Genetic Variants of Human UGT1A3: Functional Characterization and Frequency Distribution in a Chinese Han Population

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Received February 9, 2006; accepted May 30, 2006

ABSTRACT:

UDP-glucuronosyltransferase 1A3 (UGT1A3) contributes to glucuronidation of many important endogenous compounds and xenobiotics, including some flavonoids. Recently, a total of six single nucleotide polymorphisms (SNPs) have been identified in the human UGT1A3 gene. Among them, four SNPs (A17G, Q6R; T31C, W11R; C133T, R45W; and T140C, V47A) cause amino acid substitutions. Variants caused by these SNPs showed an activity change in estrone metabolism, whereas their activities toward other substrates were not examined. In the present study, three common flavonoids, quercetin, luteolin, and kaempferol, were used as substrates for glucuronidation by wild-type and variant UGT1A3s. Our results demonstrated that the activities of three variants, UGT1A3.2, UGT1A3.3, and UGT1A3.5, were remarkably lower than that of UGT1A3.1. In contrast, UGT1A3.4 exhibited an increase in glucuronidation efficiency of approximately 4 times and a clear preference to quercetin 7- and 3-hydroxyl groups. The frequency distributions of UGT1A3 alleles and SNPs in UGT1A3 in a Chinese Han population were statistically different from the reported value in German-Caucasians (p < 0.05). UGT1A3 variants have an altered glucuronidation activity toward quercetin, luteolin, and kaempferol and may alter human susceptibility to flavonoid exposure.

UDP-glucuronosyltransferase 1A3 (UGT1A3) is a phase II drug metabolism enzyme localized in the endoplasmic reticulum and the nuclear envelope (Radominska-Pandya et al., 1999). It catalyzes the conversion of hydrophobic endogenous compounds and xenobiotics to hydrophilic glucuronides and hence plays a major role in metabolism and detoxification (Miners and Mackenzie, 1991; Wells et al., 2004).

The UGT genes are highly polymorphic. Recently, Ehmer et al. (2004) and Iwai et al. (2004) have identified six novel single nucleotide polymorphisms (SNPs) in exon 1 of human UGT1A3 gene (Fig. 1). Of these, four cause amino acid substitutions (A17G, Q6R; T31C, W11R; C133T, R45W; and T140C, V47A), and the remaining two are silent (G81A, E27E and A477G, A159A). The missense variants showed a significant difference in their estrone metabolism activity (Iwai et al., 2004). Considering a wide substrate spectrum of UGT1A3 (Green et al., 1998), and the fact that the activity alteration for a given enzyme could be substrate-specific, it is worth conducting a further functional evaluation of these UGT1A3 variants with other important substrates.

Flavonoids are dietary antioxidants widely existent in fruits, vegetables, cereals, dry legumes, chocolate, and beverages (Scalbert et al., 2000). Experimental studies support an important role of flavonoids in the prevention of cardiovascular diseases and cancers (Galati et al., 2000; Kris-Etherton and Keen, 2002; Vita, 2005). Since glucuronidation is an essential pathway for the elimination and biological activity of flavonoids (da Silva et al., 1998), genetic polymorphisms of UGTs are potentially of toxicological and physiological importance on the pharmacokinetics, pharmacologic effects, and toxicity of flavonoids (Wittig et al., 2001). In the present study, wild-type and variant human UGT1A3s were expressed in a Bac-to-Bac baculovirus expression system. Three common flavonoids, quercetin, luteolin, and kaempferol, were used as substrates to characterize their glucuronidating capacity. Regioselectivity in quercetin metabolism by these UGT1A3 variants was also observed. Finally, the frequency distribution of UGT1A3 polymorphisms in a Chinese Han population was reported in this work.

Materials and Methods

Materials. Polymerase chain reaction (PCR) primers and a blood genomic DNA isolation kit were obtained from Sangon (Shanghai, China). Restriction endonucleases, DNA molecular marker, and T4 ligase were obtained from Promega (Madison, WI). A MutanBest kit was obtained from Takara (Tokyo, Japan). Cellfectin reagent, pFastBac1 vector, DH10Bac-competent cells, and Grace’s medium were obtained from Invitrogen (Calsbad, CA). Spodoptera frugiperda Sf9 insect cells were kind gifts from YangShengTang Pharmaceutical (Hangzhou, China). Fetal bovine serum was purchased from Hyclone (Logan, UT). Mouse anti-his antibody and peroxidase-conjugated goat anti-mouse secondary antibody were supplied by GE Healthcare Bio-Sciences (Little Chalfont, Buckinghamshire, UK) and Zhongshan Biotechnology (Guangdong, China), respectively. The polyvinylidene fluoride (PVDF) membrane was acquired.
from AMRESCO (Solon, OH). Uridine diphosphate glucuronic acid (UDPGA), d-saccharic acid 1,4-lactone monohydrate, Brij 58, alamethicin, and β-glucuronidase were provided by Sigma (St. Louis, MO). Quercetin, luteolin, and kaempferol were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), and their purities were 99%.

**Construction of UGT1A3 Variants.** All five reported UGT1A3 enzymes were constructed in the present study: UGT1A3.1, UGT1A3.2, UGT1A3.3, UGT1A3.4, and UGT1A3.5. A UGT1A3*1 gene had been obtained in our laboratory previously, with an AflII site and a XhoI site at two sides of the coding sequence and a his-tag ahead of the stop codon. In this research, using pGEM-T-UGT1A3*1 as the template (Chen et al., 2005a), UGT1A3 alleles were generated by site-directed mutagenesis following the instructions of the MutanBest kit. Oligonucleotide primers for introducing nucleotide transversions are shown in Table 1. To ensure that no mistake happened during the mutagenesis, all PCR products were verified by DNA sequencing.

**Expression of UGT1A3s.** Expression of UGT1A3s was performed in an insect Bac-to-Bac system as we described previously (Chen et al., 2005a). In brief, the UGT1A3 gene was excised from the pGEM-T vector, subcloned into the pFastBac1 vector, and transformed into *Escherichia coli* DH10Bac. PCR with UGT1A3-specific primer and M13 primer was used to confirm the transposition of UGT1A3 into the bacmid. The minipreparation of bacmid-UGT1A3 was transferred into S9 cells. After 72 h, the first passage of the bacmid was transfected into Sf9 cells. After 72 h, the first passage of UGT1A3/H11032-diaminobenzidine detection solution for 10 min. The relative expression was transferred onto a PVDF membrane, and probed with the mouse anti-his antibody. In the same loading amount was separated by a 12% SDS-polyacrylamide gel, UGT1A3.1 preparation as a standard. The cell homogenate of each UGT1A3 enzyme, a semiquantitative Western blot method was established using the GeneQuant (GE Healthcare Bio-Sciences) at 280 nm and stored at 80°C until further analysis. Transfection was used at a flow rate of 1 ml/min, with 52%, 52%, or 54% methanol (volume percentage) in 0.02 M phosphoric acid (pH 2.0) for quercetin, luteolin, and kaempferol, respectively. Peaks were detected at 368 nm for quercetin, 350 nm for luteolin, and 365 nm for kaempferol. Glucuronidases were identified with an Agilent 1100 liquid chromatography-mass spectrometry system (Agilent Technologies, Palo Alto, CA) equipped with an electrospray ionization source and operated in a negative ion mode. The enzymatic kinetic assay of each UGT1A3 enzyme was conducted under conditions linear with time and protein concentration. Kinetic parameters were calculated from three independent expressions according to our previous work (Chen et al., 2005a).

**Glucuronidation Assay.** Some preliminary experiments were performed to determine the optimal incubation condition. The effects of pH value (7.0, 7.4, 7.8, 8.0, 8.2, and 8.6), β-glucuronidase inhibition reagent (d-saccharic acid 1,4-lactone monohydrate), and de-lacteny reagents (alamethicin and Brij 58) on the glucuronidation assay were evaluated. A typical incubation mixture (100 μl of total volume) contained 100 mM Tris-HCl buffer, pH 8.0, 10 mM MgCl2, 2 mM UDPGA [the UDPGA apparent *Km* is 200 μM–300 μM with the various UGT isofoms as previously determined (Green et al., 1998)], 15 μg/mg pro alamethicin, 5 mM d-saccharic acid 1,4-lactone monohydrate, 0.5 mg/ml cell homogenate, and 0.1 mM flavonoids. The reaction was initiated by adding UDPGA, incubated at 37°C, and terminated with 290 μl of methanol. After adding an internal standard (morin for quercetin, quercetin for kaempferol, and kaempferol for luteolin), the mixture was centrifuged to remove precipitated protein. Twenty-five microliters of supernatant were injected into an LC-10A HPLC system (Shimadzu, Kyoto, Japan), equipped with two LC-10AD pumps, a UV detector, and a Diamonsil C18 column (5-μm particle size, 250 mm × 4.6 mm). Data acquisition and integration were performed using an H5200 chromatography workstation. The mobile phase was used at a flow rate of 1 ml/min, with 52%, 52%, or 54% methanol (volume percentage) in 0.02 M phosphoric acid (pH 2.0) for quercetin, luteolin, and kaempferol, respectively. Peaks were detected at 368 nm for quercetin, 350 nm for luteolin, and 365 nm for kaempferol. Glucuronides were identified with an Agilent 1100 liquid chromatography-mass spectrometry system (Agilent Technologies, Palo Alto, CA) equipped with an electrospray ionization source and operated in a negative ion mode. The enzymatic kinetic assay of each UGT1A3 enzyme was conducted under conditions linear with time and protein concentration. Kinetic parameters were calculated from three independent expressions according to the Lineweaver-Burk equation. (For detailed concentrations of each tested flavonoid, see Tables 3–5.)

**Regioselectivity of UGT1A3s to Quercetin.** Regioselectivity of UGT1A3 variants to quercetin was measured using enzymatic kinetic parameters according to our previous work (Chen et al., 2005a). In brief, each monoglucuronide was separated and collected from the HPLC effluent and quantified by determination of the amount of quercetin released after hydrolysis. The iden-

**TABLE 1**

Oligonucleotide primers for site-directed mutagenesis using UGT1A3*1-pGEM-T as template

<table>
<thead>
<tr>
<th>Mutant nucleotides in italic and bold.</th>
<th>SNPs</th>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>A17G</td>
<td>CCGTTGCCCGCGCA</td>
<td>GAATGCCTGGGCAATCTGTAG</td>
<td></td>
</tr>
<tr>
<td>T31C</td>
<td>TCCCCCGCGCCGCGGCA</td>
<td>ACCCGAGTGCCTGCAGCCTCA</td>
<td></td>
</tr>
<tr>
<td>C133T</td>
<td>GCATGTCGGAAGCTTCGCGGGA</td>
<td>TGGACGAGTGCCTGCAGCAT</td>
<td></td>
</tr>
<tr>
<td>T140C</td>
<td>ATGCGGGAAGGCTTCGCGGGA</td>
<td>GCTGAGCAGTGCTGCAGC</td>
<td></td>
</tr>
</tbody>
</table>
PCRs, respectively. PCR was performed in 20 allele-specific primers (Table 2) was designed and added into two parallel experiments. At the first step, genomic DNA was amplified for 20 cycles with two PCR products were chosen for mutation detections. An aliquot of 2.5 ml of venous blood was drawn from each volunteer into a tube containing 0.3 ml of sodium citrate and stored at 20°C until further analysis. Genomic DNA was extracted with a UNIQ-10 column blood isolation kit, following the manufacturer’s specifications, and treated with sodium methoxide solution and unmelted so-
orothoperoxidase-conjugated anti-mouse antibody (1:5000) was used as the secondary antibody. Immunocomplexes were exposed to 3,3’-diaminobenzidine detection solution for 10 min. The relative expression of UGT1A3s was measured with a universal hong image system (Bio-Rad Laboratories) using Quantity One 1-D analysis software. Typical yields of DNA were determined with UV spectra in methanol and some diagnostic reagents (sodium methoxide solution and unmelted sodium acetate solution).

**DNA Isolation.** One hundred twenty-five unrelated healthy Chinese Han adults were included in this study. All these recruitments were from the clinical laboratory in the Second Affiliated Hospital of Zhejiang University. The research protocol was approved by the Ethics Committee of Zhejiang University. An aliquot of 2.5 ml of venous blood was drawn from each volunteer into a tube containing 0.3 ml of sodium citrate and stored at –80°C before DNA isolation. Genomic DNA was extracted with a UNIQ-10 column blood genomic DNA isolation kit, following the manufacturer’s specifications, and quantified by measuring absorbance at 260 nm and 280 nm with a GeneQuant spectrophotometer (GE Healthcare Bio-Sciences). Typical yields of DNA were 15 ng/μl~25 ng/μl, and A260/A280 ratios were 1.7~1.8. Samples were stored in the Tris-EDTA buffer (pH 8.0) at –20°C until further analysis.

**Distributions of UGT1A3 Alleles and SNPs in UGT1A3 in a Chinese Han Population.** Two-step allele-specific amplification (ASA) was developed for mutation detections. At the first step, genomic DNA was amplified for 20 cycles with two UGT1A3-specific primers: AAGAAAAGCAATGTA- CAGGC/TACCTTATTTCCCACCCACTTC. Ten PCR products were chosen randomly for sequencing and as positive controls for the second step. The second amplification was performed following an allele specific procedure. For each SNP in UGT1A3, two parallel reactions were performed. A pair of allele-specific primers (Table 2) was designed and added into two parallel PCRs, respectively. PCR was performed in 20 μl of reaction mixture containing 1.75 mM MgCl2, 0.2 mM dNTPs, 0.3 μM each primer (Table 2), 0.1 μl of product of the first step, 0.5 U of TaqDNA polymerase. After an initial denaturation at 94°C for 2 min, the DNA was amplified by 25 cycles of 20 s at 94°C and 60 s at an optimized temperature (Table 2). All PCR processes were performed on a Mastercycler gradient (Eppendorf, Hamburg, Germany). Special attention was paid to keep the same load and reaction conditions for two parallel PCRs. After PCR, amplification products were electrophoresed on a 1.5% agarose gel containing ethidium bromide. The images were scanned and analyzed with a universal hong gel image system (Bio-Rad Laboratories). Predicted lengths of fragments are also shown in Table 2. For the second step, 10 positive controls (sequenced samples) and 2 negative controls (reactions containing no template) were performed. The result of analysis of each example was agreed to by two individual operations.

**Data Analysis.** SNP frequencies were calculated by gene counting. Student’s t test was used for statistical comparisons. A value of p < 0.05 was considered to be statistically significant. All tests in the present study were two-sided.

### Results

**Expression of UGT1A3s.** In the present study, five UGT1A3 sequences were obtained by mutagenesis and confirmed by sequencing. Their amino acid sequences correspond to those of all known UGT1A3s in the population: UGT1A3.1, UGT1A3.2, UGT1A3.3, UGT1A3.4, and UGT1A3.5. To investigate the catalytic activity of the UGT1A3 variants in vitro, we established a Bac-to-Bac insect expression system, which has been used for functional characterization of many UGT alleles (Jinno et al., 2003) and applied in our laboratory for expression of UGT1A3, UGT1A4, UGT1A6, UGT1A9, and UGT2B7 (Qian et al., 2004; Zheng et al., 2004; Chen et al., 2005a). The optimized expression conditions were a multiplicity of infection value of 5 and an infection time of 72 h. Typical concentrations of cell homogenates were 15 mg/ml~18 mg/ml. Expressions were finally confirmed by Western blot. We used anti-his as the primary antibody, so that the immunoreactivity of UGT1A3s would not change with mutations. The linear range of Western quantification was 0.2 mg~0.5 mg of cell homogenate. We blotted one sample of each UGT1A3 on the same membrane. Three individual membranes

### Table 2

ASA conditions for detection of each SNP in UGT1A3

<table>
<thead>
<tr>
<th>SNPs Allele-Specific Primer</th>
<th>Allele-Nonspecific Primer</th>
<th>Annealing and Extension Temperature</th>
<th>Length of Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 AGATGGCCACAGGCTCTCAGAGGACGAGCTTT</td>
<td>AGCCACACAGGACGAGCTTT</td>
<td>68.5</td>
<td>bp</td>
</tr>
<tr>
<td>31 CGACCTTGGCAGCAAGGACGAGCTTT</td>
<td>AGGCCACAGGACGAGCTTT</td>
<td>68.5</td>
<td>391</td>
</tr>
<tr>
<td>81 CGACCAACACCTTTCCAGCCACACCAACCTT</td>
<td>AGGCAACACCTTTCCAGCCACACCAACCTT</td>
<td>68.5</td>
<td>289</td>
</tr>
<tr>
<td>133 CGGCACAGCTCCACGGCAGGACGAGCTTT</td>
<td>AGGCCACAGCTCCACGGCAGGACGAGCTTT</td>
<td>68.5</td>
<td>339</td>
</tr>
<tr>
<td>140 CGCACTCTGGCAGCAAGGACGAGCTTT</td>
<td>AGGCCACAGCTCTGGCAGCAAGGACGAGCTTT</td>
<td>68.5</td>
<td>388</td>
</tr>
<tr>
<td>470 CTCAGCTCTGCAAGGACGAGCTTT</td>
<td>AGGCCACAGCTCTGCAAGGACGAGCTTT</td>
<td>68.5</td>
<td>268</td>
</tr>
<tr>
<td>72.8</td>
<td>387</td>
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</table>

### Table 3

Kinetic parameters of UGT1A3 enzymes to quercetin (n = 3)

<table>
<thead>
<tr>
<th>UGT1A3 Enzymes</th>
<th>Fm</th>
<th>Vmax</th>
<th>Vmax/Km</th>
<th>Percentage of UGT1A3</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT1A3.1</td>
<td>39.40 ± 2.10</td>
<td>1974 ± 65</td>
<td>50.19 ± 1.42</td>
<td>100</td>
</tr>
<tr>
<td>UGT1A3.2</td>
<td>16.34 ± 1.35</td>
<td>90.30 ± 1.19</td>
<td>5.55 ± 0.38</td>
<td>11</td>
</tr>
<tr>
<td>UGT1A3.3</td>
<td>39.56 ± 1.64</td>
<td>450.9 ± 7.0</td>
<td>11.41 ± 0.30</td>
<td>23</td>
</tr>
<tr>
<td>UGT1A3.4</td>
<td>21.00 ± 1.82</td>
<td>4877 ± 237</td>
<td>233.0 ± 15.9</td>
<td>464</td>
</tr>
<tr>
<td>UGT1A3.5</td>
<td>11.05 ± 1.00</td>
<td>427.6 ± 31.6</td>
<td>38.76 ± 1.23</td>
<td>77</td>
</tr>
</tbody>
</table>
was linear with time up to 90 min. The apparent $K_m$, $V_{max}$, and catalytic efficiencies ($V_{max}/K_m$) shown in Tables 3 to 5 represent a composite of all glucuronides formed using quercetin, luteolin, or kaempferol as substrate. Glucuronidation activities of 100, 11, 23, 464, and 77% to quercetin were observed for UGT1A3.1, UGT1A3.2, UGT1A3.3, UGT1A3.4, and UGT1A3.5, respectively. It suggests statistically significant differences among five UGT1A3s ($p < 0.05$). The UGT1A3.4 also improved the function in glucuronidation of luteolin and kaempferol, with a relative efficiency ($V_{max}/K_m$) approximately 4 times that of UGT1A3.1, whereas UGT1A3.2 and UGT1A3.3 led to a considerable decrease of glucuronidation, to less than 20%. It explained the reason why only one glucuronide was identified in our previous activity assay of quercetin with UGT1A3.2 (Chen et al., 2005b). Compared with UGT1A3.2 and UGT1A3.3, UGT1A3.5 showed a relatively mild decrease in glucuronidation efficiency for all tested flavonoids.

It is interesting that regioselectivities of UGT1A3s to hydroxyl groups in quercetin were also significantly different (Table 6). UGT1A3.3 showed an obvious preference to 3'-glucuronidation. It catalyzed 3'-glucuronidation with more than 5 times the efficiency of 7-glucuronidation. UGT1A3.2, UGT1A3.4, and UGT1A3.5 produced much less 4'-glucuronide and 3'-glucuronide than 7-glucuronide and 3-glucuronide. Especially for the latter two, almost no 4'-glucuronide and 3'-glucuronide were detected after incubation.

**Distributions of UGT1A3 Alleles and SNPs in UGT1A3 in a Chinese Han Population.** The 125 genomic DNA samples from whole blood were screened by ASO to examine the distribution frequency of UGT1A3 alleles and SNPs in UGT1A3 in a Chinese Han population (Tables 7 and 8). Sequences of 10 positive controls were identical with the results of sequencing. The prevalence of T31C, G81A, and T140C mutants was 26.8%, 26.8%, and 10.4% of donors, respectively, percentages that are significantly different from those reported for German-Caucasians ($p < 0.05$) (Ehmer et al., 2004). German-Caucasians have much higher distribution frequencies (65% to T31C, 65% to G81A, and 58% to T140C) than the Chinese Han population has, whereas the other three SNPs (A17G, C133T, and A477G) reported in Japanese (Iwai et al., 2004) but not in German-Caucasians were also detected in the Chinese Han population. Significant distribution differences of UGT1A3 alleles were observed between the German-Caucasian and Chinese Han populations except UGT1A3*3a. These findings reveal that the types and incidence of UGT1A3 alleles and SNPs in UGT1A3 have significant race differences. Such a difference is relatively weak between Japanese and the Chinese Han population, with C133T having a significant difference ($p < 0.05$) in the SNP distribution and UGT1A3*4 showing a statistically significant frequency difference between the Chinese Han population and Japanese. In the previous report (Iwai et al., 2004), the 477 substitution is always found to be together with two other SNPs.
(T31C and G81A) in the Japanese population, whereas some subjects without the 477 substitution also show 31 and 81 mutations in a Chinese Han population. This result leads to the significant distribution difference of UGT1A3*2a and UGT1A3*3a between the Chinese Han population and Japanese.

**Discussion**

Genetic polymorphisms have been reported for several functional human UGT genes (Maruo et al., 2005), including UGT1A1 (Bosma, 1995; Maruo et al., 1999), UGT1A3 (Ehmer et al., 2004; Iwai et al., 2004), UGT1A4 (Ehmer et al., 2004), UGT1A6 (Ciotti et al., 1997), UGT1A7 (Guillemette et al., 2000), UGT1A8 (Huang et al., 2002), UGT1A10 (Jinno et al., 2003), UGT2B4 (Levesque et al., 1999), UGT2B7 (Jin et al., 1993), and UGT2B15 (Levesque et al., 1997). These genetic variants may cause different phenotypes and play an important role in drug efficacy and xenobiotic toxicity, as well as in hormonal regulation and pathogenesis. For example, mutant UGT1A1 alleles lead to inherited diseases of bilirubin metabolism (Ritter et al., 1992; Bosma et al., 1994; Labrune et al., 2002). UGT1A6, UGT1A7, UGT1A9, UGT2B7, and UGT2B15 polymorphisms have been proposed for toxicological and clinical significance (Bhasker et al., 2000; Vogel et al., 2001; Miners et al., 2002; Carlini et al., 2005; Chung et al., 2005). Novel UGT1A10 polymorphisms are associated with orolaryngeal carcinoma risk (Elahi et al., 2003). Recently, in German-Caucasians and in a Japanese population, six UGT1A3 SNPs have been identified (Ehmer et al., 2004; Iwai et al., 2004). Some UGT1A3 variants show significantly different activities in estrone metabolism. However, their activities toward other substrates have not been determined. Since flavonoids are common constituents of foods of plant origin and display positive effects on many important diseases, such as cardiovascular diseases and cancers (Galati et al., 2002; Kris-Etherton and Keen, 2002; Vita, 2005), three widely existing flavonoids were used in this study as the substrates for glucuronidation to increase understanding of UGT1A3 variants.

Although statistically significant differences in catalytic activities of UGT1A3s to flavonoids were observed in the present study, results are not the same as observations reported for estrone glucuronidation (Iwai et al., 2004). The UGT1A3 variants clearly showed different activity alteration, depending on substrates. UGT1A3.5 exhibited a mild decrease in both flavonoids and estrone. UGT1A3.3 had a mild increase in estrone glucuronidation but a considerable decrease in flavonoid glucuronidation. A distinct activity difference was observed in UGT1A3.2 and UGT1A3.4. The former, having a 369% activity in estrone metabolism, showed a decreased activity in the metabolism of flavonoids to approximately 10%. The latter showed approximately 4 times glucuronidation efficiency to flavonoids of UGT1A3.1, with a decreased activity to 70% to estrone. These results indicate that amino acid residues at positions 45 and 47 are a key region of UGT1A3 protein. Polymorphism research on UGT1A4, which shares more than 93% similarity in DNA sequence with UGT1A3 (Green et al., 1998), also indicated such an assumption. An L48V UGT1A4 mutant completely lost dihydrotestosterone glucuronidation activity (Ehmer et al., 2004) and gave twice the efficiency for clozapine as the wild-type UGT1A4. Its efficiencies for some important clinical drugs, transandrosterone, imipramine, cyproheptadine, and tigogenin, also indicated such an assumption. An L48V UGT1A4 mutant completely lost dihydrotestosterone glucuronidation activity (Ehmer et al., 2004) and gave twice the efficiency for clozapine as the wild-type UGT1A4. Its efficiencies for some important clinical drugs, trans-androsterone, imipramine, cyproheptadine, and tigogenin, also changed (Mori et al., 2005). Attention should be paid to mutations in this region in the structural and functional research of UGT1A3.

It is of interest to observe a regioselectivity variation in quercetin metabolism by the UGT1A3 variants. It has been reported that different quercetin glucuronides showed different free radical scavenging, antioxidant, antithrombotic, and anticarcinogenic activities (Moon et al., 2001; Silva et al., 2002). The quercetin 7-glucuronide and 3-glucuronide were the main active forms exerting the physiological activity in vivo (Morand et al., 1998). In the previous study, we have described that UGT1A3.1 played its minor role in quercetin physiological activities by preferring low-activity 3'- and 4'-glucuronides to high-activity 7-glucuronide and 3-glucuronide (Chen et al., 2005a). In the present work, our data showed that three variants

**TABLE 7**

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Nucleotide Change</th>
<th>Amino Acid Change</th>
<th>Frequency in a Chinese Han Population (n = 250)</th>
<th>Frequency in Japanese (n = 200)</th>
<th>Frequency in German-Caucasians (n = 162)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Wild-Type Mutant</th>
<th>Amino Acid Change</th>
<th>Frequency in a Chinese Han Population (n = 250)</th>
<th>Frequency in Japanese (n = 200)</th>
<th>Frequency in German-Caucasians (n = 162)</th>
</tr>
</thead>
</table>

* Frequencies in Japanese were published by Iwai et al. (2004).
* Frequencies in German-Caucasians were published by Ehmer et al. (2004).
* Statistically different (p < 0.05) versus frequency in a Chinese Han population, two-sided Student’s t test.

**TABLE 8**

<table>
<thead>
<tr>
<th>UGT1A3 Alleles</th>
<th>Nucleotide Change</th>
<th>Amino Acid Change</th>
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</tr>
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* Statistically different (p < 0.05) versus frequency in a Chinese Han population, two-sided Student’s t test.
* —, unpublished data.
FUNCTION AND DISTRIBUTION OF HUMAN UGT1A3 VARIANTS

Chen YK, Chen SQ, Li X, and Zeng S (2005a) Quantitative regioselectivity of glucuronidation.


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