Exposure Levels and Cytochrome P450 1A2 Activity, but not *N*-Acetyltransferase, Glutathione *S*-Transferase (GST) *M1* and *T1*, Influence Urinary Mutagen Excretion in Smokers[1]

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Abstract

We investigated the polymorphic enzymes cytochrome P450 1A2 (CYP1A2), *N*-acetyltransferase (NAT2), glutathione *S*-transferase (GST) M1 (*GSTM1*), and T1 (*GSTT1*) in relation to cigarette smoking-associated urinary mutagenicity detected on YG1024 *Salmonella typhimurium* strain with S9 mix in 97 smokers. In each subject, cigarette smoke intake was checked by analysis of urinary nicotine plus its metabolites. NAT2 and CYP1A2 phenotypes were determined by the molar ratio of urinary caffeine metabolites detected by high-performance liquid chromatography, and *GSTT1* and *GSTM1* genotypes were determined by PCR. An increase in urinary mutagenicity was significantly related to levels of exposure to cigarette smoke and CYP1A2 *N*-hydroxylation activity (linear multiple regression analysis $t = 4.51$ and $P < 0.001$ and $t = 3.09$ and $P = 0.003$; $F = 6.31$, $P < 0.001$). Urinary mutagenicity was significantly higher in CYP1A2 extensive metabolizer smokers ($n = 49$) than in CYP1A2 poor metabolizer ones ($n = 48$; 2176 ± 1525 *versus* 1384 ± 1206 revertants/mmol creatinine, Mann-Whitney *U*-test, $z = 2.65$, $P < 0.001$). The highest mutagenic activity was seen in subjects CYP1A2 extensive metabolizer/NAT2 slow acetylators ($n = 29$) with respect to the other phenotype combinations ($n = 68$; 2392 ± 1660 *versus* 1525 ± 1238 revertants/mmol creatinine, Mann-Whitney *U*-test, $z = 2.37$, $P = 0.017$). NAT2 acetylation activity was slightly but inversely related to urinary mutagenicity, and the association was not significant. No effect of *GSTM1* and *GSTT1* genotypes in lowering (detoxifying) urinary mutagens was found. The significant enhancement of urinary mutagenicity associated with increased CYP1A2 activity, as already seen for diet-caused urinary mutagenicity, allows for many analogies between the process of mutagen formation derived from cooked meat and that from cigarette smoke condensate. In conclusion, the intensity of tobacco smoke exposure, modulated by CYP1A2 activity, is the major determinant of mutagenic urine among smokers, whereas *GSTM1* and *GSTT1* genotypes have no influence on this biomarker. This study suggests that CYP1A2 should definitely be determined in future studies involving urinary mutagenicity in cases in which smoking is a factor.

Introduction

Mainstream tobacco smoke is a complex mixture containing at least 4000 compounds, both volatile and particulate, including several carcinogenic/mutagenic agents. CSC,[3] the particulate matter of mainstream smoke, is active in several short-term tests for genotoxicity (1) including the *Salmonella*/microsome assay (2). Smokers' CSC intake may range from a few to some hundreds of milligrams per day. After smoking, CSC and/or its metabolites rapidly appear in the urine, in which they have been detected as increased mutagenic activity after urine concentration on adsorbents (3, 4).

Many mutagenic compounds or their metabolites from CSC may be responsible for the mutagenicity of smokers' urinary extracts. These mutagens are relatively not polar aromatic compounds, mainly have two or more aromatic rings, and belong to three major classes of genotoxins [PAHs, HAAs, and AAs (5–7)]. The daily intake of these compounds by a heavy smoker has been estimated at a maximum amount of hundreds of nanograms for AAs 2-naphtylamine and 4-aminobiphenyl and some micrograms for PAHs benzo(*a*)pyrene and benzo(*a*)anthracene and HAAs 2-amino-1-methyl-6 phenylimidazo[4,5-b]pyridine and α-aminocarboline (8). The urinary levels of these compounds and their metabolites, considered singly, cannot explain the finding of increased urinary mutagenicity in smokers.

After being inhaled, tobacco smoke condensate undergoes several metabolic transformations of activation/detoxification in various organs (mainly the liver) before its unmetabolized form or its metabolites appear in urine. CYP1A2 and NAT2 enzymes have been identified as being involved in the activation and detoxification of AAs and HAAs (9). Recently, two genetic polymorphisms (C734A and G2964A) of CYP1A2 have been identified (10, 11) as being associated with the high

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[3] The abbreviations used are: CSC, cigarette smoke condensate; AA, aromatic amine; AFMU, 5-acetylamino-6-formyl-amino-3-methyluracile; CYP1A2, cytochrome P450 1A2; GST, glutathione *S*-transferase; HAA, heterocyclic aromatic amine; PAH, polycyclic aromatic hydrocarbon; NAT2, *N*-acetyltransferase; 17U, 1,7-dimethyluric acid; 1U, 1-methyluric acid; 1X, 1-methylxanthine; EM, extensive metabolizer; PM, poor metabolizer; CV, coefficient of variation.

inducibility of enzyme activity in smokers, whereas a bimodal distribution of CYP1A2 activity in smokers and nonsmokers has been suggested (12–14). NAT2 polymorphism, which divides human populations into slow and fast acetylators, has been well known for a long time (15). PAHs undergo several metabolic transformations, and their reactive intermediates are detoxified by GSTs. The μ *(GSTMI)* and θ (*GSTT1*) members of the GST multigene family are involved in the detoxification of several tobacco smoke-derived carcinogens, including intermediate metabolites of PAHs, and deletion variants at both these loci, associated with a lack of detoxifying function, are well known (16). Polymorphisms of these metabolizing enzymes can thereby modulate the presence of tobacco smoke mutagens in urine.

The aim of this study was to investigate the influence of CYP1A2 and NAT2 phenotypes and *GSTM1* and *GSTT1* genotypes on modulating the presence of mutagens in the urine of cigarette smokers.

Materials and Methods

Subjects. A total of 97 healthy smokers comprised the sample population. Exclusion criteria were pregnancy, liver or kidney diseases, caffeine intolerance, antibiotic therapy and/or use of urinary disinfectants within the past 3 months, and occupational exposure to PAHs or other genotoxicants. For each subject, personal data were collected by means of a questionnaire. Subjects were informed of the study's purpose and instructed about the protocol. All participants gave their informed consent. All information regarding participants was rendered anonymous after collection of data and blood and urine samples.

Study Design. This study was conducted between October 2000 and February 2001. Subjects were asked to abstain from consumption of charcoaled and pan-fried meat, alcohol-containing beverages, and any foods or beverages containing methylxanthines for 24 h before as well as during the day of the study. Participants were also instructed to keep their urine in a refrigerated dark place until sample transfer to the laboratory, where it was then stored at −20°C until analysis. In addition, subjects were asked for a blood sample for genotype analysis. On the day of the experiment, after emptying their bladders in the morning, at 7 a.m. the subjects ingested an instant coffee beverage containing 140 mg of caffeine (2 packets of Nescafé) in about 250 ml of water. Urine was collected for 6 h after the caffeine dose in 500-ml bottles preloaded with 6 ml of 6N HCl. Acidification of urine (pH 2) was further checked before urine storage at −20°C. Urine samples for the analysis of mutagenicity and nicotine and its metabolites (at least 250 ml) were collected in the late afternoon. The compliance of each volunteer with these instructions had been checked with the questionnaire.

Analysis of Urinary Nicotine plus Metabolites. Urine concentration of nicotine plus metabolites was determined colorimetrically by the diethylthiobarbituric acid extraction method (17), based on the Koenig reaction. Diethylthiobarbituric acid, used as condensing agent, gives a pink product that can easily be extracted in ethyl acetate. Absorbance was measured spectrophotometrically at a wavelength of 532 nm. This simple method is very useful to estimate active tobacco smoke exposure, and, as also seen in our previous study (18), the value of urinary nicotine plus metabolites correlates better with urinary mutagenicity than urinary cotinine levels. In each urine sample, nicotine plus metabolites were adjusted for urinary creatinine, determined according to the Boehringer-Mannheim colorimetric test, based on the reaction of creatinine with picrate in alkaline medium.

Urinary Mutagenicity. Urine samples were concentrated in glass columns (1.5 × 10 cm) packed with washed XAD-2 resin (4 g/100 ml urine; Ref. 5) and eluted with 15 ml of acetone. Dried extracts were resuspended in DMSO (250 ml of urine/ml DMSO) and placed in the dark at −20°C. Urine samples were assayed on YG1024 strain using the plate incorporation preincubation technique in the presence of Aroclor 1254-induced rat liver S9 (50 μl/plate; Ref. 19). This bacterial strain is a derivative of the TA98 frameshift mutation-sensitive strain and overexpresses *O*-acetyltransferase enzymatic activity and detects aromatic and heterocyclic amino compounds and their hydroxylamino derivatives more efficiently (20, 21). Briefly, at least five doses different from zero were assayed in duplicate on strain YG1024, ranging from 0.7 to 12.5 ml for the urine samples. Mutagenic activity was the slope of the linear portion of the dose-response curve calculated by the linear regression method and was expressed as the number of revertants/millimole of creatinine. Extracts were assayed only in the presence of S9.

Determination of CYP1A2 and NAT2 Phenotypes by Caffeine Metabolite Analysis. Caffeine and its metabolites were extracted from urine samples according to the procedure described by Berthou *et al.* (22). Recoveries calculated for caffeine metabolites were 76%, 82%, 70%, and 94% for AFMU, 1U, 1X, and 17U, respectively. The CV for repeated analyses of each compound on different days was not higher than 4%. Repeated analysis on different days of a subgroup of 30 urine samples did not exceed 8% CV for a single metabolite and 12% CV for molar metabolite ratios. The molar ratio of urinary caffeine metabolites was used to determine the CYP1A2 and NAT2 phenotypes as follows: CYP1A2 = (AFMU + 1X + 1U)/17U (23); and NAT2 = AFMU/(AFMU + 1X + 1U) (24). An (AFMU + 1X + 1U)/17U ratio $<$ and $\geq$5.5 (median value of our smoking population) defined CYP1A2 PMs and EMs, whereas an AFMU/(AFMU + 1X + 1U) ratio $<$ and $\geq$0.3 defined NAT2 slow and rapid acetylators (25).

Genotype Analysis. DNA was isolated from peripheral blood samples (5 ml) collected in EDTA tripotassium salt tubes using QIAamp DNA blood mini-kits (Qiagen, Milan, Italy). A multiplex PCR method was used to detect the presence or absence of the *GSTM1* and *GSTT1* genes, according to the protocol described previously (26). This PCR method had both *GSTM1*- and *GSTT1*-specific primer pairs in the same amplification mixture and included a third primer pair for β-globin as an internal positive PCR control. The *GSTT1* (480 bp), β-globin (285 bp), and *GSTM1* (215 bp) amplification products were resolved in an ethidium bromide-stained 2% agarose gel. The absence of the *GSTM1*- or *GSTT1*-specific fragment indicated the corresponding null genotype (*0/*0), whereas the β-globin-specific fragment confirmed the presence of amplifiable DNA in the reaction mixture.

Statistical Analysis. Statistical comparisons between various groups were made using nonparametric tests (Mann-Whitney *U*-test and Spearman correlation test). Multiple linear regression analysis was used to assess the influence of exposure to tobacco smoke (evaluated by the urinary levels of nicotine plus its metabolites in mg/mmol creatinine), NAT2 and CYP1A2 phenotypes, and *GSTM1* and *GSTT1* genotypes (independent variables) on urinary mutagenicity (revertants/mmol creatinine; dependent variable). NAT2 and CYP1A2 phenotypes and *GSTM1* and *GSTT1* genotypes were considered dichotomous variables attributed a value of 1 or 0 referring to NAT2 rapid

Table 1 Characteristics of smoking sample population

Characteristics	
No. of subjects	97
Gender	
Female	41
Male	56
Age (yrs)	
Mean ± SD	38 ± 12
Range	18–69
Cigarettes/day	
Mean ± SD	18 ± 8
Range	7–50
Nicotine and its metabolites (mg/mmol creatinine)	
Mean ± SD	0.75 ± 0.54
Range	0.07–3.56
Urinary mutagenicity (revertants/mmol creatinine)	
Mean ± SD	1784 ± 1425
Range	0–6709
CYP1A2 activity[a]	
Mean ± SD	5.89 ± 5.93
Range	1.68–15.32
PM	48 (50%)
EM	49 (50%)
NAT2 activity[b]	
Mean ± SD	0.24 ± 0.02
Range	0.05–0.62
Slow	69 (71%)
Rapid	28 (29%)
GSTM1 genotypes	
Active	42 (43%)
*0/*0	55 (57%)
GSTT1 genotypes	
Active	72 (75%)
*0/*0	24 (25%)

[a] An (AFMU + 1X + 1U)/17U ratio < and ≥ 5.5 (median value) defined PM and EM CYP1A2 phenotypes.
[b] An AFMU/(AFMU + 1X + 1U) ratio < and ≥ 0.3 defined NAT2 slow and rapid acetylators.

and slow acetylators, CYP1A2 EM and PM, *GSTM1* active and *0/*0, and *GSTT1* active and *0/*0, respectively. Analysis was carried out using the BMDP package (27).

Results

Table 1 shows the characteristics of the examined population (number of subjects, age, sex, number of cigarettes/day, frequencies of NAT2 rapid and slow acetylators, CYP1A2 EMs and PMs, and *GSTM1* and *GSTT1* active and *0/*0 subjects). The levels (mean ± SD and range) of nicotine plus its metabolites and of mutagenic activity in urine samples, together with CYP1A2 [(AFMU + 1X + 1U)/17U] and NAT2 [AFMU/(AFMU + 1X + 1U)] ratios (mean ± SD and range), are also reported.

CYP1A2 activity in smokers was distributed over a wide range; metabolite ratios were 1.68–15.32, with a median value of 5.5. In our population, the percentages of slow acetylators and *GSTM1*- and *GSTT1*-null subjects were 71%, 57%, and 25%, respectively, with frequencies similar to those already reported for Caucasian populations. Urinary mutagenicity ranged from very low values (no detectable mutagenic activity in urine) to more than 6700 revertants/mmol creatinine, and values of urinary nicotine plus metabolites also ranged from values comparable with those of nonsmokers to 3.56 mg/mmol creatinine. Both the latter parameters were quite well correlated (Spearman correlation coefficient (Rho) = 0.52, $P < 0.001$).

Table 2 Nicotine plus its metabolites and urinary mutagenicity according to NAT2 and CYP1A2 phenotypes and *GSTM1* and *GSTT1* genotypes in 97 cigarette smokers

Pheno-genotype	Subjects *N* (%)	Nicotine plus metabolites (mg/mmol creatinine; mean ± SD)	Urinary mutagenicity[a] (revertants/mmol creatinine)
CYP 1A2			
PM	48 (50%)	0.76 ± 0.64	1384 ± 1206
EM	49 (50%)	0.76 ± 0.42	2176 ± 1525**
NAT2			
Slow	69 (73%)	0.77 ± 0.58	1844 ± 1520
Rapid	28 (27%)	0.72 ± 0.44	1636 ± 1172
GSTM1			
Active	42 (43%)	0.64 ± 0.43*	1775 ± 1454
*0/*0	55 (57%)	0.86 ± 0.43	1817 ± 1418
GSTT1			
Active[b]	72 (75%)	0.79 ± 0.56	1834 ± 1476
*0/*0	24 (25%)	0.66 ± 0.48	1536 ± 1205
CYP1A2 EM/NAT2 slow	29 (30%)	0.78 ± 0.41	2392 ± 1660*
Other combinations[c]	68 (70%)	0.78 ± 0.41	1525 ± 1238

[a] On YG1024 + S9.
[b] *GSTT1* was not determined in one subject.
[c] CYP1A2 EM/NAT2 rapid, CYP1A2 PM/NAT2 rapid, and CYP1A2 PM/NAT2. Statistical comparisons: urinary mutagenicity (revertants/mmol creatinine) CYP1A2 PM *versus* EM and CYP1A2 EM/NAT2 slow *versus* other combinations Mann-Whitney *U* test, $z = 2.65$, **, $P < 0.001$ and 2.37; *, $P = 0.017$; nicotine plus its metabolites *GSTM1 *0/*0 versus* active Mann-Whitney *U*-test, $z = 2.34$, *, $P = 0.02$.

Table 2 shows urinary mutagenicity levels (range and mean ± SD) of the 97 smokers, according to NAT2 and CYP1A2 phenotypes and *GSTM1* and *GSTT1* genotypes, together with the mean ± SD levels of exposure to tobacco smoke evaluated by the urinary excretion of nicotine plus metabolites. Urinary mutagenicity was significantly higher in CYP1A2 EM smokers ($n = 49$) than in CYP1A2 PM ones ($n = 48$; 2176 ± 1525 *versus* 1384 ± 1206 revertants/mmol creatinine, Whitney *U*-test, $z = 2.65$, $P < 0.001$). In NAT2 slow acetylators, urinary mutagenicity was slightly higher than that in the NAT2 rapid acetylator smokers but was not statistically significant. However, urinary mutagenicity was significantly higher in smokers with the combination of NAT2 slow/CYP1A2 EM ($n = 29$) than in the other combinations of the two phenotypes ($n = 68$; 2392 ± 1660 *versus* 1525 ± 1238 revertants/mmol creatinine, Mann-Whitney *U*-test, $z = 2.37$, $P = 0.017$). No difference in urinary nicotine plus metabolites, the biomarker of exposure to tobacco smoke, in urinary samples from these subgroups of smokers was noted. No increased urinary excretion of mutagens was seen in null GST subjects, despite the fact that *GSTM1*0/*0* smokers had a significantly higher exposure to tobacco mutagens than *GSTM1* active smokers (0.86 ± 0.43 *versus* 0.64 ± 0.43 mg nicotine plus metabolites/mmol creatinine, Mann-Whitney *U*-test, $z = 2.34$, $P = 0.02$).

Table 3 shows the results of linear multiple regression analysis of the influence of cigarette smoke exposure (evaluated by urinary nicotine plus metabolites, CYP1A2 and NAT2 phenotypes, and *GSTM1* and *GSTT1* genotypes) on the urinary mutagenicity of the 96 smokers. Urinary mutagenicity levels were significantly related mainly to cigarette smoke exposure and, to a lesser extent, to CYP1A2 phenotypes ($F = 6.31$, $P < 0.001$; $t = 4.51$, $P < 0.001$; $t = 3.09$, $P = 0.003$; partial contribution to $r^2 = 16.7\%$ and 7.9%, respectively). NAT2 acetylation activity was slightly but inversely related to urinary mutagenicity, and the association was not significant. In the regression analysis, no appreciable contribution of the GST

Table 3 Influence of nicotine and its metabolites, CYP1A2 and NAT2 phenotypes and *GSTM1* and *GSTT1* genotypes on urinary mutagenicity in 96 smokers: results of multiple linear regression analysis

	Nicotine and its metabolites	CYP1A2	NAT2	*GSTM1*	*GSTT1*
b[a]	1091	815	−370	196	264
SE(*b*)	242	263	289	268	300
t	4.51	3.09	1.28	0.73	0.88
P	<0.001	0.003	0.205	0.467	0.382
Partial r^2	16.7%	7.9%	1.3%	0.4%	0.3%

[a] Coefficients of regression (*b*), corresponding standard error (SE(*b*)), *t* test of partial significance, and partial explained variance (r^2) were estimated for each term included in the model. $F = 6.31$, $P < 0.001$.

active genotype to the lowering of urinary mutagenicity in smokers was observed.

Discussion

In this study, we investigated polymorphic enzymes CYP1A2 and NAT2 (phenotype) and *GSTM1* and *GSTT1* (genotype), involved in the metabolism of several genotoxic compounds, in relation to cigarette smoking-associated urinary mutagenicity.

An increase in S9-mediated urinary mutagenicity in smokers, related to levels of tobacco smoke exposure, was easily detectable with the YG1024 strain, as observed previously by other authors (28–30).

The CYP1A2 activity of our smoking population had a very wide interindividual variability and was significantly different from that of nonsmokers, as reported by many authors (31–36) and by us as well (37). CYP1A2 is an inducible enzyme. Besides smoking, PAHs, HAAs, and certain dietary components (38) are known to induce enzyme activity.

Our results indicate that an increase in urinary mutagenicity was significantly related to CYP1A2 activity in smokers, also confirming other authors' reports (39). Although CYP1A2 is expressed mainly in the liver, considering its great relevance in the activation of many environmental carcinogens (*i.e.*, conversion of aromatic or heterocyclic amines to their proximate mutagenic *N*-hydroxy derivatives), CYP1A2 activity may be a risk factor for the development of cancers in other tissues, targets for activated carcinogens. In a previous study, the CYP1A2 phenotype was significantly associated with increased risk of nonoccupational urinary bladder cancer (40). In another case-control study, persons with the high inducibility variant C734A polymorphism in intron 1 of *CYP1A2* genotype were overrepresented in bladder cancer, but only if they were smokers or had slow *NAT2* genotypes (41).

In agreement with other authors (29, 42), we did not find any clear effect of NAT2 phenotype on cigarette smoke-induced urinary mutagenicity. NAT2 slow acetylation alone, in workers professionally exposed to AAs (43), or in combination with *GSTM1* in smoking coke-oven workers (44) has been shown to increase mutagenic activity. The slight increase in urinary mutagenicity in the subgroup of slow acetylators (which, in the present work, was significant only if extensive CYP1A2 activity was present) may indicate that aromatic and heterocyclic amine *N*-hydroxy derivatives from tobacco smoke do not undergo sequestration as stable DNA adducts in organs where NAT2 *O*-acetyltransferase is active and/or detoxification via NAT2 *N*-acetylation in the liver (45). Certainly, we cannot exclude the role of other conjugation pathways, *e.g.*, glucuronidation, in the urinary elimination of tobacco-derived mutagenic aryl/heterocyclic amines.

Neither the *GSTM1* nor *GSTT1* genotype influenced urinary mutagenicity in smokers. Only one report deals with urinary mutagenicity in smokers with *GSTM1*-null genotype (29). A significant increase in S9-mediated urinary mutagenicity, detected with YG1024 and TA 98 *Salmonella* strains, has been reported in smokers with *GSTM1*-null genotype compared with *GSTM1* active ones, but the small number of observations and the poor control of smoke exposure (6 of 7 *GSTM1*-null subjects were heavy smokers, but only 5 of 10 were *GSTM1* active) may explain this discrepancy. The influence of the *GSTM1*-null genotype on increasing mutagenic activity in humans highly exposed to PAHs alone has been reported by our research group (46). One consequence of the present results is that the contribution to smokers' urinary mutagenicity of PAHs, the *GSTM1*-related detoxification pathway of which is well-known, is slight.

Urinary mutagens in smokers are a complex mixture containing indirectly acting mutagens, the identification of which is quite far from being achieved, although some attempts have been made (47). Most of smokers' urinary mutagens have been found in the relatively nonpolar chemical acid extractable fraction, which contains both PAHs and HAAs (48, 49). PAHs and their metabolites have been detected in small quantities in smokers' urine (50–53), much lower than the detection limit of many urinary mutagenesis assays (54). Instead, the bacterial mutagenic potency of some HAAs is extraordinarily high (55). Because smokers' urinary mutagens act by means of a frameshift mechanism and can easily be extracted with "cotton bleu," and their activity is abolished by nitrite treatment, it has been suggested that they are primary AAs including HAAs (56, 57). Moreover, later studies showed that the mutation spectrum of cigarette smoke more closely resembles that of AAs than that of PAHs (58). Tobacco pyrolysis is one indispensable step in the formation of mutagenic substances in condensate (59–61), which depends on combustion temperature (62) and tobacco protein contents (63). Subjects who use tobacco but do not burn it or burn only a little of it do not show detectable urinary mutagenic activity (64), or their values are greatly reduced (30). The process has many analogies with that of the formation of mutagens in cooked meat (65), and both these types of environmental exposure give rise to frameshift urinary mutagens in man that are evident only after metabolic activation (5, 66). Previously, we showed the significant enhancement of urinary mutagenicity associated with increased CYP1A2 activity in diet-caused urinary mutagenicity (37).

In conclusion, the intensity of tobacco smoke exposure modulated by CYP1A2 activity is the major determinant of mutagenic urine among smokers, whereas *GSTM1* and *GSTT1* genotypes have no influence on this biomarker. This study suggests that CYP1A2 should definitely be determined in fu-

ture studies involving urinary mutagenicity in cases in which smoking is a factor.

References

1. DeMarini, D. M. Genotoxicity of tobacco smoke and tobacco smoke condensate. Mutat. Res., *114:* 59–89, 1983.

2. Kier, L. D., Yamasaki, E., and Ames, B. N. Detection of mutagenic activity in cigarette smoke condensates. Proc. Natl. Acad. Sci. USA, *71:* 4159–4163, 1974.

3. Kobayashi, H., and Hayatsu, H. A time-course study on the mutagenicity of smoker's urine. Gann, *75:* 489–493, 1984.

4. Kado, N. Y., Manson, C., Eisenstadt, E., and Hsieh, D. P. H. The kinetics of mutagen excretion in the urine of cigarette smokers. Mutat. Res., *157:* 227–233, 1985.

5. Yamasaki, E., and Ames, B. N. Concentration of mutagens from urine by absorption with the non-polar resin XAD-2: cigarette smokers have mutagenic urine. Proc. Natl. Acad. Sci. USA, *74:* 3555–3559, 1977.

6. Hayatsu, H., Oka, T., Wakata, A., Ohara, Y., Hayatsu, T., Kobayashi, H., and Arimoto, S. Adsorption of mutagens to cotton bearing covalently bound trisulfocopper-phthalocyanine. Mutat. Res., *119:* 233–238, 1983.

7. Rannug, A., Olsson, M., Aringer, L., and Brunius, G. An improved standardized procedure for urine mutagenicity testing. *In:* H. Bartsch, K. Hemminki, and I. K. O'Neill (eds.), Methods for Detecting DNA Damaging Agents in Humans: Applications in Cancer Epidemiology and Prevention, IARC Sci. Pub. No. 89, pp. 396–400. Lyon, France: IARC, 1988.

8. IARC. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Tobacco Smoking, Vol. 38. Lyon, France: IARC, 1986.

9. Kadlubar, F. F. Biochemical individuality and its implications for drug and carcinogen metabolism: recent insights from acetyltransferase and cytochrome P4501A2 phenotyping and genotyping in humans. Drug Metab. Rev., *26:* 37–46, 1994.

10. Sachse, C., Brochmoller, J., Bauer, S., and Roots, I. Functional significance of a C > A polymorphism in intron I of the cytochrome P450 *CYP1A2* gene tested with caffeine Br. J. Clin. Pharmacol., *47:* 445–449, 1999.

11. Han, X. M., Ou-Yang, D. S., Lu, P. X., Jiang, C. H., Shu, Y., Chen, X. P., Tan, Z. R., and Zhou, H. H. Plasma caffeine metabolite ratio (17X/137X) *in vivo* associated with G-2964A and C734A polymorphisms of human *CYP1A2*. Pharmacogenetics, *11:* 429–435, 2001.

12. Schrenk, D., Brockmeier, D., Morike, K., Bock, K. W., and Eichelbaum, M. A distribution study of CYP1A2 phenotypes among smokers and non-smokers in a cohort of healthy Caucasian volunteers. Eur. J. Clin. Pharmacol., *53:* 361–367, 1998.

13. Nakajima, M., Yokoi, T., Mizutani, M., Shin, S., Kadlubar, F. F., and Kamataki, T. Phenotyping of CYP1A2 in Japanese population by analysis of caffeine urinary metabolites: absence of mutation prescribing the phenotype in the *CYP1A2* gene. Cancer Epidemiol. Biomark. Prev., *3:* 413–421, 1994.

14. Fuhr, U., and Rost, K. L. Simple and reliable CYP1A2 phenotyping by the paraxanthine/caffeine ratio in plasma and in saliva. Pharmacogenetics, *4:* 109–116, 1994.

15. Evans, D. A. P. *N*-Acetyltransferase. Pharmacol. Ther., *42:* 157–234, 1989.

16. Coles, B., and Ketterer, B. The role of glutathione and glutathione transferases in chemical carcinogenesis. Crit. Rev. Biochem. Mol. Biol., *25:* 47–70, 1990.

17. Peach, H., Ellard, G. A., Jenner, P. J. and Morris, R. W. A simple, inexpensive urine test of smoking. Thorax, *40:* 351–357, 1985.

18. Granella, M., Priante, E., Nardini, B., Bono, R., and Clonfero, E. Excretion of mutagens, nicotine and its metabolites in urine of cigarette smokers. Mutagenesis, *11:* 207–211, 1996.

19. Maron, D., and Ames, B. N. Revised methods for the *Salmonella* test. Mutat. Res., *113:* 173–215, 1983.

20. Watanabe, M., Ishidate, M., Jr., and Nohmi, T. Sensitive method for the detection of mutagenic nitroarenes and aromatic amines: new derivatives of *Salmonella typhimurium* tester strains possessing elevated *O*-acetyltransferase levels. Mutat. Res., *234:* 337–348, 1990.

21. Einisto, P., Watanabe, M., Ishidate, M., and Nohmi, T. Mutagenicity of 30 chemicals in *Salmonella typhimurium* strains possessing different nitroreductase or *O*-acetyltransferase activities. Mutat. Res., *259:* 95–102, 1991.

22. Berthou, F., Ratanasavanh, D., Alix, D., Carlhant, D., Riche, C., and Guillouzo, A. Comparison of caffeine metabolism by slices, microsomes and hepatocyte cultures from adult human liver. Xenobiotica, *19:* 401–417, 1989.

23. Campbell, M. E., Spielberg, S. P., and Kalow, W. A urinary metabolite ratio that reflects systemic caffeine clearance. Clin. Pharmacol. Ther., *42:* 157–165, 1987.

24. Tang, B. K., Kadar, D., Qian, L., Iriah, J., Yip, J., and Kalow, W. Caffeine as a metabolic probe: validation of its use for acetylation phenotyping. Clin. Pharmacol. Ther., *49:* 648–657, 1991.

25. Berthou, F., Guillois, B., Riche, C., Dreano, Y., Jacqz-Aigrain, E., and Beaunes, P. H. Interspecies variations in caffeine metabolism related to cytochrome P4501A2 enzymes. Xenobiotica, *22:* 671–680, 1992.

26. Hirvonen, A., Saarikoski, S. T., Linnainmaa, K., Koskinen, K., Husgafvel-Pursiainen, K., Mattson, K., and Vainio, H. Glutathione *S*-transferase and *N*-acetyltransferase genotypes and asbestos-associated pulmonary disorders. J. Natl. Cancer Inst. (Bethesda), *88:* 1853–1856, 1996.

27. Dixon, W. J. BMDP Statistical Software Manual. Berkeley, CA: University of California Press, 1992.

28. Einistö, P., Nohmi, T., Watanabe, M., and Ishidate, M., Jr. Sensitivity of *Salmonella typhimurium* YG1024 to urine mutagenicity caused by cigarette smoking. Mutat. Res., *245:* 87–92, 1990.

29. Hirvonen, A., Nylund, L., Kociba, P., Husgafvel-Pursiainen, K., and Vainio, H. Modulation of urinary mutagenicity by genetically determined metabolism in smokers. Carcinogenesis (Lond.), *15:* 813–815, 1994.

30. Smith, C. J., McKarns, S. C., Davis, R. A., Livingston, S. D., Bombick, B. R., Avalos, J. T., Morgan, W. T., and Doolittle, D. J. Human urine mutagenicity study comparing cigarettes which burn or primarily heat tobacco. Mutat. Res., *361:* 1–9, 1996.

31. Sesardic, D., Boobis, A. R., Edwards, R. J., and Davies, D. S. A form of cytochrome P450 in man, orthologous to form d in the rat, catalyses the *O*-deethylation of phenacetin and is inducible by cigarette smoking. Br. J. Clin. Pharmacol., *26:* 363–372, 1988.

32. Kalow, W., and Tang, B. K. Caffeine as a metabolic probe: exploration of the enzyme-inducing effect of cigarette smoking. Clin. Pharmacol. Ther., *49:* 44–48, 1991.

33. Rasmussen, B. B., and Brosen, K. Determination of urinary metabolites of caffeine for the assessment of cytochrome P4501A2, xanthine oxidase, and *N*-acetyltransferase activity in humans. Ther. Drug Monit., *18:* 254–262, 1996.

34. Tantcheva-Poor, I., Zaigler, M., Rietbrock, S., and Fuhr, U. Estimation of cytochrome P-450CYP1A2 activity in 863 healthy Caucasians using a saliva-based caffeine test. Pharmacogenetics, *9:* 131–144, 1999.

35. Nordmark, A., Lundgren, S., Cnattingius, S., and Rane, A. Dietary caffeine as a probe agent for assessment of cytochrome P4501A2 activity in random urine samples. Br. J. Clin. Pharm., *47:* 397–402, 1999.

36. Chung, W. G., Kang, J. H., Park, C. S., Cho, M. H., and Cha, Y. N. Effect of age and smoking on *in vivo* CYP1A2, flavin-containing monooxygenase, and xanthine oxidase activities in Koreans: determination by caffeine metabolism. Clin. Pharmacol. Ther., *67:* 258–266, 2000.

37. Pavanello, S., Simioli, P., Mastrangelo, G., Lupi, S., Gabbani, G., Gregorio, P., and Clonfero, E. Role of metabolic polymorphisms NAT2 and CYP1A2 on urinary mutagenicity after a pan-fried hamburger meal. Food Chem. Toxicol., *40:* 1139–1144, 2002.

38. Lampe, J. W., King, I. B., Li, S., Grate, M. T., Barale, K. V., Chen, C., Feng, Z. D., and Potter, J. D. Brassica vegetables increase and apiaceous vegetables decrease cytochrome P450 1A2 activity in humans: changes in caffeine metabolite ratios in response to controlled vegetable diets. Carcinogenesis (Lond.), *21:* 1157–1162, 2000.

39. Sinues, B., Saenz, M. A., Lanuza, J., Bernal, M. L., Fanlo, A., Juste, J. L., and Mayayo, E. Five caffeine metabolite ratios to measure tobacco-induced CYP1A2 activity and their relationships with urinary mutagenicity and urine flow. Cancer Epidemiol. Biomark. Prev., *8:* 159–166, 1999.

40. Lee, S., Jang, I., Shin, S. G., Lee, K. H., Yim, D. S., Kim, S. W., Oh, S. J., and Lee, S. H. CYP1A2 activity as a risk factor for bladder cancer. J. Korean Med. Sci., *9*: 482–489, 1994.

41. Brockmoller, J., Cascorbi, I., Kerb, R., Sachse, C., and Roots, I. Polymorphisms in xenobiotic conjugation and disease predisposition. Toxicol. Lett., *103:* 173–183, 1998.

42. Bartsch, H., Caporaso, N., Coda, M., Kadlubar, F., Malaveille, C., Talaska, G., Tannenbaum, S. R., and Vineis, P. Carcinogen hemoglobin adducts, urinary mutagenicity, and metabolic phenotype in active and passive cigarette smokers. J. Natl. Cancer Inst. (Bethesda), *82:* 1826–1831, 1990.

43. Sinuès, B., Pèrez, J., Bernal, M. L., Sáenz, M. A., Lanuza, J., and Bartolomé, M. Urinary mutagenicity and *N*-acetylation phenotype in textile industry workers exposed to arylamines. Cancer Res., *52:* 4885–4889, 1992.

44. Gabbani, G., Hou, S-M., Nardini, B., Marchioro, M., Lambert, B., and Clonfero, E. *GSTM1* and *NAT2* genotypes and urinary mutagens in coke oven workers. Carcinogenesis (Lond.), *17:* 1677–1681, 1996.

45. Hein, D. W., Rustan, T. D., Ferguson, R. J., Doll, M. A., and Gray, K. Metabolic activation of aromatic and heterocyclic *N*-hydroxyarylamines by wild-type and mutant recombinant human NAT1 and NAT2 acetyltransferases. Arch. Toxicol., *68:* 129–133, 1994.

46. Gabbani, G., Pavanello, S., Nardini, B., Tognato, O., Bordin, A., Veller-Fornasa, C., Bezze, G., and Clonfero, E. Influence of metabolic genotype *GSTM1* on levels of urinary mutagens in patients treated topically with coal tar. Mutat. Res., *440:* 27–33, 1999.

47. Connor, T. H., Ramanujam, V. M. S., Ward, J. B., and Legator, M. S. The identification and characterization of a urinary mutagen resulting from cigarette smoke. Mutat. Res., *113:* 161–172, 1983.

48. Putzrath, R. M., Langley, D., and Eisenstadt, E. Analysis of mutagenic activity in cigarette smokers' urine by high performance liquid chromatography. Mutat. Res., *85:* 97–108, 1981.

49. Mure, K., Hayatsu, H., Takeuchi, T., Takeshita, T., and Morimoto, K. Heavy cigarette smokers show higher mutagenicity in urine. Mutat. Res., *373:* 107–111, 1997.

50. Venier, P., Clonfero, E., Cottica, D., Gava, C., Zordan, M., Pozzoli, L., and Levis, A. G. Mutagenic activity and polycyclic aromatic hydrocarbon levels in urine of workers exposed to coal tar pitch volatiles in an anode plant. Carcinogenesis (Lond.), *6:* 749–752, 1985.

51. Clonfero, E., Jongeneelen, F., Zordan, M., and Levis, A. G. Biological monitoring of human exposure to coal tar. Urinary mutagenicity assays and analytical determination of polycyclic aromatic hydrocarbons metabolites in urine. *In*: H. Vainio, M. Sorsa, and A. J. McMichael (eds.), Complex Mixtures and Cancer Risk, IARC Sci. Pub. No. 104, pp. 215–222. Lyon, France: IARC, 1990.

52. Jongeneelen, F. J., Bos, R. P., Anzion, R. B., Theuws, J. L., and Henderson, P. T. Biological monitoring of polycyclic aromatic hydrocarbons. Metabolites in urine. Scand. J. Work Environ. Health, *12:* 137–143, 1986.

53. Heudorf, U., and Angerer, J. Urinary monohydroxylated phenanthrenes and hydroxypyrene: the effects of smoking habits and changes induced by smoking on monooxygenase-mediated metabolism. Int. Arch. Occup. Environ. Health, *74:* 177–183, 2001.

54. Granella, M., and Clonfero, E. Sensitivity of different bacterial assays in detecting mutagens in urine of humans exposed to polycyclic aromatic hydrocarbons. Mutat. Res., *268:* 131–137, 1992.

55. Smith, C., Payne, V., Doolittle, D. J., Debnath, A. K., Lawlor, T., and Hansch, C. Mutagenic activity of a series of synthetic and naturally occurring heterocyclic amines in *Salmonella*. Mutat. Res., *279:* 61–73, 1992.

56. Peluso, M., Castegnaro, M., Malaveille, C., Talaska, G., Vineis, P., Kadlubar, F. F., and Bartsch, H. ^{32}P-postlabelling analysis of DNA adducted with urinary mutagens from smokers of black tobacco. Carcinogenesis (Lond.), *11:* 1307–1311, 1990.

57. Bartsch, H., Malaveille, C., Friesen, M., Kadlubar, F. F., and Vineis, P. Smoking and urinary bladder cancer: molecular dosimetry studies in smokers of black and blond tobacco implicate aromatic amines. Eur. J. Cancer, *29A:* 1199–1207, 1993.

58. DeMarini, D. M., Shelton, M. L., and Levine, J. G. Mutation spectrum of cigarette smoke condensate in *Salmonella*: comparison to mutations in smoking-associated tumors. Carcinogenesis (Lond.), *16:* 2535–2542, 1995.

59. Mizusaki, S., Okamoto, H., Akiyama, A., and Fukuhara, Y. Relation between chemical constituents of tobacco and mutagenic activity of cigarette smoke condensate. Mutat. Res., *48:* 319–325, 1977.

60. Matsumoto, T., Yoshida, D., Mizusaki, S., and Okamoto, H. Mutagenicities of the pyrolyzates of peptides and proteins. Mutat. Res., *56:* 281–288, 1978.

61. DeBethizy, J. D., Borgerding, M. F., Doolittle, D. J., Robinson, J. H., McManus, K. T., Rahn, C. A., Davis, R. A., Burger, G. T., Hayes, J. R., and Reynolds, J. H. Chemical and biological studies of a cigarette that heats rather than burns tobacco. J. Clin. Pharmacol., *30:* 755–763, 1990.

62. White, J. L., Conner, B. T., Perfetti, T. A., Bombick, B. R., Avalos, J. T., Fowler, K. W., Smith, C. J., and Doolittle, D. J. Effect of pyrolysis temperature on the mutagenicity of tobacco smoke condensate. Food Chem. Toxicol., *39:* 499–505, 2001.

63. Clapp, W. L., Fagg, B. S., and Smith, C. J. Reduction in Ames *Salmonella* mutagenicity of mainstream cigarette smoke condensate by tobacco protein removal. Mutat. Res., *446:* 167–174, 1999.

64. Curvall, M., Romert, L., Norlen, E., and Enzell, C. R. Mutagen levels in urine from snuff users, cigarette smokers and non tobacco users. A comparison. Mutat. Res., *188:* 105–110, 1987.

65. Nagao, M., Honda, M., Seino, Y., Yahagi, T., and Sugimura, T. Mutagenicities of smoke condensates and the charred surfaces of fish and meat. Cancer Lett., *2:* 221–226, 1977.

66. Baker, R., Arlauskas, A., Bonin, A., and Angus, D. Detection of mutagenic activity in human urine following fried pork or bacon meals. Cancer Lett., *16:* 81–89, 1982.

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