A (G590A), NAT2*7A/B (G857A) and NAT2*14A (G191A).

The main characteristics of patients with colorectal carcinoma and controls are presented in Table 1. After grouping according to protein intake, the individuals with high protein intake were at risk of developing

Table 3. NAT2 phenotypes and the risk of developing colorectal carcinoma

Acetylator type	Control group *n* (%)	Patient group *n* (%)	OR (CI)*
NAT2*5A Fast	40 (48.7)	62 (49.6)	
NAT2*5A Slow	42 (52.3)	63 (50.4)	1.80 (0.71–2.89)
NAT2*6A Fast	42 (52.3)	65 (52.0)	
NAT2*6A Slow	40 (48.7)	60 (48.0)	0.63 (0.31–1.26)
NAT2*7A/B Fast	50 (60.9)	78 (62.4)	
NAT2*7A/B Slow	32 (39.1)	47 (37.6)	1.37 (0.67–2.80)
NAT2*14A Fast	31 (37.8)	72 (57.6)	
NAT2*14A Slow	51 (62.2)	53 (42.4)	3.03 (1.56–5.86)

*ORs (odds ratio), CI (confidence interval) from conditional logistic regression.
n, number in sample.

Table 4. The association between cigarette consumption, high protein intake and NAT2 polymorphisms in colorectal carcinoma and controls

Variable	Patient *n* (%)	Control *n* (%)	OR (95% CI)*
Cigarette consumption			
NAT2*5A (fast)	29 (23.2)	18 (19.1)	1.38 (0.64–2.98)
NAT2*6A (fast)	34 (27.2)	24 (25.5)	0.88 (0.29–2.63)
NAT2*7A/B (fast)	38 (30.4)	24 (25.5)	0.96 (0.33–2.76)
NAT2*14A (fast)	34 (27.2)	19 (20.2)	1.47 (0.77–2.79)
High protein intake			
NAT2*5A (fast)	35 (28.0)	19 (20.2)	1.47 (0.70–3.09)
NAT2*6A (fast)	36 (28.8)	20 (21.3)	0.66 (0.20.2.12)
NAT2*7A/B (fast)	43 (34.4)	19 (20.2)	2.06 (1.10–3.86)
NAT2*14A (fast)	35 (28.0)	12 (12.8)	2.65 (1.29–5.46)

*ORs (odds ratio), CI (confidence interval) from conditional logistic regression.
n, number in sample.

of colorectal cancer (OR = 1.73; 95% CI, 1.10–3.07; Table 2). The individuals who smoked 20 cigarettes/day, were not found to be significantly at risk (OR = 1.21; 95% CI, 0.69–2.12; Table 2).

The frequency of the NAT2*14A (fast) genotype in patients with colorectal cancer (57.6%) showed a statistically significant increase compared with the among frequency the control group (37.8%). The NAT2*14A (fast) genotype was associated with a three-fold increased risk of developing colorectal cancer (OR = 3.03; 95% CI, 1.56–5.86). The distribution of the genotypes with NAT2*5A (fast), NAT2*6A and NAT2*7A/B polymorphisms did not differ significantly between patient and control groups (Table 3).

After grouping according to level of protein intake, the NAT2*7A/B (fast) and NAT2*14A (fast) genotypes were found to be a risk factor for colorectal cancer (OR = 2.06, 95% CI, 1.10–3.86; OR = 2.65; 95% CI, 1.29–5.46). There were no significant differences in the distributions of any other NAT2 polymorphisms and high protein intake. Also the fast acetylator high risk phenotypes (heterozygotes and mutant) of the other NAT2 polymorphisms and cigarette consumption were not associated with an increase in the risk of developing colorectal cancer (Table 4).

DISCUSSION

Acetylation polymorphism is a common inherited variation in human drug and carcinogen metabolism. Because N-acatytransferase (NAT2) is important for the detoxification and/or bioactivation of drugs and carcinogens, this polymorphism has important implications in the therapeutics of and susceptibility to cancer.[3] Acetylation polymorphism and resultant division into the fast and free acetylator is caused by the occurrence of wild-type allele NAT2 gene and its mutant forms. A given person shows the fast acetylation phenotype if at least one allele NAT2 is wild. The presence of mutation in both alleles of the NAT2 gene is manifested by the free acetylation phenotype (slow acetylator).[2,12]

In 1996, an estimated 876 000 new cases of colorectal cancer occurred worldwide: 445 000 in males and 431 000 in females. Less than one-third of colorectal cancer cases occur in developing countries. In developed countries, colorectal cancer is the second most common cancer in both sexes.[13] Human epidemiological studies suggest that the rapid acetylator phenotype may be associated with a higher incidence of colorectal cancer, but with a decreased risk of bladder cancer.[5,8–12]

Heterocyclic amines are associated with colorectal cancer in rodents but are poor substrates for N-acetylation. Thus, a biologically plausible mechanistic hypothesis suggests that rapid NAT1 and/or NAT2 acetylators are more readily able to convert N-hydroxy-heterocyclic amines within the colon to their ultimate carcinogenic forms, thereby predisposing the rats to colorectal cancer. Human colon cytosols activate N-hydroxy heterocyclic amine carcinogens to a DNA adduct catalysed by N-acetyltransferases. Because human populations are genetically heterogeneous, and exposure to heterocyclic amines are difficult to estimate, it is not surprising that the results are inconsistent.[5]

Several studies reported that the rapid NAT2 acetylator phenotype increased risk of colorectal carcinoma.[8,13–17] Hubbard *et al.*[18] reported that the NAT2 slow phenotype increased the risk of colorectal cancer

among subjects less than 70 years old. In contrast, Slattery *et al.*[19] reported that fast/intermediate acetylator phenotypes are associated with increased of colorectal carcinoma among older women (aged > 67 years). Lee *et al.*[20] reported that the rapid acetylation genotype of NAT2 was associated with cancer occurring on the right side of the colon. They also showed that the NAT2*7A allele was seen more frequently in distal cancer. Bell *et al.*[21] found an association between the NAT1*10 allele and colorectal cancer, and the risk was highest among NAT2 rapid acetylators. Le Marchand *et al.*[22] showed that there was an association between red meat intake and NAT2 genotype, but that there was a nine-fold increase in colorectal carcinoma risk for heavy smokers who preferred their red meat well-done and had a rapid metabolic phenotype for NAT2. They also showed that cigarette smoking may increase the risk for colon carcinoma by inducing CYP1 A2.[22] In another study they found that preference for well-done red meat was associated with an 8.8-fold increased risk of colorectal carcinoma among heavy smokers with the NAT2 and CYP1A2 rapid phenotypes.[23] Another study also showed a higher risk for colorectal cancer in individuals who consumed well-done meat and possessed both the NAT1*10 allele and rapid acetylator NAT2 genotype.[24]

According to our study, high protein intake is associated with an increased risk of colon cancer development (OR = 1.73; 95% CI, 1.10–3.07). Although only the NAT14 fast type showed increased risk for colorectal carcinoma in persons with a normal diet (OR = 3.03; 95% CI, 1.56–5.86), when the high protein diet is considered, the NAT7A/B fast genotype was also found to be associated with an increased risk (OR = 2.65; 95% CI, 1.29–5.46). Smoking status did not differ in our control and patient groups. Our data and that of others[22–24] suggest that exposure to carcinogens through consumption of high protein quantities increases the risk of colorectal carcinoma only in genetically susceptible individuals.

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