N-ACETYLTANSFERASE 2 (NAT2) AND GLUTATHIONE S-TRANSFERASE \( \mu \) (GSTM1) IN BLADDER-CANCER PATIENTS IN A HIGHLY INDUSTRIALIZED AREA

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**N-Acetyltransferase 2 (NAT2) and Glutathione S-Transferase μ (GSTM1) in Bladder-cancer Patients in a Highly Industrialized Area**

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The study was designed to assess occupational and non-occupational risk factors in patients with urothelial carcinomas in an area of former coal, iron, and steel industries, with special regard to the impacts of polymorphic enzymes involved in the metabolism of aromatic amines (N-acetyltransferase 2, NAT2) and of polycyclic aromatic hydrocarbons (glutathione S-transferase μ, GSTM1). Inpatients with bladder cancer (n = 179) were interviewed for occupations ever engaged in for more than six months, and for bladder cancer risk factors in general. NAT2 was phenotyped by high-pressure liquid chromatography of caffeine metabolites in urine. The NAT2 status was additionally evaluated by genotyping 88 of these patients. Eighty-nine patients were genotyped for GSTM1. Of the 179 bladder-cancer patients, 115 (64%) were slow acetylators. In 70% of the subgroup of 89 patients, GSTM1 was negative, suggesting an impact of polycyclic aromatic hydrocarbons (PAHs) in bladder-cancer carcinogenesis in the general population in this area. Contrary to an ordinal distribution of the acetylator status in underground coal miners (18 slow acetylators out of 32), GSTM1 was negative in 16 of 19 of these coal miners. Five of six coke-oven workers were slow acetylators; GSTM1 was negative in all four genotyped coke-oven workers. Twelve of 17 patients formerly exposed to colorants were slow acetylators. Distributions of NAT2 (59% slow acetylators) and GSTM1 (54% GSTM1 negative) were normal in businessmen and administrative officers among the occupationally non-exposed bladder-cancer patients. The results are consistent with the view that a slow-acetylator status and lack of the GSTM1 gene are individual risk factors for bladder cancer in persons occupationally exposed to aromatic amines and PAHs. Aromatic amines may be connected with induction of bladder cancer in persons who have been in contact with azo dyes and in coke-oven workers. PAHs may also contribute to elevated bladder-cancer risks in coke-oven workers and in underground coal miners. **Keywords**: bladder cancer; N-acetytransferase 2; glutathione S-transferase μ; occupational risk factors; aromatic amines; polycyclic aromatic hydrocarbons; genetic predisposition.

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Bladder cancer is the second most common urologic tumor among men and the most common urologic tumor among women in Germany. The association between elevated bladder-cancer risk and occupational exposure to aromatic amines, first described by Rehn,1 has been well established. Between 1978 and 1994, 506 victims of bladder cancer were compensated as having an occupational disease (BK 1301) in Germany.2 There is a discrepancy between the numbers of occupationally induced bladder cancers estimated on the basis of epidemiologic data and the numbers of cases actually reported/compensated as occupational bladder-cancer cases.3 Doll and Peto4 estimate that 10% of all bladder cancers in men and 5% in women are of occupational origin.

Workers occupationally exposed to aromatic amines or to bioavailable azo dyes (which are cleaved in the human to the parent aromatic amines) are subject to elevated risks of bladder cancer.5-7 Moreover, case-control studies also show higher-than-normal risks of bladder cancer in occupations without overt exposures to proven carcinogenic aromatic amines, e.g., in coal miners.8-13 The influence of the genetically determined enzyme polymorphism of NAT2 in European populations exposed to aromatic amines has been established (for reviews, see Risch et al.14 and Golka et al.15).

In 1986, Seidegard et al.16 observed that smokers who had lung cancer more frequently lacked GSTM1-associated enzyme activity than did controls. The GSTM1 isoenzyme is likely to be involved in detoxifying intermediates of polycyclic aromatic hydrocarbons (PAHs) by conjugation to glutathione. In 1998, Bell et al.17 showed an association between the absence of the GSTM1 gene and an increased risk of bladder cancer in smokers. The assumption that GSTM1 was involved in

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the detoxification of polycyclic aromatic hydrocarbons was later substantiated by Hirvonen et al., who detected increased mutagenicity in the urine of smokers lacking the GSTM1 gene, compared with nonsmokers.

The present study provides further evidence of the impacts of genetic polymorphisms of NAT2 among workers exposed to aromatic amines and of GSTM1 among persons exposed to PAHs, on the basis of an examination of the bladder-cancer cases of a large urology department in a traditional German industrial area.

MATERIALS AND METHODS

Bladder-cancer Patients

The hospital-based study was conducted between January 1992 and June 1995. The subjects of the study were 179 inpatients (137 men, 42 women, mean age 66 years, SD 10.8 years) of the Department of Urology, Staatliche Kliniken Dortmund, located in the East Ruhr area, a region formerly dominated by the coal, iron, and steel industries.

In these patients, urothelial carcinomas of the bladder (transitional-cell carcinomas) had first been histologically diagnosed between 1971 and 1995. All patients were treated by transurethral tumor resection. Either local BCG or mitomycin instillation or partial or radical cystectomy was performed, depending on tumor stage and grade.

Written consent was obtained from all participating patients. Bladder tumors other than those of the urothelium (e.g., squamous-cell carcinomas, adenocarcinomas, sarcomas) were excluded from this study. The patients participating in this study were of European origin. All patients were always examined by the same physician (ThR), using a questionnaire within a standardized interview. Detailed medical histories, smoking habits, current drug intakes, occupational histories (jobs ever done for more than six months), and occupational exposures to possible or known bladder carcinogens were recorded.

The 179 patients reported 439 jobs. Occupations were classified based on the official list of occupations of the German Federal Office of Statistics.

NAT2 Phenotyping

The "caffeine test" method of Grant et al. was used for NAT2 phenotyping. There was no dietary or drug restriction. The patient drank one to two cups of coffee, and spot urine was obtained about two hours later. A second spot urine sample was obtained from 78 of the 179 patients after four hours or with a repeat test on the occasion of a second hospitalization. For persons from whom two or more samples were analyzed, a mean value was entered into the further evaluations; no difference in individual phenotyping results based on these parallel samples was observed in any case (see the results section).

Spot urine was immediately adjusted to pH 3.5 by addition of small quantities of 1M HCl, and was stored in 10-mL plastic tubes at -18°C until processing. Individual storage times did not exceed six weeks. The caffeine metabolites AFMU (5-acetylamin0-6-formylamino-3-methyluracil; acetylated metabolite) and 1X (1-methylxanthine; non-acetylated metabolite) were quantitated by high-pressure liquid chromatography (HPLC), using the standard-addition procedure. Standard 1X was a commercial product of Sigma (Deisenhofen, Germany); AFMU was synthesized as described by Roehrkasten et al., according to a modification of the method of Fink et al. The HPLC procedure is described in detail elsewhere.

NAT2 Genotyping

Genotyping for rapid and slow alleles of the NAT2 gene was performed on genomic lymphocyte DNA of anticoagulated blood samples taken from a subgroup of 89 of the 179 patients. The DNA was prepared by phenol extraction according to standard methods and analyzed by polymerase chain reaction with the use of restriction enzymes, according to the procedure of Cascorbi et al.

The new nomenclature of alleles of Vatis et al. was used.

GSTM1 Genotyping

Genotyping for the GSTM1 gene was performed on genomic lymphocyte DNA taken from anticoagulated blood samples from a subgroup of 89 patients, isolated as described above and analyzed by PCR, according to the method of Bell et al.

RESULTS

Correspondence between NAT2 Genotypes and Phenotypes

The "caffeine test" for NAT2 phenotyping, as introduced by Grant et al., has facilitated clinical studies of acetylator status. This test, however, requires careful consideration of the antimode of the urinary caffeine metabolite ratio (AFMU/1X) that distinguishes between slow and rapid acetylators. Eighty-five of the bladder cancer patients were therefore genotyped, in addition to the phenotyping.

As in the preceding study of Golka et al., the antimode of the AFMU/1X ratio discriminating between slow and rapid acetylator phenotypes was found to be 1.0. Only seven of the 89 patients, four of whom had the alleles 4/5B, showed a discrepancy between genotype and phenotype; in three of these seven cases, the molar ratio AFMU/1X characterizing the phenotype deviated
substantially from the expected range on the basis of the genotype (Fig. 1).

**Distribution of NAT2 Phenotypes**

The prevalences of 64% slow acetylators (115 persons) in the entire group of 179 men and women bladder-cancer patients combined and of 66% slow acetylators (90 persons) among the men is, in general, consistent with findings in other studies of similar design.14,35-37 It is somewhat higher than that in a previous study of Golka et al.15 where 55% slow acetylators were found among 196 bladder-cancer patients in Leverkusen, Germany, and is comparable to that of the study of Brockmoeller et al.,25 who found 62% slow acetylators among 374 bladder-cancer patients in Berlin, Germany.

Patient age, gender, and drug intake and histologic tumor stage (Ta/T1 vs T2-T4) and tumor grade had no obvious impact on the distribution of the acetylator phenotypes. Contrary to the findings in a majority of published studies, a higher percentage of slow acetylators, 72%, was found in the subgroup of smokers. The distribution of the slow-acetylator phenotype was normal in 41 diseased, but occupationally non-exposed, businessmen and administrative officers (59%). Seventeen bladder-cancer patients reported occupational exposures to colorants, and 12 of them (71%) were slow acetylators. Five of six coke-oven workers were slow acetylators (83%). Normal distributions of slow acetylators were seen in other subgroups of bladder-cancer patients who were likely to have been subject to occupational exposures to polycyclic aromatic hydrocarbons: 32 underground coal miners (56%), 24 workers exposed to fumes (54%), and 17 workers exposed to tar (65%) (Fig. 2).
Distribution of GSTM1 Genotype

The GSTM1 gene was absent in 70% (62 persons) of a subgroup (including both men and women) of 89 bladder-cancer patients in this study. The percentage of GSTM1-negative persons in our study group differed consistently from the percentages found in newborn infants in the East Ruhr Area (54%) and an adult population in Berlin (50%).

Patient age, gender, and smoking habits and histologic tumor stage (Ta/T1 vs T2–T4) and grade had no obvious impact on the distribution of GSTM1 gene. The distribution of GSTM1-negative persons in the 13 occupationally non-exposed businessmen and administrative officers (54%) was normal.

All four genotyped coke-oven workers, 16 of 19 underground coal miners (84%), ten of 13 workers exposed to fumes (77%), and five of eight workers exposed to tar (62%) were GSTM1-negative (Fig. 3).

DISCUSSION

Previous investigations in bladder-cancer patients of European origin have consistently revealed small excesses of slow acetylators, compared with the distribution in healthy controls. The positioning of the antimode of the ratio of caffeine metabolites in NAT2 phenotyping studies using Grant’s “caffeine test” discriminating between slow and fast acetylators is of outstanding methodologic importance. Antimode figures used in phenotyping studies range from 0.48 to 1.0 due to differences in the analytic procedures used.

In order to rule out possible influences such as interactions of xenobiotics with caffeine metabolism, e.g., by drugs, dietary factors, tobacco smoking, or other factors, we validated the antimode for the “caffeine test” by additional genotyping.

A recent study confirmed the observations of Cartwright et al. and Lewalter and Milsche that an overrepresentation of slow acetylators in groups of bladder-cancer patients is an indicator of the probability of past occupational contacts with aromatic amines. In the present study, occupational exposures to chemically defined aromatic amines could not be observed, because the chemical industry now or formerly producing aromatic amines is not located in the study area. However, it is well established that bioavailable azo dyes that are based on carcinogenic aromatic amines are cleaved in the human organism. We found that 12 of 17 bladder-cancer patients who reported occupational exposures to colorants, e.g., paints and lacquers, were slow acetylators. This higher-than-normal percentage (71%) confirms the hypothesis that exposures to certain colorants should be considered a possible risk factor for bladder cancer.

Because of the geographic location of the study patients in an area of former coal, iron, and steel industries, it was of particular interest to concentrate on subgroups of patients who had occupational backgrounds in these industries. For workers in some of these occupations, e.g., coke-oven workers and underground coal miners, increased bladder cancer risks had been found in case–control studies.

The carcinogenic potential of occupational exposure to PAHs, so far as lung cancer is concerned, has been well established, although such a potential for the bladder as target organ is not so obvious. Nevertheless, the known elevation of the risk of bladder cancer in smokers is likely to be associated with exposure to PAHs.

Coke-oven workers are exposed to small amounts of aromatic amines in the coke-oven emissions, but this alone cannot explain the observed elevated bladder-cancer risk. On the other hand, these workers are primarily exposed to high concentrations of PAHs. Although this occupational group was very small in our investigation, the distributions of the slow-acetylator phenotype (5 of 6) and the GSTM1 genotype (4 of 4) are well in line with the assumption that elevated bladder cancer risks in coke-oven workers are due to a combined effect of aromatic amines (tumor induction) and PAHs (tumor promotion).

A limited number of case–control studies have revealed increased bladder-cancer risks in underground coal miners. In our subgroup of 32 underground coal miners suffering from bladder cancer, an ordinary distribution of the slow-acetylator phenotype was found. Thus, a relevant impact of occupational exposure to aromatic amines in the origin of bladder cancer in underground coal miners is not likely. By contrast, the percentage of underground coal miners lacking the GSTM1 gene was impressively higher than normal (84%), both in comparison with the general population in this area (54% of 170 newborn infants studied by Kempkes et al.) and in comparison with 400 adults in Berlin. Such a shift in GSTM1 distribution in underground miners suffering from bladder cancer points to a primary role of PAH in the elevation of the risk of bladder cancer in this occupation.

CONCLUSIONS

The results of this study are consistent with the view that slow-acetylator status and absence of the GSTM1 gene are individual risk factors in persons significantly exposed to aromatic amines and to polycyclic aromatic hydrocarbons (PAHs), respectively. In the population studied, aromatic amines are likely to be a causative factor in the induction of bladder cancer in persons who have formerly handled certain azo dyes and in coke-oven workers. Polycyclic aromatic hydrocarbons are probably also involved in the elevated cancer risks of coke-oven workers, and may be regarded as the carcinogenic principle leading to bladder cancer in underground coal miners, according to the results presented.
References


