

Sex Steroid Levels and Cortical Bone Size in Young Men Are Associated with a Uridine Diphosphate Glucuronosyltransferase 2B7 Polymorphism (H²⁶⁸Y)

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Context: Sex steroids are involved in the regulation of pubertal cortical bone expansion in males. *In vitro* studies have indicated that the enzyme uridine diphosphate glucuronosyltransferase (UGT) 2B7 has the capacity to glucuronidate sex steroids and their metabolites.

Objective: Our objective was to determine the impact of the H²⁶⁸Y polymorphism in the *UGT2B7* gene on interindividual variation of serum levels of sex steroids and cortical bone dimensions.

Participants: The population-based cohort Gothenburg Osteoporosis and Obesity Determinants study consists of 1068 young adult Swedish men (age 18.9 yr).

Main Outcome Measures: Serum levels of sex steroids and the three major glucuronidated androgen metabolites, androstane-3 α ,17 β -diol-17glucuronide, androstane-3 α ,17 β -diol-3glucuronide, and androsterone-glucuronide, were analyzed. Cortical and trabecular volumetric bone mineral density and cortical bone size were measured by peripheral quantitative computer tomography.

Results: Serum levels of testosterone (YY 9% over HH; $P < 0.01$), dihydrotestosterone (YY 10% over HH; $P < 0.01$), and estradiol (YY 8% over HH; $P < 0.01$) were associated with the UGT2B7 H²⁶⁸Y polymorphism. The polymorphism was associated with androstane-3 α ,17 β -diol-17glucuronide and androstane-3 α ,17 β -diol-3glucuronide ($P < 0.01$), but not with androsterone-glucuronide serum levels. In addition, the UGT2B7 H²⁶⁸Y polymorphism was an independent predictor of cortical bone size, reflected by periosteal circumference and cortical moment of inertia ($P < 0.01$), in both the weight-bearing tibia and nonweight-bearing radius.

Conclusions: The UGT2B7 H²⁶⁸Y polymorphism is independently associated with cortical bone size and serum sex steroid levels in young adult men. Subjects homozygous for the Y allele had higher serum testosterone and larger cortical bone size than subjects homozygous for the H allele. However, the underlying mechanism behind these associations is unknown and has to be studied further. (*J Clin Endocrinol Metab* 92: 3697–3704, 2007)

PEAK BONE MASS is higher in men than in women. This is mainly due to the fact that the bones are larger in men as a result of a more pronounced pubertal periosteal bone expansion in men compared with women (1, 2). This difference in cortical bone size between men and women is likely due to differences in sex steroid exposure. Androgens stimulate periosteal bone expansion in males during sexual maturation (3), and a recent study using the androgen receptor (AR) inactivated mouse model has demonstrated that the AR is required for this effect (4).

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Abbreviations: ADT, Androsterone; ADTG, ADT-glucuronide; AR, androgen receptor; CSA, cross-sectional area; CV, coefficient of variation; DHT, dihydrotestosterone; 3 α -diol, androstane-3 α ,17 β -diol; E₁, estrone; E₁S, E₁ sulfate; E₂, estradiol; EC, endosteal circumference; epiT, epitestosterone; 3G, androstane-3 α ,17 β -diol-3glucuronide; 17G, androstane-3 α ,17 β -diol-17glucuronide; GC-MS, gas chromatography-mass spectrometry; PC, periosteal circumference; pQCT, peripheral quantitative computer tomography; T, testosterone; UGT, uridine 5'-diphosphate glucuronosyltransferase; vBMD, volumetric bone mineral density.

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The bioactive androgens testosterone (T) and dihydrotestosterone (DHT) can be inactivated directly by conjugation with glucuronic acid. An alternative pathway is transformation of DHT by hydroxysteroid dehydrogenase enzymes, resulting in two main metabolites, namely androstane-3 α ,17 β -diol (3 α -diol) and androsterone (ADT) (Fig. 1), which have a lower affinity for the AR (5), but these changes are reversible and do not lead to termination of the androgenic signal (6). However, conjugation of 3 α -diol and ADT with polar cofactors, such as glucuronic acid, is an irreversible step that causes steric hindrance of the parental molecule, abolishing its affinity for the AR (Fig. 1) (7). Conjugation of androgens with glucuronic acid has been suggested to play a role in the regulation of intracellular levels of unconjugated steroids as well as their biological activities in tissues (8, 9). It is now well established that uridine diphosphate glucuronosyltransferase (UGT) 2B7, UGT2B15, and UGT2B17 are the three major enzymes responsible for glucuronidation of all androgens and their metabolites in humans (8). *In vitro* studies have demonstrated that UGT2B7 conjugates the 3 α -hydroxy position of 3 α -diol, but not the 17 β -hydroxy position. ADT is a consistently good substrate for UGT2B7,

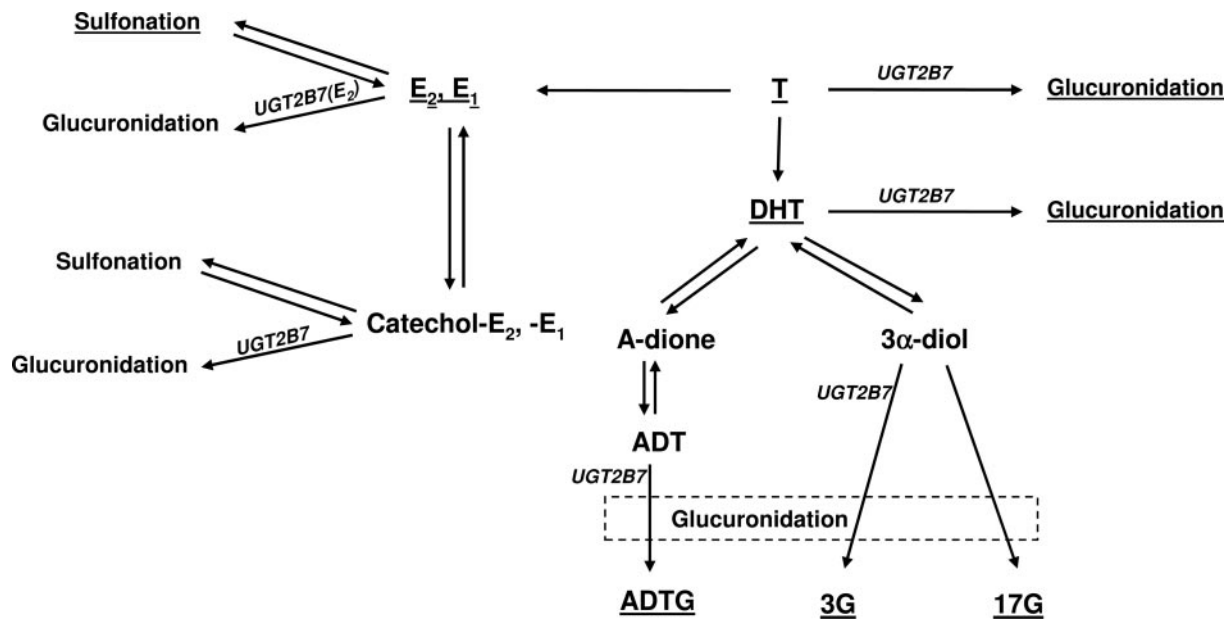


FIG. 1. Conjugation of estrogens and androgens. The *underlined* sex hormones and metabolites are analyzed in the present study. The conjugation by UGT2B7 in the metabolic pathway is indicated. A-dione, Androstenedione; Catechol- E_2 , - E_1 , 2- and 4-hydroxyestradiols and 2- and 4-hydroxyestrone; 3 α -diol, androstane-3 α ,17 β -diol.

whereas T and DHT, which only have a hydroxyl group at the 17 β -position, are poor substrates for UGT2B7.

Estrogen sulfates represent a form of estrogen storage that acts as a precursor of estradiol (E_2) and estrone (E_1), whereas glucuronidation by UGTs leads to complete inactivation of estrogens (10). UGT2B7 glucuronidates not only androgen but also some estrogen metabolites (Fig. 1) (11). The *in vivo* role of UGT2B7 for the regulation of circulating, as well as local, levels of androgens, estrogens, and their metabolites is unknown. A polymorphism H²⁶⁸Y has been described in the UGT2B7 gene, resulting in an amino acid change within the substrate binding site (12, 13).

The aim of the present study was to determine the impact of the UGT2B7 H²⁶⁸Y polymorphism on serum levels of sex steroids and cortical bone dimensions in young adult men at the age of peak bone mass.

Subjects and Methods

Study subjects

The Gothenburg Osteoporosis and Obesity Determinants (GOOD) study was initiated to determine environmental and genetic factors involved in the regulation of bone and fat mass (14). Study subjects were randomly identified using national population registers, contacted by telephone, and asked to participate. Men aged 18.9 ± 0.6 yr ($n = 1068$) from the greater Gothenburg area were included. Of the contacted candidates, 48.6% agreed to participate and were included. Informed consent was obtained from all participants.

Assessment of covariates

Height and weight were measured using standard equipment. Standardized questionnaires were used to collect information about amount of physical activity, nutritional intake, and smoking. Calcium intake was calculated using information about amount of dairy product intake.

Peripheral quantitative computer tomography (pQCT)

A pQCT device (XCT-2000; Stratec Medizintechnik, Pforzheim, Germany) was used to scan tibia and radius of the nondominant leg and

arm, respectively (14). The cortical volumetric bone mineral density (vBMD) (mg/cm^3), cortical bone mineral content (mg/mm), cortical cross-sectional area (CSA) (mm^2), endosteal and periosteal circumference (EC and PC, respectively), and cortical thickness (mm) were measured scanning the diaphysis (at 25% the bone length in the proximal direction of the distal end of the bone) of the radius and tibia. Trabecular vBMD (mg/cm^3) was measured scanning the metaphysis (at 4% the bone length in the proximal direction of the distal end of the bone) of these bones. The coefficients of variation (CVs) were less than 1% for all measurements.

Dual x-ray absorptiometry

Lean mass of whole body was assessed using Lunar Prodigy dual x-ray absorptiometry (GE Lunar Corp., Madison, WI).

Serum analyses using gas chromatography-mass spectrometry (GC-MS) ($n = 1068$).

The validated GC-MS system was used for analysis of T (limit of detection 0.05 ng/ml, intraassay CV 2.9%, interassay CV 3.4%), DHT (limit of detection 0.02 ng/ml, intraassay CV 3.1%, interassay CV 4.1%), E_2 (limit of detection 2.00 pg/ml, intraassay CV 1.5%, interassay CV 2.7%), and E_1 (limit of detection 8.00 pg/ml, intraassay CV 1.8%, interassay CV 1.7%) (15). Analytes and internal standard were detected using a HP5973 quadrupole mass spectrometer equipped with a chemical ionization source.

Serum analyses using liquid chromatography-tandem mass spectrometry ($n = 1068$)

Androsterone-glucuronide (ADTG) (limit of detection 2.00 ng/ml, intraassay CV 3.1%, interassay CV 3.7%), 3 α -diol-3glucuronide (3G) (limit of detection 0.50 ng/ml, intraassay CV 10.3%, interassay CV 10.7%), 3 α -diol-17glucuronide (17G) (limit of detection 0.50 ng/ml, intraassay CV 4.6%, interassay CV 5.3%), and E_1 sulfate (E_1S) (limit of detection 0.075 ng/ml, intraassay CV 3.2%, interassay CV 4.4%) were analyzed by a validated liquid chromatography-tandem mass spectrometry method using TurbolonSpray (15).

Urinary analyses using GC-MS ($n = 115$)

Urinary glucuronides [T, DHT, and epitestosterone (epiT)] were determined by GC-MS. One microgram of internal standard (methyltes-

tosterone) was added to each 2.0 ml urine, and steroids were extracted after hydrolysis of conjugates with β -glucuronidase as described (16) with minor modifications (17). Within and between assay CVs were less than 7% and less than 10%, respectively, for all steroids analyzed.

Serum analyses of SHBG, LH, FSH, and IGF-I

Serum SHBG was measured by IRMA (limit of detection 1.3 nmol/liter, intraassay CV 3%, interassay CV 7%; Orion Diagnostics, Espoo, Finland). Free T was calculated as previously described (18, 19). Serum LH was measured by ELISA (limit of detection 0.10 mIU/ml, intraassay CV 6%, interassay CV 7%; Diagnostic Systems Laboratories, Inc., Webster, TX). Serum FSH was measured by IRMA (limit of detection 0.11 mIU/ml, intraassay CV 3%, interassay CV 6%; Diagnostic Systems Laboratories, Inc.). Serum IGF-I levels were measured by double-antibody, IGF-binding protein-blocked RIA (20).

DNA isolation and genetic polymorphism analysis

Genomic DNA was isolated from whole blood (DNA Purigene kit; Gentra Systems, Inc., Minneapolis, MN). The rs7439366 polymorphism of the UGT2B7 gene was successfully genotyped in 1013 study subjects using gene-specific PCR forward primer 5'-AGCTGACGTATGGCT-TATTCGAA-3', reverse primer 5'-GGGTTTGGCAGGTTTGCA-3', VIC (TTCAGTTTCCTCATCCACT), and FAM (TTCAGTTTCATATC-CACTCT) labeled probes [probes as in Bhasker *et al.* (13), with minor modification of the C-specific sequence]. After PCR amplifications (GeneAmp 9700; Applied Biosystems, Stockholm, Sweden), the PCR products were analyzed [7900HT Sequence Detection System (ABI PRISM); Applied Biosystems]. The UGT2B7 H²⁶⁸Y nomenclature is as previously described (21). The H allele is in some reports denoted as UGT2B7*1 and the Y allele as UGT2B7*2 (13).

Enzymatic assays

UGT2B7 proteins were stably expressed in HEK-293 cell systems, and microsomal fractions were prepared as previously described (22). For accurate assessment of the quantitative differences in glucuronidation activity for variant alloenzymes, HEK-293-derived cell lines were characterized for UGT protein expression by Western blot analysis (data not shown) as previously described (11). Enzymatic assays were performed with 40–60 μ g total microsomal proteins in 100 μ l reaction volume containing 50 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 2.5 μ g/ml pepstatin, 0.5 μ g/ml leupeptin, 10 μ g/ml phosphatidylcholine, and 1 mM uridine diphosphate glucuronic acid. Varying concentrations of T, DHT, ADT, and 3 α -diol ranging from 1–200 μ M were incubated at 37 C. The assays were stopped after 60 min, by adding 100 μ l ice-cold methanol and then centrifuged at 14,000 g for 10 min to remove proteins before mass spectrometry analysis. Relative glucuronidation activities are expressed as picomole per minute per UGT level, which was obtained after dividing the absolute activity of UGT (picomole per minute per milligram) by the protein expression level obtained by Western blot analysis. High-performance liquid chromatography/tandem mass spectrometry of the sex steroids from the enzymatic analyses was performed as previously described (23).

Statistical analysis

Continuous variables were compared using ANOVA followed by Tukey's *post hoc* test. Regression analyses using height, weight, age, smoking habits, calcium intake, physical activity, and the UGT2B7 genotype (coded as HH = 1, HY = 2, and YY = 3) as covariates were used to estimate the independent contribution of the UGT2B7 H²⁶⁸Y polymorphism to cortical bone parameters. Standardized β -values were used. Values are given as mean \pm SD. For enzymatic parameters, a nonparametric Student's *t* test was used to analyze for significant difference between K_m of UGT2B7 H- and Y-variant using the JMP V5.0.2 software (SAS Institute Inc., Cary, NC).

Results

The genotype frequencies of the H²⁶⁸Y polymorphism were 24.2% for HH, 49.0% for HY, and 26.7% for YY, con-

tributing to allele frequencies of 48.7% for H and 51.2% for Y. The frequency distribution was similar to those previously reported (13, 24). The genotype frequencies were in Hardy-Weinberg equilibrium. The UGT2B7 H²⁶⁸Y polymorphism was not associated with age, height, weight, physical activity, smoking, or calcium intake (data not shown). Descriptive characteristics of age, anthropometrics, sex hormone levels, and bone variables are given in Table 1.

The UGT2B7 polymorphism is associated with serum levels of both androgens and estrogens

Serum levels of the bioactive, nonglucuronidated androgens T (YY 9% over HH; *P* < 0.01) and DHT (YY 10% over HH; *P* < 0.01) were significantly associated with the UGT2B7 H²⁶⁸Y polymorphism (Table 2).

Furthermore, the associations between the UGT2B7 H²⁶⁸Y polymorphism and serum levels of the three major glucuronidated androgen metabolites in men were investigated.

TABLE 1. Characteristics of the study subjects

Variables	
Age (yr)	18.9 \pm 0.6
Height (cm)	181.4 \pm 6.8
Weight (kg)	73.8 \pm 11.9
Physical activity (h/wk)	4.3 \pm 5.3
Smoking (%)	8.7
Calcium intake (mg/d)	1,095 \pm 724
Serum analyses	
T (ng/ml)	4.69 \pm 1.52
Free T (ng/ml)	0.13 \pm 0.04
DHT (ng/ml)	0.31 \pm 0.11
E ₂ (pg/ml)	18.7 \pm 6.2
Free E ₂ (pg/ml)	0.40 \pm 0.14
E ₁ (pg/ml)	22.6 \pm 7.7
E ₁ S (ng/ml)	0.54 \pm 0.36
ADTG (ng/ml)	61.2 \pm 35.4
3G (ng/ml)	1.52 \pm 0.92
17G (ng/ml)	4.03 \pm 2.08
SHBG (nmol/liter)	20.4 \pm 7.41
LH (mIU/ml)	8.19 \pm 8.14
FSH (mIU/ml)	2.30 \pm 3.18
IGF-I (ng/ml)	267 \pm 74
Urine analyses	
T (ng/ μ mol creatinine)	5.73 \pm 3.37
DHT (ng/ μ mol creatinine)	1.28 \pm 0.87
T/epiT	2.11 \pm 2.04
pQCT	
Tibia cortical CSA (mm ²)	270 \pm 34
Tibia cortical thickness (mm)	4.43 \pm 0.51
Tibia cortical PC (mm)	75.0 \pm 4.9
Tibia cortical EC (mm)	47.2 \pm 5.5
Tibia cortical vBMD (mg/cm ³)	1,156 \pm 20
Tibia cortical MI (mm ⁴)	24,098 \pm 5,907
Tibia cortical MR (mm ³)	1,653 \pm 312
Tibia trabecular vBMD (mg/cm ³)	266 \pm 34
Radius cortical CSA (mm ²)	96 \pm 12
Radius cortical thickness (mm)	2.92 \pm 0.27
Radius cortical PC (mm)	42.1 \pm 3.0
Radius cortical EC (mm)	23.7 \pm 3.1
Radius cortical vBMD (mg/cm ³)	1,165 \pm 23
Radius cortical MI (mm ⁴)	2,442 \pm 688
Radius cortical MR (mm ³)	306 \pm 61
Radius trabecular vBMD (mg/cm ³)	219 \pm 41

Values are given as means \pm SD. The number of subjects was 115 for urine analyses and 1,068 for all other variables. MI, Moment of inertia; MR, moment of resistance.

TABLE 2. Sex steroid levels according to the UGT2B7 H²⁶⁸Y polymorphism

	HH (n = 246)	HY (n = 495)	YY (n = 272)	P value
Serum analyses				
T (ng/ml)	4.49 ± 1.48 ^b	4.67 ± 1.46	4.89 ± 1.54	0.008
Free T (ng/ml)	0.12 ± 0.04 ^a	0.12 ± 0.04	0.13 ± 0.04	0.018
DHT (ng/ml)	0.29 ± 0.11 ^b	0.31 ± 0.11	0.32 ± 0.12	0.010
E ₂ (pg/ml)	18.3 ± 6.0 ^a	18.3 ± 6.0 ^b	19.8 ± 6.8	0.006
Free E ₂ (pg/ml)	0.39 ± 0.13 ^a	0.39 ± 0.13 ^a	0.42 ± 0.15	0.012
E ₁ (pg/ml)	22.1 ± 7.0	22.5 ± 8.0	23.6 ± 8.1	NS
E ₁ S (ng/ml)	0.53 ± 0.33	0.52 ± 0.33	0.59 ± 0.43	NS
ADTG (ng/ml)	61.5 ± 35.5	59.7 ± 32.0	61.8 ± 31.0	NS
3G (ng/ml)	1.51 ± 0.85	1.43 ± 0.77 ^a	1.63 ± 0.95	0.002
17G (ng/ml)	3.92 ± 2.07 ^a	3.86 ± 1.96 ^b	4.37 ± 2.21	0.004
SHBG (nmol/liter)	19.7 ± 7.3	20.6 ± 7.3	20.7 ± 7.6	NS
LH (mIU/ml)	9.01 ± 12.56	7.56 ± 5.65	8.40 ± 6.96	NS
FSH (mIU/ml)	2.55 ± 6.00	2.12 ± 1.14	2.33 ± 1.65	NS
IGF-I (ng/ml)	272 ± 79	264 ± 72	267 ± 73	NS
Urine analyses				
T (ng/μmol creatinine)	6.06 ± 2.61	5.46 ± 3.64	5.97 ± 3.36	NS
DHT (ng/μmol creatinine)	1.03 ± 0.70	1.33 ± 0.93	1.47 ± 0.92	NS
T/epiT	2.34 ± 2.53	1.96 ± 1.86	2.28 ± 2.08	NS

Values are given as means ± SD. NS, Nonsignificant.

^a *P* < 0.05 vs. YY.

^b *P* < 0.01 vs. YY.

The polymorphism was associated with 17G levels (YY 15% over HH; *P* < 0.01) and 3G levels (YY 14% over HY; *P* < 0.01), but not with ADTG levels in serum (Table 2).

Urine levels (mainly glucuronidated) of T and DHT are indicators of glucuronidation of these two androgens. Urinary levels of neither T nor DHT were associated with the UGT2B7 H²⁶⁸Y polymorphism (Table 2). Furthermore, the urinary T to epiT ratio was not influenced by the UGT2B7 H²⁶⁸Y amino acid change.

The UGT2B7 H²⁶⁸Y polymorphism was associated with both total and free E₂ (total E₂ YY 8% over HH, *P* < 0.05; free E₂ YY 8% over HH, *P* < 0.01). Serum levels of SHBG, FSH, and LH were not significantly associated with the UGT2B7 H²⁶⁸Y polymorphism (Table 2).

Enzymatic activities of UGT2B7 variants toward androgens

Previous work showed that T and DHT are not substrates for UGT2B7, whereas their metabolites 3α-diol and ADT can be conjugated. Levels of conjugation obtained with T, E₂, and DHT substrates were extremely low (data not shown) and did not allow the calculation of kinetic parameters for these substrates. ADTG and 3G were detected and, as expected, 17G was absent. The velocity of conjugation of ADT by the variants of UGT2B7 remains in the same range, but the affinity of the Y variant is higher for ADT, resulting in efficacy of conversion 1.5-fold higher than that for the H variant (*P* <

0.05), whereas formation of 3G was not influenced by the UGT2B7 H²⁶⁸Y change (Table 3).

The UGT2B7 polymorphism is an independent predictor of cortical bone size

Because the sex steroid levels were associated with the UGT2B7 H²⁶⁸Y polymorphism and we recently reported that sex steroids are associated with the cortical bone size of the subjects in the same cohort (14), we investigated if the UGT2B7 H²⁶⁸Y polymorphism was associated with cortical bone dimensions. The UGT2B7 H²⁶⁸Y polymorphism was a significant independent predictor of cortical bone size (PC and EC) of both the weight-bearing tibia and the nonweight-bearing radius (Tables 4 and 5). The larger cortical bone size in YY than in HH subjects was reflected by higher cortical moment of inertia and cortical moment of resistance. The UGT2B7 H²⁶⁸Y polymorphism was not associated with the cortical and the trabecular vBMD in tibia or radius (Tables 4 and 5).

Inclusion of neither total nor free levels of T or E₂ as additional covariates in the regression model had any substantial impact on the association between the UGT2B7 H²⁶⁸Y polymorphism and cortical bone dimensions (Table 5; data not shown).

Because serum IGF-I levels have been positively associated with cortical bone size (25), we investigated if the UGT2B7

TABLE 3. *In vitro* analyses of enzymatic activities of UGT2B7 variants toward androgens

	ADTG			3G		
	Apparent K _m (μM)	Relative V _{max} (pmol/min/UGT level)	CL _{int} (μl/min/mg)	Apparent K _m (μM)	Relative V _{max} (pmol/min/UGT level)	CL _{int} (μl/min/mg)
H variant	12.34 ± 1.17	646.99 ± 14.52	52.43	17.37 ± 2.84	325.21 ± 28.19	18.72
Y variant	7.36 ± 0.36 ^a	587.30 ± 5.77	79.80	30.50 ± 8.07	516.50 ± 90.69	16.93

Values are means ± SD. CL_{int}, Intrinsic clearance; K_m, substrate concentration at half of the maximum velocity (V_{max}).

^a *P* < 0.05 vs. H variant.

TABLE 4. Bone size and vBMD according to UGT2B7 H²⁶⁸Y polymorphism

	HH (n = 246)	HY (n = 495)	YY (n = 272)	P value
Tibia				
Cortical CSA (mm ²)	267 ± 26	271 ± 28	272 ± 28	0.053
Cortical thickness (mm)	4.42 ± 0.47	4.44 ± 0.48	4.42 ± 0.48	0.703
Cortical PC (mm)	74.4 ± 3.5	75.0 ± 3.8	75.6 ± 3.8 ^b	0.001
Cortical EC (mm)	46.6 ± 4.7	47.1 ± 4.9	47.9 ± 4.9 ^b	0.011
Cortical vBMD (mg/cm ³)	1,157 ± 21	1,156 ± 20	1,156 ± 18	0.805
Cortical MI (mm ⁴)	23,327 ± 4,181	24,196 ± 4,564 ^a	24,732 ± 4,602 ^b	0.002
Cortical MR (mm ³)	1,610 ± 223	1,661 ± 246 ^a	1,686 ± 248 ^b	0.001
Trabecular vBMD (mg/cm ³)	266 ± 32	265 ± 31	267 ± 33	0.706
Radius				
Cortical CSA (mm ²)	95 ± 10	96 ± 11	97 ± 11	0.118
Cortical thickness (mm)	2.92 ± 0.26	2.93 ± 0.26	2.91 ± 0.25	0.676
Cortical PC (mm)	41.7 ± 2.6	42.1 ± 5.6	42.3 ± 2.7 ^a	0.014
Cortical EC (mm)	23.2 ± 3.0	23.6 ± 2.8	24.0 ± 2.9 ^b	0.014
Cortical vBMD (mg/cm ³)	1,166 ± 23	1,164 ± 22	1,165 ± 19	0.687
Cortical MI (mm ⁴)	2,358 ± 597	2,439 ± 596	2,500 ± 624 ^a	0.028
Cortical MR (mm ³)	299 ± 53	306 ± 55	312 ± 55 ^a	0.017
Trabecular vBMD (mg/cm ³)	220 ± 40	218 ± 39	222 ± 39	0.401

Bone parameters are given as means ± SD. MI, Moment of inertia; MR, moment of resistance.

^a *P* < 0.05 vs. HH.

^b *P* < 0.01 vs. HH.

polymorphism was associated with serum IGF-I levels. However, serum IGF-I levels were not associated with the UGT2B7 polymorphism (Table 2).

Cortical bone size is also often associated with muscle mass and lean mass. However, no significant association between lean mass and the UGT2B7 polymorphism was found in the present study (data not shown).

Discussion

A major determinant of the lower incidence of fractures in men compared with women is that men have a larger cortical bone area as a result of a more pronounced pubertal cortical bone expansion, resulting in a higher bone strength in men than in women (2). Here, we demonstrate that a polymorphism in the UGT2B7 gene, resulting in an amino acid

change, is associated with cortical bone size and serum sex steroid levels in young adult men.

Several genes involved in the synthesis or inactivation of sex steroids or responsiveness to sex steroids might modulate sex steroid action in the skeleton. Most previous studies, investigating the genetic influence of sex steroid-related genes on skeletal parameters, have focused on genes involved in sex steroid synthesis and responsiveness (26, 27), whereas not many have reported associations between polymorphisms in genes involved in inactivation of sex steroids and skeletal parameters (28). In the present study, we investigated a polymorphism in the UGT2B7 gene, which via an irreversible glucuronidation is involved in the inactivation and subsequent elimination of both estrogens and androgens and their metabolites. The main finding is that the

TABLE 5. The UGT2B7 H²⁶⁸Y polymorphism as an independent predictor of bone size

	Crude		T-adjusted		E ₂ -adjusted	
	β	P value	β	P value	β	P value
Tibia						
Cortical CSA (mm ²)	0.058	0.022	0.060	0.018	0.067	0.008
Cortical thickness (mm)	−0.001	0.970	0.005	0.869	0.008	0.778
Cortical PC (mm)	0.092	<0.001	0.088	<0.001	0.095	<0.001
Cortical EC (mm)	0.082	0.003	0.075	0.007	0.079	0.005
Cortical vBMD (mg/cm ³)	−0.018	0.553	−0.024	0.441	−0.027	0.378
Cortical MI (mm ⁴)	0.085	<0.001	0.084	0.001	0.090	<0.001
Cortical MR (mm ³)	0.086	<0.001	0.086	0.001	0.093	<0.001
Trabecular vBMD (mg/cm ³)	0.017	0.568	0.017	0.559	0.014	0.635
Radius						
Cortical CSA (mm ²)	0.052	0.064	0.050	0.080	0.059	0.038
Cortical thickness (mm)	−0.017	0.583	−0.016	0.592	−0.014	0.645
Cortical PC (mm)	0.080	0.004	0.076	0.007	0.087	0.002
Cortical EC (mm)	0.086	0.003	0.082	0.006	0.091	0.002
Cortical vBMD (mg/cm ³)	−0.020	0.509	−0.022	0.462	−0.029	0.343
Cortical MI (mm ⁴)	0.074	0.008	0.071	0.011	0.082	0.003
Cortical MR (mm ³)	0.080	0.004	0.079	0.005	0.089	0.002
Trabecular vBMD (mg/cm ³)	0.020	0.512	0.018	0.560	0.011	0.720

Linear regression including the UGT2B7 H²⁶⁸Y polymorphism (coded as HH = 1, HY = 2, YY = 3), smoking, amount of present physical activity, age, height, weight, calcium intake without (crude), or with adjustment for serum T (T-adjusted) or with adjustment for serum E₂ (E₂-adjusted). Standardized β and P values are presented. MI, Moment of inertia; MR, moment of resistance.

UGT2B7 H²⁶⁸Y polymorphism is an independent predictor of cortical bone size and of indicators of cortical bone strength as a result of an impact on PC. However, the possible role of the association found between the UGT2B7 H²⁶⁸Y polymorphism and cortical bone size in determining fracture risk remains to be studied.

In vitro studies with stably transfected cells have indicated that T, E₂, and DHT are no major substrates of UGT2B7, whereas their metabolites 4-hydroxycatecholestrogens, ADT, and 3 α -diol are clearly conjugated by UGT2B7 (11, 29). In the present study, serum levels of E₂ and the two androgens, T and DHT, were all associated with the UGT2B7 H²⁶⁸Y polymorphism. This finding indicates that UGT2B7 is of importance for the regulation of the circulating levels of bioactive sex steroids. Furthermore, this also suggests that the UGT2B7 H²⁶⁸Y polymorphism is functional or in linkage with another functional polymorphism in the *UGT2B7* gene. Previous *in vitro* studies have demonstrated that UGT2B7 conjugates only at the 3 α -hydroxy position of 3 α -diol (Fig. 1) (8), and, therefore, we believe that the higher 17G levels in YY than in HH subjects are a consequence of the higher T and DHT levels in YY subjects, and not due to variations in glucuronidation of the 17 β -hydroxy position of 3 α -diol. This is also confirmed by the increase in 3G levels, which is likely the result of higher serum T levels. ADTG in serum was not associated with the UGT2B7 H²⁶⁸Y amino acid change, indicating that, despite likely increases in substrate ADT production, the presence of the high-activity Y allele does not cause augmentation of serum ADTG levels, thus suggesting that the UGT2B7 polymorphism YY is more associated with 17-hydroxysteroids than 17-ketosteroids (Fig. 1). Because it was previously reported that a mutation in the *UGT2B7* gene promoter, which occurs only in the Y allele, might significantly decrease expression of the gene (30), it may be possible that the amount of enzyme is lower in subjects with the YY genotype.

Because the present and previous *in vitro* studies demonstrate that UGT2B7 does not glucuronidate T, DHT, or E₂ efficiently, the higher levels of these sex steroids in subjects with the YY genotype than in subjects with the HH genotype must be due to indirect effects either caused by increased synthesis or decreased degradation of sex steroids in YY subjects. The impact on T and free T levels corresponds to a 9 and 8% increase, respectively, for subjects homozygous for the Y allele compared with individuals homozygous for the H allele. The clinical significance of this relatively modest difference in T levels between genotypes remains to be investigated. The elevated 3G and 17G, but unchanged ADTG, levels in YY subjects suggest that the degradation of androgens was at least not reduced in these subjects. In addition, urinary T and DHT levels are not altered by the UGT2B7 polymorphism, indicating that the pathway of kidney elimination remains unchanged. Because UGT2B7 is expressed in the brain, one may speculate that the UGT2B7 H²⁶⁸Y polymorphism might affect the conversion of DHT metabolites locally in this tissue, resulting in affected secretion of LHRH and LH, and, consequently, altered serum T in the circulation. However, this notion is not supported by the present finding that serum LH levels were not significantly associated with the UGT2B7 H²⁶⁸Y polymorphism. In addition, one

might speculate that the local androgenic environment in either the testis or adrenal gland might be affected by the UGT2B7 H²⁶⁸Y polymorphism, resulting in altered secretion of androgens/androgen precursors from these tissues. However, further studies are required to explain the exact mechanism by which the UGT2B7 H²⁶⁸Y polymorphism affects serum levels of sex steroids. Yet, it is clear that in YY subjects, the T pathway instead of the androstenedione pathway is preponderant, and the mechanism is likely located at the level of synthesis, namely the 17 β -hydroxysteroid dehydrogenase balance. Nonetheless, the present study clearly demonstrates that the UGT2B7 H²⁶⁸Y polymorphism is associated with serum levels of T, DHT, and E₂ in men, making it an interesting candidate polymorphism for future association studies in sex steroid-related conditions.

The effects of androgens on the skeleton can be exerted either directly through the AR or indirectly through aromatization to estrogens, and further through estrogen receptor- α and/or - β interactions (31). All three of these sex steroid receptors are expressed in bone (31) and experimental animal studies, using sex steroid receptor inactivated transgenic mouse models, have indicated that each of these three receptors mediates site-specific skeletal effects on bone (32–35). Moreover, recent data have shown that androgens stimulate periosteal cortical bone formation (3) and that this requires a functional AR (4). Furthermore, we have recently reported that serum T is positively associated with the cortical bone size of the young adult men investigated in the present study (14), supporting the notion that androgens increase the cortical bone size in men. Therefore, it is conceivable that any possible biological effects on bone size of the UGT2B7 H²⁶⁸Y polymorphism are, at least partly, mediated by the alteration in circulating T levels in adulthood or during pubertal growth. Because the YY subjects had both higher T levels and larger PC than the HH subjects, we investigated if the impact of the UGT2B7 H²⁶⁸Y polymorphism on cortical bone size was mediated via serum T. Inclusion of serum T levels as a covariate in the regression model had no substantial impact on the association between the UGT2B7 H²⁶⁸Y polymorphism and PC, suggesting that present T levels in these young adult men at the age of peak bone mass do not solely mediate the genetic polymorphism effect on bone parameters. This indicates that a possible effect of the UGT2B7 H²⁶⁸Y polymorphism on T levels during pubertal growth could be of greater importance. This notion is supported by the fact that most of the cortical bone expansion occurs during pubertal growth, whereas periosteal bone expansion after puberty is limited (2). In addition, inclusion of E₂ levels in the regression model did not affect the association between the polymorphism and bone parameters.

It is established that the urinary T to epiT ratio, commonly used in international antidoping test programs, is considerably lower in Asians than Caucasians, leading to difficulties in interpretation of the test results (36). We have recently demonstrated that the differences in T excretion between Korean and Swedish men are strongly associated with a UGT2B17 polymorphism (36), supporting the notion that genetic variations in UGTs are of importance for the ethnic differences in the T to epiT ratio. Interestingly, there is an approximately equal distribution of subjects homozygous

for each allele of the UGT2B7 H²⁶⁸Y polymorphism in Caucasians, whereas subjects homozygous for the H allele were over 10-fold more prevalent than Y homozygotes in an Asian population (13). In the present study, neither the urinary T levels nor the T to epiT ratio were associated with the UGT2B7 H²⁶⁸Y polymorphism, arguing against that this polymorphism is involved in ethnic differences of the T to epiT ratio. Some, but not all, studies demonstrate that serum levels of androgens are higher in Caucasian than Asian men (37, 38). Interestingly, YY subjects in the present study had higher serum androgen levels than HH subjects. Because the Y allele is less common in Asian compared with Caucasian populations (13), one might speculate that the UGT2B7 H²⁶⁸Y polymorphism could contribute to the suggested ethnic variations in serum androgen levels.

We have also investigated the impact of two other polymorphisms in major glucuronidation enzymes [the D⁸⁵Y polymorphism in the UGT2B15 gene (39) and a deletion polymorphism in the UGT2B17 gene (40)] in this population. However, these polymorphisms were not associated with any bone parameters, but similar to the UGT2B7 polymorphism, they were both associated with serum levels of the glucuronidated androgen metabolite 17G (own unpublished data).

A limitation of the present study is the low number of samples available for urinary analysis. Thus, the absence of associations with urinary parameters might be due to a lack of statistical power.

In conclusion, the UGT2B7 H²⁶⁸Y polymorphism is an independent predictor of cortical bone size and is associated with serum sex steroid levels in young adult men. However, the underlying mechanism behind these associations is unknown and has to be studied further. One may speculate that affected sex steroid levels during pubertal development might contribute to the association between the UGT2B7 H²⁶⁸Y polymorphism and cortical bone size in men.

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