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# Relationship of *SULT1A1* Copy Number Variation with Estrogen Metabolism and Human Health

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## Abstract

Human cytosolic sulfotransferase 1A1 (SULT1A1) is considered to be one of the most important SULT isoforms for metabolism, detoxification, and carcinogenesis. This theory is driven by observations that SULT1A1 is widely expressed in multiple tissues and acts on a wide range of phenolic substrates. *SULT1A1* is subject to functional common copy number variation (CNV) including deletions or duplications. However, it is less clear how *SULT1A1* CNV impacts health and disease. To better understand the biological role of *SULT1A1* in human health, we genotyped CNV in 14,275 Marshfield Clinic patients linked to an extensive electronic health record. Since *SULT1A1* is linked to steroid metabolism, select serum steroid hormones were measured in 100 individuals with a wide spectrum of *SULT1A1* CNV genotypes. Furthermore, comprehensive phenome-wide association studies (PheWAS) were conducted using diagnostic codes and clinical text data. For the first time, individuals homozygous null for *SULT1A1* were identified in a human population. Thirty-six percent of the population carried >2 copies of *SULT1A1* whereas 4% had ≤ 1 copy. Results indicate *SULT1A1* CNV was negatively correlated with estrone-sulfate to estrone ratio predominantly in males (E1S/E1; p=0.03, r=−0.21) and may be associated with increased risk for common allergies. The effect of *SULT1A1* CNV on circulating estrogen metabolites was opposite to the predicted CNV-metabolite trend based on enzymatic function. This finding, and the potential association with common allergies reported herein, warrants future studies.

### Keywords

PheWAS; TextWAS; allergy; estrogen; sulfation; metabolism

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**Declaration of interest**

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

## 1. Introduction

Human cytosolic sulfotransferase 1A1 (*SULT1A1*) is one of four genes in the *SULT1A* subfamily (*SULT1A1*, *1A2*, *1A3*, and *1A4*) that is mapped to the short arm of chromosome 16 [1–4]. This chromosomal region is rich in repetitive sequences and segmental duplications [3, 5], as demonstrated by a large ancestral duplication containing *SULT1A3*/*SULT1A4* [3] and a highly polymorphic copy number variant (CNV) resulting in the complete deletion and duplication of *SULT1A1* [6]. SULT1A1 is hypothesized to be one of the most important SULT isoforms. This assumption is driven by observations that SULT1A1 is widely expressed in multiple human tissues, and biochemical experiments demonstrate that SULT1A1 activity plays an important role in the metabolism, bioactivation, and detoxification of procarcinogens, medications, and steroid hormones, including estrogens [7–9].

In addition to the biochemical properties of SULT1A1, early functional genetic studies have identified coding and regulatory single-nucleotide polymorphisms (SNPs) that are associated with SULT1A1 transcription, translation, and enzyme activity [10–14]. Many of these SNPs have been associated with a wide spectrum of clinical phenotypes [15]. For example, variant SULT1A1*2 has been reported to be associated with cancer risk in various organs and tissues in different ethnic groups [16–21], but other research groups have found conflicting evidence [22]. Since these studies often fail to consider the functional CNV [6, 23, 24], interpreting the relevance of these associations is challenging. Furthermore, *SULT1A1* CNV may have an impact on SNP genotype quality when there can be between 0–6 copies of SULT1A1 with varying dosages of SNP alleles. A few studies have begun to assess the relevance of *SULT1A1* CNV with clinical phenotypes [5, 10, 14, 25–27] and metabolism. These studies are often limited in scope, sample size, and do not capture extreme CNV genotypes [23, 24].

Given the observed importance of CNV in SULT1A1 activity, biochemical relevance of SULT1A1 with respect to metabolism, and potential role in human health, we conducted a large-scale screen of *SULT1A1* CNV in 14,725 patients of European descent linked to an extensive electronic health record (EHR). To better understand the potential metabolic impact of SULT1A1, we selected 100 individuals with a wide range of *SULT1A1* genotypes (0–6 copies) and correlated those genotypes with serum estrogen levels. To further assess the potential clinical relevance of CNV, we associated *SULT1A1* genotype with over 30,000 phenotypes by a phenome-wide association study (PheWAS) and a text-wide association study (TextWAS). This research builds on the relevance of *SULT1A1* CNV in estrogen metabolism, identified individuals who are homozygous deleted for this important enzyme, and describes how *SULT1A1* CNV may be associated with phenotypes linked to common allergies.

## 2. Materials and methods

### 2.1 Population

All subjects studied came from Marshfield Clinic's Personalized Medicine Research Project (PMRP) and have been described previously [28–31]. PMRP is a homogenous cohort of adult patients who are predominantly Caucasian, 77% claiming German ancestry, and have on average over 30 years of EHR data. In total, all individuals over the current age of 40 (14,275 participants) were genotyped for *SULT1A1* copy number. The electronic medical records of individuals with rare variants of *SULT1A1* and 100 randomly selected individuals taking Nasacort for nasal allergies were reviewed manually by a trained study coordinator. This study was approved by the Marshfield Clinic Institutional Review Board. Written and informed consent was acquired for all participants prior to study enrollment.

## 2.2 Copy number variation genotyping

Fluorescent-based semi-quantitative PCR was used to genotype *SULT1A1* CNV [6]. A set of PCR primers were designed to co-amplify a 212 bp fragment within exons 2 and 3 of *SULT1A1* (NM_177534) and a 208 bp fragment within exons 3 and 4 of *SULT1A2* (NM_177528). *SULT1A2* was used as an internal two copy control. PCR products were analyzed on an ABI3130 DNA analyzer (Foster City, CA). Copy number was estimated by calculating the height ratio of the 212 bp amplicon of *SULT1A1* to the reference 208 bp amplicon of *SULT1A2*. Samples with a ratio of 0, ~0.5, ~1.0, ~1.5, ~2.0, ~2.5, and ~3.0 were defined as having a *SULT1A1* copy number of 0, 1, 2, 3, 4, 5, and 6, respectively (Figure 1). Assuming there are four possible alleles for *SULT1A1* CNV (0–3 copies), and seven observed CNV genotypes (0–6 copies), an expectation-maximization algorithm was utilized to estimate allele frequencies. The initial allele frequency was set at 0.25 for all alleles and the model converged after 13 iterations with a sum of all errors across the alleles of 5.6E-7.

## 2.3 Steroid measurements

**2.3.1 Sample collection—**To understand the potential biological involvement of SULT1A1 activity on estrogen metabolism, we quantified estrogen metabolites in serum from 100 PMRP patients. This included 50 males between the ages of 40 to 50 and 50 premenopausal females between the ages of 30 to 40; age is defined as age at blood draw. All individuals with rare CNV genotypes in their respective age group (0, 5 and 6 copies) were selected whereas 10 randomly selected individuals from each of the remaining common copy number categories (1–4 copies) were selected for analysis. No individual was pregnant within a year of blood draw, on hormone replacement therapy, or had a history of breast or prostate cancer.

**2.3.2 Sample processing and steroid measurements—**All sample processing and liquid chromatography–mass spectrometry (LC-MS) analysis was performed by researchers blinded to sample identity and CNV genotype. Measurement of the products of SULT1A1 estrogen metabolites, specifically free estrone (E1) and sulfated estrone (E1S) were conducted. As a physiological negative control, free dehydroepiandrosterone (DHEA) and sulfated dehydroepiandrosterone (DHEA-S) were analyzed, as these compounds are generated primarily by adrenal synthesis and undergo sulfation by SULT2A1 [32].

Steroid levels were measured on an Ultimate 3000 quaternary UHPLC coupled to a Q Exactive Plus mass spectrometer operating in the negative ion mode (conjugates) and positive mode (unconjugated steroids). LC-MS Optima grade solvents (water, methanol, acetonitrile, and acetic acid) were purchased from Fisher Scientific (Pittsburg, PA). DHEA-S, E1S, E1, DHEA as well as stable isotope-labeled standards [$^{2}H_{5}$]-DHEA-S and [$^{2}H_{4}$]-E1S were purchased from Sigma (St. Louis, MO) and were tested before analysis for cross-contamination for hormones measured here. Girard P reagent was from Tokyo Chemical Industry Company, LTD (Tokyo, Japan). Stable isotope-labeled [$^{13}C_{3}$]-E1 98% purity and [$^{2}H_{5}$]-DHEA 97% purity were from Cambridge Isotope Labs (Andover, MA). Double charcoal stripped human serum from Golden West Biologicals, Inc. (Temecula, CA, USA) was used as a surrogate matrix for calibrators and quality control and contained no detectable levels of any analytes.

Levels of unconjugated steroids were measured using a validated and previously published protocol of Girard P derivatization for keto-steroids [33, 34]. For conjugated steroid analysis, internal standard solutions containing 10 pg/μL [$^{2}H_{5}$]-DHEA-S and 10 pg/μL [$^{2}H_{4}$]-E1S in methanol were added (20 μL) to each sample of serum (100 μL). These samples were diluted with 320 μL of methanol, vortexed, and centrifuged at 15,000 × *g* for 10 minutes to precipitate insoluble components. Supernatants were transferred to new microcentrifuge tubes and evaporated to dryness under nitrogen then re-suspended in 100 μL solution of 95:5 water:methanol. Following resuspension, samples were vortexed, centrifuged at 15,000 × *g* for 5 minutes, and transferred to injection vials (90 μL total sample). A 10 μL aliquot of sample with 20 pg of each internal standard was injected on the column and analyzed via LC-MS/HRMS. LC separation of derivatized sample components was performed using a Waters XBridge C18 column (3.5 μm particle size, 2.1 × 150 mm) stored at 40ºC in a column heater with a two solvent gradient where solvent A was water with 0.2 mM ammonium fluoride and solvent B was methanol. The LC gradient was set at 0.2 ml/min flow 5% B for 1 min increasing in solvent concentration to 50% B at 5 min, with an increase in flow rate and solvent concentration to 0.225 ml/min and 90% B at 20 min and holding these parameters to 25 min, followed by re-equilibration at starting conditions from 26 to 30 min. MS analysis was performed using alternating full scan with data independent analysis at a 1 m/z isolation widow looped three times based on appearance of $[M\text{-}H]^{-}$ of each analyte and internal standard for a total of 18 scans. Peak integration was performed from the full scan at a 5 ppm window with a confirming ion from the MS/HRMS scan (10 ppm window) using the matched stable isotope labeled internal standard. Standard curves were linear within the range of the samples and quality control samples falling within 20% coefficient of variation across the analytical runs. The limits of quantitation (LOQ) were conservatively set at 10 times the lowest non-zero standard curve point since the signal intensity was zero in multiple analyte channels for the matrix blanks. LOQs for selected hormones were as follows: E1: 0.75 pg/mL, DHEA: 3.75 pg/mL, E1S: 0.24 ng/mL, and DHEA-S: 15.6 ng/mL. Selected samples were re-injected over the storage time during analysis to confirm stability within 5% of the original values. One sample was lost during processing due to defective glassware.

**2.3.3 Correlation analysis of SULT1A1 CNV and estrogen metabolism—**Since all metabolite levels were highly skewed, (|skew| > 0.6 checked by manual observation of histograms of hormone levels, with the exception of DHEA-S/DHEA in men only), Spearman rank correlations were used to examine the relationships among free compounds, conjugated analytes, paired free/conjugate ratios, and copy number stratified by sex. To determine if there was a significant difference between compound levels by copy number, the data was analyzed using non-parametric Kruskal-Wallis tests stratified by sex. Less than 2% of free steroids (E1, DHEA) had values that fell below the limit of detection. All statistical analyses were performed using SAS version 9.3.

### 2.4 Association methods

Because SULT1A1 genotype may be involved in estrogen metabolism and human health, a PheWAS was conducted. The phenome was defined by ICD9 coding extracted from patient EHR data using standard methods as described previously [29–31]. Individuals whose medical records contained ICD9 codes inclusive of three levels of resolution defined by ICD9 code suffix (for example, ICD9 720, 720.8, 720.89) were designated as a case for a particular condition, whereas individuals with no record of the broadest code (e.g., 720) were classified as controls. Due to privacy concerns, only those phenotypes that were observed >9 times within the cohort were assessed. Utilizing this approach, there were 6,910 phenotypes extracted from the EHR. Special attention was given toward two codes defining breast cancer (ICD9 174, malignant neoplasm of breast; and ICD9 233.0, carcinoma in situ of breast) in females only and 115 codes that define adverse drug events (E930–E949, E850–E858, except E850.1, E854.1) [35]. As an alternative to PheWAS, a TextWAS was conducted with methodologies reported previously [29]. Briefly, all clinical notes were broken down into four possible combinations of word strings which included unigrams (one word), bigrams (two adjacent words), trigrams (three adjacent words), and quad-grams (four adjacent words). All word strings were then cross referenced with the National Library of Medicine's unified Medical Language System medical dictionary. In total, the text-based phenome consisted of 23,382 clinically relevant terms (word strings). Individuals with a given word string were considered cases for that word string while all others were considered controls. Logistic regression using CNV as a continuous variable was conducted in both PheWAS and TextWAS. Sex and EHR length were included in the analysis as covariates. The p-value of CNV was generated using Wald statistics. All associations were conducted by Plink v1.9 (http://pngu.mgh.harvard.edu/purcell/plink/) [36] and R i386 3.1.0 (http://www.R-project.org/) [37].

## 3. Results

### 3.1 Correlation between SULT1A1 CNV and estrogen sulfation

*SULT1A1* CNV ranged from zero to six copies in the study population. Among the 14,872 individuals genotyped, approximately 4% could not be definitively defined due to *1A1/1A2* ratios falling between copy number bins. Of the remaining 14,275 subjects, 605 individuals (4%) carried less than two copies, 9,108 individuals (64%) had two copies, and 4,562 individuals (32%) carried three or more copies of *SULT1A1*. The frequency for the common CNV is similar to previous reports [6]. For the first time, 11 individuals (0.08%) were identified as homozygous deleted and 12 individuals (0.08%) had six copies of *SULT1A1* (Figure 1). Given there were seven observed genotypes (0–6 copies), it was assumed that there were predominantly 4 possible alleles of *SULT1A1* on any given chromosome (0–3 copies). Under this assumption, the frequency of the null allele was estimated at 2.6% while the frequencies for two and three copies of *SULT1A1* alleles were estimated at 17% and 1.2%, respectively.

To assess the physiological significance of *SULT1A1* CNV with respect to estrogen metabolism, E1 and E1S serum levels were measured from 50 males and 50 females, including those with extreme CNV genotypes. Estrogen levels from one female were identified as an extreme outlier and were excluded from further analysis. Spearman correlation analysis of *SULT1A1* CNV against measurements of E1 and E1S in all individuals and after adjusting for sex indicated that *SULT1A1* CNV was not individually associated with E1 or E1S levels; however, *SULT1A1* CNV was significantly correlated with E1S/E1 levels (p= 0.035, r=−0.21). Further analyses were performed with data from females and males separately. In males, *SULT1A1* CNV was significantly correlated with E1S/E1 levels (p=0.025, r=−0.32). This association was not as pronounced in females but had a similar direction of effect (p=0.15, r=−0.21) (Table 1). E1S levels in males were significantly different among different copy number groups (p= 0.012). As expected, no significant associations were detected between *SULT1A1* CNV and levels of DHEA and DHEA-S.

### 3.2 Association analysis

SNPs in *SULT1A1* have been associated with numerous phenotypes with varying and often conflicting results [16–22, 38]. Few studies have assessed the functionally relevant CNV. To understand the potential clinical impact of *SULT1A1* CNV, we leveraged extensive EHR data linked to all 14,275 individuals. Initial focus was directed toward the 11 individuals who were homozygous null for *SULT1A1*. After manual chart review, none of these individuals had any common or unusual phenotypic patterns. Therefore, it is unlikely the homozygous null genotype results in an unusual congenital abnormality. Because *SULT1A1* CNV was associated with baseline E1S/E1 ratios and SNPs in *SULT1A1* have previously been associated with breast cancer [20, 21], we further evaluated the relevance of *SULT1A1* CNV to breast cancer risk by analyzing the medical records of females diagnosed with ICD9 174, malignant neoplasm of breast (640 cases) and ICD9 233.0, carcinoma in situ of breast (210 cases). In this population, neither ICD9 code was associated with *SULT1A1* CNV (p=0.24 and 0.69, respectively).

Given SULT1A1’s reported involvement in the metabolism of numerous drugs [9], we further evaluated 115 ICD9 codes that define adverse drug events [35]. The strongest association was between *SULT1A1* CNV and “Anticoagulants causing adverse effects in therapeutic use” (ICD9 E934.2, p=0.0024, OR=0.64 [0.48–0.85] (Supplementary Table 1). Given the number of tests, this association was not statistically significant (p<4.3E-4 assuming α<0.05, 115 tests/phenotypes).

To further assess the impact of *SULT1A1* CNV on thousands of phenotypes, a PheWAS was conducted. Based on ICD9 codes to define cases and controls [29–31], no phenotype passed a conservative Bonferroni threshold (p<7.2E-6, assuming α < 0.05 and 6910 tests/phenotypes). The top associations included ICD9 616.3 defining abscess of Bartholin’s gland (p= 0.00020, OR=2.0[1.4–2.9]) followed by ICD9 379.92 defining swelling or mass of eye (p= 0.00021, OR=1.7[1.3–2.3]) (Figure 2, Supplementary Table 2); the relevance of these association is uncertain. Because it has been demonstrated that clinical text data can provide complementary data to ICD9 coding and provide additional phenotypic specificity [29], we conducted a TextWAS that associated 23,382 medical terms with *SULT1A1* CNV genotype. In this analysis, the top association was for the term "Nasacort" (1,455 cases; p=5.8E-7, OR 0.8[0.73–0.87]) (Figure 2, Supplementary Table 2). This association passed a conservative experiment-wise Bonferroni threshold (p<2.2E-6 assuming $\alpha$<0.05, 23,382 tests/phenotypes), and further passed a study-wise Bonferroni threshold when considering the total number of tests from both the TextWAS and PheWAS (p<1.7E-6 assuming $\alpha$<0.05, 30,292 tests/phenotypes) (Figure 2). Nasacort is an over-the-counter nasal spray containing triamcinolone, an adrenocortical steroid commonly used to treat nasal allergy symptoms such as sinusitis and rhinitis. An additional top TextWAS result included "Claritin," another over-the-counter drug frequently taken to treat common allergy symptoms (p=3.7E-4 OR 0.9[0.85–0.95]; Supplemental Table 2). Manual chart review of 100 randomly selected individuals with "Nasacort" indicated that none of the patients had any adverse drug reactions though approximately 15% and 42% with Nasacort documented were in reference to the treatment of sinusitis and rhinitis, respectively. The term "allergic rhinitis" extracted from TextWAS data was moderately associated with *SULT1A1* genotype (p=0.028) whereas the ICD9 code defining rhinitis (ICD9 477) from PheWAS data had a suggestive association (p=0.057).

## 4. Discussion

SULT1A1 is proposed to be involved in a variety of pathophysiologic processes such as drug metabolism, cancer, hormone regulation, and neurotransmitter biology [5]. Multiple coding and promoter variants have been associated with human disease with varying results. Many of these studies were conducted without considering the common CNV, even when functional studies suggest *SULT1A1* CNV may have a significant influence on enzyme activity [6, 10]. Only recently have other research groups begun to assess the impact of *SULT1A1* CNV on disease risk and estrogen metabolism.

Our results suggest that increasing copies of *SULT1A1* are inversely associated with E1S/E1 levels in males and, to a lesser degree, in females. Interestingly, *SULT1A1* copy number has been previously associated with male breast cancer risk [26]. Our associations may agree with Moyer, *et al.* who reported that increased copies of SNP alleles driven by the *SULT1A1* CNV are associated with lower E2S/E2 levels. These associations were only observed in women treated with oral conjugated equine estrogen [24]. In combination with Moyer *et al.*, our findings seem counterintuitive since increased copies of *SULT1A1* would result in increased SULT1A1 enzyme activity and should result in increased levels of E1S and E2S levels. It may be hypothesized that intracellular estrogen levels have an inverse relationship to circulating estrogens and that *SULT1A1* CNV may influence this dichotomy. It should be noted that SULT1E1 has been reported to be the primary conjugating enzyme for estrogen sulfation at physiological concentrations with two reported genetic variants that influence enzyme activity, but *SULT1E1* genetics may not have large influences on estrogen metabolism variability given reported functional variants are predominantly rare (minor allele frequency </= 1%) [39]. Although our estrogen metabolite experiments were the first to evaluate extreme CNV genotypes, larger population studies with sufficient power to

evaluate both the CNV and other potential functional variants in *SULT1A1* [16, 17, 22] in different ethnicities are required. This may be particularly relevant when evaluating extreme duplication events, which are more common in populations with African compared to European ancestries [6, 10]. Such studies should consider standardized sample collection to account for circadian rhythms and can stratify by therapeutic drugs such as aromatase inhibitors or tamoxifen, in combination with further functional studies, to better understand the relationship between *SULT1A1* genotype and estrogen metabolism. Likewise, further studies on other substrates of SULT1A1 would be highly informative to understanding the paradoxical relationship between CNV and metabotype.

To identify individuals with extreme CNV genotypes for the estrogen experiments required widespread screening. For the first time, individuals who were homozygous null for *SULT1A1* were observed but these individuals presented with no overt phenotype. This finding corresponds to phenotypic analysis of *SULT1A1* mouse knockouts that are viable and have no outwardly apparent phenotype. However, the absence of functional SULT1A1 enzyme in mouse knockouts may have an influence on the reduction of DNA adducts [40, 41], warranting future investigation into the effects of extreme *SULT1A1* CNV.

Because all individuals genotyped were linked to extensive phenotypic data, we conducted the first PheWAS/TextWAS for *SULT1A1*. Based on ICD9 coding, no association passed statistical significance and there was no evidence for association with female breast cancer risk. Although ICD9 coding has repeatedly been shown to be effective in defining case-control groups for thousands of phenotypes [15, 30, 42], ICD9 coding is primarily applied for billing purposes in the United States and can be limited in the phenotypes it captures. To address these limitations, it has been shown that clinical text data can complement ICD9 coding and provide additional phenotypic information [29]. In our TextWAS, we identified a statistically significant association with the term “Nasacort” (p=5.83E-7). As described previously, Nasacort, also known as triamcinolone, is an over-the-counter medication often used to treat allergy symptoms including rhinitis. Although text data can often provide additional phenotypic information not acquired by ICD9 coding, it can be more difficult to interpret text data without context. In relation to Nasacort, this challenge may be further exacerbated given many patients likely self-medicate with this over-the-counter drug. Association data from text and ICD9 coding related to rhinitis may support *SULT1A1* CNV involvement in risk for symptoms related to common allergies. Future disease specific studies that consider both CNV and SNP genotyping will be required to better understand this potential relationship.

## 5. Conclusions

In conclusion, this study is the first large-scale screen of *SULT1A1* CNV that identified individuals that were null for *SULT1A1*. Metabolic analysis of circulating hormones in the context of *SULT1A1* CNV identified a significant inverse relationship with E1/E1S concentration primarily in males. Further PheWAS/TextWAS results suggest *SULT1A1* CNV may be related to the treatment of common allergies. These results build on the growing importance of *SULT1A1* CNV on metabolism and human health.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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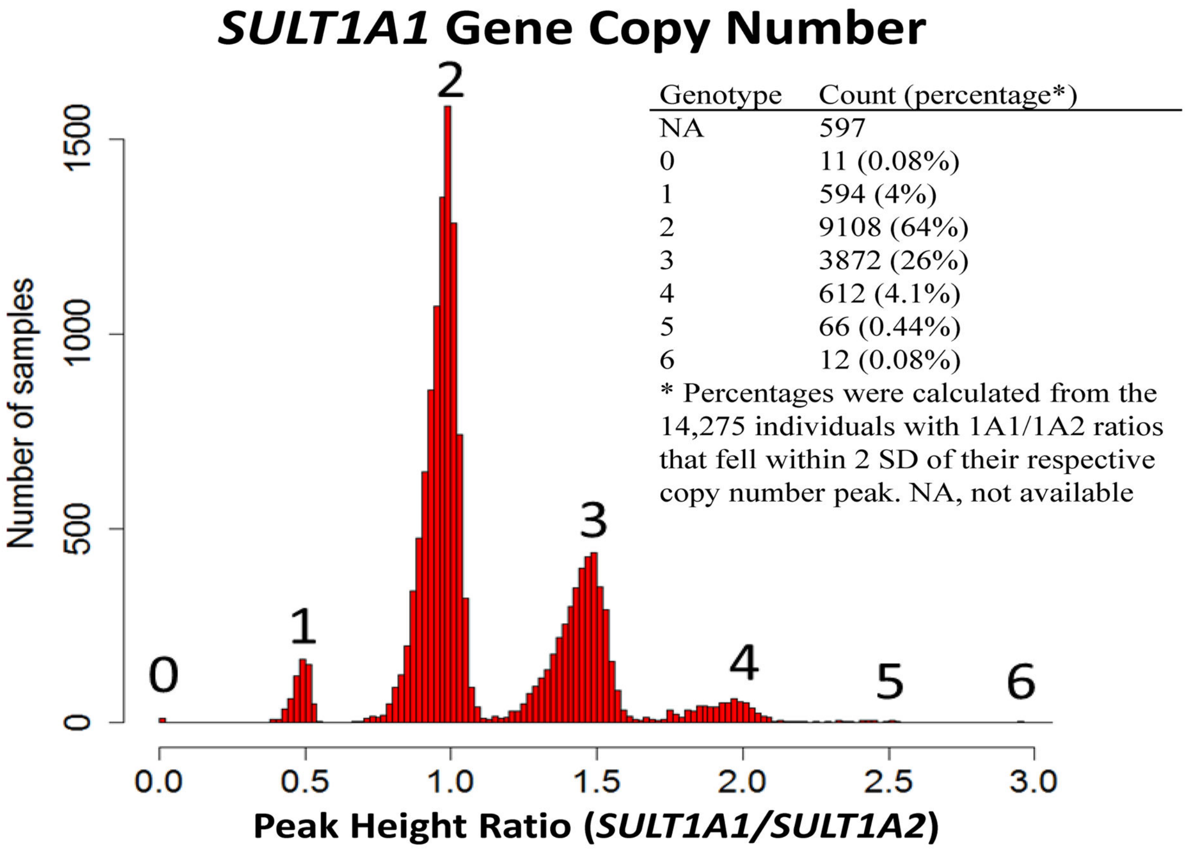

| Genotype | Count (percentage*) |
|---|---|
| NA | 597 |
| 0 | 11 (0.08%) |
| 1 | 594 (4%) |
| 2 | 9108 (64%) |
| 3 | 3872 (26%) |
| 4 | 612 (4.1%) |
| 5 | 66 (0.44%) |
| 6 | 12 (0.08%) |

* Percentages were calculated from the 14,275 individuals with 1A1/1A2 ratios that fell within 2 SD of their respective copy number peak. NA, not available

**Figure 1. Histogram of *SULT1A1/SULT1A2* ratios for all individuals genotyped**

Each peak with 1A1/1A2 ratios of 0, ~0.5, ~1.0, ~1.5, ~ 2.0, ~2.5, and ~3.0 define those individuals with 0, 1, 2, 3, 4, 5, and 6 copies of *SULT1A1*, respectively.

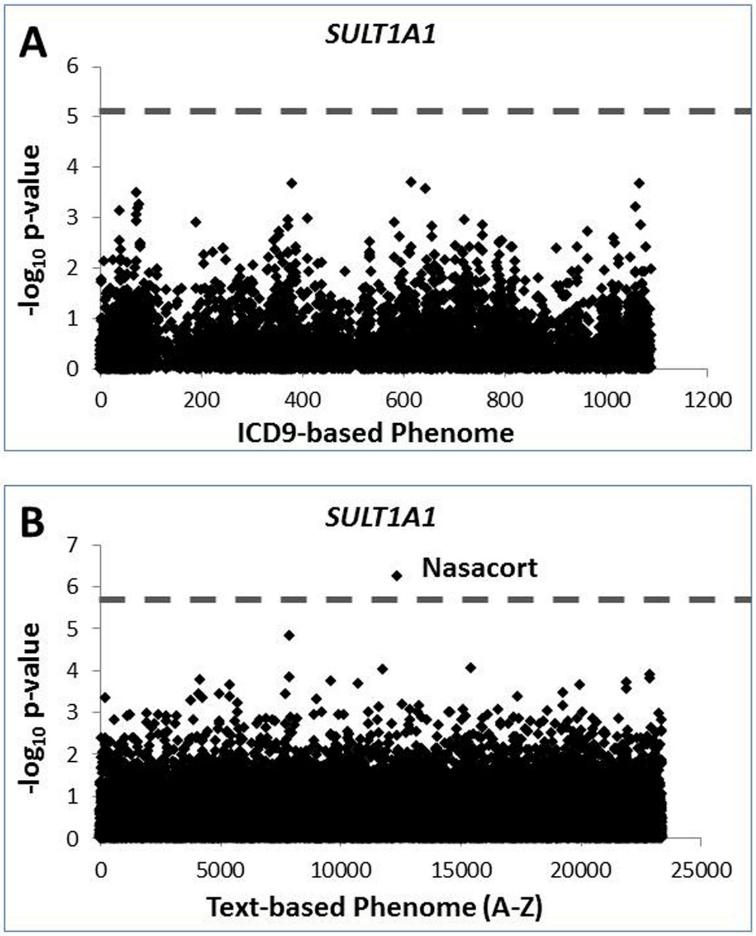

**Figure 2. Manhattan plot for (A) PheWAS and (B) TextWAS of *SULT1A1* CNV**

Dotted lines represent thresholds for statistical significance for the respective experiment.

## Table 1

Correlation between *SULT1A1* CNV and Estrogen sulfation

| **Gender (sample size)** | **Metabolite** | **Categories of CNV (Mean±SD)** | | | | | | | **r (p-value)** |
|---|---|---|---|---|---|---|---|---|---|
| | | **CP0<br>(n=3F, 2M)** | **CP1<br>(n=10F, 10M)** | **CP2<br>(n=10F, 10M)** | **CP3<br>(n=10F, 10M)** | **CP4<br>(n=10F, 10M)** | **CP5<br>(n=6F, 6M)** | **CP6<br>(n=0F, 2M)** | |
| Male (n=50) | E1 (pg/mL serum) | 25.18±15.34 | 25.34±18.57 | 48.02±45.49 | 52.03±46.30 | 48.71±28.53 | 41.68±21.88 | 27.55±6.07 | 0.23 (0.11) |
| | E1S (ng/mL serum) | 2.92±0.36 | 2.82±0.77 | 2.72±1.44 | 4.03±1.38 | 2.21±0.77 | 2.51±0.77 | 1.17±0.021 | −0.22 (0.11) |
| | E1S/E1 | 147.81 ± 104.27 | 182.61±137.54 | 80.63±45.73 | 142.38±114.90 | 83.12±80.11 | 75.84±43.85 | 43.57±8.84 | −0.32 (**0.025**) |
| | DHEA (pg/mL serum) | 24614.97±2953.30 | 21104.87±9556.66 | 37211.83±26096.38 | 30202.69±22522.43 | 31417.76±24561.34 | 32065.90±8646.91 | 40043.00±1870.94 | 0.26 (0.06) |
| | DHEAS (ng/mL serum) | 2491.77±511.21 | 3740.32±5343.70 | 13158.48±24639.23 | 8316.33±14730.70 | 2826.07±3308.61 | 3952.67±3909.26 | 2804.18±448.25 | −0.005 (0.97) |
| | DHEAS/DHEA | 100.71± 8.69 | 284.73± 613.71 | 345.25± 434.25 | 256.08± 322.08 | 119.67± 147.13 | 118.24± 104.96 | 70.37± 14.48 | −0.20 (0.16) |
| Female (n=49) | E1 | 68.61±39.60 | 57.86±52.49 | 74.08±46.69 | 77.69±45.60 | 175.07±186.32 | 84.89±86.97 | NA | 0.19 (0.20) |
| | E1S | 4.38±3.06 | 2.72±1.53 | 2.88±0.48 | 3.05±0.93 | 3.56±2.29 | 2.78±0.92 | NA | 0.03 (0.85) |
| | E1S/E1 | 70.74 ±34.23 | 300.85 ±481.30 | 55.63±39.31 | 68.99±66.23 | 40.17±45.87 | 92.57±84.91 | NA | −0.21 (0.15) |
| | DHEA | 19644.86±9322.50 | 47853.93±38728.78 | 32841.50±18937.03 | 33987.81±23845.05 | 46801.92±54419.97 | 16745.35±6075.99 | NA | −0.17(0.25) |
| | DHEAS | 927.29±243.11 | 3353.70±3109.54 | 1751.68±1036.42 | 1114.11±506.36 | 1458.83±779.25 | 1167.37±381.91 | NA | −0.17 (0.26) |
| | DHEAS/DHEA | 54.33± 30.07 | 76.54± 61.26 | 67.60± 44.77 | 39.52± 17.04 | 44.52± 25.88 | 79.81± 40.99 | NA | −0.04 (0.77) |
| Female + Male (n=99) | E1 | 51.23±37.54 | 40.64±40.72 | 61.05±46.82 | 64.86±46.62 | 115.21±148.05 | 61.32±61.43 | 27.55±6.07 | 0.14 (0.17) |
| | E1S | 3.79±2.32 | 2.77±1.18 | 2.80±1.05 | 3.54±1.25 | 2.88±1.80 | 2.65±0.82 | 1.17±0.021 | −0.10 (0.31) |
| | E1S/E1 | 101.57 ± 71.32 | 238.25±338.39 | 68.13±43.44 | 105.69±98.73 | 60.51±66.26 | 84.20±62.62 | 43.57±8.84 | −0.21 (**0.035**) |
| | DHEA | 21632.90±7283.24 | 34479.40±30692.70 | 35026.66±22304.34 | 32095.25±22657.93 | 39109.84±41843.38 | 24405.63±10713.59 | 40043.00±1870.94 | 0.04 (0.71) |
| | DHEAS | 1553.08±910.58 | 3547.01±4259.76 | 7154.90±17452.69 | 4715.22±10796.22 | 2142.45±2442.32 | 2560.02±3021.36 | 2804.18±448.25 | −0.08 (0.43) |
| | DHEAS/DHEA | 72.88± 33.41 | 180.64± 437.72 | 206.43± 332.50 | 142.10± 242.06 | 82.09± 109.81 | 99.03± 78.58 | 70.37± 14.48 | −0.08 (0.41) |

Abbreviations: CP0, CP1, CP2, CP3, CP4, CP5, and CP6 stands for the number of copy number variants 1–6 for SULT1A1; r (p-value), Spearman’s rank correlation coefficient and p-value.