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SHORT COMMUNICATION

Implication of arylamine *N*-acetyltransferase (*NAT2*) polymorphism on levels of tumour markers CEA, AFP, CA 125, CA 19.9, and CA 15.3

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Polymorphic arylamine *N*-acetyltransferase 2 (NAT2) is responsible for metabolizing various drugs, precarcinogens and other xenobiotics. The acetylator status of an individual may influence susceptibility to certain diseases including malignancies. Numerous serum tumour markers were used for the diagnosis and management of certain types of cancers and their serum levels may be affected by several factors. A group of 60 unrelated Turkish subjects (non-smokers) was studied to determine whether an individual's acetylator status has an impact on certain tumour marker levels. The *NAT2* genotype was characterized by polymerase chain reaction-restriction fragment length polymorphism at nucleotid positions 191, 282, 341, 481, 590, 803, and 857. Serum concentrations of carcinoembryonic antigene (CEA), α-fetoprotein (AFP), CA19-9, CA 15-3, and CA 125 were determined in each subject. Forty-two subjects were genotyped as slow (70 %) and 18 (30 %) as rapid acetylators. We found that CEA serum levels among rapid acetylators were approximately 30 % higher than in the slow acetylator group ($p < 0.02$). AFP, CA19-9, CA 15-3, and CA 125 serum levels were not altered significantly with an individual's acetylation status. The results suggest that *NAT2* genotype may have an impact on concentrations of some tumour markers in subjects without malignancies and other known factors such as smoking that can affect their serum levels.

Keywords: *N*-acetyltransferase, polymorphism, tumour markers, molecular epidemiology.

Introduction

The human arylamine *N*-acetyltransferase (NAT2) polymorphism results in different rates of biotransformation of various drugs, such as isoniazid, procainamide and sulphonamides as well as certain precarcinogens and other xenobiotics (Evans 1992, Hein *et al.* 1993). An individual's acetylator status can be determined either phenotypically with test drugs such as caffeine (Grant *et al.* 1984), or genotypically according to at least seven point-mutations recently described for *NAT2* genes (Vatsis *et al.* 1995). There are considerable interindividual and interethnic differences in *NAT2* activity. For example, the frequency of slow-acetylator genotypes is around 8 % in the Japanese population

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(Mashimo *et al.* 1992) but about 75 % in certain African populations (reviewed by Evans (1992)). The frequency of slow-acetylator genotype was found to be 57.4 % in a Turkish population (Aynacioglu *et al.* 1997), similar to Middle European Caucasians (Cascorbi *et al.* 1995, Mrozikiewicz *et al.* 1996).

The acetylator status of an individual affects significantly the therapeutic efficacy and occurrence of side effects of drugs that are acetylated by this enzyme. Moreover, NAT2 activity may influence susceptibility to certain diseases such as lupus erythematosus and Gilbert's syndrome (see Evans 1992) as well as malignancies such as colorectal, bladder, hepatocellular, breast or lung cancers (Gonzalez and Idle 1994, Agundez *et al.* 1996, Ambrosone *et al.* 1996, Brockmöller *et al.* 1996, Cascorbi *et al.* 1996, Gil and Lechner 1998).

Tumour markers as indicators of tumour-progression responses of cancer treatment have increasingly been used in recent years (Wu 1996). Among them, carcinoembryonic antigene (CEA), α-fetoprotein (AFP), CA 15-3, CA 125, and CA 19-9 are widely employed for the diagnosis and management of hepatocellular, colorectal, breast, ovarian, and pancreas carcinoma, respectively. However, most tumour markers are not specific enough for a given type of cancer and not sufficiently sensitive to detect small tumours (Kinzler and Vogelstein 1995).

As it has been shown that conditions such as smoking and certain benign diseases may influence the levels of some tumour markers (Begent 1984) and *NAT2* is responsible for metabolizing certain xenobiotics to ultimate carcinogens that also may affect a tumour marker level, the aim of the present study was to investigate whether an individual's acetylation status may influence and alter certain serum tumour marker concentrations.

Material and methods

Subjects

A group of 60 (39 female, 21 male) unrelated subjects from south-east Anatolia, Gaziantep, Turkey, was studied after their informed and written consent. All subjects were outpatients, who were sent for routine biochemical investigations to the laboratory of Biochemistry and Clinical Biochemistry from the Departments of Gastro-enterology, Urology, Endocrinology, Gynaecology and Dermatology of the Gaziantep University, Faculty of Medicine. This study group was chosen randomly from 303 volunteers who had been genotyped recently for *NAT2* (Aynacioglu *et al.* 1997). All subjects were non-smokers, without known malignant diseases and also without benign diseases such as cirrhosis, pulmonary emphysema, and ulcerative colitis which may have an impact on serum tumour marker levels.

Genotyping and identification of NAT2 mutations

Venous blood samples (5–10) ml, drawn in EDTA as anticoagulant were obtained from each subject and stored at –20 ºC until transported to the Institute of Clinical Pharmacology, University Clinic Charité, Humboldt University of Berlin, Germany. DNA was extracted from leucocytes manually by standard three-step phenol/chloroform extraction and stored at +4 °C until further analysis. The 1211 base pair (bp) fragments containing the coding region of the *NAT2* gene were amplified by a polymerase chain reaction (PCR). Seven mutation sites were identified by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis as described by Cascorbi *et al.* (1995, 1996). Alleles were classified according to Vatsis *et al.* (1995). Briefly, after amplifying 1211 bp fragments using primers 5'-GTCACACGAGGAAATCAAATGC (sense) and 5'-GTTTTCTAGCATGAATCACTCTGC (antisense), digestion with *Msp*I/*Alu*I, *Kpn*I and *Bam*HI was performed to detect mutations at nt positions 191, 481, and 857, respectively. For evaluation of mutations at positions 282 and 341, incubation with *Fok*I and *Dde*I was performed, respectively, after amplifying a 442-bp fragment from the 1211-bp PCR fragment with primers 5'-GTCACACGAGGAAATCAAATGC (sense) and 5'-ACCCAGCATCGACAAT-GTAATTCCTGCCCTCA (antisense). A 421-bp fragment was amplified also from the 1211-bp fragment using primers 5'-CCTGGACCAAATCAGGAGAG (sense) and 5'-ACACAAGGGTTTATTTTGTTCC (antisense). The amplification products were incubated with *Taq*I and *Dde*I for detection of mutations at nt 590 and 803, respectively.

Table 1. Demographic data and NAT2 genotypes of the 60 subjects investigated.

	Slow acetylators	Rapid acetylators
Mean age	41 years	39 years
Sex	28 females,14 males	11 females, 7 males
NAT2 Genotype		
**5B/*6A*	14 (23.3 %)	–
**5B/*5B*	9 (15.0 %)	–
**6A/*6A*	8 (13.3 %)	–
**5C/*6A*	6 (10.0 %)	–
**5B/*7B*	5 (8.3 %)	–
**4/*5B*	–	12 (20.0%)
**4/*6A*	–	6 (10.0%)
Total	42 (70.0%)	18 (30.0%)

Table 2. Serum tumour marker levels of the slow and the rapid acetylator genotype groups. Values are given as mean ±SEM.

Tumour markers	Slow acetylators (A) $n = 42$	Rapid acetylators (B) $n = 18$
CEA (ng ml^{-1})	2.28 ±0.45	3.14 ±0.49[a]
AFP (IU ml^{-1})	4.80 ±2.56	1.20 ±0.21
CA 19-9 (U ml^{-1})	12.86 ±3.04	15.51 ±3.45
CA 15-3 (U ml^{-1})	37.46 ±3.30	31.60 ±5.42
CA 125 (U ml^{-1})	12.59 ±1.33	16.41 ±4.91

[a] A versus B $p < 0.02$ (z: 2.284).

Serum tumour marker analysis and routine biochemical assays

Approximately 4 ml of the blood samples drawn into plain tubes were separated from the cells with centrifugation at 1500 *g* for 10 min. Routine biochemical assays were performed on the same day and a portion of the sera was stored at –20 °C until tumour marker analysis. Serum CEA, AFP, and CA 19-9 levels were determined with solid-phase, two-site chemiluminescent enzyme immunometric assay using Immulite CEA, DPC®; AFP, DPC®; and GIM-MA, DPC®, respectively. Serum CA 15-3 levels were determined with solid-phase, sequential two-site chemiluminescent enzyme immunometric assay using Immulite BR-MA, DPC® and CA 125 levels were analysed with solid-phase, competitive chemiluminescent enzyme immunometric assay using Immulite OM-MA, DPC®. Serum total protein (TP), albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP), glucose and total bilirubine (TB) assays were performed with Beckman Synchron CX5 automated analyser and reactives.

Statistical analysis

Statistical comparison of tumour marker levels according to acetylation genotype was performed using the Mann–Whitney *U*-test. Two-tailed *p*-values were taken. SPSS and MedCalc® statistics program was used for statistical evaluation.

Results

Characteristics of the slow and rapid acetylator groups

From 60 subjects investigated, 42 (70 %) were found to be slow and 18 (30 %) to be rapid acetylator genotypes of *NAT2* (table 1).

Tumour marker levels of the slow and rapid acetylators

Table 2 presents the serum CEA, AFP, CA19-9, CA 15-3, and CA 125 levels of distinct *NAT2* genotypes and the comparison of the slow and rapid acetylator

groups. Serum CEA levels of the rapid acetylator group were found to be significantly higher than the slow acetylator group ($p < 0.02$). The other tumour marker levels were not altered statistically significantly with an individual's acetylation status. Tumour marker levels of detected *NAT2* genotypes are given in table 3, but no specific associations were observed.

Discussion

This study shows that an individual's *NAT2* genotype may have an impact on certain tumour marker serum concentrations. Although AFP, CA19-9, CA 15-3, and CA 125 serum levels were not altered significantly with an individual's acetylation status, a 30 % increase in CEA serum levels was found amongst rapid acetylators. Certain chemical substances such as smoke, alcohol, and asbestos may influence CEA levels, like other tumour markers (Herbeth and Bagrel 1980, Begent 1984, Lutz *et al.* 1997). Smokers show a two fold increase of CEA levels compared with non-smokers, which is attributable to chemical components of tobacco (Begent 1984). CEA is a glycoprotein synthesized mainly in intact colon mucosa and also in other tissues (Cooper *et al.* 1989). It could be conceivable that certain chemicals or metabolites may affect colorectal tissue and increase CEA levels. Interestingly, *N*-acetyltransferase and the associated *N*-hydroxyarylamine *O*-acetyl transferase (OAT) activity was found to be higher in colonic mucosa in contrast to the low level of this latter activity in the bladder (Hein 1988). Moreover, it is suggested that the colonic mucosa activates hydroxyarylamines and *O*-acetylation of *N*-hydroxy arylamine metabolites converts them into DNA-reactive derivatives which could predispose rapid acetylators to colon cancer (Lang *et al.* 1986, Caporaso *et al.* 1991, Caporaso and Goldstein 1995, Gil and Lechner 1998). These mechanisms could explain partly our result that serum CEA levels are relatively higher in rapid acetylators. On the other hand more than 90 % of the Japanese population has the rapid genotype of *NAT2*, but the incidence of colorectal carcinoma is relatively low compared with American Caucasians. The tendency for an increase in incidence of colorectal carcinoma in immigrants and their sons from Japan to the United States indicates that the presence of the appropiate *NAT2* genotype in addition to nutritional habits and environmental factors may trigger such a situation (Wu 1996). CEA levels are also elevated in lung cancer patients (Begent 1984). It has been shown that carriers of the *NAT2*4/*4* genotype, which have the highest acetylation capacity, are at increased risk of developing lung cancer

Table 3. Serum levels of tumour markers among detected *NAT2* genotypes of 60 subject. Values are given as mean ±SEM.

NAT2 Genotypes	*n*	CEA (ng ml^{-1})	AFP (IU ml^{-1})	CA 19-9 (U ml^{-1})	CA 15-3 (U ml^{-1})	CA 125 (U ml^{-1})
Rapid						
**4/*6A*	6	3.33 ±0.83	0.78 ±0.16	13.70 ±5.80	39.20 ±11.50	22.42 ±9.64
**4/*5B*	12	3.23 ±0.66	1.46 ±0.30	17.01 ±4.75	29.05 ±6.42	14.02 ±6.24
Slow						
**5B/*6A*	14	3.23 ±1.21	7.81 ±7.03	19.01 ±7.94	37.12 ±5.63	11.51 ±1.73
**6A/*6A*	8	2.01 ±0.40	5.35 ±4.59	6.97 ±2.55	33.86 ±5.05	14.96 ±5.77
**5B/*5B*	9	1.80 ±0.38	1.59 ±0.40	13.80 ±4.59	42.51 ±9.70	13.06 ±1.80
**5B/*7B*	5	2.50 ±1.09	5.08 ±4.11	13.32 ±5.38	43.94 ±8.99	12.26 ±2.24
**5C/*6A*	6	0.81 ±0.08	0.96 ±0.19	3.85 ±0.87	26.58 ±7.07	10.95 ±2.07

(Cascorbi *et al.* 1996). These findings could also support the association between rapid *NAT2* genotype and relatively higher CEA levels.

A recent study reported that cigarette-smoking postmenopausal women with the slow acetylator phenotype are as much as four-fold more likely to develop breast cancer than carriers of the rapid acetylator allele (Ambrosone *et al.* 1996). Although our studied group consists of non-smokers and a mixture of men and women, CA 15-3 levels, a marker used especially for breast cancer diagnosis and prognosis, tend to increase among slow acetylators, but this increase was not found to be statistically significant. Further investigations with larger and more appropriate groups should clarify this issue.

In conclusion, our findings suggest that *NAT2* genotypes may influence concentrations of CEA, but no impact was observed on other tumour markers investigated. However, it could be possible that ethnic and environmental differences may have an impact on serum tumour marker levels, according to genotypic characteristics that are associated with xenobiotic metabolism.

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