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Genetic polymorphisms of *N*-acetyltransferase 2 and colorectal cancer risk

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Abstract

AIM: To identify the distribution of *N*-acetyltransferase 2 (NAT2) polymorphism in Hebei Han Chinese and the effects of the polymorphism on the development of colorectal cancer.

METHODS: We performed a hospital-based case-control study of 237 healthy individuals and 83 colorectal cancer patients of Hebei Han Chinese. DNA was extracted from peripheral blood and cancer tissues. The genotypes of the polymorphisms were assessed by PCR-restriction fragment length polymorphism(RFLP).

RESULTS: There were four NAT2 alleles of WT, M1, M2, and M3 both in the healthy subjects and in the patients, and 10 genotypes of WT/WT, WT/M1, WT/M2, WT/M3, M1/M1, M1/M2, M1/M3, M2/M2, M2/M3, M3/M3. M2 allele was present in 15.61% of healthy subjects and 29.52% of patients ($\chi^2 = 15.31$, $P < 0.0001$), and M3 allele was present in 30.59% of healthy subjects and 16.87% of patients ($\chi^2 = 25.33$, $P < 0.0001$). There were more WT/M2 ($\chi^2 = 34.42$, $P < 0.0001$, odd ratio = 4.99, 95%CI = 2.27-9.38) and less WT/M3 ($\chi^2 = 3.80$, $P = 0.03$) in the patients than in the healthy subjects. In 70.3% of the patients, there was a difference in NAT2 genotype between their tumors and blood cells. Patients had more WT/M2 ($\chi^2 = 5.11$, $P = 0.02$) and less M2/M3 ($\chi^2 = 4.27$, $P = 0.039$) in their blood cells than in the tumors. Furthermore, 53.8% (7/13) of M2/M3 in tumors were from WT/M2 of blood cells.

CONCLUSION: There is a possible relationship between the NAT2 polymorphisms and colorectal cancer in Hebei Han Chinese. The genotype WT/M2 may be a risk factor for colorectal cancer.

Key words: NAT2 gene; Colorectal cancer; RFLP

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INTRODUCTION

Colorectal cancer is one of the common tumors in Hebei Province of China. *N*-acetyltransferase 2 (NAT2) is polymorphic and catalyzes both *N*-acetylation (usually deactivation) and *O*-acetylation (usually activation) of a variety of heterocyclic amine drugs and carcinogens^[1]. Heterocyclic amines which are found mainly in well-cooked meat, require metabolic activation to function as mutagens and animal carcinogens. Enzymes such as cytochrome P4501A2 (CYP1A2) and NAT2 perform this task. Genetic studies in humans have shown that individuals may be classified as rapid or slow acetylators according to the rates at which drugs are acetylated by NAT2^[2]. NAT2 polymorphism is a representative genetic trait of individual's susceptibility to several cancers^[3]. Epidemiological studies suggest that the NAT2 acetylation polymorphisms modify the risk of developing carcinoma of urinary bladder, colorectal and breast cancer, head and neck cancer, pulmonary and prostatic cancer. Associations between slow NAT2 acetylator genotypes and urinary bladder cancer as well as between rapid NAT2 acetylator genotypes and colorectal cancer are the most consistently reported^[3]. However, whether there is a true association between colorectal cancer and NAT2 polymorphism is controversial. Human epidemiological studies suggest that rapid acetylator phenotypes may be associated with higher incidences of colorectal cancer in American populations^[4]. Researchers in UK^[5] found that no significant differences in NAT2 allelic frequencies (WT, M1, M2, M3 alleles) or genotypes are observed between colorectal cancer patients and the healthy population. Ethnic differences exist in NAT2 genotype frequencies that may be a factor in cancer incidence, emphasizing the need to investigate the distribution frequency of NAT2 genotypes and the association of their polymorphism with colorectal cancer in Hebei Han Chinese population.

We identified the distribution of NAT2 polymorphisms in Chinese healthy individuals and colorectal cancer patients from Hebei Province. We also performed a case-control study to investigate whether the NAT2 genetic polymorphism was a risk factor for colorectal cancer in these Chinese population.

MATERIALS AND METHODS

Subjects

We conducted a case-control study of colorectal cancer patients between April 2001 and April 2003. Both patients and controls were Hebei Han Chinese. Eighty-three patients (46 males, 37 females) with histopathologically confirmed diagnosis of colorectal cancer, were recruited from the Department of Surgery at Hebei No. 4 Hospital. Their mean age was 47.5 years (ranging 25-77 years). The control group consisted of 237 (124 males, 113 females) healthy individuals selected from the blood donors in Hebei Province Blood Center. Their average age was 42.6 years (ranging 20-80 years).

DNA extraction

The peripheral blood was treated with EDTA. Tumor samples were obtained from colorectal cancer patients and immediately frozen in liquid nitrogen and stored at -80 °C until used. Genomic DNA was isolated from peripheral leukocytes by PEL-FREEZE reagent protocol or from the tumor samples by the standard procedure of proteinase K/RNase digestion and phenol/chloroform extraction.

Typing NAT2 polymorphism

PCR-RFLP was performed to investigate the NAT2 allele. PCR was performed in 40 µL reaction mixture containing 50-200 ng DNA, 20 mmol/L of each primer, 1.25 mmol/L of dNTPs, 25 mmol/L of MgCl₂, 1 PCR buffer, 5U of thermostable Taq DNA polymerase using a programmable thermocycler. The primer sequences were 5'-GGA ACA AAT TGG ACT TGG-3' and 5'-TCT AGC ATG AAT CAC TCT GC-3'. PCR conditions were 5 min at 95 °C; 35 cycles of 1 min at 95 °C, 1 min at 57 °C and 1.5 min at 72 °C; followed by a final extension for 5 min at 72 °C. After amplification, the 1 093 bp PCR-products were digested with *Kpn*I (M1 allele), *Bam*HI (M3 allele), *Msp*I/*A*luI (M4 allele) for 16-18 h at 37 °C and *Taq*I (M2 allele) for 16-18 h at 65 °C separately in a 25 µL final volume. Digested fragments were then separated by electrophoresis on 20 g/L agarose gel (M1, M3) or 40 g/L agarose gel (M2, M4) and visualized by ethidium bromide staining. All the experiments included positive and negative controls for each studied polymorphism.

Statistical analysis

Statistical analysis was performed with SAS 6.0 for Windows. Hardy-Weinberg equilibrium test was carried out using statistical software for linkage analysis. χ^2 and Fisher's exact test were used for comparisons of frequencies. Associations were expressed as odd ratios (OR) with 95% confidence interval (95%CI). $P < 0.05$ was considered statistically significant.

RESULTS

Polymorphism of NAT2 gene in Hebei Han Chinese

The results of genotype analysis are shown in Figure 1. In 237 healthy and 83 colorectal cancer patients of Hebei Han Chinese, there were four kinds of NAT2 alleles including wild-type gene WT allele and polymorphisms of M1, M2, and M3 alleles (Table 1). No M4 polymorphism was found. Ten genotypes including WT/WT, WT/M1, WT/M2, WT/M3, M1/M1, M1/M2, M1/M3, M2/M2, M2/M3, M3/M3 and their frequencies in both groups are shown in Table 2. M3 (59.67%) was the major polymorphism in healthy group. The distribution of NAT2 genotypes in healthy group was consistent with Hardy-Weinberg equilibrium ($\chi^2 = 7.2533$, $g = 8$, $P > 0.5$). In healthy group, 71.3% and 28.7% were classified as rapid-acetylator genotypes (WT/WT, WT/Mx, $x = 1, 2, 3$) and slow-acetylator genotypes (Mx/Mx), respectively. The frequency of WT/M3 (29.1%) in the rapid-acetylator genotypes was the highest.

The frequency of allele M2 (29.5%) was the highest in the 83 patients, 18 (21.7%) were slow-acetylator genotypes (Mx/Mx), and 65 (78.3%) were rapid-acetylator genotypes (WT/WT, WT/Mx), 35 (53.8%) of them being WT/M2 (Table 2).

Association of NAT2 polymorphism with susceptibility to colorectal cancer in Hebei Han Chinese

M3 allele was present in 30.59% (154/474) of the healthy subjects and in 16.87% (28/166) of the patients ($\chi^2 = 25.33$, $P < 0.0001$). The M2 allele frequency was significantly different between healthy (15.61%) and patient groups (29.52%) ($\chi^2 = 15.31$, $P < 0.0001$). There was no difference in rapid-acetylator or slow-acetylator frequency between healthy and patient groups. However, there was a significant

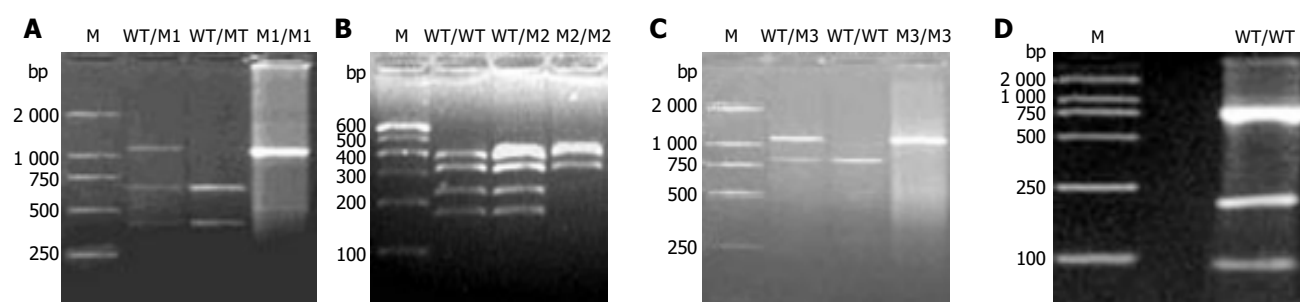


Figure 1 NAT2 genotype analysis by PCR-RFLP. M: DNA Marker (100, 250, 500, 750, 1 000, 2 000 bp); **A**: digest with *Kpn*I (M1 allele) shows wildtype genotype (lane 2: 660 and 433 bp, WT/WT), variant genotype (lane 3: 1 093 bp, M1/M1) and heterozygote genotype (lane 1: 1 093, 660 and 433 bp, WT/M1); **B**: digest with *Taq*I (M2 allele) shows wildtype genotype (lane 1: 380, 317, 226 and 170 bp, WT/WT), variant genotype (lane 3: 396, 380 and

317 bp, M2/M2) and heterozygote genotype (lane 2: 396, 380, 317, 226 and 170 bp, WT/M2); **C**: digest with *Bam*HI (M3 allele) shows wildtype genotype (lane 2: 811 and 282 bp, WT/WT), variant genotype (lane 3: 1 093 bp, M3/M3) and heterozygote genotype (lane 1: 1 093, 811 and 282 bp, WT/M3); **D**: digest with *Msp*I/*A*luI (M4 allele) shows wildtype genotype (lane 1: 759, 189, 91 and 53 bp, WT/WT).

Table 1

Alleles	Healthy controls		Patients	
	Number	Frequency	Number	Frequency
WT	231	0.4874	80	0.4819
M1	24	0.0506	9	0.0542
M2	74	0.1561 ^{1b}	49	0.2952
M3	145	0.3059 ^{2d}	28	0.1687
M4	0	0	0	0
SUM	474	1.0000	166	1.0000

¹ $\chi^2=15.31$, ^b $P<0.0001$ and ² $\chi^2=25.33$, ^d $P<0.0001$ vs patients.

Table 2

	Rapid-acetylase genotype					Slow-acetylase genotype						
	WT/WT	WT/M1	WT/M2	WT/M3	Sum	M1/M1	M1/M2	M1/M3	M2/M2	M2/M3	M3/M3	Sum
Controls <i>n</i>	62	9	29	69	169	1	4	9	5	31	18	68
%	26.2	3.8	12.2	29.1	71.3	0.4	1.7	3.8	2.1	13.1	7.6	28.7
Patients <i>n</i>	15	1	35	14	65	1	2	4	3	6	2	18
%	18.1	1.2	42.2	16.9	78.3	1.2	2.4	4.8	3.6	7.2	2.4	21.7
χ^2	2.20	0.64	34.42	3.80	1.54	0.00	0.00	0.007	0.12	2.06	2.01	1.54
<i>P</i>	0.14	0.42	<0.0001	0.03	0.22	1.00	1.00	0.94	0.73	0.15	0.16	0.22

difference between WT/M2 ($\chi^2=34.42$, $P<0.0001$) and WT/M3 ($\chi^2=3.80$, $P<0.05$) compared to the healthy controls. The risk associated with combinations of NAT2 genotypes is shown in Table 3. The genotype WT/M2 might be a risk factor for colorectal cancer (OR = 4.99, 95%CI = 2.27-9.38).

Comparable analysis of WT/M2 and M2/M3 genotypes in blood cells and cancer tissues of colorectal cancer patients

In 70.4% of patients, there was a difference in NAT2 genotype between tumor tissues and blood cells. The frequency of NAT2 genotypes, especially WT/M2 and M2/M3, was significantly different between tumor tissues and blood cells ($\chi^2=5.11$, $P<0.05$; $\chi^2=4.27$, $P<0.05$, Table 4). The frequency of genotype M2/M3 was 9.3% in

peripheral blood cells and 24.1% in cancer tissue. Furthermore, 53.8% (7/13) of the tumors had M2/M3 while the corresponding blood cells had WT/M2.

DISCUSSION

Human NAT2 is a genetic polymorphism. Different genotypes (WT/WT, WT/Mx, Mx/Mx, x = 1, 2, 3, 4) are composed of different NAT2 alleles (wild type allele WT, mutation allele Mx). According to the intensity of acetyl action, a different genotype shows a different phenotype (rapid-acetylase genotype and slow-acetylase genotype). Genotypes composed of any two mutation alleles (Mx/Mx) show slow-acetylase genotypes, while those consisted of WT/WT or WT/Mx show rapid-acetylase genotypes. Bell

Table 3

Genotypes	Control		Patients		OR (95%CI)
	<i>n</i>	%	<i>n</i>	%	
WT/WT	62	26.2	15	18.1	1.00
WT/M2	29	12.2	35	42.2	4.99 (2.27-9.38)
WT/M1	9	3.8	1	1.2	0.46 (0.05-3.92)
WT/M3	69	29.1	14	16.9	0.84 (0.38-1.88)
WT/WT and WT/Mx	169	71.3	65	78.3	1.59 (0.85-2.99)
Mx/Mx	68	28.7	18	21.7	1.09 (0.51-2.35)

x = 1, 2, 3, 4, OR: odds ratio; CI: confidence interval.

Table 4

	WT/M2		M2/M3		Others	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Blood cells	23	42.6	5	9.3	26	48.2
Cancer tissues	12	22.2 ¹	13	24.1 ²	29	53.7

¹ $\chi^2=5.11$, $P=0.020$ and ² $\chi^2=4.27$, $P=0.039$ vs blood cells.

et al.^[6], reported that there are significant differences in the frequency of WT, M1, and M4 alleles between Caucasian and African-Americans, while the frequency of M2 and M3 alleles is similar. The M4 allele is not detectable in Caucasian-Americans, while 18% African-Americans carry at least one M4 allele. The frequencies of NAT2 WT, M1, M2, M3, and M4 alleles in Singapore Chinese are 0.51, 0.07, 0.32, 0.10, and 0.00, respectively^[7]. The proportions of rapid and slow acetylators do also vary remarkably between ethnic groups as well as between populations of different geographical origins^[8]. For example, the percentage of slow acetylators is 5% in Canadian Eskimos, over 80% in Egyptians and 90% in Moroccans^[9,10]. Singapore Chinese have 72% of rapid-acetylator genotypes. In our study, four NAT2 alleles (WT, M1, M2, M3) and ten NAT2 genotypes (WT/WT, WT/M1, WT/M2, WT/M3, M1/M1, M1/M2, M1/M3, M2/M2, M2/M3, M3/M3) were found in Hebei Han Chinese. M4 was not present in Hebei Han Chinese, while 29.1% of healthy controls carried at least one M3 allele. Hebei Han Chinese had 71.3% of rapid-acetylator genotypes. The frequencies of the NAT2 M4 allele and rapid-acetylator genotypes are similar in both Hebei Han Chinese and Singapore Chinese, but there are significant differences in the frequencies of WT, M1, M2, and M3 between the two populations.

NAT2 is an important enzyme for the detoxification and/or bioactivation of several carcinogenic arylamines, its activity directly affects metabolic response of carcinogen *in vivo* and intensity of toxic reaction^[11]. It was reported that the polymorphism of NAT2 gene is associated with the occurrence of colorectal cancer^[12]. Genetic epidemiological studies suggest that rapid acetylator phenotype may be associated with the higher incidence of colorectal cancer in the UK populations^[12]. Lee *et al.*^[7], also showed that the rapid acetylator genotype is associated with cancer occurring in Singapore Chinese. However, our results suggest that rapid-acetylator genotype or slow-acetylator genotype does not appear to be associated with colorectal cancer development, but there is an association between the WT/M2 genotype and colorectal cancer. The M2 allele was more frequent in the patients than in the healthy subjects ($\chi^2 = 15.31$, $P < 0.0001$). Odds ratio (OR) for genotype WT/M2 was 4.99 (95%CI = 2.27-9.38). The data suggest that genotype WT/M2 may be a risk factor for colorectal cancer in Hebei Han Chinese.

Schnakenberg *et al.*^[13], detected the loss of heterozygosity in slow and rapid acetylator genotypes in bladder cancer patients. They observed allelic loss at the NAT2 locus in 11 of 60 tumor samples (18.3%), but not in controls. The

present study also demonstrated changes of NAT2 genotypes in 70.4% of the tumor specimens. The rising M2/M3 in tumors seems to be transformed from genotype WT/M2 of blood cells, and also suggests that WT/M2 may be a risk factor for colorectal cancer. Our findings may contribute to the early diagnosis of colorectal cancer.

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