SULT2A1 gene copy number variation is associated with urinary excretion rate of steroid sulfates

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INTRODUCTION

Human cytosolic sulfotransferases (SULT) enzymes catalyze the sulfate conjugations of many xenobiotics and endogenous compounds such as hormones. These reactions result in increased water solubility and enhanced urinary excretion of the conjugates. To date, 12 human SULT genes have been identified. Among these SULT1A1 (also known as P-PST) is an enzyme that has been well studied in relation to hormone related disease and detoxification of polycyclic aromatic hydrocarbons (1, 2). Another SULT member SULT2A1 (also known as DHEA ST) is highly expressed in human liver and catalyzes the sulfation of dehydroepiandrosterone (DHEA), the major steroid precursor in humans (3, 4). DHEA itself is a weak androgen which is converted into more active androgens in peripheral tissues (5, 6).

Large inter-individual variations in circulatory levels of DHEAS have been observed. Three SULT2A1 single nucleotide polymorphisms altering the amino acid sequence have been identified and associated with altered SULT2A1 activity and DHEA:DHEAS ratio in the circulation (7). Circulatory DHEAS is considered to represent the androgen pool and high concentration of DHEAS is strongly associated with testosterone-glucuronide concentrations in urine, both at baseline (15) and after the administration of a supra-physiological dose of testosterone (16). Here we aim to investigate if SULT2A1 display variation in copy numbers in a Swedish population, and if there is an association with SULT1A1 and SULT2A1 CNV genotype and the urinary excretion of different androgen sulfates prior to and after the administration of testosterone.

MATERIALS AND METHODS

SUBJECTS AND DESIGN

Study subjects included 30 healthy male volunteers aged 18–50 years. The study population has been described in more detail elsewhere (16). All participants gave informed consent consistent with the approval of the Ethics Review Board, Karolinska Institutet, Stockholm. The participants were given 500 mg testosterone
enanthate as a single intra muscular (i.m) dose of Testoviron Depot (kindly provided by Schering Nordiska AB, Solna, Sweden) equivalent to 360 mg testosterone. Urine samples were collected for analyses before administration (day 0) and on days 1–9, 11, 13, and 15 after dose. All samples were collected between 07:00 and 11:00 h. Adverse drug reactions were monitored during the study period. No major adverse drug reactions were registered. No follow-up was needed. The study was conducted according to the Helsinki Declaration and the ICH Harmonized Tripartite Guideline for Good Clinical Practice.

URINARY ANALYSIS
The concentrations of sulfated androgens were analyzed using a LC-MS/MS method as previously described (10). Briefly, 1 mL and 20 IL ISTD samples were added to SPE Oasis HLB 96-well plates and washed with 0.1% acetic acid, 0.1% ammonia, and 10% MeOH. The analytes were eluted with acetone and after evaporation the residue was reconstituted with 20% MeOH. Waters Acquity ultra performance liquid chromatographic (UPLC) system was used to perform the separation on Waters Acquity UPLC BEH RP18 column 50 9 2.1 mm with 1.7 lm particles. The mobile phases were 5 mM NH4Ac adjusted by ammonia to pH 9.6 (A) and MeOH (B). A Waters Micromass Quattro Premier triple-quadrupole instrument (Waters Associates, Manchester, UK) operating with fast polarity switching in multiple reaction mode (MRM) was used to detect the target analytes.

SULT1A1 AND SULT2A1 GENE COPY NUMBER VARIATION
Genomic DNA was extracted from 200 µL blood using Qiagen Mini Kit. Twenty nanograms genomic DNA was used in each reaction together with 2X TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA, USA). The SULT1A1 and SULT2A1 CNV polymorphisms were analyzed by real-time polymerase chain reaction analysis using premade assays Hs04461762_cn and Hs03013147_cn (Applied Biosystems). Expression of albumin was quantified as an endogenous control as described (17). Both reactions were run in 15 µL reactions. The PCR profile consisted of an initial denaturation step at 95°C for 10 min followed by 40 cycles of 92°C for 15 s and 60°C for 1 min.

DATA EVALUATION
Integration, calibration, and data evaluation was performed by the TargetLynx software (Waters Associates, Manchester, UK). The between subject variation in urine dilution was corrected for by dividing the concentration values by the urinary creatinine (cr) concentration, which was determined by colorimetric analysis (DRI Creatinine-Detect Test; Thermo Fisher Scientific, Waltham, MA, USA). The areas under the curve (AUC) of the different urinary steroids were calculated using the trapezoidal rule. Statistical analyses were performed by Kruskal Wallis followed by Dunn’s Multiple Comparison Test, since the data were not normally distributed.

RESULTS
GENE COPY NUMBER ANALYSIS
The distribution of SULT1A1 copy numbers were; individuals with one gene copy (i.e., one deletion) N = 6 (20%), two copies N = 16 (53%), and three copies N = 8 (26%), respectively. The distribution of SULT2A1 copy numbers were; individuals with one copy N = 7 (23%), two copies N = 9 (60%), and three gene copies N = 4 (13%), respectively.

SULT1A1 AND SULT2A1 CNV AND URINARY BASELINE LEVELS OF STEROID SULFATES
There was a significant correlation between urinary concentration of DHEAS (p = 0.02) and androsteroneS (p = 0.01) and the number of SULT2A1 CNV, Figures 1A,B. Individuals displaying one SULT2A1 gene copy excreted lower levels of DHEAS and androsteroneS (8.8 and 22.9 ng/µmol cr) compared to individuals with two copies (44.5 and 53.6 ng/µmol cr) and three SULT2A1 gene copies (48.5 and 60.1 ng/µmol cr). There were no significant correlation between SULT2A1 CNV and urinary concentration of testosteroneS and etiocholanoloneS, Figures 1C,D. There was no significant association between SULT1A1 CNV and urinary levels of any androgen sulfates investigated.

SULT2A1 CNV AND URINARY AUC OF STEROID SULFATES POST TESTOSTERONE ADMINISTRATION
The mean AUC for DHEAS during 15 days post dose were 44.5, 279, and 300 ng/µmol cr in individuals with one, two, and three gene copies, respectively (p = 0.046), Figure 2A. The mean AUC for testosteroneS during 15 days were 2.2, 7.4, and 11.6 nmol/µmol cr in individuals with one, two, and three gene copies, respectively (p = 0.019), Figure 2B. There were no significant correlations between urinary AUC for etiocholanoloneS, androsteroneS, and SULT2A1 CNV, Figures 2C,D. There were no significant association between SULT1A1 CNV and urinary AUC of androgen sulfates investigated.

DISCUSSION
Piper et al. showed that there is a large inter-individual variation (500-fold) in the urinary excretion rate of DHEAS (11). However, there are no studies investigating the inter-individual variability of urinary levels of DHEAS in relation to genotype. We show for the first time that SULT2A1 CNV is functional and that the number of SULT2A1 copies is associated with the baseline concentration of DHEAS, as well as the baseline concentration of the DHEA metabolite androsterone. In vitro studies have shown that in addition to DHEA, SULT2A1 also possesses high sulfation activity toward androsterone (4). There was lower sulfation activity in individuals with one SULT2A1 copy compared to individuals with two or more copies for all steroid metabolites investigated except etiocholanoloneS. Our results demonstrate that SULT2A1 exerts activity not only toward DHEA but also toward several DHEA metabolites in vivo.

After administration of exogenous testosterone to healthy volunteers, the urinary excretion rate of sulfate metabolites was significantly associated with SULT2A1 CNV. The urinary concentration of DHEAS and testosteroneS decreased approximately 50 and 80% respectively, after the administration of testosterone enanthate. This is probably a result of a feedback mechanism on the hypothalamic-pituitary axis. However, a significant SULT2A1 genotype association with the excretion of both DHEAS and testosteroneS, even at the low concentrations observed post dose further strengthen the finding that DHEA and testosterone are substrates of SULT2A1 in vivo. On the other hand the urinary
FIGURE 1 | The mean urinary concentration of (A) DHEAS (B) androsteroneS (C) testosteroneS and (D) etiocholanoloneS in different SULT2A1 genotype panels. The androgen conjugate concentration was normalized against creatinine (cr) concentration. Individuals with two and three SULT2A copy number variation excrete higher levels of DHEAS and androsteroneS.

FIGURE 2 | Urinary (A) DHEAS (B) testosteroneS (C) etiocholanoloneS and (D) androsteroneS excretion (ng/µmol) for 15 days in the different SULT2A1 genotype groups after an i.m injection of 500 mg testosterone enanthate.
levels of the testosterone metabolites androsteroneS and etiocholanoloneS increased as a result of the administrated testosterone. The association between CNV of SULT2A1 and AUC of androsteroneS was not statistically significant. However as the study sample was small with only seven individuals with one gene copy, the power might have been insufficient to demonstrate any significance. Our AUC results are in agreement with our baseline finding, supporting that these androgen metabolites are sulfated by SULT2A1 in vivo.

The allele distribution of SULT1A1 was in agreement with a previous study reporting a 25% frequency of three SULT1A1 copies in Caucasians (13) but opposite to other studies reporting a 5% occurrence of one copy number (13, 18). There was no correlation between CNV of SULT1A1 and the urine concentration of the steroid sulfates. Even though SULT1A1 has been shown to be involved in hormone metabolism, this enzyme has been shown to be more important in the sulfation of estrogens (2).

For ethical and medical reason, testosterone was administered to men only. Sulfation of androgens is important also in women, and genetic variation in different SULTs has been associated with altered risk for PCOS and breast cancer treatment (19, 20) and it would be of interest to study this functional CNV in relation to the metabolism of endogenous and exogenous androgens in females in future studies.

DHEAS is found at high concentrations in the circulation and has in addition to hormone related cancers and PCOS also been shown to be involved in the risk of cardiovascular diseases (21, 22). SULT2A1 expression is down-regulated in hepatocellular carcinoma and correlated with higher grade and stage of cancer (23). Additionally, SULT2A1 has been shown to be involved in the metabolism and activation of carcinogenic compounds and drugs (24, 25). It is possible that SULT2A1 CNV polymorphism may contribute to individual variation in response to drugs that undergo sulfation as well as altered risk for cancer and other diseases.

Variability in CNV is a common phenomenon in the human genome, and has been observed for a number of enzymes involved in biotransformation, i.e., UGT2B17, CYP2D6, and GSTM (26). This is the first time SULT2A1 CNV has been studied in a human population, and our results confirm that this putative polymorphism exists. According to the provider (Applied Biosystems) of the SULT2A1 gene copy assay used in this study the deletion consists of 2849 bp at the 5′-end of the SULT2A1 gene, at chromosome position 19q13.33. Jung et al. identified a 150 000 bp homozygous SULT2A1 gene deletion at position 19q13.32 in a pulmonary inflammatory myofibroblastic tumor (27). It is possible that different CNV exists throughout the SULT2A1 gene. Further studies are warranted in order to characterize the genetic make-up of this CNV and the phenotypic consequences.

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