BRIEF REPORT

The Uridine Diphosphate Glucuronosyltransferase 2B15 D85Y and 2B17 Deletion Polymorphisms Predict the Glucuronidation Pattern of Androgens and Fat Mass in Men

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Context: Previous in vitro studies have demonstrated that the UDP glucuronosyltransferase (UGT)2B15 and UGT2B17 glucuronidate androgens and their metabolites.

Objective: Our objective was to determine in vivo whether the UGT2B15 D85Y and the UGT2B17 deletion polymorphisms predict androgen glucuronidation and body composition.

Participants: Two population-based cohorts including young adult (n = 1068; age = 18.9 yr) and elderly (n = 1001; age = 75.3 yr) men were included in the study.

Main Outcome Measures: Serum and urine levels of testosterone (T) and dihydrotestosterone (DHT) were measured by gas chromatography-mass spectrometry, and serum levels of the major glucuronidated androgen metabolites androstane-3α,17β-diol(androstanediol)-3-glucuronide, androstanediol-17-glucuronide, and androsterone-glucuronide were measured by liquid chromatography-tandem mass spectrometry. Body composition was measured by dual-energy x-ray absorptiometry.

Results: Both the UGT2B15 D85Y and the UGT2B17 deletion polymorphisms were associated with serum levels of androstanediol-17-glucuronide (P < 0.001) but not with levels of androstanediol-3-glucuronide or androsterone-glucuronide in both cohorts. Glucuronidation of T and DHT was associated with the UGT2B17 deletion but not with the UGT2B15 D85Y polymorphism, suggested by strong associations between the deletion polymorphism and urine levels of these two hormones. Both polymorphisms were associated with several different measures of fat mass (P < 0.01). The UGT2B17 deletion polymorphism was associated with insulin sensitivity (P < 0.05) as indicated by the homeostasis model assessment index.

Conclusions: The UGT2B15 D85Y and the UGT2B17 deletion polymorphisms are both predictors of the glucuronidation pattern of androgens androgen metabolites. Our findings indicate that UGT2B17 is involved in 17-glucuronidation of mainly T but also of DHT and androstanediol and that UGT2B15 is involved in the 17-glucuronidation of androstanediol. Furthermore, these two polymorphisms are predictors of fat mass in men. (J Clin Endocrinol Metab 92: 4878–4882, 2007)

CONJUGATION OF ANDROGENS with glucuronic acid has been suggested to play a role in the regulation of the intracellular levels of unconjugated androgens as well as their biological activities in tissues (1, 2).

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Abbreviations: CV, Coefficient of variation; Del, deletion; DHT, dihydrotestosterone; DXA, dual-energy x-ray absorptiometry; HOMA, homeostasis model assessment; T, testosterone; UGT, UDP glucuronosyltransferase; WT, wild type.

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(Y85) amino acid change at position 85 (4). A 150-kb deletion polymorphism spanning the whole UGT2B17 gene has been identified (5, 6).

The aim of the present study was to determine in vivo whether the D8Y polymorphism of the UGT2B15 gene and/or the deletion polymorphism of the UGT2B17 gene predict the glucuronidation pattern of androgens/androgen metabolites and body composition in men.

Subjects and Methods

Study subjects

Young adult men from the population-based Gothenburg Osteoporosis and Obesity Determinants (GOOD) study (n = 1068, 18.9 ± 0.5 yr of age) (7) and elderly men from the Gothenburg part of the population-based MrOS Sweden cohort (n = 1001, 75.3 ± 3.2 yr of age) (8) were included. Information about prevalent diabetes mellitus (n = 110) was obtained through questionnaires in the elderly. Informed consent was obtained from all study participants.

Dual-energy x-ray absorbtiometry (DXA)

Body composition was assessed using Lunar Prodigy DXA for the young adult men (GE Lunar Corp., Madison, WI) or Hologic QDR 4500/A-DELPHI for the elderly men (Hologic, Waltham, MA).

Assessment of sex hormones in serum

Serum levels were measured by validated high-sensitivity gas chromatography-mass spectrometry [T limit of detection of 0.05 ng/ml and interassay coefficient of variation (CV) of 3.4% dihydrotestosterone (DHT) limit of detection of 0.02 ng/ml and interassay CV of 4.1%] and liquid chromatography-tandem mass spectrometry (androsterone-glucuronide limit of detection of 2.00 ng/ml and interassay CV of 3.7%, androstanediol-3-glucuronide limit of detection of 0.50 ng/ml and interassay CV of 10.7%, androstanediol-17-glucuronide limit of detection of 0.50 ng/ml and interassay CV of 5.3%) as previously described (9, 10). The assay parameters (n = 1100) were spot validated with an interassay CV of less than 4%. Homeostasis model assessment (HOMA) index was calculated as the product of fasting serum insulin level (micro-units per milliliter) and fasting plasma glucose level (millimoles per liter) divided by 22.5.

Assessment of sex hormones in urine

Urinary unconjugated steroids (typically < 1% of glucuronide fraction) including T and DHT plus their glucuronides were determined by gas chromatography-mass spectrometry (n = 449, randomized sub-sample of the Gothenburg part of MrOS) after hydrolysis of the conjugates with β-glucuronidase as previously described (11, 12). Interassay CV was less than 10% for all steroids analyzed. Urine samples were spot collections (before 0900 h). Serum samples in the GOOD study were nonfasting samples obtained over the whole day.

TABLE 1. The UGT2B17 deletion and the UGT2B15 D8Y polymorphisms as predictors of sex steroids and SHBG in elderly men

<table>
<thead>
<tr>
<th>Table 1: UGT2B17 deletion and UGT2B15 D8Y polymorphisms as predictors of sex steroids and SHBG in elderly men</th>
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<tbody>
<tr>
<td><strong>Serum parameters</strong></td>
</tr>
<tr>
<td>T (ng/ml)</td>
</tr>
<tr>
<td>Androsterone (ng/ml)</td>
</tr>
<tr>
<td>Androstanediol-17-glucuronide (ng/ml)</td>
</tr>
<tr>
<td>Urine parameters</td>
</tr>
<tr>
<td>T (ng/μmol creatinine)</td>
</tr>
<tr>
<td>Testosterone</td>
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<tr>
<td>Values are given as means ± SD. Serum hormone levels were compared by Student’s t test and P values by ANOVA. G, Glucuronide; NS, nonsignificant.</td>
</tr>
</tbody>
</table>

| P < 0.05 vs. young adult men. |
| P < 0.05 vs. Del/WT for UGT2B17 deletion and P < 0.05 vs. YY for UGT2B15 D8Y. |
| P < 0.05 vs. Del/WT for UGT2B15 D8Y. |
dence of linkage between the UGT2B15 D85Y and the UGT2B17 deletion polymorphisms.

Serum and urine analyses

Serum levels of the glucuronidated androgen metabolite androstanediol-17-glucuronide, but not those of androstanediol-3-glucuronide or androsterone-glucuronide, were associated with the UGT2B17 deletion polymorphism in both elderly and young adult men (Tables 1 and 2). Androstanediol-17-glucuronide levels were lower for subjects with the Del/WT and WT/WT genotypes (Table 1). The UGT2B17 deletion polymorphism was strongly associated with the urinary levels of T and moderately associated with urinary DHT (Table 1). Both urinary T and DHT were lower for the Del/Del subjects than for the Del/WT and WT/WT subjects (Table 1). Furthermore, the urinary T to epitestosterone ratio was strongly associated with the UGT2B17 deletion polymorphism.

Androstanediol-17-glucuronide levels were clearly associated with the UGT2B15 D85Y polymorphism in both young adult and elderly men (Tables 1 and 2). The serum levels of androstanediol-17-glucuronide were higher for subjects with the DD and the DY genotypes than for subjects with the YY genotype. Serum DHT and SHBG levels but not T levels were slightly higher for the DD than in the YY subjects. We conclude that subjects with the DD genotype probably have a more efficient UGT2B15 enzyme for 17-glucuronidation of androstanediol than the YY subjects.

Earlier in vitro studies have indicated that UGT2B17 has the capacity to glucuronidate androstanediol at the 17β-hydroxy position (resulting in androstanediol-17-glucuronide) and androsterone at the 3α-hydroxy position (resulting in androsterone-glucuronide) (2). However, the present in vivo study of a naturally occurring gene inactivation of the UGT2B17 gene demonstrates that UGT2B17 is a rather selective enzyme in the glucuronidation of androgen metabolites, enhancing the 17β-glucuronidation of androstanediol but not the 3α-glucuronidation of androstanediol or androsterone (Fig. 1).

UGTs glucuronidate not only androgen metabolites but also T and DHT. The present finding of a major role of UGT2B17 for T excretion in elderly men (Fig. 1) confirms our previous results in young adult men (13) and is consistent with previous in vitro reports showing that T is a good substrate for UGT2B17 (15) but not for UGT2B15 (2). In addition, the present finding that urine DHT was associated with the UGT2B17 deletion polymorphism but not with the UGT2B15 D85Y polymorphism (Fig. 1) is supported by previous studies investigating enzymatic activity in vitro (2, 14). Interestingly, the clear association between the UGT2B15 D85Y polymorphism and androstanediol-17-glucuronide levels was accompanied by slightly affected serum levels of SHBG and DHT in the young adult men, suggesting that this polymorphism might affect androgen-dependent phenotypes. The mechanism behind the affected SHBG levels in the young adult men is an open question, but it might be related to the lower capacity to conjugate androstanediol-17-glucuronide.

Body composition analyses

Several indicators of body fat (body weight, body mass index, total body fat, and trunk fat; P < 0.05) were associated with the UGT2B17 deletion polymorphism in the elderly cohort and with the UGT2B15 D85Y polymorphism in the young adult cohort (Table 3). Some of the parameters reflecting fat mass (total body fat percent and arm fat; P < 0.05) were associated with the UGT2B15 D85Y polymorphism also in the elderly cohort. Subjects with the UGT2B15 YY genotype had a higher amount of fat than the subjects with the DY and DD genotypes, and subjects with the UGT2B17 Del/Del genotype had a higher amount of fat than the subjects with the Del/WT and WT/WT genotypes (Table 3; P < 0.05). In addition, the UGT2B17 deletion polymorphism was a predictor of serum insulin and HOMA index in the elderly cohort (Table 3; P < 0.05).

TABLE 2. The UGT2B17 deletion and the UGT2B15 D85Y polymorphisms as predictors of sex steroids and SHBG in young adult men

<table>
<thead>
<tr>
<th>Serum parameters</th>
<th>All subjects</th>
<th>UGT2B17 deletion</th>
<th>UGT2B15 D85Y</th>
<th>Value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 46)</td>
<td>(n = 569)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T (ng/ml)</td>
<td>4.69 ± 1.52</td>
<td>4.76 ± 1.53</td>
<td>4.81 ± 1.46</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>DHT (ng/ml)</td>
<td>0.31 ± 0.11</td>
<td>0.31 ± 0.12</td>
<td>0.31 ± 0.11</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Androsterone-G (ng/ml)</td>
<td>61.2 ± 35.4</td>
<td>61.7 ± 28.5</td>
<td>63.0 ± 35.8</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Androstanediol-3G (ng/ml)</td>
<td>1.52 ± 0.92</td>
<td>1.58 ± 0.81</td>
<td>1.58 ± 0.98</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Androstanediol-17G (ng/ml)</td>
<td>4.03 ± 2.08</td>
<td>3.28 ± 2.13</td>
<td>4.16 ± 2.09a</td>
<td>&lt;0.001 NS</td>
<td></td>
</tr>
<tr>
<td>SHBG (nmol/liter)</td>
<td>20.4 ± 7.4</td>
<td>20.4 ± 6.9</td>
<td>20.1 ± 7.0</td>
<td>NS</td>
<td></td>
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<tr>
<td></td>
<td>(n = 535)</td>
<td></td>
<td></td>
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<tr>
<td>D (n = 222)</td>
<td>4.87 ± 1.63</td>
<td>4.72 ± 1.53</td>
<td>4.53 ± 1.38</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Y (n = 304)</td>
<td>0.31 ± 0.11</td>
<td>0.31 ± 0.12b</td>
<td>0.31 ± 0.11b</td>
<td>0.29 ± 0.10b</td>
<td>0.048 NS</td>
</tr>
<tr>
<td>Androsterone-G (ng/ml)</td>
<td>60.4 ± 39.8</td>
<td>63.2 ± 35.1</td>
<td>58.5 ± 32.6</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Androstanediol-3G (ng/ml)</td>
<td>1.49 ± 0.86</td>
<td>1.54 ± 0.86</td>
<td>1.51 ± 0.90</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Androstanediol-17G (ng/ml)</td>
<td>4.69 ± 2.22b</td>
<td>4.27 ± 2.06b</td>
<td>3.13 ± 1.67b</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>SHBG (nmol/liter)</td>
<td>21.9 ± 7.66</td>
<td>20.5 ± 7.6</td>
<td>19.1 ± 6.76</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Values are given as means ± sd. Serum hormone levels were compared by Student's t test and P values by ANOVA. G, Glucuronide; NS, nonsignificant.

a P < 0.05 vs. Del/Del for UGT2B17 deletion and P < 0.05 vs. YY for UGT2B15 D85Y.

b P < 0.05 vs. Del/WT for UGT2B17 deletion and P < 0.05 vs. DY for UGT2B15 D85Y.

Discussion

We here demonstrate that both the UGT2B15 D85Y polymorphism and the UGT2B17 deletion polymorphism have an impact on the glucuronidation pattern of androgens/androgen metabolites.

The present in vivo data, showing that the UGT2B15 D85Y polymorphism is strongly associated with serum levels of androstanediol-17-glucuronide but not androstanediol-3-glucuronide or androsterone-glucuronide (Fig. 1) support earlier in vitro findings that UGT2B15 specifically conjugates the 17β-hydroxy position of androstenediol (14). Furthermore, the results indicate that the G to T polymorphism in the UGT2B15 gene is functional. Because the serum levels of androstanediol-17-glucuronide were higher in the DD than in the YY subjects, we conclude that subjects with the DD genotype probably have a more efficient UGT2B15 enzyme for 17-glucuronidation of androstanediol than the YY subjects.

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The UGT2B15 D85Y polymorphism is coded as YY, and the UGT2B17 deletion polymorphism is coded as WT/WT. The regression analysis regarding insulin, glucose, and HOMA index was performed to determine the impact of these two polymorphisms on fat mass. The results indicate that the UGT2B15 deletion polymorphism was not associated with fat mass in the young adult men, whereas the impact of the UGT2B17 deletion polymorphism was not associated with fat mass in the elderly men (Fig. 1), suggesting that these two polymorphisms are predictors of the glucuronidation of androstanediol. Furthermore, these two polymorphisms are linked to the regulation of fat mass. Hence, the UGT2B15 D85Y and the UGT2B17 deletion polymorphisms are predictors of the glucuronidation pattern of androgens/androgen metabolites. Our findings indicate that UGT2B17 is involved in 17-glucuronidation of androstanediol, whereas UGT2B15 is involved in the 17-glucuronidation of androstanediol. Hence, the UGT2B15 D85Y and the UGT2B17 deletion polymorphisms might have an impact on the local androgen/androgen metabolite levels in fat tissue and/or other tissues of importance for fat homeostasis and thereby affect fat mass. However, additional functional studies are required to determine how these two polymorphisms are linked to the regulation of fat mass.

Parameters were investigated using linear regression, including age as covariate. Subjects with known diabetes mellitus were excluded from the regression analysis regarding insulin, glucose, and HOMA index. The UGT2B17 deletion polymorphism is coded as YY > DY > DD. Standardized β and P values are presented. Central fat distribution is calculated as trunk fat/total body fat × 100. ND, Not determined.

In conclusion, The UGT2B15 D85Y and the UGT2B17 deletion polymorphisms are predictors of the glucuronidation pattern of androgens/androgen metabolites. Our findings indicate that UGT2B17 is involved in 17-glucuronidation of mainly T but also of DHT and androstanediol, whereas UGT2B15 is involved in the 17-glucuronidation of androstanediol. Hence, the UGT2B15 D85Y and the UGT2B17 deletion polymorphisms might have an impact on the local androgen/androgen metabolite levels in fat tissue and/or other tissues of importance for fat homeostasis and thereby affect fat mass. However, additional functional studies are required to determine how these two polymorphisms are linked to the regulation of fat mass.

The cohort is unknown, but one may speculate that the UGT2B15 D85Y polymorphism affects the local androgen environment in selected tissues, which in turn results in a regulation of SHBG levels. However, one cannot exclude a direct influence on SHBG abundance, which secondarily changes androgen metabolism.

Several measures of fat mass were associated with the UGT2B15 D85Y polymorphism in both the young adult and the elderly men and with the UGT2B17 deletion polymorphism in the elderly men (Fig. 1), suggesting that these two UGTs, with the capacity to alter the local androgenic environment, might affect fat mass homeostasis. In contrast, the UGT2B17 deletion polymorphism was not associated with fat mass in the young adult men, indicating that the impact of this polymorphism on fat mass either is age dependent or of inconsistent nature.

It is impossible from this association study to determine the causality of the association between the polymorphisms and fat mass. Because UGT2B15 is highly expressed in adipose tissue (16), one may speculate that a substantial part of the UGT2B15 activity is adipose tissue derived and/or that the UGT2B15 D85Y polymorphism affects local androgen/androgen metabolite levels in adipose tissue, which in turn affects the fat mass. In contrast to UGT2B15, no major expression of UGT2B17 has been described in fat tissue (16). The UGT2B17 deletion polymorphism, but not the UGT2B15 D85Y polymorphism, was associated not only with fat mass but also with insulin sensitivity, indicating that the metabolic consequences of these two polymorphisms at least partly differ.

In conclusion, The UGT2B15 D85Y and the UGT2B17 deletion polymorphisms are predictors of the glucuronidation pattern of androgens/androgen metabolites. Our findings indicate that UGT2B17 is involved in 17-glucuronidation of mainly T but also of DHT and androstanediol and that UGT2B15 mainly is involved in the 17-glucuronidation of androstanediol. Furthermore, these two polymorphisms are predictors of fat mass in men. Additional studies are required to determine the clinical significance of the reported associations between the UGT2B15 D85Y/UGT2B17 deletion polymorphisms and glucuronidation patterns of androgens because this cannot be determined by the present investigation.

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