Correlation between UDP-Glucuronosyltransferase Genotypes and 4-(Methylnitrosamo)-1-(3-Pyridyl)-1-Butanone Glucuronidation Phenotype in Human Liver Microsomes

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ABSTRACT

The nicotine-derived tobacco-specific nitrosamine, 4-(methylnitrosamo)-1-(3-pyridyl)-1-butane (NNK), is one of the most potent and abundant procarcinogens found in tobacco and tobacco smoke, and glucuronidation of its major metabolite, 4-(methylnitrosamo)-1-(3-pyridyl)-1-butanol (NNAL), is an important mechanism for 4-(methylnitrosamo)-1-(3-pyridyl)-1-butane detoxification. Significant interindividual variability in urinary NNAL glucuronide formation has been observed in smokers and tobacco chewers. To determine whether genetic variations may play a role in this interindividual variability, NNAL-glucuronidating activities were analyzed in 78 human liver microsomal specimens and compared with the prevalence of missense polymorphisms in the two major NNAL-glucuronidating enzymes UGT1A4 and UGT2B7. In vitro assays using liver microsomal specimens from individual subjects demonstrated a 70- and 50-fold variability in NNAL-N-Gluc and NNAL-O-Gluc formation, respectively, and a 20-fold variability in the ratio of NNAL-N-Gluc: NNAL-O-Gluc formation. Microsomes from subjects with a homozygous polymorphic UGT1A4*/4*/UGT1A4*/4* genotype exhibited a significantly higher (P < 0.05) level of NNAL-N-Gluc activity compared with microsomes from subjects with the wild-type UGT1A4*/4*/UGT1A4*/4* genotype, and a significantly higher (P < 0.05) number of subjects with liver microsomes having high NNAL-N-Gluc formation activity contained the UGT1A4*/4*/UGT1A4*/4* genotype. Microsomes from subjects with the homozygous polymorphic UGT2B7*12*/UGT2B7*12* genotype exhibited a significantly lower level (P < 0.025) of NNAL-O-Gluc activity when compared with microsomes from subjects with the wild-type UGT2B7*12*/UGT2B7*12* genotype, and a significantly higher (P < 0.05) higher number of subjects with liver microsomes having low NNAL-O-Gluc formation activity contained the UGT2B7*12*/UGT2B7*12* genotype. These data suggest that the UGT1A4 codon 24 and UGT2B7 codon 268 polymorphisms may be associated with altered rates glucuronidation and detoxification of NNAL in vivo.

INTRODUCTION

The nicotine-derived tobacco-specific nitrosamine 4-(methylnitrosamo)-1-(3-pyridyl)-1-butane (NNK) is one of the most potent and abundant procarcinogens found in tobacco and tobacco smoke (1, 2). NNK levels in tobacco smoke are 3–15 times higher than that of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), is an important mechanism for 4-(methylnitrosamino)-1-(3-pyridyl)-1-butane detoxification. Significant interindividual variability in NNAL glucuronide formation has been observed in smokers and tobacco chewers. To determine whether genetic variations may play a role in this interindividual variability, NNAL-glucuronidating activities were analyzed in 78 human liver microsomal specimens and compared with the prevalence of missense polymorphisms in the two major NNAL-glucuronidating enzymes UGT1A4 and UGT2B7. In vitro assays using liver microsomal specimens from individual subjects demonstrated a 70- and 50-fold variability in NNAL-N-Gluc and NNAL-O-Gluc formation, respectively, and a 20-fold variability in the ratio of NNAL-N-Gluc: NNAL-O-Gluc formation. Microsomes from subjects with a homozygous polymorphic UGT1A4*/4*/UGT1A4*/4* genotype exhibited a significantly higher (P < 0.05) level of NNAL-N-Gluc activity compared with microsomes from subjects with the wild-type UGT1A4*/4*/UGT1A4*/4* genotype, and a significantly higher (P < 0.05) number of subjects with liver microsomes having high NNAL-N-Gluc formation activity contained the UGT1A4*/4*/UGT1A4*/4* genotype. Microsomes from subjects with the homozygous polymorphic UGT2B7*12*/UGT2B7*12* genotype exhibited a significantly lower level (P < 0.025) of NNAL-O-Gluc activity when compared with microsomes from subjects with the wild-type UGT2B7*12*/UGT2B7*12* genotype, and a significantly higher (P < 0.05) higher number of subjects with liver microsomes having low NNAL-O-Gluc formation activity contained the UGT2B7*12*/UGT2B7*12* genotype. These data suggest that the UGT1A4 codon 24 and UGT2B7 codon 268 polymorphisms may be associated with altered rates glucuronidation and detoxification of NNAL in vivo.
preliminary studies has been linked to increased risk of for prostate cancer (26). In studies examining UGT family IA variants, the ‘TATA’ box polymorphism in the promoter region of UGT1A1, commonly associated with Gilbert’s syndrome, is associated with reduced function in the UGT1A1 transcripational promoter (22) and has been implicated in increased risk for breast cancer (27) and with decreased formation of the glucuronide conjugate of the important benz(a)pyrene metabolite, BaP-7,8-dihydrodiol-, in liver microsomes (28). Results from other studies suggest that UGT1A7-specific genetic variants are associated with reduced UGT1A7 metabolic function (23) and are strongly linked to increased risk for orolaryngeal cancer (29), as well as hepatocellular carcinoma (30), whereas a Glu>Lys polymorphism at codon 139 of the UGT1A10 gene has been linked to risk for orolaryngeal cancer (25).

The goal of the present study was to determine whether genetic variations in the two major NNAL-glucuronidating enzymes, UGT1A4 and UGT2B7, could potentially play a role in the interindividual variability observed in the levels of NNAL-N-Gluc and NNAL-O-Gluc formation in human liver microsomes (16) and potentially the variability in the ratios of both NNAL-Gluc:NNAL and NNAL-N-Gluc:NNAL-O-Gluc observed in the urine of smokers and snuff users (15, 17). In this article, results are presented demonstrating a correlation between a newly identified polymorphism in codon 24 (Pro->Thr) of the UGT1A4 gene and NNAL-N-Gluc formation and the codon 268 (His->Tyr) polymorphism in the UGT2B7 gene and NNAL-O-Gluc formation in human liver microsomes.

MATERIALS AND METHODS

Chemicals and Materials. UDPGA, D-t-2-lsophosphatidyl choline palmital C16:0, and β-glucuronidase were purchased from Sigma (St. Louis, MO). NNAL was obtained from Toronto Research Chemicals (Toronto, Ontario, Canada), 14C-UDPGA (specific activity: 300 mCi/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO), TaqDNA polymerase (H9252) from Innotech Corp., San Leandro, CA), whereas Geneticin was purchased from Medicult (Herndon, VA).

Tissues and Cell Lines. Normal human liver tissues and genomic DNA specimens were provided by the Tissue Procurement Facility at the H. Lee Moffitt Cancer Center from individuals (n = 78) undergoing surgery for resection of hepatocellular carcinoma, with matching tissue and DNA obtained from each subject. Tissue samples were quick-frozen at -70°C within 2 h after surgery. Liver microsomes were prepared through differential centrifugation as previously described (31) and were stored (10–20 mg protein/ml) at -70°C, with total protein concentrations measured using the BCA assay (Pierce Research). Dideoxy sequencing was performed at the DNA Sequencing Facility in the Department of Genetics at the University of Pennsylvania School of Medicine.

For dideoxy sequencing, PCR products were purified after electrophoresis in 1% agarose using the QIAEX II gel extraction kit (Qiagen, Valencia, CA). Dideoxy sequencing was performed at the DNA Sequencing Facility in the Department of Genetics at the University of Pennsylvania School of Medicine, using the same sense and antisense primers as were used for PCR amplification as well as an internal antisense primer (1AAS2; 5'-CACACAACCTAT-GAAGG-3') corresponding to nucleotides +983 through +1008 relative to the UGT1A4 translation start site.

PCR amplifications were routinely performed in a 50-μl reaction volume containing 100–250 ng of purified genomic DNA in 1X HotMaster Taq Buffer with 0.05 mM of each of deoxynucleotide triphosphates, 20 pmol of both sense and antisense UGT1A4 primers, and 2.5 units of TaqDNA polymerase. PCR amplifications were performed essentially as described above using UGT1A4- and UGT2B1-overexpressing cell line homogenates (1.5 mg of protein) or UGT1A4- or UGT2B1-overexpressing baculosomes or cell homogenates (10, 16, 33). UGT1A4- and UGT2B7 polymorphisms were assessed by PCR-RFLP analysis. PCR amplifications were performed essentially as described above using the following primers: 1AAS1 and 1AAS2 (UGT1A4) (producing a PCR-amplified product of 567 bp), and 2B7S1 (sense; 5'-TGCTACATCT-TCTAAACC-3') and 2B7AS1 (antisense; 5'-CTCTGGAATATCTGGACT-3') for UGT2B7 (producing a PCR-amplified product of 580 bp). PCR annealing temperatures were at 59°C and 58°C for UGT1A4 and UGT2B7, respectively. The UGT1A4 codon 24 polymorphism was examined by digestion with the HPY188III restriction enzyme (37°C, overnight), the UGT1A4 codon 48 polymorphism by digestion with the SnaI restriction enzyme (37°C, overnight).
and two bands (319 and 248 bp) for the wild-type analysis of levels of NNAL-Gluc formation for multiple genotypes. The results of these analyses are presented in Table 1.

Statistical Analysis. Bivariate analysis included the \( \chi^2 \) test or Fisher's exact test (used as appropriate) for examining differences in genotype frequencies and the Student's \( t \) test for the examination of continuous variables (i.e., levels of NNAL-Gluc formation). The \( \chi^2 \) test for trend was used for the analysis of levels of NNAL-Gluc formation for multiple genotypes.

RESULTS

Significant variability in total urinary NNAL-Gluc and urinary NNAL-N-Gluc:NNAL-O-Gluc ratios were observed between individual subjects in previous studies (15). To determine whether variability in NNAL-Gluc formation exists for human liver specimens from individual subjects, NNAL glucuronidation assays were performed for microsomes prepared from liver specimens from 78 different subjects. Significant variability in the ratio of NNAL-N-Gluc:NNAL-O-Gluc was observed for liver microsomes from individual subjects, with a ratio range of 0.16–3.2 (Table 1). The mean (±SD) NNAL-N-Gluc: NNAL-O-Gluc ratio for the 78 specimens was 1.61 ± 0.59. Representative chromatograms demonstrating this variability are shown in Fig. 1. Significant variability was also observed between liver microsomal specimens from individual subjects in terms of absolute levels of glucuronidated metabolites formed, with a range of 0.48–25.5 and 0.27–21.5 pmol/mg microsomal protein \(^{-1}\)min\(^{-1}\) for NNAL-N-Gluc and NNAL-O-Gluc, respectively.

Previous studies have shown that the hepatic enzyme, UGT1A4, is the only known UGT that exhibits NNAL-N-glucuronidating activity (16). To determine whether there are polymorphisms in the UGT1A4 gene that could contribute to the variability in NNAL-N-Gluc levels observed in liver microsomes from different individuals, dideoxy sequencing was performed on genomic DNA from liver specimens from the 10 subjects whose liver microsomes exhibited the five highest and five lowest levels of NNAL-N-Gluc formation. The family IA locus codes for 8 known functional UGT enzymes and is composed of divergent and individually regulated exon 1 sequences that transcribe for mRNAs that are alternatively spliced onto the 5'-end of the sequence encoded by the common UGT exons 2–5 region. Because UGT mRNAs consist of a unique region encoded by exon 1 and a region encoded by exons 2–5 that is common for all family IA UGTs, only UGT1A4 exon 1 was examined in this analysis.

In addition to one silent polymorphism at codon 268 (CGG>CCA), two missense polymorphisms were identified by DNA sequencing analysis: one at codon 24 (CCC>ACC) resulting in an amino acid change from Pro>Thr, and one at codon 48 (TTG>GTG) resulting in an amino acid change from Leu>Val (Fig. 2A). Both polymorphisms were identified in the high-activity NNAL-N-glucuronidating group and were confirmed by RFLP analysis, with the RFLP pattern consisting of three bands (266, 246, and 55 bp) for the UGT1A4<sup>24Pro</sup> variant and two bands (512 and 55 bp) for the wild-type UGT1A4<sup>24Pro</sup> allele (Fig. 2B) and one band (567 bp) for the UGT1A4<sup>48Val</sup> variant and two bands (319 and 248 bp) for the wild-type UGT1A4<sup>48Val</sup> allele (Fig. 2C).

To determine whether there is a potential link between UGT1A4 genotype and the variability in NNAL-N-Gluc activity observed in human liver microsomes, RFLP analysis was used to screen for the codon 24 polymorphism using DNA from the 78 liver specimens previously analyzed for NNAL glucuronidation. Eight (10%) of the 78 subjects were heterozygous for the UGT1A4<sup>24Thr</sup> variant, whereas an additional 2 subjects (3%) exhibited the homozygous polymorphic UGT1A4<sup>24Thr/UGT1A4<sup>24Thr</sup></sup> genotype (Table 2). The allelic frequency for the UGT1A4<sup>24Thr</sup> variant in this cohort was 0.077. Two of the 78 subjects were heterozygous for the UGT1A4<sup>48Val</sup> variant, whereas an additional subject exhibited the homozygous polymorphic

**Table 1 NNAL-N-Gluc and NNAL-O-Gluc formation in microsomes prepared from the livers of 78 individual subjects**

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
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<tbody>
<tr>
<td>NNAL-N-Gluc formation (pmol \cdot mg^{-1}\cdot min^{-1})</td>
<td>10.5 ± 5.8</td>
<td>0.48–25.5</td>
</tr>
<tr>
<td>NNAL-O-Gluc formation (pmol \cdot mg^{-1}\cdot min^{-1})</td>
<td>6.7 ± 1.3</td>
<td>0.27–21.5</td>
</tr>
<tr>
<td>Ratio of NNAL-N-Gluc to NNAL-O-Gluc</td>
<td>1.6 ± 0.59</td>
<td>0.16–3.21</td>
</tr>
</tbody>
</table>

Fig. 1. High-pressure liquid chromatography analysis of NNAL-Gluc formation by human liver microsomes. Human liver microsomes (0.5 mg of protein) were incubated at 37°C for 2 h with 5 mM NNAL and 4 mM \(^{14}\)C-UDPGA as described in “Materials and Methods.” A. \(^{14}\)C-labeled NNAL-N-Gluc and NNAL-O-Gluc standards. B and C, \(^{14}\)C-labeled metabolites from incubations using human liver microsomal specimens 13 and 30, respectively. D, \(^{14}\)C-labeled metabolites from incubations using liver microsomal specimen 13 after subsequent incubation with β-glucuronidase.
Table 2. NNAL-N-glucuronidation levels in liver microsomes in subjects stratified by UGT1A4 genotype

| UGT1A4 genotype | No. of subjects | NNAL-N-Gluc formation (pmol · min⁻¹ · mg⁻¹ protein) | NNAL-N-Gluc total NNAL-Gluc | Ratio | P
|------------------|-----------------|-----------------------------------------------|----------------------------|-------|-------
| Pro²⁴Thr         | 68              | 10.0 ± 5.7                                  | Referent                  | 0.59 ± 0.12      | Referent
| Pro²⁴Pro         | 8               | 13.2 ± 5.5                                   | 0.13                      | 0.56 ± 0.10      | 0.98
| Thr²⁴Thr         | 2               | 16.3 ± 4.1                                   | 0.12                      | 0.67 ± 0.13      | 0.33
| Pro²⁴Thr + Thr²⁴Thr | 10             | 13.9 ± 5.2                                   | 0.047                     | 0.61 ± 0.10      | 0.66

a Levels of NNAL-N-Gluc formation determined for 78 subjects for whom UGT1A4 genotype could be obtained.

b A significant (P < 0.001) trend towards increasing NNAL-N-Gluc formation was observed with UGT1A4 genotypes containing increasing numbers of variant UGT1A4²⁴Thr alleles as determined by trend test.

c P-values determined by Student’s t test, with comparisons made versus wild-type UGT1A4 Pro²⁴Pro genotype.

d Numbers represent means ± SD for three experiments.
Table 3  UGT1A4 genotypes and alleles versus NNAL N-glucuronidation phenotype

<table>
<thead>
<tr>
<th>NNAL-N-glucuronidating phenotype</th>
<th>UGT1A4 genotype</th>
<th>UGT1A4 allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pro&lt;sup&gt;24Pro&lt;/sup&gt;</td>
<td>Pro&lt;sup&gt;24Thr&lt;/sup&gt; + Thr&lt;sup&gt;24Thr&lt;/sup&gt;</td>
</tr>
<tr>
<td>Slow</td>
<td>37</td>
<td>2</td>
</tr>
<tr>
<td>Rapid</td>
<td>31</td>
<td>8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>10</td>
</tr>
</tbody>
</table>

<sup>a</sup> Subjects were stratified at the median level of NNAL-N-Glc formation.
<sup>b</sup> A significantly (P = 0.017) higher number of subjects with the rapid NNAL-N-glucuronidating phenotype contained at least one UGT1A4<sup>24Thr</sup> allele as compared with subjects with the slow NNAL-N-glucuronidating phenotype as determined by the χ² test.
<sup>c</sup> A significantly (P = 0.007) higher number of UGT1A4<sup>24Thr</sup> alleles were from subjects with the rapid NNAL-N-glucuronidating phenotype as compared with subjects with the slow NNAL-N-glucuronidating phenotype as determined by the χ² test.
<sup>d</sup> Two of these subjects were homozygous for the UGT1A4<sup>24Thr</sup> allele. All other subjects in this study with at least one UGT1A4<sup>24Thr</sup> allele were heterozygous.

The UGTs are a superfamily of enzymes that glucuronidate many xenobiotics and endogenous compounds (36). Both the O- and N-glucuronides of NNAL-Glc have been observed in the urine of smokers (15, 17, 37), never-smokers exposed to environmental tobacco smoke (38, 39), as well as tobacco chewers (15, 40), and both glucuronides were shown to be formed in human liver microsomes (10, 16). Significant interindividual variability in the ratios of both NNAL-Glc:NNAL (15) was observed in the urine of smokers and snuff users, suggesting that individuals may differ greatly in their ability to detoxify NNK and forming different N-glucuronidases. In addition, variability in the levels of NNAL-N-Glc and NNAL-O-Glc formation was also observed in in vitro assays of human liver microsomal specimens (16). In the present study, large differences in the ratio of NNAL-N-Glc:NNAL-O-Glc, as well as in the absolute levels of NNAL-N-Glc and NNAL-O-Glc were observed in in vitro assays of a series of liver samples.

Table 4  NNAL O-glucuronidation levels in liver microsomes in subjects stratified by UGT2B7 genotype

<table>
<thead>
<tr>
<th>UGT2B7 genotype</th>
<th>No. of subjects</th>
<th>NNAL-O-Glc formation&lt;br&gt;pmol · mg&lt;sup&gt;-1&lt;/sup&gt; · min&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
<th>NNAL-O-Glc/total NNAL-Glc</th>
<th>Ratio</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>His&lt;sup&gt;268&lt;/sup&gt;His</td>
<td>18</td>
<td>7.3 ± 2.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Referent</td>
<td>0.39 ± 0.10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Referent</td>
<td>0.18 ± 0.08&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>His&lt;sup&gt;268&lt;/sup&gt;Tyr</td>
<td>38</td>
<td>7.1 ± 3.9</td>
<td>0.77</td>
<td>0.41 ± 0.11</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Tyr&lt;sup&gt;268&lt;/sup&gt;Tyr</td>
<td>18</td>
<td>5.3 ± 2.3</td>
<td>0.02</td>
<td>0.41 ± 0.14</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>His&lt;sup&gt;268&lt;/sup&gt;Tyr + Tyr&lt;sup&gt;268&lt;/sup&gt;Tyr</td>
<td>56</td>
<td>6.5 ± 3.5</td>
<td>0.36</td>
<td>0.41 ± 0.12</td>
<td>0.49</td>
<td></td>
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</tbody>
</table>

<sup>a</sup> Levels of NNAL-O-Glc formation determined for 74 subjects from whom UGT2B7 genotype could be obtained.<n> <sup>b</sup> P-values determined by Student’s t test, with comparisons made versus UGT2B7 His<sup>268</sup>His genotype.<n> <sup>c</sup> Numbers represent means ± SD for three experiments.

Table 5  UGT2B7 genotypes and alleles versus NNAL O-glucuronidating phenotype

<table>
<thead>
<tr>
<th>NNAL-O-glucuronidating phenotype&lt;sup&gt;a&lt;/sup&gt;</th>
<th>UGT2B7 genotype&lt;sup&gt;b&lt;/sup&gt;</th>
<th>UGT2B7 allele&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>His&lt;sup&gt;268&lt;/sup&gt;His</td>
<td>His&lt;sup&gt;268&lt;/sup&gt;Tyr</td>
</tr>
<tr>
<td>Lowest</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Low</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>High</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Highest</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>38</td>
</tr>
</tbody>
</table>

<sup>a</sup> Subjects were stratified by quartiles according to levels of NNAL-O-Glc formation.<n> <sup>b</sup> A significantly (P = 0.0044) higher number of subjects from the lowest NNAL-N-glucuronidating activity group contained the UGT2B7<sup>268Tyr</sup>/UGT2B7<sup>268Tyr</sup> genotype as compared to subjects from the highest NNAL-N-glucuronidating activity group as determined by the Fisher’s exact test.<n> <sup>c</sup> A significantly (P = 0.017) higher number of variant UGT2B7<sup>268Tyr</sup> alleles were from subjects from the lowest NNAL-N-glucuronidating activity group as compared with subjects from the highest NNAL-N-glucuronidating activity group as determined by the χ² test.
results from our laboratory demonstrating that the variant UGT2B7Tyr test substrates (41). These results are also consistent with preliminary N-glucuronidates NNAL to form the glucuronide. Individual subjects in this study. These data are consistent with the individual UGT2B7 variants as determined by the glucuronidation activities, respectively, in liver microsomes from the present study, a larger study will be necessary to evaluate the role of this polymorphism on liver microsomal glucuronidation activities.

Both the UGT2B7 codon 268 and UGT1A4 codon 24 polymorphisms were significantly associated with NNAL-O-Gluc and NNAL-N-Gluc-forming activities, respectively, in liver microsomes from individual subjects in this study. These data are consistent with the fact that UGT2B7 glucuronidates NNAL to form the O-glucuronide, whereas UGT1A4 glucuronidates NNAL to form the N-glucuronide. The phenotype:genotype correlation observed in the present study for the UGT2B7 codon 268 polymorphism is consistent with the decrease in glucuronidating activity observed for cell lines overexpressing the UGT2B7 variants as determined by in vitro assays against a variety of test substrates (41). These results are also consistent with preliminary results from our laboratory demonstrating that the variant UGT2B7^2Thr isoform exhibits decreased NNAL-glucuronidating activity as compared with the wild-type UGT2B7^*10 in in vitro assays using cell homogenates overexpressing UGT2B7 variants. However, studies by Bhasker et al. (35) have shown that the rates of human liver microsomal glucuronidating activity against androsterone, menthol, and morphine were not linked to UGT2B7 genotype. These results may be due to cross-reactivity of other hepatic UGTs against these same compounds, a pattern observed by Fang et al. (28) for the UGT1A1*28 allelic variant and altered liver microsomal glucuronidating activity against (-)-BaP-7,8-dihydroni. In vitro studies will be required to confirm the functional significance of the UGT1A4 codon 24 polymorphism.

In conclusion, the results from the present study demonstrate a clear relationship between genetic variations in UGT genes and resulting tissue activities against NNAL, the major carcinogenic metabolite of the potent and abundant tobacco carcinogen, NNK. A potential role for either UGT2B7 or UGT1A4 genotype has as yet to be defined for organs/tissues that are targets for NNK exposure (e.g., tobacco-related cancers). The effect of UGT2B7 or UGT1A4 genotype on cancer risk may be most pronounced for those organs/tissues where there is exposure to NNK and where these UGTs are expressed. Although no significant expression of UGT2B7 was observed in the aerodigestive tract (42), low levels of expression of UGT2B7 were observed in lung (10, 43). Although previous studies have shown no significant level of expression of UGT1A4 in either the aerodigestive tract or lung (42), a hepatic UGT1A4 (or UGT2B7) genotype-associated effect on overall NNK detoxification and cancer risk cannot be excluded for these organ/tissue sites. Additional studies examining the role of UGT2B7 and UGT1A4 genotype on susceptibility to tobacco-related cancers will be necessary to better evaluate the role of UGT gene variations on cancer risk.

ACKNOWLEDGMENTS

We thank Marek Wolek and the staff at the Tissue Procurement Facility at the H. Lee Moffitt Cancer Center for providing tissue specimens and corresponding genomic DNA and medical chart data from the same subjects.

REFERENCES


1196
Corrections

In the article by A-K. Olsson et al., titled “A Fragment of Histidine-Rich Glycoprotein Is a Potent Inhibitor of Tumor Vascularization,” which appeared in the January 15, 2004 issue of Cancer Research (pp. 599–605), the color contrast in Figure 6B was insufficient to illustrate the authors’ findings. The correct figure appears below.

In the article by D. Wiener et al., titled “Correlation between UDP-Glucuronosyltransferase Genotypes and 4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanone Glucuronidation Phenotype in Human Liver Microsomes,” which appeared in the February 1, 2004 issue of Cancer Research (pp. 1190–1196), the titular phenotype should have been identified as butanol. The correct title is “Correlation between UDP-Glucuronosyltransferase Genotypes and 4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanol Glucuronidation Phenotype in Human Liver Microsomes.”

Fig. 6. Paxillin in focal adhesions is affected by histidine-rich glycoprotein (HRGP) treatment. A, cells were treated with HRGP (100 ng/ml), as indicated, and paxillin was immunoprecipitated from the cell lysate. Immunoblotting was performed for paxillin (top panel) and phospho-tyrosine (middle panel). To verify equal loading, the cell lysate was blotted for β-actin (bottom panel). IP, immunoprecipitation. B, bovine adrenal cortex capillary endothelial cells treated with vascular endothelial growth factor (VEGF, 10 ng/ml), fibroblast growth factor (FGF)-2 (10 ng/ml), and HRGP (100 ng/ml) as indicated were fixed after 10 min and stained with anti-paxillin antibody (green). Nuclei (blue) were stained by Hoechst 33342. Bar indicates 20 μm.
Correlation between *UDP-Glucuronosyltransferase* Genotypes and 4-(Methylnitrosamino)-1-(3-Pyridyl)-1-Butanone Glucuronidation Phenotype in Human Liver Microsomes

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