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Associations between polymorphisms in glucuronidation and sulfation enzymes and sex steroid concentrations in premenopausal women in the United States

Mellissa Yong[a,b], **Stephen M. Schwartz**[a,b], **Charlotte Atkinson**[c], **Karen W. Makar**[a], **Sushma S. Thomas**[a], **Frank Z. Stanczyk**[d], **Kim C. Westerlind**[e], **Katherine M. Newton**[f], **Victoria L. Holt**[a,b], **Wendy M. Leisenring**[a], and **Johanna W. Lampe**[a,b,*]

[a]Fred Hutchinson Cancer Research Center, 1100 Fairview Ave, Seattle, WA 98109 USA

[b]University of Washington Department of Epidemiology, Seattle, WA 98195 USA

[c]Department of Oral and Dental Science, University of Bristol, Lower Maudlin Street, Bristol, BS1 2LY UK

[d]Keck School of Medicine, University of Southern California, Los Angeles, CA 90033 USA

[e]AMC Cancer Research Center, 1600 Pierce Street, Lakewood, CO 80214 USA

[f]Group Health Research Institute, 1730 Minor Ave, Suite 1600, Seattle, WA 98101 USA

Abstract

Glucuronidation, catalyzed by UDP-glucuronosyltransferases (UGT) and sulfation, catalyzed by sulfotransferases (SULT), are pathways through which sex steroids are metabolized to less active compounds. These enzymes are highly polymorphic and genetic variants frequently result in higher or lower activity. The phenotypic effects of these polymorphisms on circulating sex steroids in premenopausal women have not yet been investigated. One hundred and seventy women ages 40-45 years had a blood sample drawn during the follicular phase of the menstrual cycle for sex steroid measures and to obtain genomic DNA. Urine was collected for 2-hydroxy (OH) estrone (E_1) and 16α-OH E_1 measures. Generalized linear regression models were used to assess associations between sex steroids and polymorphisms in the *UGT1A* and *UGT2B* families, *SULT1A1*, and *SULT1E1*. Women with the *UGT1A1(TA7/TA7)* genotype had 25% lower mean estradiol (E_2) concentrations compared to the wildtype *(TA6/TA6)* (p = 0.02). Similar associations were observed between *SULT1A1(R213/H213)* and E_1 (13% lower mean E_1 concentration vs. wildtype; p-value = 0.02) and *UGT2B4(E458/E458)* and dehydroepiandrosterone (DHEA) (20% lower mean DHEA vs. wildtype; p-value = 0.03). The *SULT1E1(A/C)* and the *UGT1A1(TA7)-UGT1A3(R11)* haplotypes were associated with reduced estrogen concentrations. Further study of *UGT* and *SULT* polymorphisms and circulating sex steroid measures in larger populations of premenopausal women is warranted.

*Corresponding author: Fred Hutchinson Cancer Research Center, Cancer Prevention Program, Public Health Sciences, 1100 Fairview Ave, M4-B402, Seattle, WA 98109 USA; Phone: 206-667-680; Fax: 206-667-7850; jlampe@fhcrc.org .

Keywords

Uridine diphosphoglucuronosyltransferases; sulfotransferases; estrogens; androgens; premenopausal women

Introduction

Circulating sex steroids are implicated in the etiology of certain cancers (e.g., breast, ovary, and endometrium) and other conditions with high morbidity (e.g., endometriosis and polycystic ovarian syndrome) arising in hormone-sensitive tissues [1-3]. Thus, the determinants of circulating concentrations of estrogens and androgens, and of estrogen metabolism, may be associated with risk of a number of hormone-dependent conditions. Conjugation of sex steroids via glucuronidation [catalyzed by UDP-glucuronosyltransferases (UGT)] [4] and sulfation [catalyzed by sulfotransferases (SULT)] [5] is a major pathway for estrogen and androgen clearance in humans. Steroid hormone conjugation may therefore represent an important regulator of sex steroid activity. UGT(1A1, 1A3, 2B4, 2B7) [6], and SULT(1A1, 1E1) [7] have been shown to be involved in the biotransformation of estrogens and their oxidative metabolites, and UGT2B15 and UGT2B17 have been implicated in androgen metabolism [8,9]. The genes for many UGT and SULT enzymes that are capable of contributing to the conjugation of estrogens and catechol estrogens, as well as androgens, harbor common and functionally significant genetic polymorphisms [10]. Such polymorphisms may ultimately affect the clearance of, and exposure to, endogenous and exogenous estrogens and androgens.

The functional relevance of *UGT* and *SULT* polymorphisms to steroid hormone concentrations in vivo has only been studied in postmenopausal women and men; these studies have shown that *UGT* and *SULT* polymorphisms influence estrogen and androgen concentrations [11,12]. Further, several studies also suggest that these polymorphisms influence risk of hormone-dependent cancers such as breast and prostate cancer [11-17]. To date, no studies have examined the relationship between *UGT* and *SULT* polymorphisms and serum steroid hormone concentrations and urinary catechol estrogen metabolites in premenopausal women. Specifically, there is a lack of studies that evaluate associations between circulating estrogens and *UGT1A* haplotypes, despite strong linkage disequilibrium between functional polymorphisms in multiple *UGT1A* family genes. Similarly, even though *SULT1E1* shows the highest affinity for estrogens among members of the known human SULT enzymes [7] and is highly expressed in normal human mammary epithelial cells [18], no studies have yet investigated the association between *SULT1E1* polymorphisms and sex hormone concentrations in premenopausal women. Other UGTs and SULTs also have been shown to catalyze the glucuronidation of estrogens and androgens, but some of these enzymes are expressed exclusively in extrahepatic tissues, including the small intestine, biliary tract, esophagus, and colon (e.g., 1A7, 1A8, 1A10) [19] or the genetic polymorphisms in them are rare. In the selection of the UGT and SULT genetic variants for our study, data from in vitro kinetic studies were considered [20-22]. For example, Nagar et al. [23] described the differences in sulfation activity toward estrogens, including estradiol, 2-hydroxyestradiol, and 2-methoxyestradiol, of the *His/Arg213* alleles, with *Arg213* showing greater catalytic activity compared to *His213*.

We hypothesized that alleles that code for enzymes with higher conjugating activity resulting in increased clearance of endogenous sex steroids [*UGT1A3(R11)*, *UGT1A3 (V47)*, *UGT2B7(Y268)*, and *UGT2B15(Y85)*] would be associated with lower circulating hormone concentrations. Likewise, alleles that code for enzymes with lower conjugating activity

resulting in lower clearance of endogenous sex steroids [*UGT1A1(TA7), UGT2B17(null)*, and *SULT1A1(H213)*] would be associated with higher circulating hormone concentrations.

Materials and methods

Study population

As described in detail elsewhere [24], women were recruited from within Group Health (GH), a large integrated health plan in Washington State. Premenopausal women ages 40-45 years who had undergone a screening mammogram in the previous 10 months, and who were not using exogenous hormones, were identified from the GH Breast Cancer Screening Program [25] and recruited based on the Breast Imaging Reporting and Data System (BI-RADS®) density score [26] assigned to their most recent screening mammogram. Our aim was to recruit approximately similar numbers of women with a BI-RADS® density score of 1 or 2 (combined as one group, where 1 = almost entirely fat and 2 = scattered fibroglandular densities), 3 (heterogeneously dense), and 4 (extremely dense). We established eligibility criteria to include only premenopausal women who 1) were not perimenopausal, defined as skipped ≥1 periods in the previous 12 months, 2) were not currently taking exogenous sex steroids, 3) had not used sex steroids at all in the six months prior to the screening mammogram, and 4) had not used sex steroids for a month or more in the 6-12 months prior to the screening mammogram. A history of sex steroid hormone use in our study population was defined as the use of oral contraceptives, or sex steroid hormone patches, injections, or implants, or intrauterine devices containing progesterone at any time prior to the six-month period before the screening mammogram. All study procedures were approved by the Institutional Review Boards of the Fred Hutchinson Cancer Research Center (FHCRC) and GH, and all study participants provided written informed consent.

Blood and urine sample collection

Clinic visits were scheduled to occur during the follicular phase (days 5 through 9) of the menstrual cycle. At the clinic visit, an early morning blood sample was drawn following an overnight fast and participants also provided a spot urine sample. Collection, storage, and transportation of samples are described in detail elsewhere [27].

Serum steroid hormone and SHBG concentration analyses

Estrone (E_1), estradiol (E_2), testosterone (T), androstenedione (A), and dehydroepiandrosterone (DHEA) were quantified by sensitive and specific radioimmunoassays with preceding organic solvent extraction and Celite column partition chromatography steps [28,29]. The assay sensitivities were 14.8 pmol/L, 7.3 pmol/L, 0.05 nmol/L and 0.10 nmol/L for E_1, E_2, T and A, respectively. The interassay coefficients of variation ranged from 8% to 13%.

Urinary estrogen metabolite concentration analyses

Urinary 2-hydroxyestrone (2-OH E_1) and 16α-hydroxyestrone (16α-OH E_1) were measured using a commercially available competitive, solid-phase enzyme-linked immunoassay (ESTRAMET, ImmunaCare, Corp., Bethlehem, PA). Intra-assay and inter-assay coefficients of variation for 2-OHE_1 were 4.4% and 8.8%, respectively; for 16!-OHE_1, they were 5.1% and 9.2%, respectively.

Genotyping of UGT1A, UGT2B, SULT1A1, and SULT1E1 polymorphisms

A total of 11 polymorphisms were genotyped from DNA extracted from the buffy coat fraction: *UGT1A1(TA6/TA7)* – rs8175347, *UGT1A3(W11R)* – rs3821242, *UGT1A3(V47A)* – rs6431625, *UGT2B4(D458E)* – rs13119049, *UGT2B7(H268Y)* – rs7439366,

UGT2B15(D85Y) – rs1902023, *SULT1A1(R213H)* – rs9282861, *SULT1E1*: *I169A>G* – rs3775768, *I1(−73)G>C* – rs4149530, and *(−10)C>G* – rs1220702, and a deletion in *UGT2B17* using a variety of polymerase chain reaction-based methods, including size dependent-separation, restriction fragment length polymorphism, sequencing, and fluorescent allelic discrimination (TaqMan™) as described in detail elsewhere [14].

Data analysis

Chi square tests were used to assess Hardy-Weinberg equilibrium for each of the genotypes. Measures of central tendency and categorical distributions were calculated to describe the characteristics of the study population, and initial comparisons of hormone concentrations among genotypes were done using non-model based approaches including simple means and t-test. Because the hormone and urinary catechol estrogen metabolite measures were highly skewed, log-transformations of these values were performed.

As described previously [14], we inferred two-locus haplotypes involving *UGT1A1(TA6/TA7)*, *UGT1A3(W11R)*, and *UGT1A3(V47A)*; our sample size was too small to obtain stable estimates for a three-locus haplotype analysis. For *SULT1E1*, we selected three single nucleotide polymorphisms (*SULT1E1(I169A>G)*, *SULT1E1[I1(−73)G>C]*, and *SULT1E1[I5(−10)C>G]*) found by Adjei et al. to distinguish the most common haplotypes (>5% allele frequency) in a Caucasian-American population [21]. Of the three *SULT1E1* single nucleotide polymorphisms genotyped, *SULT1E1[I5(−10)C>G]*) did not occur as frequently relative to the other two [minor allele frequency (MAF) = 11%] and did not contribute to the delineation of any common haplotype. Among the two remaining *SULT1E1* single nucleotide polymorphisms, *SULT1E1(I169A>G)*, MAF=0.27%; *SULT1E1[I1(−73)G>C]*, MAF=22%), we identified three haplotypes in our study population.

For each gene (*UGT1A, SULT1E1*), we performed a global test of all the haplotypes versus no haplotypes using a likelihood-ratio test for each hormone. We then fit a generalized linear model with additive haplotype effects under Hardy-Weinberg equilibrium to test for an association between each of the inferred *UGT1A1-UGT1A3* and *SULT1E1* haplotypes and hormone measure [30].

For our models, characteristics were selected as covariates if they were associated with both genotypes and sex steroids. Previous studies have reported ethnicity to be associated with *UGT* and *SULT* genotypes [31-33]. Lampe et al. [31] showed that both allele and genotype frequencies of the *UGT1A1(TA6/TA7)* genotypes varied by race (i.e., Caucasians vs. Asians). Race/ethnicity has also been shown to be associated with hormone concentrations [34]; thus, this characteristic (categorized as Asian, Caucasian, Other) was included in our final model.

Genotypes were coded on an ordinal scale [homozygous wildtype (wt/wt) = 0, heterozygous (wt/v) = 1, and homozygous variant (v/v) = 2] to model allele dosage effects with the wt/wt genotype as the reference category. If no gene dosage effect was observed, genotype was evaluated by using a dichotomous variable to indicate whether the participant was a carrier (i.e., wt/v or v/v) of the variant allele. Adjusted generalized linear regression models were fit and the mean estimates were back-transformed to obtain the geometric mean values and 95% confidence intervals (CIs) of the sex steroids and urinary catechol estrogen metabolites by genotype. A test for trend was conducted using adjusted linear regression between the ordinal genotype measures and hormone and estrogen metabolite measures.

All analyses were conducted using STATA/SE (version 9.0; STATACorp LP, College Station, TX), with the exception that haplotypes were inferred and tested using Hapstat

(Software for the statistical analysis of haplotype-disease association; Copyright © 2006-2008 Tammy Bailey, Danyu Lin and the University of North Carolina at Chapel Hill) [30]. A two-sided p-value of < 0.05 was considered statistically significant.

Results

A total of 189 (93%) study participants were willing to have stored biological samples used for future studies. Of these participants, 176 (93%) had a buffy coat available for extracting DNA. Among the 176 women with a buffy coat available, four had E_2 concentrations > 1,468 pmol/l. Concentrations at this level are considered periovulatory and not typical of concentrations seen between days 5 and 9 of the menstrual cycle; as such, we excluded these four women from our analyses. Two participants had blood samples that were drawn outside of their follicular phase and were also excluded. Therefore, a total of 170 women were included in our analyses.

The mean age (SD) of the study participants was 42.4 (1.4) y and the majority had had one or more pregnancies, had a history of sex steroid hormone use (i.e., contraceptive hormones provided orally or as patches, injections, and implants, or intrauterine devices containing progesterone), were non-smokers (never or former), white, and highly educated (Table 1). The haplotype frequencies of *UGT1A* and *SULT1E1*, which satisfied Hardy-Weinberg equilibrium, and all genotype frequencies with nonsignificant chi-square tests at p-value>0.05 for each hormone and urinary catechol estrogen metabolite are presented in Tables 2-4.

After adjusting for ethnicity, inverse associations were observed between estrogens and polymorphisms in *UGT1A1* and *SULT1A1* (Table 2). Individuals with the *UGT1A1(TA7/TA7)* genotype had a 25% lower E_2 concentration compared to the wildtype (*TA6/TA6*) (p-value = 0.02; p trend = 0.09). For individuals carrying the *H213* allele of SULT1A1 (*R213/H213* + *H213/H213*), an 11% lower E_1 concentration compared to the wildtype was observed (p-value = 0.02). A trend for lower E_1 concentrations with increasing number of *H213* alleles was not significant.

Individuals with the *UGT2B4(E458/E458)* genotype had a 20% lower DHEA concentration compared to the wildtype (p-value = 0.03; p trend = 0.08) [Table 3]. No association was observed between the *UGT2B17(null/null)* genotype and circulating T concentrations. A 10% lower T concentration was observed for women with the *SULT1A1(R213/H213)* genotype compared to the *SULT1A1(R213/R213)* genotype (p-value = 0.04). However, there was no indication of a linear trend (p-trend = 0.94).

No statistically significant associations were shown between the urinary catechol estrogen metabolites and any of the individual polymorphisms (data not shown). An inverse association was observed between 16α-OH E_1 and the *UGT1A1(TA7)-UGT1A3(R11)* haplotype (p-value = 0.04) (Table 4). Results from a likelihood ratio test comparing a model with the two-locus haplotype effects of UGT1A (full model) to the model with no haplotypes (reduced model) showed that the model without the haplotypes provided an adequate fit to the data for all the sex steroids.

Compared to *SULT1E1(A-G)*, inverse associations were shown between the *SULT1E1(A-C)* haplotype and E_1 and E_2. Log(E_1) and log E_2 concentrations were 0.96 pmol/l (95% CI: −1.55, −0.33, p-value = 0.003) and 1.06 (95% CI: −2.09, 0) pmol/l lower for women with the *SULT1E1(A-C)* haplotype compared to *SULT1E1(A-G)* (Table 4). Likelihood ratio tests comparing the full model with all the *SULT1E1* haplotypes to the model with no haplotypes showed that the model without the haplotypes provided an adequate fit to the data for all the sex steroids.

Discussion

In this well-characterized population of healthy premenopausal women, we assessed the associations between follicular phase circulating steroid hormone concentrations and urinary catechol estrogen metabolites and polymorphisms and / or haplotypes in the *UGT1A, UGT2B, SULT1A1*, and *SULT1E1* genes. Previous studies of UGT and SULT genotypes and steroid hormone concentrations have been conducted only in postmenopausal women. As such, our study provides unique data on the relation of these functional polymorphisms to circulating steroid hormone concentrations before the menopausal transition.

We observed an inverse association between the *UGT1A1(TA7/TA7)* genotype and circulating E_2 concentrations. UGT1A1 is known to glucuronidate estriol, E_2, and catechol estrogens [35,36]. Findings from functional analyses of the transcriptional promoter activity in breast and liver cells showed that the *UGT1A1(TA7)* allele has a 30% reduction in gene transcription and reduced *UGT1A1* gene expression compared to the wildtype allele [*UGT1A1(TA6)*] [37-39]. Thus, we hypothesized that carriers of the *TA7* allele should have higher concentrations of circulating estrogens. Our finding that E_2 concentration was 25% lower in premenopausal women with the *UGT1A1(TA7/TA7)* genotype compared with those with the *UGT(TA6/TA6*) genotype appear to contradict this hypothesis. However, strong linkage disequilibrium between the *TA7* allele of *UGT1A1* and *UGT1A6(T181A, R184S)* has been previously reported [31,40], and the *UGT1A1(TA7)* allele also has been shown to be in linkage disequilibrium with polymorphisms in *UGT1A3* [41], as our data demonstrate.

We did not observe strong statistically significant associations between the *UGT1A1(TA7)-UGT1A3(R11)* and *UGT1A1(TA7)-UGT1A3(A47)* haplotypes and E_2, 2-OH E_1, and 16α-OH E_1. The magnitude of the differences in these estrogen measures that we observed according to *UGT1A3* genotype is consistent with the greater clearance of estrogen expected in women with the *R11* and *A47* alleles [22]. Similar to our previous findings on the *UGT1A1-UGT1A3* haplotypes and mammographic density [14], our results suggest that at the *UGT1A* locus, the coinheritance of *UGT1A3(R11)* and / or *UGT1A3(V47A)*, or other variation on the haplotype containing these alleles, has the strongest influence on circulating estrogen concentrations. Because we did not have a large enough sample size to obtain stable estimates for a three-locus haplotype analysis, we were only able to infer two-locus haplotypes between *UGT1A1* and *UGT1A3* for our study population. Thus, haplotype analysis with UGT1A and hormone measures in a larger population of premenopausal women is warranted as it remains to be determined whether these alleles or others that are in linkage disequilibrium with the *TA7* allele most strongly influence serum estrogens and urinary estrogen metabolites.

We also observed an inverse association between carriers of the *H213* allele of *SULT1A1* and E_1 concentrations compared to the wildtype genotype (*R213/R213*). The *H213* allele is consistently associated with both low sulfation activity and low thermostability [42]. In our study, carriers of the *H213* allele had 10% lower E_1 concentrations compared to the wildtype allele. Given that the *SULT1A1(H213/H213)* genotype is associated with lower capacity to sulfate E_2 and catechol estrogens compared to the wildtype [23], we hypothesized that premenopausal women with the *H213* allele would have higher circulating estrogen concentrations. Our results (and those of others [43]), are opposite to what we hypothesized. Alleles for *SULT1A1* have been shown to be in linkage disequilibrium with alleles for *SULT1A2* [43], and possibly *SULT1A3* [44]. In addition, polymorphisms in the 5′-flanking region of the *SULT1A1* gene were shown to be in linkage disequilibrium with, and attenuate the effect of, the *SULT1A1(H213)* allele [45]. These findings suggest that haplotype analysis with *SULT1A* would shed more light on the association between *SULT1A* and circulating estrogen concentrations.

We also observed inverse associations between the *SULT1E1(A-C)* haplotype and E_1 and E_2. *SULT1E1* shows the highest affinity for estrogens among known members of the human SULT enzymes [7], and is highly expressed in normal human mammary epithelial cells [18]. Our findings should be interpreted with caution given that we did not observe all of the expected haplotypes that were identified in Adjei et al. [7], from which we selected our SNPs. To the extent that haplotypes were not observed due to the inclusion of insufficient tagSNPs, individuals with missing haplotypes may have been misclassified as having the measured haplotypes. We were also unable to determine other common polymorphisms in our study population because we did not sequence the *SULT1E1* gene in our study. Thus, it is possible that we did not select an optimal set of SNPs for our study population. To our knowledge, this is the first study examining associations between *SULT1E1* haplotypes and hormone concentrations in premenopausal women. Additional studies are needed to confirm our findings and investigate the functional implications of *SULT1E1* haplotypes on hormone concentrations.

Modest significant and non-significant associations between polymorphisms in the *UGT2B* genes and circulating androgen concentrations were also shown. UGT2B4 is expressed in a wide range of tissues and was reported to conjugate 4-OH E_1, androstane-3α, 17β-diol (3α-diol), and androsterone (ADT) [46]. The effect of the *UGT2B4(E458)* polymorphism has not previously been studied in vivo, despite the location of the single amino acid change in the putative cosubstrate binding domain [10]. In vitro studies using bile acids, phenol derivatives, and catechol estrogens as substrates suggest no functional impact of this variation on gene product function [46]; however the impact of this polymorphism on androgen glucuronidation and properties such as enzyme stability has not been evaluated. Our results suggest that in vivo the *UGT2B4(E458/E458)* genotype is associated with higher glucuronidation activity, thereby leading to reduced concentrations of circulating androgen metabolites.

We did not find a statistically significant association between the androgens and polymorphisms in *UGT2B7*, *UGT2B15* and *UGT2B17*, which have been shown to conjugate testosterone and androgen metabolites in the liver and several extrahepatic tissues [8,47]. Results from Lazarus et al. [48] suggest that the UGT2B17 deletion, which results in the absence of a 150kb genomic interval spanning the entire gene, is significantly associated with lower glucuronidation activities in human liver microsomes. Furthermore, Jakobsson et al. [32] showed that the UGT2B17 deletion is strongly associated with no or negligible amounts of urinary testosterone glucuronide concentrations in men. In our study, women with the *UGT2B17(null/null)* genotype had a borderline significantly higher testosterone concentration of 13.3% compared to women with the *UGT2B17(not null/not null)* genotype. This is in the direction hypothesized based on the in vitro and in vivo studies mentioned above. A potential explanation for our non-significant findings could be the lower concentrations of androgens in women compared to men [total serum testosterone concentration: 0.5-2.5 nmol/l versus 9-25 nmol/l, respectively [49]]. Against this background of low androgen concentrations, the polymorphisms in *UGT2B7*, *2B15*, and *2B17* may not sufficiently reduce activity to a minimal threshold that would affect circulating androgen concentrations. Another possible explanation implicates the link between the deletion polymorphism in *UGT2B17* and production of UGT2B15 [50]. Jakobsson et al. [51] showed that individuals with the *UGT2B17(null/null)* genotype had 4.5 times more UGT2B15 mRNA compared to individuals with the *UG2B17(not null/not null)* genotype, suggesting that the lack of the UGT2B17 enzyme may be compensated for by an increase in UGT2B15 transcription.

Our study has some limitations. It can be argued that follicular phase hormone measures may not be representative of sex steroid concentrations for premenopausal women

throughout the menstrual cycle. However, it has been shown that a reasonable characterization of inter-individual differences in premenopausal E_2 concentrations can be obtained with single blood samples taken between days 5 and 9 (i.e., early to mid-follicular phase of the menstrual cycle) [52]. Further, it has been suggested that among premenopausal women, a single blood measurement can reliably categorize average concentrations of androgens and estrone sulfate over at least a 3-y period [53].

It is often difficult to extrapolate results of in vitro cell- or tissue-specific UGT kinetic studies to genotype-phenotype associations in vivo. Furthermore, given the complexity and challenges of studying these associations in vivo, we also are limited in our ability to assess whether supposed small changes in activity or expression by the variants in vitro translate to changes in physiological hormone concentrations. It is possible that the lack of an association is not because the polymorphism does not change enzyme function, but because the effect of the single enzyme variant may be too small to detect in vivo where functional redundancy in UGT and SULT enzymes compensates for the less active variant enzyme.

Because most women were white and well-educated and all were members of a health plan, our findings may be generalizable only to similar populations of women. Another limitation is our small sample size, which restricted our ability to explore potential interactions between genotypes. Given that many comparisons were made, it is possible that some of the statistically significant findings may have occurred by chance. The associations between *SULT1A1* and circulating E_1 and T concentrations for example, were significant only with the heterozygote genotypes with non-significant p trend values. Thus, these results were very likely to have occurred by chance. Adjustment for multiple testing using the Holm step-down procedure [54] showed no statistically significant results, suggesting that further study in larger population samples of premenopausal women is warranted.

Measuring all the polymorphisms involved in steroid hormone metabolism was beyond the scope of this study. Nonetheless, to date, no studies have evaluated relationships between *UGT* and *SULT* polymorphisms and circulating sex steroids in healthy, premenopausal women, and results from our study can be used as important preliminary data for determining approaches for future, larger-scale molecular epidemiologic studies that aim to capture variation in all the relevant sex-hormone metabolizing enzymes.

In summary, in this population of premenopausal women, E_2, E_1, and DHEA were associated with polymorphisms in *UGT1A1*, *SULT1A1* and *UGT2B4*. The estrogens were also shown to be inversely associated with *SULT1E1* haplotypes. More studies in larger population samples of premenopausal women examining the role of polymorphisms and haplotypes in steroid hormone pathway genes as predictive markers of circulating sex steroids and urinary catechol estrogen metabolites are needed to confirm these genotype-phenotype relationships and improve the generalizability of these findings.

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Refereces

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Table 1

Characteristics of study population: Group Health, Seattle, WA, 2004 - 2005 (N = 170[a])

Age, y (mean, SD)	42.4	(1.4)
Age at menarche, y (mean, SD)	12.8	(1.3)
Age at first birth[b], y (mean, SD)	28.8	(5.9)
BMI, kg/m^2 (mean, SD)	25.7	(4.5)
Height, m (mean, SD)	1.65	(0.07)
Weight, kg (mean, SD)	70.1	(12.8)
Waist:Hip ratio (mean, SD)	0.79	(0.06)
Number of pregnancies (n, %)		
None	49	(28.8)
1	24	(14.1)
2+	94	(55.3)
History of breast-feeding[b] (n, %)	97	(81.5)
History of hormone use (n, %) (e.g., oral contraceptives, hormone patches, hormone injections, hormone implants, intrauterine devices containing progesterone)	120	(70.6)
Smoking status (n, %)		
Current	8	(4.7)
Former	45	(26.5)
Never	115	(67.6)
Race / ethnicity (n, %)		
Asian	13	(7.6)
White	147	(86.5)
Other	8	(4.7)
Years of school completed (n, %)		
≤ 12	12	(7.1)
13 – 15	45	(26.5)
16	47	(27.6)
≥ 17 years	64	(37.6)
Income (n, %)		
≤ $49,999	25	(14.7)
$50,000 - $75,000	41	(24.1)
> $75,000	81	(47.6)
Prefer not to answer	21	(12.4)

[a] Numbers may not add up to 170 for some characteristics due to missing values

[b] Among parous women only (n = 119), number of parous women does not add up to 119 for the variable “Number of pregnancies” due to a missing value for a parous woman

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Table 2

Adjusted[a] generalized regression analysis: Geometric mean circulating estrogen concentrations by genotype

		Hormone[b]							
		E_1 (pmol/l)				E_2 (pmol/l)			
Genotype	**n**	**Geometric mean (95% CI)**	**p-value**	**% diff**	**p trend**	**Geometric mean (95% CI)**	**p-value**	**% diff**	**p trend**
UGT1A1									
6/6	86	255 (237, 274)	Ref	Ref	0.82	378 (338, 421)	Ref	Ref	0.09
6/7	69	265 (246, 285)	0.46	+4.0		363 (318, 415)	0.66	−3.8	
7/7	15	234 (195, 282)	0.40	−8.1		284 (228, 353)	0.02	−24.9	
6/7 + 7/7	84	259 (242, 277)	0.74	+1.7		348 (309, 391)	0.31	−7.9	
UGT1A3									
W11/W11	63	248 (228, 269)	Ref	Ref	0.60	364 (323, 411)	Ref	Ref	0.49
W11/R11	74	268 (248, 289)	0.17	+8.2		375 (329,429)	0.75	+3.0	
R11/R11	33	252 (225, 281)	0.84	+1.5		331 (278, 393)	0.37	−9.2	
W11/R11 + R11/R11	107	263 (247, 280)	0.27	+6.0		361 (325, 401)	0.91	−1.0	
UGT1A3									
V47/V47	75	248 (229, 269)	Ref	Ref	0.61	363 (324, 407)	Ref	Ref	0.53
V47/A47	71	271 (253, 290)	0.10	+9.1		376 (329, 430)	0.69	+3.6	
A47/A47	24	249 (216, 286)	0.91	−0.9		321 (261, 396)	0.32	−11.4	
V47/A47 + A47/A47	95	264 (248, 281)	0.22	+6.5		362 (323, 405)	0.96	−0.4	
UGT2B4									
D458/D458	94	260 (242, 281)	Ref	Ref	0.45	374 (335, 418)	Ref	Ref	0.67
D458/E458	67	254 (237, 274)	0.68	−2.2		340 (298, 388)	0.28	−9.2	
E458/E458	9	240 (204, 281)	0.36	−8.0		415 (322, 535)	0.47	+10.9	
D458/E458 + E458/E458	76	253 (236, 270)	0.57	−2.9		348 (308, 392)	0.39	−7.0	
UGT2B7									
H268/H268	45	268 (244, 296)	Ref	Ref	0.05	356 (307, 413)	Ref	Ref	0.19
H268/Y268	85	262 (244, 283)	0.70	−2.3		397 (354, 445)	0.24	+11.6	

		Hormone[b]							
		E_1 (pmol/l)				E_2 (pmol/l)			
Genotype	**n**	**Geometric mean (95% CI)**	**p-value**	**% diff**	**p trend**	**Geometric mean (95% CI)**	**p-value**	**% diff**	**p trend**
Y268/Y268	40	234 (214, 257)	0.05	−12.7		304 (258, 358)	0.17	−14.6	
H268/Y268 + Y268/Y268	125	253 (239, 268)	0.32	−5.6		365 (332, 401)	0.74	+3.0	
UGT2B15									
D85/D85	34	255 (231, 281)	Ref	Ref	0.10	336 (280, 404)	Ref	Ref	0.60
D85/Y85	92	272 (253, 291)	0.31	+6.5		394 (357, 434)	0.14	+17.0	
Y85/Y85	44	230 (210, 253)	0.15	−9.5		322 (268, 386)	0.73	−4.4	
D85/Y85 + Y85/Y85	136	258 (243, 273)	0.83	+1.2		369 (338, 403)	0.36	+9.9	
UGT2B17									
Not null/Not null	70	254 (234, 276)	Ref	Ref	0.52	352 (307, 402)	Ref	Ref	0.46
Not null/Null	73	256 (236, 276)	0.91	+0.6		363 (323, 409)	0.72	+3.3	
Null/Null	27	270 (243, 298)	0.37	+6.1		389 (315, 482)	0.43	+10.7	
Not null/Null + Null/Null	100	259 (243, 276)	0.74	+1.8		370 (334, 408)	0.58	+4.8	
SULT1A1									
R213/R213	80	274 (254, 295)	Ref	Ref	0.08	390 (344, 444)	Ref	Ref	0.16
R213/H213	64	239 (219, 260)	0.02	−12.8		335 (294, 383)	0.11	−14.2	
H213/H213	26	253 (227, 282)	0.24	−7.6		348 (295, 410)	0.29	−11.0	
R213/H213 + H213/H213	90	243 (227, 260)	0.02	−11.4		339 (305, 376)	0.10	−13.2	

[a] adjusted for ethnicity

[b] E_1 – estrone, E_2 – estradiol

Table 3

Adjusted[a] generalized linear regression analysis: Geometric mean circulating androgen concentrations by genotype

		Hormone[b]											
		A (nmol/l)				T (nmol/l)				DHEA (nmol/l)			
Genotype	**n**	**Geometric mean (95% CI)**	**p-value**	**% diff**	**p trend**	**Geometric mean (95% CI)**	**p-value**	**% diff**	**p trend**	**Geometric mean (95% CI)**	**p-value**	**% diff**	**p trend**
UGT1A1													
6/6	86	3.21 (3.00, 3.42)	Ref	Ref	0.30	0.88 (0.81, 0.95)	Ref	Ref	0.90	12.6 (11.4, 14.0)	Ref	Ref	0.85
6/7	69	3.24 (3.04, 3.49)	0.76	+1.5		0.83 (0.77, 0.89)	0.30	−5.4		12.0 (10.8, 13.4)	0.50	−4.9	
7/7	15	3.49 (3.14, 3.91)	0.15	+9.6		0.93 (0.81, 1.07)	0.44	+6.3		13.9 (12.1, 16.1)	0.26	+10.3	
6/7 + 7/7	84	3.28 (3.11, 3.49)	0.53	+2.9		0.85 (0.79, 0.90)	0.49	−3.5		12.4 (11.3, 13.5)	0.73	−2.3	
UGT1A3													
W11/W11	63	3.24 (2.97, 3.52)	Ref	Ref	1.00	0.88 (0.81, 0.96)	Ref	Ref	0.38	12.4 (11.0, 14.1)	Ref	Ref	0.96
W11/R11	74	3.28 (3.07, 3.49)	0.87	+0.9		0.87 (0.81, 0.94)	0.86	−1.0		12.6 (11.3, 13.9)	0.90	+1.0	
R11/R11	33	3.21 (2.93, 3.56)	0.96	−0.3		0.82 (0.74, 0.91)	0.32	−6.4		12.4 (11.0, 14.2)	0.99	+0.1	
W11/R11 + R11/R11	107	3.24 (3.97, 3.42)	0.92	+0.5		0.85 (0.81, 0.91)	0.61	−2.8		12.5 (11.6, 13.6)	0.92	+0.7	
UGT1A3													
V47/V47	75	3.21 (2.97, 3.46)	Ref	Ref	0.68	0.88 (0.81, 0.96)	Ref	Ref	0.56	12.4 (11.2, 13.8)	Ref	Ref	0.78
V47/A47	71	3.28 (3.07, 3.49)	0.66	+2.2		0.85 (0.79, 0.91)	0.47	−4.0		12.4 (11.2, 13.8)	0.98	+0.2	
A47/A47	24	3.28 (2.90, 3.70)	0.76	+2.1		0.86 (0.75, 0.97)	0.70	−2.8		12.8 (11.0, 15.0)	0.72	+3.4	
V47/A47 + A47/A47	95	3.28 (3.11, 3.46)	0.65	+2.2		0.85 (0.80, 0.91)	0.47	−3.7		12.6 (11.5, 13.7)	0.88	+1.0	
UGT2B4													

		Hormone[b]											
		A (nmol/l)				T (nmol/l)				DHEA (nmol/l)			
Genotype	**n**	**Geometric mean (95% CI)**	**p-value**	**% diff**	**p trend**	**Geometric mean (95% CI)**	**p-value**	**% diff**	**p trend**	**Geometric mean (95% CI)**	**p-value**	**% diff**	**p trend**
D458/D458	94	3.28 (3.07, 3.49)	Ref	Ref	0.35	0.86 (0.81, 0.93)	Ref	Ref	0.71	13.0 (11.9, 14.2)	Ref	Ref	0.08
D458/E458	67	3.21 (2.97, 3.49)	0.69	−2.0		0.87 (0.80, 0.95)	0.87	+0.9		12.1 (10.8, 13.6)	0.33	−6.8	
E458/E458	9	3.00 (2.69, 3.32)	0.12	−9.2		0.78 (0.62, 0.99)	0.44	−9.3		10.4 (8.61, 12.5)	0.03	−20.3	
D458/E458 + E458/E458	76	3.21 (2.97, 3.42)	0.54	−2.9		0.86 (0.79, 0.93)	0.95	−0.3		11.9 (10.7, 13.2)	0.19	−8.5	
UGT2B7													
H268/H268	45	3.24 (2.93, 3.59)	Ref	Ref	0.84	0.86 (0.77, 0.95)	Ref	Ref	0.64	12.9 (11.4, 14.5)	Ref	Ref	0.84
H268/Y268	85	3.24 (3.07, 3.46)	0.98	+0.2		0.88 (0.82, 0.95)	0.69	+2.7		12.2 (11.1, 13.5)	0.53	−4.8	
Y268/Y268	40	3.21 (2.90, 3.56)	0.84	−1.5		0.82 (0.74, 0.92)	0.63	−3.7		12.6 (10.8, 14.7)	0.85	−1.9	
H268/Y268 + Y268/Y268	125	3.24 (3.07, 3.42)	0.96	−0.3		0.86 (0.82, 0.92)	0.91	+0.7		12.4 (11.4, 13.4)	0.59	−3.9	
UGT2B15													
D85/D85	34	3.32 (3.04, 3.63)	Ref	Ref	0.57	0.87 (0.78, 0.97)	Ref	Ref	1.00	13.5 (11.8, 15.6)	Ref	Ref	0.46
D85/Y85	92	3.24 (3.04, 3.46)	0.66	−2.4		0.85 (0.79, 0.92)	0.73	−2.2		12.1 (11.0, 13.4)	0.23	−10.2	
Y85/Y85	44	3.21 (2.93, 3.49)	0.56	−3.6		0.87 (0.79, 0.96)	0.97	−0.3		12.5 (11.0, 14.2)	0.41	−7.8	
D85/Y85 + Y85/Y85	136	3.21 (3.07, 3.42)	0.58	−2.8		0.86 (0.81, 0.91)	0.79	−1.6		12.2 (11.3, 13.2)	0.24	−9.5	
UGT2B17													
Not null/not null	70	3.35 (3.07, 3.63)	Ref	Ref	0.75	0.85 (0.79, 0.92)	Ref	Ref	0.26	13.2 (11.8, 14.7)	Ref	Ref	0.57
Not null/Null	73	3.07 (2.90, 3.24)	0.10	−8.1		0.84 (0.77, 0.91)	0.81	−1.3		11.5 (10.5, 12.6)	0.06	−12.8	

		Hormone[b]											
		A (nmol/l)				T (nmol/l)				DHEA (nmol/l)			
Genotype	**n**	**Geometric mean (95% CI)**	**p-value**	**% diff**	**p trend**	**Geometric mean (95% CI)**	**p-value**	**% diff**	**p trend**	**Geometric mean (95% CI)**	**p-value**	**% diff**	**p trend**
Null/Null	27	3.49 (3.11, 3.87)	0.58	+4.1		0.96 (0.86, 1.08)	0.07	+13.3		13.6 (11.3, 16.4)	0.76	+3.3	
Not null/Null + Null/Null	100	3.18 (3.00, 3.35)	0.24	−5.6		0.87 (0.81, 0.93)	0.76	+1.6		12.0 (11.0, 13.0)	0.14	−9.6	
SULT1A1													
R213/R213	80	3.35 (3.14, 3.56)	Ref	Ref	0.74	0.90 (0.83, 0.96)	Ref	Ref	0.94	13.0 (11.7, 14.4)	Ref	Ref	0.18
R213/H213	64	3.07 (2.86, 3.32)	0.12	−7.7		0.80 (0.73, 0.87)	0.04	−10.8		12.6 (11.4, 13.8)	0.65	−3.1	
H213/H213	26	3.38 (3.00, 3.80)	0.81	+1.6		0.94 (0.82, 1.08)	0.50	+5.5		11.1 (9.06, 13.5)	0.16	−14.8	
R213/H213 + H213/H213	90	3.18 (2.97, 3.38)	0.27	−5.1		0.84 (0.78, 0.90)	0.21	−6.3		12.1 (11.1, 13.2)	0.32	−6.7	

[a] adjusted for ethnicity

[b] A – androstenedione, T – testosterone, DHEA – dehydroepiandrosterone

Table 4

Adjusted[a] generalized linear regression analysis: Coefficient of log hormone concentrations by UGT1A and SULT1E1 haplotypes

		Hormone[b]						
		log(E_1) (pmol/l)	log(E_2) (pmol/l)	log(A) (nmol/l)	log(T) (nmol/l)	log(DHEA) (nmol/l)	log(2-OH E_1) (nmol/mmol cr)	log(16α-OH E_1) (nmol/mmol cr)
Haplotype	**Frequency**	**Coefficient (95% CI) p-value**	**Coefficient (95% CI) p-value**	**Coefficient (95% CI) p-value**	**Coefficient (95% CI) p-value**	**Coefficient (95% CI) p-value**	**Coefficient (95% CI) p-value**	**Coefficient (95% CI) p-value**
UGT1A1(TA6/TA7)-UGT1A3(W11R)								
TA6-W11	0.58	Ref	Ref	Ref	Ref	Ref	Ref	Ref
TA6-R11	0.13	0.18 (−0.18, 0.59) 0.34	0.22 (−0.40, 0.84) 0.49	−0.17 (−0.49, 0.17) 0.34	−0.002 (−0.006, 0.001) 0.23	−0.03 (−0.52, 0.48) 0.94	−0.17 (−0.10, 0.06) 0.67	−0.03 (−0.09, 0.02) 0.26
TA7-R11	0.29	0 (−0.30, 0.30) 0.99	−0.33 (−0.77, 0.15) 0.16	0.07 (−0.14, 0.35) 0.48	0 (−0.003, 0.002) 0.73	0.03 (−0.35, 0.38) 0.89	−0.04 (−0.10, 0.02) 0.17	−0.46 (−0.09, 0) 0.04
UGT1A1(TA6/TA7)-UGT1A3(V47A)								
TA6-V47	0.65	Ref	Ref	Ref	Ref	Ref	Ref	Ref
TA6-A47	0.06	0.30 (−0.18, 0.81) 0.23	0.51 (−0.29, 1.32) 0.21	−0.10 (−0.56, 0.31) 0.60	−0.002 (−0.007, 0.002) 0.35	0.07 (−0.59, 0.76) 0.80	0.07 (−0.29, 0.17) 0.17	0.02 (−0.05, 0.10) 0.53
TA7-A47	0.29	0 (−0.30, 0.30) 0.98	−0.33 (−0.77, 0.15) 0.16	0.10 (−0.14, 0.35) 0.42	0 (−0.003, 0.002) 0.81	0.03 (−0.31, 0.07) 0.84	−0.03 (−0.09, 0.02) 0.27	−0.04 (−0.08, 0) 0.08
SULT1E1[c] *: (A/G), (G/C)*								
A-G	0.57	Ref	Ref	Ref	Ref	Ref	Ref	Ref
A-C	0.05	−0.96 (−1.55, −0.33) 0.003	−1.06 (−2.09, 0) 0.05	−0.31 (−0.87, 0.28) 0.30	0 (−0.007, 0.006) 0.82	0.24 (−0.66, 1.14) 0.59	−0.08 (−0.21, 0.06) 0.29	−0.01 (−0.10, 0.08) 0.81

		Hormone[b]						
		log(E_1) (pmol/l)	**log(E_2) (pmol/l)**	**log(A) (nmol/l)**	**log(T) (nmol/l)**	**log(DHEA) (nmol/l)**	**log(2-OH E_1) (nmol/mmol cr)**	**log(16α-OH E_1) (nmol/mmol cr)**
Haplotype	**Frequency**	**Coefficient (95% CI) p-value**	**Coefficient (95% CI) p-value**	**Coefficient (95% CI) p-value**	**Coefficient (95% CI) p-value**	**Coefficient (95% CI) p-value**	**Coefficient (95% CI) p-value**	**Coefficient (95% CI) p-value**
G-C	0.26	0.15 (−0.15, 0.44) 0.32	0.11 (−0.37, 0.59) 0.68	0.01 (−0.24, 0.28) 0.93	0 (−0.003, 0.003) 0.91	−0.28 (−0.69, 0.10) 0.15	−0.03 (−0.10, 0.03) 0.29	−0.04 (−0.08, 0.01) 0.10

[a] adjusted for ethnicity

[b] E_1 – estrone, E_2 – estradiol, A – androstenedione, T – testosterone, DHEA – dehydroepiandrosterone, OH – hydroxyl cr - creatinine

[c] refer to Adjei et al. [21]