GENETIC POLYMORPHISM OF CONJUGATING ENZYMES AND CANCER RISK: GSTM1, GSTT1, NAT1 and NAT2

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INTRODUCTION

Several enzymes of detoxification are subject to genetic polymorphisms. Unlike their Phase I counterparts, the extent of the problem with the conjugating enzymes is relatively poorly understood. It has been known however that glutathione S-transferases (GST) μ and θ are genetically polymorphic. Both GSTM1 and GSTT1 genes can be absent in certain individuals. Homozygosity for the GSTM1 null allele exists in approximately 40-65% of a normal population. Since these enzymes catalyse the conjugation of a wide variety of electrophilic compounds, including a number of carcinogens, it has been postulated that the null genotype may predispose to the development of cancer. Indeed the null genotype has been found more frequently in Caucasian and Japanese patients with lung, colorectal, stomach, bladder and laryngeal cancers. Despite this, the hypothesis remains in doubt as a number of studies have obtained conflicting results.

The situation with GSTT1 is even less clear. Like GSTM1, about 50% of a normal population are homozygous for the null genotype. The few studies looking at GSTT1 genotypes in colorectal cancers have also obtained conflicting results.

The N-acetyltransferase (NAT) enzymes are involved in either detoxification or bioactivation of carcinogenic arylamines. The gene encoding the human NAT2 enzyme is also subject to a genetic polymorphism with numerous mutant alleles already described. The homozygous mutant is phenotypically a slow acetylator. The frequency of slow acetylators vary considerably across populations, and is approximately 10-30% in Chinese and Japanese populations. The acetylator status has been variously linked to an increased risk of bladder, colon, larynx and breast cancers. The NAT1 gene has only been recently

shown to be polymorphic and has not received as much attention as NAT2.

This study describes the frequency of mutant alleles of GSTM1, GSTT1, NAT1 and NAT2 among Chinese subjects in Singapore and provides a control population against which allelic frequencies in a large cohort of colorectal patients are compared.

METHODS

GSTM1 and GSTT1

Control subjects were obtained from the Clinical Chemistry department of a local hospital. After excluding patients with a history of neoplasms, 183 blood samples were genotyped for GSTM1 and GSTT1 alleles. The leucocyte DNA was extracted from whole blood samples by the rapid method and genotyping carried out by standard polymerase chain reaction methods. A null genotype was recorded if no amplification products were obtained.

NAT1 and NAT2

The control population for this study was drawn from 187 healthy undergraduates. NAT1 and NAT 2 genotyping were carried out by polymerase chain reaction. Primers specific for wildtype DNA, NAT2*5A, NAT2*6A and NAT2*7A alleles were used. For NAT genotyping, primers specific for NAT1*3, NAT1*4, NAT1*10 and NAT1*11 were used.

Colorectal carcinoma

Colorectal patients (n = 300) were recruited from a surgical department. The histology and site of the tumour, together with the age of the patient were recorded. All samples were characterized for GST alleles but only 216 and 68 were characterized for NAT2 and NAT1 alleles respectively.

Nasopharyngeal carcinoma

DNA samples from 51 nasopharyngeal carcinomas were studied. Of these 25 were from an immortalized white cell bank and 26 were from patients seen at a throat clinic. NAT1 and NAT2 genotypes were characterized.

RESULTS

GSTM1 and GSTT1 in colorectal cancer

Age and gender were similar between the cancer and control groups. The GSTM1 null genotype was seen in 48.6% of the control population (n=183) and in 42.7% (n=300). This difference was only marginally significant and gave a odds ratio of 0.79 (95% CI = 0.53-1.16) in patients. The frequency of the null genotype however was strongly related to the site of the tumour - 54.1% (20/37), 48.3% (14/29) and 40.2% (94/234) for right, left and recto/sigmoid respectively.

The GSTM1 null genotype was also strongly related to the histological differentiation of the tumour. The frequency of the null genotype was 66.7% and 63.6% for poorly differentiated and mucinous cancers respectively, much higher than controls and moderately and well differentiated (41.0 % and 42.9%) tumours respectively.

The overall frequency of the GSTT1 null genotype in patients with colorectal cancer was also similar to controls. The frequency of the null genotype was also affected by the sites of the tumour, although the trend was in the opposite direction (48.6% in right sided tumours as compared to 56.4% in recto/sigmoid tumours). Correspondingly, right sided tumours were characterized by a higher frequency of GSTM1 null/GSTT1 positives as compared to what might be expected in a normal population (35.1% vs 21.9%). Tumour histology had no effect on the frequency of null genotype.

NAT1 and NAT2 in colorectal cancer

The frequencies of NAT2*4, NAT2*5A, NAT2*6A and NAT2*7A alleles in the control group were 0.51, 0.07, 0.32 and 0.10 respectively. The most significant difference between colorectal cancer patients and the controls was the almost doubled frequency of the NAT2*7A allele (0.19). This was almost entirely at the expense of the NAT2*6A allele (0.26).

Correspondingly, when the genotypes were examined, carriers of the NAT2*7A allele was associated with elevated risks for colorectal cancer. Of these

the highest risks were seen with subjects with two mutant alleles of which one was NAT2*7 (OR for NAT2*5A/ NAT2*7A = 4.4; OR for NAT2*6A/ NAT2*7A = 3.9). Surprisingly, homozygosity for NAT2*7A did not confer a higher risk.

No significant differences were seen in the frequencies of NAT1 alleles between controls and colorectal cancer patients.

NAT1 and NAT2 in nasopharyngeal cancer

As for colorectal cancers, the frequency of NAT2*7A (0.24) was elevated substantially giving odds ratios of 7.6 and 6.9 respectively the mutant heterozygotes NAT2*5A/ NAT2*7A and NAT2*6A/ NAT2*7A. There was also no increased risk associated with homozygosity of NAT2*7A. NAT1 mutants also did not confer an increased risk for nasopharyngeal carcinomas.

CONCLUSIONS

The hypothesis that mutations in genes coding for the activities of conjugating enzymes may be associated with elevated risks for cancer is an attractive one. However, results from several studies have not shown any major effects of these polymorphisms of cancer risk. This suggests that the hypothesis is either wrong or has not been formulated accurately. Our results show that the effect of the genetic polymorphisms on colorectal and nasopharyngeal cancer risks in Chinese is a complex one. While the overall frequency of the GSTM1 genotype was not substantially different from those on the controls, the frequency was clearly related to the site of the tumour and the histological type. Loss of GSTM1 appeared to be related primarily to right sided tumours and to those that are poorly differentiated. This finding would suggest that the pathogenesis of colorectal cancer differs according to the site of the tumour, and thus the dependance on GSTM1 activity might be expected to be different. Accordingly this would explain why different studies could have obtained variable results if site and tumour differentiation were not comparable. The inverse relationship of the null GSTT1 genotype to the site of tumour underlines this fact.

The NAT2 results possibly illustrate a different principle. While the frequency of slow acetylators did not differ substantially between cancer and controls, for both colorectal and nasopharyngeal cancers, there was a clear increase in the frequency of one particular mutant allele, NAT2*7A. It would appear that the

observed risk of cancer is related less to the NAT2 activity than to an as yet unknown factor in equilibrium with the presence of NAT2*7A. It is not clear at this stage why two such alleles do not confer any risk of either cancer but the results suggest that the mechanism involved is a complex one.

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