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BRIEF ARTICLE

N-Acetyltransferase 2 genetic polymorphisms and risk of colorectal cancer

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Abstract

AIM: To investigate the possible association between meat intake, cigarette smoking and N-acetyltransferase 2 (NAT2) genetic polymorphisms on colorectal cancer (CRC) risk.

METHODS: Patients with CRC were matched for gender and age to healthy controls. Meat intake and cigarette smoking were assessed using a specific frequency questionnaire. DNA was extracted from peripheral blood and the genotypes of the polymorphism were assessed by polymerase chain reaction-restriction fragment length polymorphism. Five NAT2 alleles were studied (WT, M1, M2, M3 and M4) using specific digestion enzymes.

RESULTS: A total of 147 patients with colorectal cancer (76 women and 90 men with colon cancer) and 212 controls were studied. The mean age of the two groups was 62 years. More than half the subjects (59.8% in the case group and 51.9% in the control group) were NAT2 slow acetylators. The odds ratio for colorectal cancer was 1.38 (95% CI: 0.90-2.12) in slow acetylators. Although the number of women was small (n = 76 in the case group), the cancer risk was found to be lower in intermediate (W/Mx) acetylators [odds ratio (OR): 0.55, 95% confidence interval (95% CI): 0.29-1.02]. This difference was not observed in men (OR: 0.56, 95% CI: 0.16-2.00). Among NAT2 fast acetylators (W/W or W/Mx), meat consumption more than 3 times a week increased the risk of colorectal cancer (OR: 2.05, 95% CI: 1.01-4.16). In contrast, cigarette smoking increased the risk of CRC among slow acetylators (OR: 1.97, 95% CI: 1.02-3.79).

CONCLUSION: The risk of CRC was higher among fast acetylators who reported a higher meat intake. Slow NAT2 acetylation was associated with an increased risk of CRC.

Key words: N-acetyltransferase 2; Polymorphism; Colorectal cancer

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers in the world. In Brazil, 13 310 new cases in men and

14 800 in women are estimated to occur in 2010[1].

N-acetyltransferase 2 (NAT2) is an enzyme found in a large number of organs such as the lungs, colon, breast, prostate, and liver. The expression of this enzyme suggests that it plays a key role in the protection against reactive molecules resulting from environmental insults not only in the liver but in all target tissues[2]. The *NAT2* gene is located in the chromosome 8p22 region and has no introns. The gene contains an 870-bp open reading frame and encodes a protein of 290 amino acids[3]. It is an important phase Ⅱ enzyme that catalyzes the acetylation of aromatic and heterocyclic amines and hydrazines present in carcinogenic compounds and medicines. Individuals can be divided into three different phenotypes based on the acetylation activity of NAT2: fast, intermediate, and slow. These phenotypes are determined by single nucleotide polymorphisms in NAT2[4].

Some NAT2 polymorphisms have been consistently associated with a reduction in acetylation activity (e.g. $T^{341}C$). The functional state of the phenotype is due to the impairment of protein translation or stability. No changes in mRNA levels are detected. For several polymorphisms, the classification as "fast" or "slow" is not final[5].

The probability of developing cancer depends on the natural response of each organism to different aggressive agents. Humans present different susceptibilities to carcinogens[6,7]. This difference in susceptibility to various environmental aggressors is related to genetic polymorphisms[8].

Studies have associated meat consumption with an increased risk of CRC[9]. Red meat, especially meat that is well done, is a source of chemical carcinogens such as heterocyclic aromatic amines, polycyclic aromatic hydrocarbons, and other products. The fast acetylation genotype is probably related to larger amounts of metabolic activators of heterocyclic aromatic amines when compared to the slow NAT2 acetylation genotype. Metabolic activators are transported to colorectal tissues through the bloodstream, causing DNA damage and mutations in tumor suppressor genes involved in the carcinogenesis of CRC[10].

The association between genetic polymorphisms in the *NAT2* gene and CRC has been studied extensively; however, the results are not conclusive, possibly because of ethnic differences and differences in the lifestyle and number of patients studied. The proportion of fast and slow acetylation phenotypes varies markedly depending on ethnicity and geographic origin[11]. Thus, there is an urgent need for studies investigating the distribution of NAT2 genotypes in different countries.

The aim of the present study was to investigate NAT2 polymorphisms in Brazilian patients from São Paulo.

MATERIALS AND METHODS

A case-control study involving 147 patients with CRC and 212 healthy subjects was carried out between March 2008 and December 2009. All patients were born in Brazil and were treated at the Oncology Division, Department of Gastroenterology, University Hospital, Universidade Federal de São Paulo (UNIFESP). The study was approved by the Ethics Committee of UNIFESP and all patients signed an informed consent form.

The patients answered a questionnaire regarding food habits and food frequency, whether they were current or former cigarette smokers, and their pattern of alcohol consumption.

Peripheral blood was collected for genomic DNA extraction. The *NAT2* gene polymorphisms were investigated by the polymerase chain reaction (PCR)-restriction fragment length polymorphism genotyping technique.

DNA extraction

Leukocyte DNA was extracted from peripheral venous blood collected with ethylenediaminetetraacetic acid using the Invisorb® Spin Blood Mini Kit.

Analysis of NAT2 genetic polymorphisms

The genotypes of the NAT2 polymorphism were analyzed as described previously[9]. Genomic DNA was amplified using the following primers: 5'-GGAACAAATTGGACTTGG-3' and 5'-TCTAGCATGAATCACTCTGC-3'. After amplification, the PCR product was digested with KpnI (M1 allele), BamHI (M3 allele) and MspI/AluI (M4 allele), and with TaqI (M2 allele). The digestion products were separated on agarose gels stained with ethidium bromide, and were then visualized under UV light[15]. The W/W and W/MX genotypes were classified as conferring the fast acetylation phenotype and the Mx/MX genotype as conferring the slow acetylation phenotype.

Statistical analysis

The Student *t*-test was used for the comparison of age between groups. Differences in the polymorphisms between the two groups were determined by the χ^2 test. This test was also used to compare clinical variables between NAT2 genotypes and alleles in the group of cancer patients. The association between the risk of developing cancer and these variables was assessed by calculating the odds ratio (OR) and 95% confidence interval (95% CI). A P value < 0.05 was considered to be statistically significant and a P value of 0.05 to 0.10 was considered to be marginally significant.

RESULTS

A case-control study including 147 patients with CRC and 212 healthy controls was conducted to determine whether NAT2 genetic polymorphisms are associated with the development of this disease. The characteristics of the cancer patients and controls are shown in Table 1. No difference in age or gender was observed between groups. Among the 147 patients with cancer, 90 (61.2%) had colon cancer and 57 (38.8%) had rectal cancer. According to the TNM classification, most patients were stage Ⅱ (44.2%) or stage Ⅲ (26.5%).

Four different NAT2 alleles were found, including the wild type (WT) and the M1, M2 and M3 polymorphisms. The M4 allele was not detected. Among healthy control

Table 1 Characteristics of the patients in both groups *n* (%)

Parameters	Patients	Control	*P*
Age (yr, ± DP)	61.9 (13.6)	62.0 (13.4)	0.96[a]
⩽ 50	30 (20.4)	31 (14.6)	0.196[b]
> 5	17 (79.5)	181 (85.4)	
Gender			
Male	71 (48.3)	85 (40.1)	0.15[b]
Female	76 (51.7)	127 (59.9)	
Tumor site			
Colon	90 (61.2)		
Rectum	57 (38.8)		
Stage			
I	23 (15.6)		
II	65 (44.2)		
III	39 (26.5)		
IV	20 (13.6)		

[a]*t* test; [b]χ^2 test.

subjects, the observed genotype frequency of the NAT2 polymorphisms were consistent with the expected frequency of the Hardy-Weinberg equilibrium (P = 0.56), suggesting that the distribution of NAT2 genotypes is adequate in the cancer-free population.

The slow acetylation phenotype predominated in the two groups (59.8% in the case group and 51.9% in the control group). No significant differences in the frequency of the NAT2 polymorphisms were observed between groups (Table 2).

The odds ratio for CRC was 1.38 (95% CI: 0.90-2.12) in slow acetylators. The M1 allele was the most frequent allele in the two groups, with a frequency of 45% in the control group and of 44.5% in the case group, followed by the WT allele (28% in the control group and 25.1% in the case group).

No significant association was observed between NAT2 polymorphism and acetylation phenotype or tumor site. Comparison of patients in TNM stage I or II *vs* stage III or IV showed a higher frequency of the slow acetylation phenotype in stage I and II patients (60.1%).

Analysis of red meat intake showed that half of the subjects consumed red meat more than 3 times a week. Subjects with the fast acetylation phenotype who consumed meat more than 3 times a week presented an increased risk of CRC (OR: 2.05, 95% CI: 1.01-4.16) (Table 3). With respect to cigarette smoking, the number of ex-smokers was marginally higher in the cancer group. Cigarette smoking increased the risk of CRC among slow acetylators (Mx/Mx) (OR: 1.97, 95% CI: 1.02-3.79) (Table 4).

DISCUSSION

CRC is one of the most common cancers. Almost 70% of CRC patients are diagnosed at age 65 years or older[12]. Most of the subjects studied here were women (n = 76, 51.7%), and the mean age was 61.9 years. These findings agree with data published by the Brazilian National Cancer Institute[1].

According to the Annual Report to the Nation on the Status of Cancer, prostate cancer is the most frequent cancer among men, followed by lung, colon and rectal cancer, except for Latin America where the incidence of CRC is slightly higher than that of lung cancer. Among women, the most frequent cancer is breast cancer, followed by lung cancer and CRC[13].

Variations in the frequency of NAT2 genotypes/phenotypes among different populations and ethnic groups have been reported in several studies carried out in different regions around the world. In this respect, a high frequency of the slow acetylator phenotype is observed in populations of European and African descent. Other populations are characterized by a high frequency of fast acetylation phenotypes, such as Japanese, Chinese and Amerindians[14-16].

In the present study, the slow acetylation phenotype was slightly more frequent in the two groups, although the difference was not statistically significant. However, when divided by gender, the fast acetylation phenotype tended to be more common among women. The M1 allele was the most frequent allele in the two groups (45% in the control group and 44.5% in the case group), followed by the WT allele (28% in the control group and 25.1% in the case group). The M4 allele was not detected. These data are consistent with the literature, which indicates a difference in the frequency of the WT, M1 and M4 alleles between Caucasians and Africans, whereas the frequency of the M2 and M3 alleles is similar. The M4 allele is detected at a rate of less than 1% in Caucasians, whereas its frequency is 18% in the African population[9,17,18].

No significant difference in NAT2 polymorphism or acetylation phenotype was observed between tumor sites. Analysis according to TNM stage showed that the slow acetylation phenotype was more frequent among stage I or II patients (60.1%) compared to stage III or IV. This finding might be explained by the fact that the NAT2 fast acetylation phenotype activates carcinogens and produces mutations more quickly, resulting in aggressive tumors. However, these findings should be analyzed carefully because of the small number of patients participating in the present study.

For a long time, genetic susceptibility to cancer has been attributed to xenobiotic exposure. This view was mainly due to the fact that the molecular mechanisms involved in carcinogenesis were not known. However, this view has changed over recent years with the advances in molecular biology. It is now known that exposure to xenobiotics and the development of cancer vary among individuals because of variations that occur at the molecular level which, in turn, are under genetic control[19]. In recent studies, lifestyle habits including alcohol and tobacco use and dietary habits (i.e. adequate protein and fiber intake) have been associated with gene mutations in an attempt to obtain more consistent results regarding cancer risk factors and prognosis. Although currently available data are controversial due to ethnic differences and differences in lifestyle, this has been the best approach to better understand carcinogenesis at the molecular level.

Smoking has been associated with several types of cancer other than lung cancer, including cancer of the oral cavity, pancreas, and kidney[20]. A recently published meta-

Table 2 Distribution of N-acetyltransferase 2 polymorphism and the risk of cancer

Genetic polymorphism NAT2[a]	Cancer, *n* (%)	Control, *n* (%)	*P*	OR (95% CI)
All	147	212		
Mx/Mx	88 (59.8)	110 (51.9)	0.19	1
W/Mx	44 (30.0)	83 (39.1)		0.66 (0.42-1.05)
W/W	15 (10.2)	19 (8.9)		0.99 (0.47-2.05)
Slow	88 (59.8)	110 (51.9)	0.17	1.38 (0-2.12)
Fast	59 (40.1)	102 (48.1)		
Female	76	127		
Mx/Mx	42 (55.2)	58 (45.6)	0.07	1
W/Mx	23 (30.2)	58 (45.6)		0.55 (0.29-1.02)
W/W	11 (14.4)	11 (8.6)		1.38 (0.55-3.48)
Slow	42 (55.2)	58 (45.6)	0.24	1.47 (0.83-2.60)
Fast	34 (44.7)	69 (54.3)		
Male	71	85		
Mx/Mx	46 (64.8)	52 (61.1)	0.67	1
W/Mx	21 (29.5)	25 (29.4)		0.95 (0.47-1.92)
W/W	4 (5.6)	8 (9.4)		0.57 (0.16-2.00)
Slow	46 (64.7)	52 (61.1)	0.76	0.86 (0.45-1.65)
Fast	25 (35.2)	33 (38.9)		

[a]Homozygous individuals with genotype W/W, and heterozygote W/Mx, are grouped into fast acetylation phenotype, while homozygous Mx/Mx are grouped in slow acetylators. The percentages of data are in parentheses. NAT2: N-acetyltransferase 2; OR: Odds ratio; 95% CI: Confidence interval.

Table 3 Comparison between meat intake and risk of of cancer in rapid acetylator patients *n* (%)

	Cancer	Control	*P*	OR	Lower[95% CI]/upper
All	59	102			
High meat intake[1]	44 (74.5)	60 (58.8)	0.06	2.05	1.01/4.16
Low meat intake[2]	15 (25.5)	42 (41.2)			

[1]More than 3 time per week; [2]Less than 3 times a week. OR: Odds ratio; 95% CI: Confidence interval.

Table 4 Correlation between genotypes and risk for cancer in smokers or ex-smokers

Genetic polymorphism	Cancer, *n* (%)	Control, *n* (%)	*P*	OR (95% CI)
NAT2[a]				
All	65	87		
Mx/Mx	40 (61.5)	39 (44.8)	0.09	1
W/Mx	22 (33.8)	39 (44.8)		1.82 (0.92-3.60)
W/W	3 (4.6)	9 (10.3)		3.08 (0.77-12.22)
Slow	40 (61.5)	39 (44.8)	0.06	1.97 (1.02-3.79)
Fast	25 (38.5)	48 (61.0)		

[a]Homozygous individuals with genotype W/W, and heterozygote W/Mx, are grouped into fast acetylation phenotype, while homozygous Mx/Mx are grouped in slow acetylators. NAT2: N-acetyltransferase 2; OR: Odds ratio; 95% CI: Confidence interval.

analysis reported a strong association between smoking and the development of CRC[12]. However, smoking is currently not recognized as a risk factor for CRC by the International Agency for Research on Cancer (IARC) or the US Surgeon General[12]. Sørensen *et al*[21] studied the association between NAT1 and NAT2 polymorphisms, smoking, meat consumption and CRC risk in 379 cancer patients and 769 healthy subjects. In that study, only the NAT1 polymorphism affected cancer risk. However, the NAT1 and NAT2 fast acetylation phenotype increased the risk of CRC among patients who smoked more cigarettes, suggesting that N-acetylation status affects the relationship between smoking and CRC risk.

In the present study, most subjects in the two groups had never smoked, but the rate of ex-smokers was higher in the case group than in the control group. Once diagnosed with cancer, individuals tend to break old habits that may affect the prognosis and treatment of the disease even if it is not possible to reverse the previous damage. The slow acetylation phenotype was more frequent among smokers of the case group, suggesting an increased risk of cancer (OR: 1.97, 95% CI: 1.02-3.79) in subjects with this phenotype. A higher frequency of the slow acetylation phenotype among patients with lung and bladder cancer has been demonstrated in other studies. Carcinogens present in tobacco are metabolized by NAT enzymes and activation of these enzymes is reduced in slow acetylators, thus increasing the risk of cancer[22].

An association between red meat consumption and a higher risk of CRC has been reported in case-control studies, prospective epidemiological studies and in a recent meta-analysis[23]. The last study suggested that this increased risk is due to the production of polycyclic aromatic hydrocarbons and heterocyclic amines when meat is cooked at high temperatures[24].

Tamer *et al*[25], studying 125 patients with CRC and 82 healthy subjects, observed an association between NAT2 polymorphisms and cancer development. In that study, high protein intake was found to be correlated with an increased risk of colon cancer (OR: 1.73, 95% CI: 1.10-3.07). Patients with the NAT2 * 14A (M4 allele) fast acetylation phenotype and high meat intake presented an increased risk of CRC (OR: 3.03, 95% CI: 1.56-5.86). In the present study, the risk of cancer was higher among patients consuming meat more than 3 times per week (OR: 1.65, 95%

CI: 1.05-2.61). The risk of CRC was increased among patients presenting the fast acetylator genotypes (W/W or W/Mx) and a high frequency of meat intake (OR: 2.05, 95% CI: 1.01-4.16).

Heterocyclic amines are formed when meat is cooked by the condensation of creatinine with amino acids. The NAT2 fast acetylation phenotypes are more readily able to convert N-hydroxy heterocyclic amines into carcinogens, a fact predisposing to cancer. Thus, heterocyclic amines require metabolic activation to induce DNA mutations and to initiate carcinogenesis. After N-oxidation, N-hydroxy aromatic and heterocyclic amines are activated (*via* O-acetylation) by NAT to acetoxy intermediates, which react spontaneously with DNA to form adducts[26,27]. The increased cancer risk observed in patients with the NAT2 fast acetylation phenotype and high meat consumption suggests that heterocyclic amines mediated by metabolic activation of NAT2 fast acetylation might be important carcinogens and increase the risk of cancer.

In conclusion, the proportion of subjects with the slow acetylation phenotype was high in this study. No association was observed between the risk of CRC and NAT2 polymorphisms. However, the slow acetylation phenotype increased the risk of CRC in smokers and the fast acetylation phenotype increased this risk among subjects with high red meat intake.

COMMENTS

Background

Colorectal cancer (CRC) is considered the fourth leading cause of cancer worldwide and is one of the most common malignancies in the West. The probability of developing cancer depends on the natural response of each organism to different exposures from various aggressors. Humans have different susceptibilities to different carcinogens and lifestyle may be a risk factor for cancer. The interaction between diet, alcoholism, cigarette smoking, obesity and physical inactivity can lead to its development.

Research frontiers

The HAAs (present in red meat, tobacco, *etc.*) are bioactivated through N-oxidation by the enzymes CYP1A2 in the liver or by CYP1A1/CYP1B1 in extra hepatic tissues. The products of this oxidation are the N-hydroxy-N, which in turn suffer the bioactivation in the liver by O-acetylation of N-acetyltransferase (NAT) enzymes (mainly NAT2) and sulfotransferase. NAT2 fast acetylation individuals may have larger amounts of metabolic activators of HAA than slow acetylation NAT2 subjects that can be transmitted to the colorectal tissues through the bloodstream, causing DNA damage and mutations.

Innovations and breakthroughs

Previous studies linking meat consumption and colorectal cancer have concluded that diets rich in meat consumption increase the risk of this disease. In the case of red meat, the degree of cooking can be a source of exposure to chemical carcinogens such as heterocyclic amines, nitrosamines and other products. This study aimed to identify the distribution of NAT2 gene polymorphism in a Brazilian population from São Paulo correlating these polymorphisms and the phenotypes of NAT2 acetylation with the consumption of red meat, alcohol and cigarette smoking and the colorectal cancer risk.

Applications

The proportion of people with the phenotype of fast and slow acetylation varies considerably depending on the ethnicity and geographic origin. This variability lead to the investigation into the frequency of NAT2 genotypes and the association of this polymorphism with colorectal cancer in a Brazilian population of São Paulo. The relationship between the mechanism of acetylation by NAT2 and susceptibility to colorectal cancer may possibly screen patients who have a higher risk of developing this disease.

Terminology

The NAT2 is an important phase Ⅱ enzyme that catalyzes the acetylation of heterocyclic aromatic amines and hydrazines, which include carcinogenic compounds and drugs. Based on the activity of NAT2 acetylation, subjects are divided into three different phenotypes: fast, intermediate and slow. Single nucleotide polymorphisms in NAT2 determine this phenotype.

Peer review

The authors examined the phenotypes and polymorphisms of NAT2 acetylation on a case-control study, involving the eating habits and lifestyle as risk modifiers of colorectal cancer development. The results indicated that cigarette smokers with slow acetylation phenotype had an increased risk of developing colorectal cancer and individuals with fast acetylation phenotype intake of red meat increased the risk of developing the disease. These results are interesting because the study of NAT2 acetylation may identify individuals with a higher risk of developing CRC in relation to environmental factors and diet.

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