EBioMedicine 28 (2018) 316–323

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

Research Paper

Acetaminophen (Paracetamol) Use Modifies the Sulfation of Sex Hormones

Isaac V. Cohen [a,b], Elizabeth T. Cirulli [a], Matthew W. Mitchell [c], Thomas J. Jonsson [c], James Yu [a], Naisha Shah [a], Tim D. Spector [d], Lining Guo [c], J. Craig Venter [a,e], Amalio Telenti [b,e,*]

[a] Human Longevity, Inc., San Diego, CA, USA
[b] Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, San Diego, CA, USA
[c] Metabolon, Inc., Durham, NC, USA
[d] Department of Twin Research and Genetic Epidemiology, King's College London, London, UK
[e] J. Craig Venter Institute, La Jolla, CA, USA

ARTICLE INFO

Article history:
Received 30 November 2017
Received in revised form 12 January 2018
Accepted 24 January 2018

Keywords:
Metabolome
Mendelian randomization
Sufotransferases
sult2a1

ABSTRACT

Background: Acetaminophen (paracetamol) is one of the most common medications used for management of pain in the world. There is lack of consensus about the mechanism of action, and concern about the possibility of adverse effects on reproductive health.
Methods: We first established the metabolome profile that characterizes use of acetaminophen, and we subsequently trained and tested a model that identified metabolomic differences across samples from 455 individuals with and without acetaminophen use. We validated the findings in a European ancestry adult twin cohort of 1880 individuals (TwinsUK), and in a study of 1235 individuals of African American and Hispanic ancestry. We used genomics to elucidate the mechanisms targeted by acetaminophen.
Findings: We identified a distinctive pattern of depletion of sulfated sex hormones with use of acetaminophen across all populations. We used a Mendelian randomization approach to characterize the role of Sulfotransferase Family 2A Member 1 (SULT2A1) as the site of the interaction. Although *CYP3A7-CYP3A51P* variants also modified levels of some sulfated sex hormones, only acetaminophen use phenocopied the effect of genetic variants of *SULT2A1*. Overall, acetaminophen use, age, gender and *SULT2A1* and *CYP3A7-CYP3A51P* genetic variants are key determinants of variation in levels of sulfated sex hormones in blood. The effect of taking acetaminophen on sulfated sex hormones was roughly equivalent to the effect of 35 years of aging.
Interpretation: These findings raise concerns of the impact of acetaminophen use on hormonal homeostasis. In addition, it modifies views on the mechanism of action of acetaminophen in pain management as sulfated sex hormones can function as neurosteroids and modify nociceptive thresholds.

Introduction

Acetaminophen, often referred to by its brand name Tylenol or the abbreviation 'APAP' or in Europe as Paracetamol, has been on the market for over 50 years and is one of the most well-known over-the-counter drugs. It is widely regarded as one of the safest analgesics in use, although its efficacy and safety profile have recently been questioned (Machado et al., 2015). According to the U.S. Food and Drug Administration, there are over 200 approved drug products containing acetaminophen as an active ingredient (Blough and Wu, 2011). Recent work suggests that acetaminophen functions as a cyclooxygenase (COX) inhibitor in the central nervous system (CNS) and via interaction with endocannabinoid and vanilloid signaling pathways (Ghanem et al., 2016). This may explain some of the calming effects experienced by some individuals taking acetaminophen and its use as a mild sedative in children. The uncertainty and growing number of proposed mechanisms raise the possibility that there are further actions involving CNS receptors.

The lack of complete understanding of the mechanism of action of acetaminophen extends to recent concerns about endocrine and reproductive effects of this drug. There are epidemiological and experimental data that suggest a possible link between intrauterine exposure to acetaminophen, disruption of hormonal homeostasis, and male urogenital malformations at birth (reviewed in (Kristensen et al., 2016)). Prenatal exposure to acetaminophen alters the masculinization of male brain and behavior in mice (Hay-Schmidt et al., 2017), and reduces testosterone production by the human fetal testis in a xenograft model (van den Driesche et al., 2015). We employed untargeted metabolomics and genomic analyses to expose overlooked effects of acetaminophen on

* Corresponding author at: J. Craig Venter Institute, La Jolla, CA, USA.
E-mail address: atelenti@jcvi.org (A. Telenti).

https://doi.org/10.1016/j.ebiom.2018.01.033

human metabolism and to identify the genetic target that mediates effects on hormonal homeostasis.

1. Methods

1.1. Study Design

For the first phase of the study, we enrolled 455 unselected active adults more than 18 years old divided into an initial 208 participant set, for training machine learning models, and a subsequent 247 participant test set, for testing and validating the accuracy of models. For the independent validation set, we included 1880 European ancestry twins enrolled in the TwinsUK registry (Hay-Schmidt et al., 2017) and 1235 African American and Hispanics enrolled in the Insulin Resistance Atherosclerosis Study (IRAS) (Wagenknecht et al., 2003). Details of the population and ethics consents are provided in Supplementary Methods.

1.2. Metabolite Profiling

The non-targeted metabolomics analysis of over 700 metabolites was performed at Metabolon, Inc. (Durham, North Carolina, USA) on a platform consisting of four independent ultra high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) methods. Detailed descriptions of the platform can be found in the supplementary notes and in published work (Dehaven et al., 2010; Evans et al., 2009). Values from multiple experimental batches were normalized into Z-scores based on a reference cohort of 42 self-reported healthy individuals ran with each batch. Any metabolites with more than 5% missingness in the original train set of 208 were excluded, bringing the final number of metabolites to 436. Measurements included acetaminophen and seven acetaminophen metabolites (Fig. 1A). We first used the identification of the parent drug 4-acetamidophenol and at least 3 metabolites (four if the parent compound was not detected) as the cutoff to call an active use of acetaminophen. We found that the precise number of metabolites used for the cutoff did not greatly vary the outcome. For example, using 3 metabolites instead of 4 only changed the classification for two people in the initial set of 455 participants. For the IRAS cohort, 3 metabolites was tested as the cutoff.

1.3. Genome Sequencing and Analysis

DNA samples were sequenced on an Illumina HiSeqX sequencer utilizing a 150 base paired-end single index read format. Reads were mapped to the human reference sequence build HG38. Variants were called using ISIS Analysis Software (v. 2.5.26.13; Illumina). The full set of metabolome data and genome sequence were available for 1,820 participants. A linear mixed model was applied to account for family structure in the cohort while testing for associations between genetic variants and metabolite levels (Lippert et al., 2011). Specific details

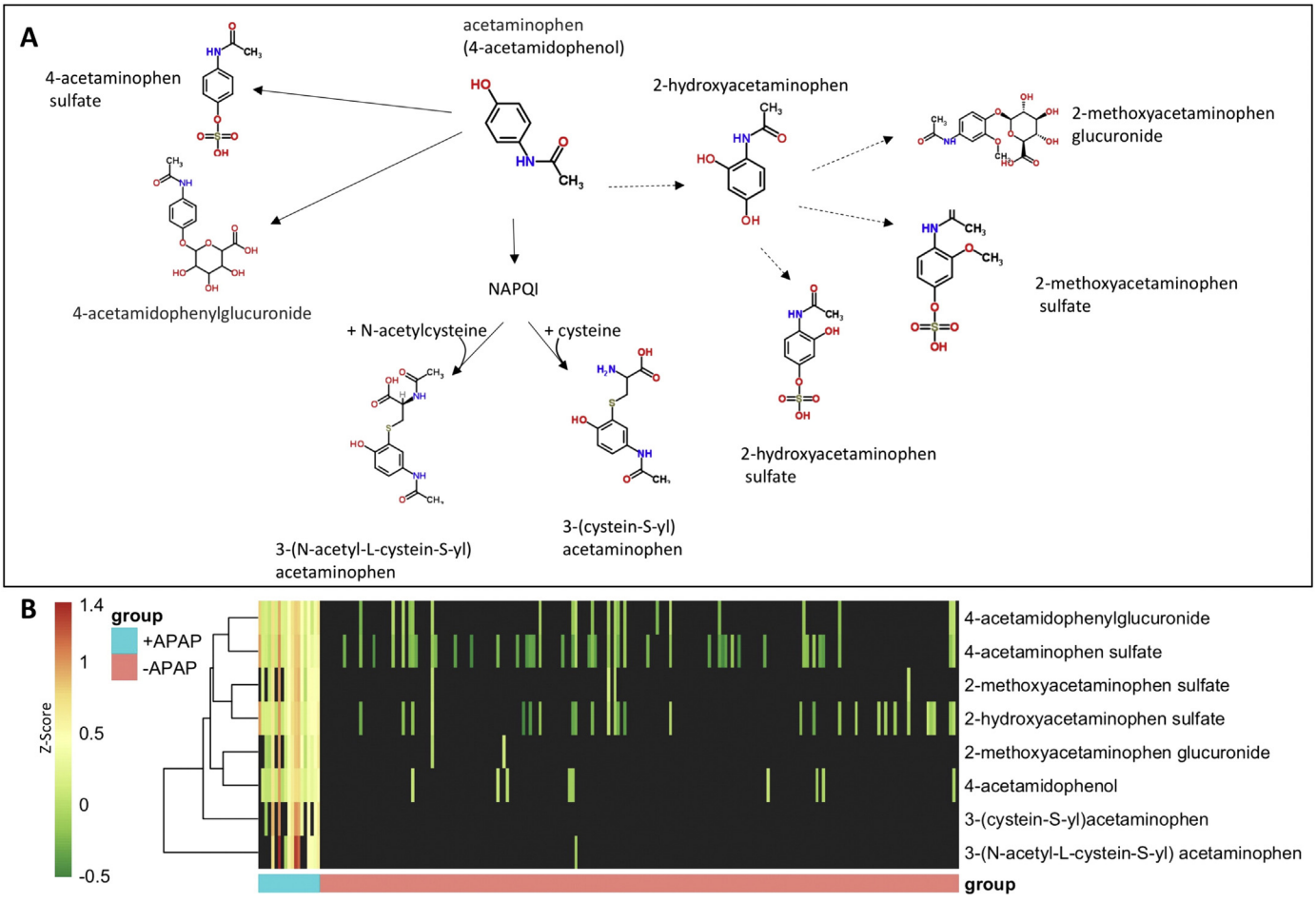

Fig. 1. Changes in the metabolome in individuals receiving acetaminophen. Panel A. Acetaminophen metabolism map. Solid pathway arrows are supported by literature reports (Moayyeri et al., 2013; Gamage et al., 2006; Evans and Davey Smith, 2015; Wang et al., 2014; Reddy, 2010). Dashed pathway arrows are proposed pathways. Panel B. Each vertical line corresponds to a participant (Training set, n = 208). Horizontal lines correspond to acetaminophen or its known metabolites. Participant metabolite measurements are shown in z-score (Red = highest concentration, Green = lowest concentration, and Grey = not observed).

have been reported elsewhere (Telenti et al., 2016; Long et al., 2017), and are summarized in Supplementary notes.

1.4. Statistical Analysis

R was used for the analysis and data manipulation. Statistical tools are described in Supplementary methods. Participants who had more than 4 measurable acetaminophen metabolites above a Z-Score of 0.05 were considered to have sufficient evidence of acetaminophen use. Acetaminophen use was used as a classifier for sPLS-DA (sparse partial least squares-discriminant analysis) machine learning. ANCOVA was used for analysis of the IRAS cohort. Linear regression comparing metabolites with age, sex, body mass index, acetaminophen status, the first genetic principal component, and genotypes were performed in R using the lm function.

2. Results

2.1. Identification of Participants Receiving Acetaminophen

The first step of the analysis was to determine which patients in a 208-person training set cohort were taking acetaminophen. Nineteen out of 208 participants were classified as highly likely to be taking acetaminophen on the basis of the identification of acetaminophen and its metabolites (Fig. 1B). The pathways of metabolism of the metabolites measured are mapped in Fig. 1A. Use of acetaminophen stratified the patients into two groups with clear support (Fig. 1B).

2.2. Effects of Acetaminophen Use on the Metabolome

Out of the 796 metabolites tracked in this experiment, the 436 with less than 5% missingness in the training set were used as variables in sPLS-DA to identify metabolites that could distinguish those who were and were not taking acetaminophen (Fig. 2A). The top loadings of the sPLS-DA were plotted as a heatmap for the training set (Suppl. Fig. S1). The signal seen in the sPLS-DA was driven by the decrease in several sulfated sex hormones. In particular, four highly correlated compounds, androstenediol (3beta,17beta) disulfate, pregnenolone sulfate, 21-hydroxypregnenolone disulfate, and pregnen-diol disulfate supported the effect of acetaminophen use on steroid hormones with sulfonated hydroxyl groups (Fig. 2B, Suppl. Table S1). These results were then confirmed in a test set of 247 participants (Fig. 2C, Suppl. Table S1).

For the initial analyses (train and test), we used a hard criterion of identification of acetaminophen and/or multiple metabolites to define active drug intake. We revisited this operational decision by assessing the relationship between the number of observed acetaminophen metabolites (as a surrogate of time from drug intake) and the inhibition of sulfation. There was a linear relationship between the number of metabolites and levels of sulfation of steroids (Suppl. Fig. S2). The plasma half-life of the parental acetaminophen is 1.5–2.5 h at the recommended doses (Mazaleuskaya et al., 2015). The observed relationship between the number of acetaminophen metabolites and decrease in various sulfated hormones is consistent with an effect of acetaminophen on sulfation that is dose and time dependent and reversible.

To elucidate the full spectrum of activity, both the test and training groups were combined in order to profile the effect of acetaminophen use on steroid metabolism. There is a clear spectrum of activity on a number of sulfated hormones. In contrast, there was no effect on cortisol, cortisone, corticosterone, epiandrosterone sulfate, and androsterone sulfate. Acetaminophen use does not appear to impact the glucuronidation of steroids (Table 1). In summary, acetaminophen use specifically decreases the levels of a unique subset of sulfated sex hormones.

2.3. Independent Validation in Multi-Ethnic Cohorts and in Twins

We first validated the contribution of acetaminophen use to the measurement of sulfated sex hormones through longitudinal visits in the European ancestry cohort (TwinsUK (Moayyeri et al., 2013)). This analysis of 1880 individuals confirmed the association of age with the decrease in the levels of sulfated hormones, and the effect of acetaminophen use at all times (Fig. 3). At any visit, a paired t test showed that the twin receiving acetaminophen had values of sulfated hormones statistically lower than those of their co-twin that was not receiving the drug (e.g., $p = 1.5 \times 10^{-11}$ for pregnen-diol disulfate; Table S2). As the majority of participants in the study were of European ancestry, we aimed to validate the analysis on 1235 Hispanic or African-American subjects from IRAS (Wagenknecht et al., 2003) (Suppl. Table S3). We observed strong associations between multiple sulfated steroids and acetaminophen status (Fig. 3, Suppl. Table S4). Overall, our analyses of 3570 individuals (including training, test, Europeans,

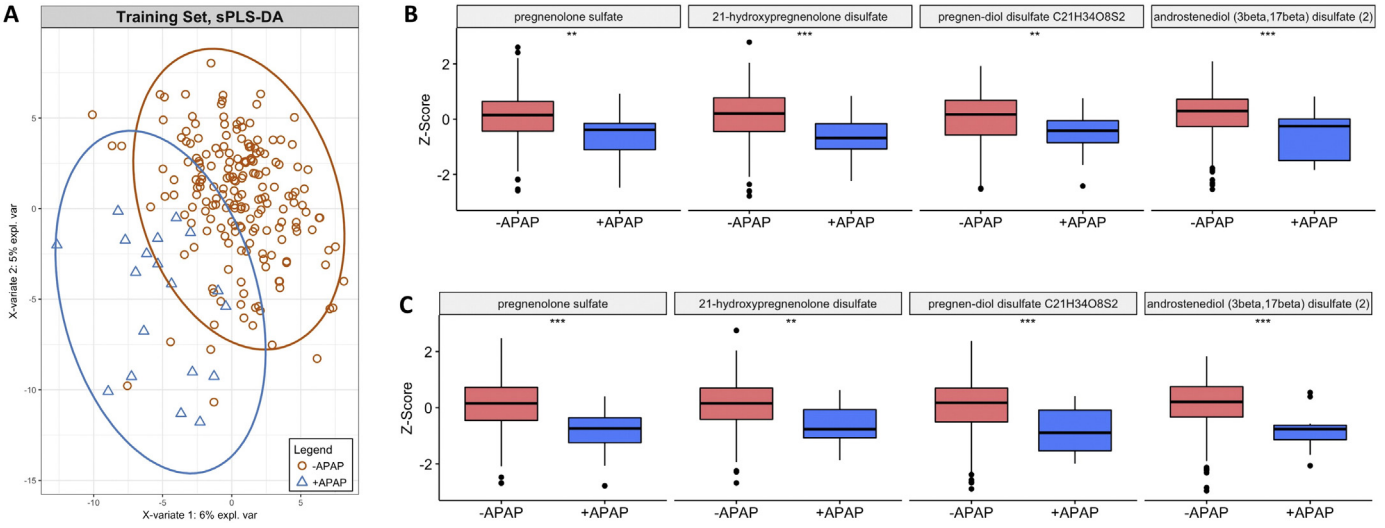

Fig. 2. Panel A. Discrimination by sPLS-DA between individuals taking (blue) and not taking (orange) acetaminophen. Panel C and D. Box and Whisker plots of training set (C) (n = 208) and test set (D) (n = 247) for selected metabolites differentially suppressed in the presence of acetaminophen and its metabolites. +APAP = very likely to be taking acetaminophen and -APAP = unlikely to be taking acetaminophen. Supplementary Table S1 lists all significant metabolites.

Table 1
Spectrum of effect of acetaminophen use on steroid metabolism on training and testing set of 455 individuals. Sulfation of multiple androgenic and progestin steroids is affected by acetaminophen use. In contrast, cortisol, cortisone, corticosterone, pregnanediol-3-glucuronide, epiandrosterone sulfate, and androsterone sulfate are not affected. Acetaminophen use does not impact the glucuronidation of steroids. Suffixes (e.g., (1)) indicate different sulfation locations on the molecular structure. Arrows indicate direction of effect for metabolites that show at least a nominal level of association ($p < 0.05$ before Bonferroni correction).

Pathway	Metabolite	Training and test sets combined (n = 455)	
		Direction change	p-value
Androgenic steroids	Dehydroisoandrosterone sulfate (DHEA-S)	↓	0.001
Androgenic steroids	16a–hydroxy DHEA 3-sulfate	↓	0.005
Androgenic steroids	Epiandrosterone sulfate		0.257
Androgenic steroids	Androsterone sulfate		0.143
Androgenic steroids	Etiocholanolone glucuronide		0.394
Androgenic steroids	5alpha-androstan-3alpha,17alpha-diol monosulfate		0.451
Androgenic steroids	Androstenediol (3beta,17beta) monosulfate (1)	↓	0.043
Androgenic steroids	Androstenediol (3beta,17beta) monosulfate (2)	↓	0.003
Androgenic steroids	Androstenediol (3beta,17beta) disulfate (1)	↓	3.74E-04
Androgenic steroids	Androstenediol (3beta,17beta) disulfate (2)	↓	8.53E-06
Androgenic steroids	Androstenediol (3alpha, 17alpha) monosulfate (2)	↓	0.005
Androgenic steroids	Androstenediol (3alpha, 17alpha) monosulfate (3)		0.270
Androgenic steroids	5alpha-androstan-3alpha,17beta-diol monosulfate (1)		0.969
Androgenic steroids	5alpha-androstan-3alpha,17beta-diol monosulfate (2)		0.436
Androgenic steroids	5alpha-androstan-3alpha,17beta-diol disulfate		0.423
Androgenic steroids	5alpha-androstan-3alpha,17beta-diol 17-glucuronide		0.256
Androgenic steroids	5alpha-androstan-3beta,17beta-diol monosulfate (2)		0.760
Androgenic steroids	5alpha-androstan-3beta,17beta-diol disulfate	↓	0.022
Androgenic steroids	5alpha-androstan-3beta,17alpha-diol disulfate		0.147
Androgenic steroids	Andro steroid monosulfate C19H28O6S (1)	↓	0.008
Corticosteroids	Corticosterone		0.714
Corticosteroids	Cortisol		0.585
Corticosteroids	Cortisone		0.098
Pregnenolone steroids	Pregnenolone sulfate	↓	1.50E-04
Pregnenolone steroids	17alpha-hydroxypregnenolone 3-sulfate		0.724
Pregnenolone steroids	21-Hydroxypregnenolone disulfate	↓	2.77E-05
Progestin steroids	Pregnanolone/allopregnanolone sulfate		0.144
Progestin steroids	Pregnen-diol disulfate C21H34O8S2	↓	1.32E-05
Progestin steroids	Pregn steroid monosulfate C21H34O5S	↓	5.46E-04
Progestin steroids	5alpha-pregnan-3beta,20beta-diol monosulfate (1)		0.178
Progestin steroids	5alpha-pregnan-3beta,20alpha-diol monosulfate (2)		0.078
Progestin steroids	5alpha-pregnan-3beta,20alpha-diol disulfate	↓	0.006
Progestin steroids	5alpha-pregnan-3(alpha or beta),20beta-diol disulfate		0.128
Progestin steroids	Pregnanediol-3-glucuronide		0.281

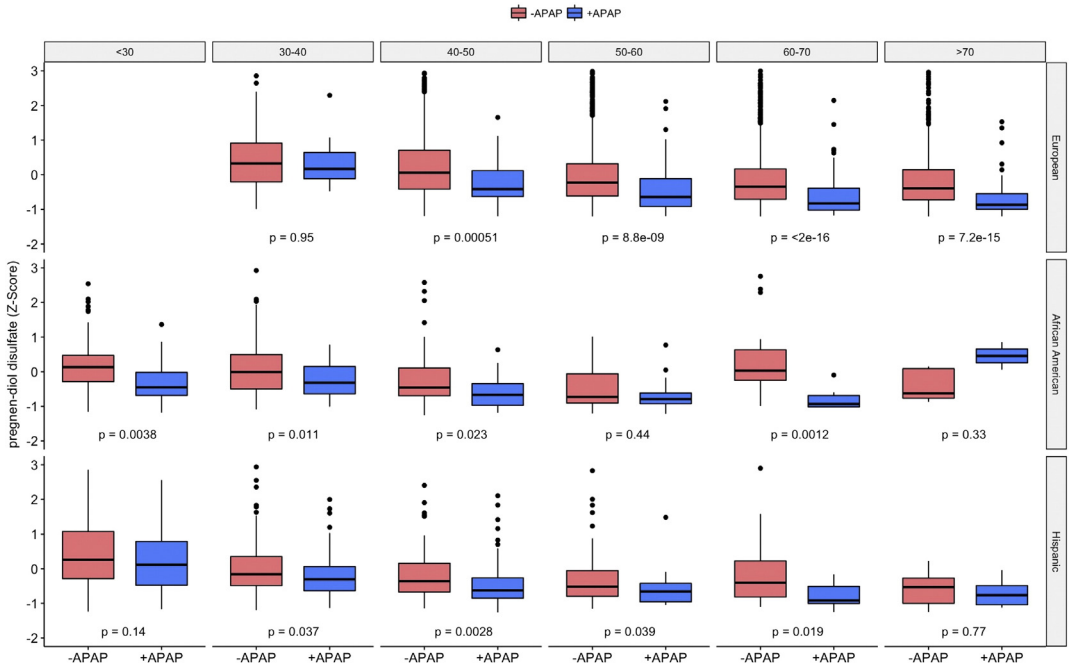

Fig. 3. Effect of acetaminophen use on independent study populations. Plots represent pregnen-diol disulfate concentration, expressed as z-scores. Effects of age and acetaminophen use on the European TwinsUK cohort (n = 1880), African Americans (n = 405) and Hispanics (n = 830). Based on the presence of acetaminophen and its metabolites. +APAP = very likely to be taking acetaminophen and -APAP = unlikely to be taking acetaminophen.

African Americans, and Hispanic participants) confirm the generalizable effect of acetaminophen use on sulfated sex hormone levels across human populations.

2.4. Analysis of Sulfation Pathways

Both acetaminophen and estrogens have been observed to be metabolized by SULT, a family of enzymes responsible for catalysis of sulfation of both exogenous and endogenous metabolites, leading to significant changes in biological activity, solubility, and renal clearance. Specifically, acetaminophen is a reported substrate of SULT1A1 (sulfotransferase 1A1) (Gamage et al., 2006). There is, however, some imprecision about the additional SULT enzymes that may be targeted by acetaminophen (Mazaleuskaya et al., 2015), and which SULT enzymes are primarily responsible for sulfation of the sex hormones (proposed to be SULT2A1 and SULT2B1 splice variants 1 and 2, reviewed in (Mueller et al., 2015)).

We first used knowledge from the literature to analyze acetaminophen's interaction with various sulfotransferases. We compared participants from the TwinsUK cohort observed on the same visit in which one twin was likely to be taking acetaminophen and the other twin was not taking the drug (137 pairs of twins). By paired *t*-test, we determined the effect of acetaminophen use on 41 sulfated metabolites that expected to reflect the activity of SULT1A1, 1A3, 1E1, 2A1, and the two splice variants of SULT2B1 (Suppl. Fig. S3). These included a representation of steroid hormones, bile salts, catechol metabolites, and cresols and phenols. The analysis shows that the metabolites with the strongest associations with acetaminophen use have been previously reported in the literature to be substrates of SULT2A1 and 2B1. Importantly, there was no statistically significant effect of acetaminophen use on any of the metabolites that were previously reported to be SULT1A1, SULT1A3, or SULT1E3 substrates. Because of the lack of precision in the literature about the reported substrates of SULTs, we elected to use genomics to independently elucidate the relevant mechanisms that are targeted by acetaminophen.

2.5. Mendelian Randomization Genomic Analysis

Mendelian randomization is a useful tool for inferring causality with biomarkers (Evans and Davey Smith, 2015). If acetaminophen interacts with the SULT primarily implicated in sulfation of sex hormones, then genetic variants in that same locus should result in similar consequences (phenocopy) on the level of sulfation that is observed while receiving acetaminophen.

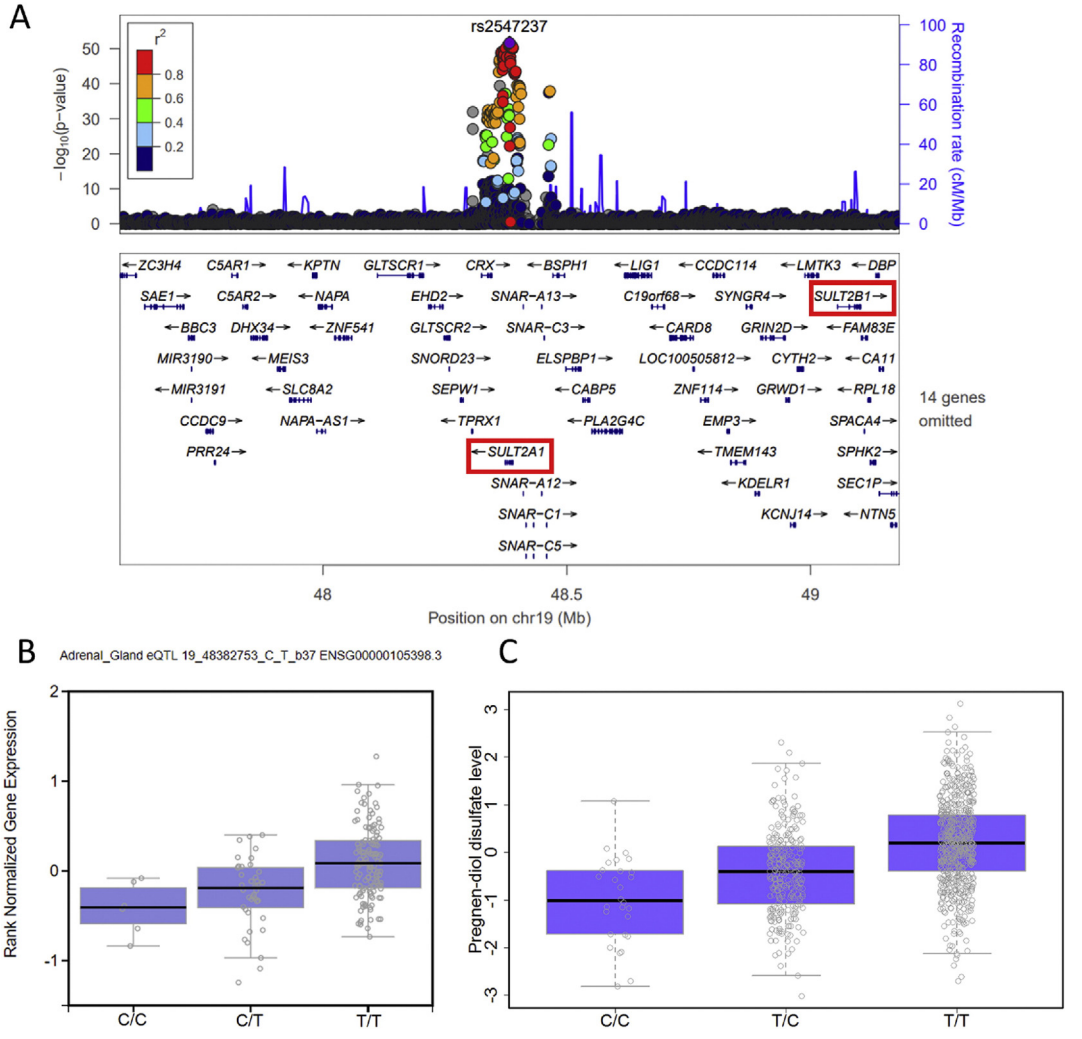

Fig. 4. Genetic mapping of the interaction. Panel A. Genome-wide association of sulfated steroids with common variants in *SULT2A1* (first red box). Shown is the association peak for pregnen-diol disulfate as a representative metabolite. There is no significant association with *SULT2B1* common variants (second red box). Panel B. The top variant, rs2547237, is a known eQTL for SULT2A1, where the minor allele (C) is associated with decreased *SULT2A1* expression in the adrenal gland ($p = 9.6 \times 10^{-8}$, available from GTEx portal). Panel C. Mean pregnen-diol disulfate level over 3 timepoints in 872 unrelated European ancestry participants. The reference allele, C, is the minor allele. Decreased expression of SULT2A1 is associated with the C genotype ($p = 2.1 \times 0^{-52}$), which corresponds to less sulfation and thus lower levels of the sulfated sex hormone pregnen-diol disulfate.

We associated the levels of pregnen-diol disulfate (a representative metabolite with the best correlation with the use of acetaminophen) with genome-wide genotypes derived from whole-genome sequencing in 1,820 individuals. We identified the most significant association ($p = 2.2 \times 10^{-52}$) with rs2547237, an intronic variant in *SULT2A1* (Fig. 4A). This particular variant is a known eQTL that associates with *SULT2A1* expression levels. The C genotype corresponds to less sulfation and thus lower levels of the sulfated sex hormone pregnen-diol disulfate (Fig. 4B and 4C). Although SULT2A1 is thought to be responsible for the metabolism of glycolithocholate sulfate, taurolithocholate 3-sulfate, taurocholenate sulfate, glycocholenate sulfate, and andro steroid monosulfate, these metabolites were not associated with *SULT2A1* variation (Suppl. Table S5). We also performed a rare coding variant analysis of SULT genes using SKAT (sequence kernel association test) but did not identify any statistically significant associations with acetaminophen-associated metabolites. Although there was no clear signal of rare variants in these genes significantly impacting metabolite levels, we did observe lower than average sulfated sex hormone levels in carriers of the *SULT2A1* variants rs11569679/Ala261Thr, chr19:47874713T>A/Asn230Ile, and chr19:47843340C>G/Arg33Thr.

In addition, a full genome-wide association analysis of common variants for all sulfated sex hormones identified multiple non-SULT associations with sulfated sex hormone levels, the most prominent of which was rs45446698 in *CYP3A7-CYP3A51P* (for example, the association with 5alpha-androstan-3beta,17beta-diol disulfate had $p = 2.9 \times 10^{-47}$) (Suppl. Table S5). In summary, common genetic variation in multiple genes, primarily *SULT2A1*, impact the levels of sulfated sex hormones.

2.6. Contribution of Acetaminophen Use, Sex, Age, SULT2A1 and CYP3A7-CYP3A51P to Levels of Sulfation

We used multivariate linear regression analysis to characterize the effects of various factors on the pregnen-diol disulfate and of 5alpha-androstan-3beta,17beta-diol disulfate levels in a subset of 872 unrelated individuals from the TwinsUK cohort. We found that decreased levels of pregnen-diol disulfate were significantly ($p < 0.01$) and independently associated with increased age, acetaminophen use, female sex, and the minor allele of *SULT2A1* (rs2547237); 5alpha-androstan-3beta,17beta-diol disulfate levels also associated with *CYP3A7-CYP3A51P* (rs45446698) (Fig. 5A and 5B). Together, age, sex, genetic variants and acetaminophen use explained 16.5%, 17.1%, and 16.3% of the total variation in pregnen-diol disulfate metabolite levels at the three timepoints. Taken separately, rs2547237 genotype explained 9.4%, 10.3%, and 7.3% of the variation; age explained 3.1%, 2.7% and 0.7%; acetaminophen use explained 2.9%, 2.9%, and 7.2%; and sex explained 1.9%, 2.0%, and 1.6%. The effect size for acetaminophen represents a fairly low bound because at each of the three time points, only 4.9-8.9% of samples were found to be positive for acetaminophen use. In permutations where 50% of the samples used acetaminophen, the proportion of variance explained rose to 15.2%, 11.1%, and 17.5% respectively at longitudinal visits 1, 2 and 3. Taking acetaminophen (beta = −0.750, −0.681, and −0.924) decreased pregnen-diol disulfate levels in a manner that was roughly equivalent to 35 years of aging (beta for years of age = −0.0217, −0.0177, and −0.0058). The co-occurrence of multiple negative factors influencing sulfation resulted in very low levels

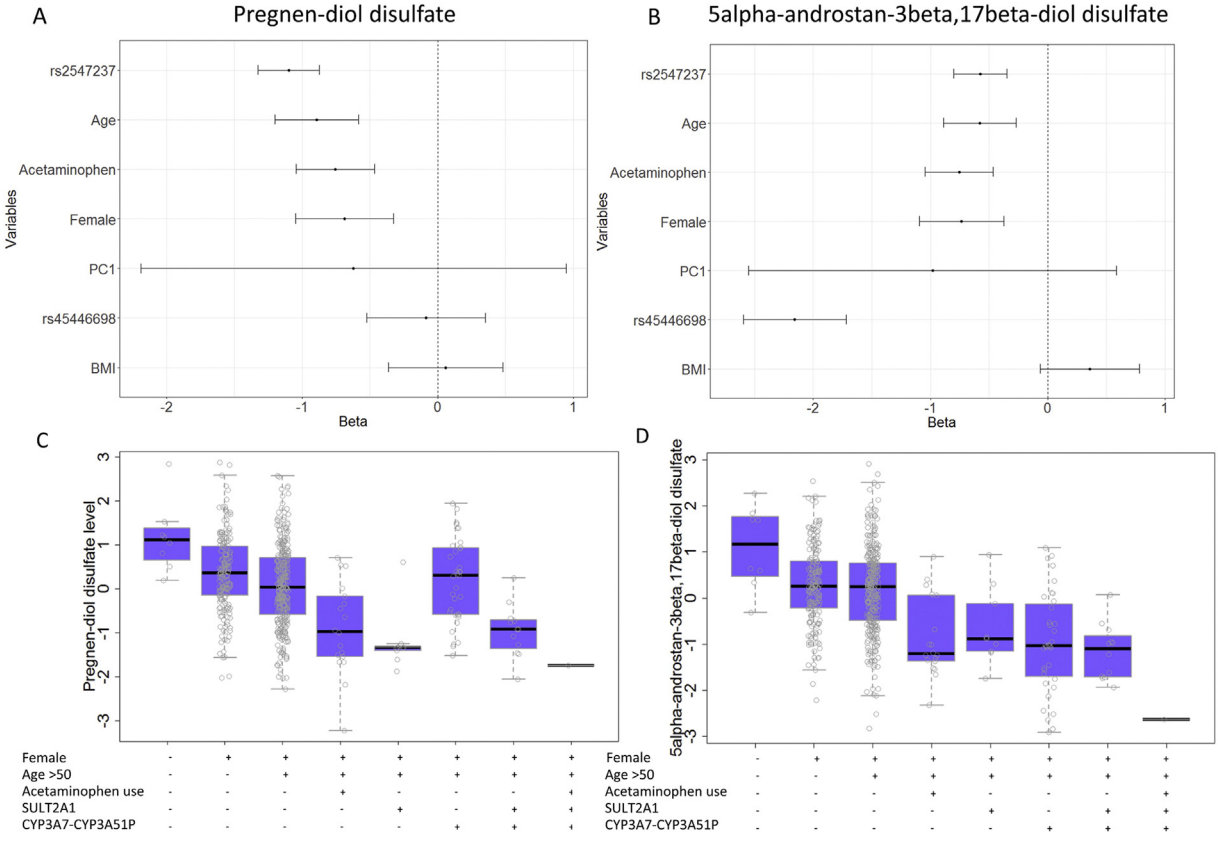

Fig. 5. Contributors to levels of representative sulfated sex hormones. Multivariate linear regression model of (Panel A) pregnen-diol disulfate levels and (Panel B) 5alpha-androstan-3beta,17beta-diol disulfate levels. Shown are the beta values from an analysis of visit 1 metabolite levels versus covariates in 872 unrelated individuals of European ancestry. To make the scaling consistent, age, the first principal component of European-specific EIGENSTRAT axes (PC1), body mass index, and genotype have been transformed to a scale from 0 to 1. Decreased levels of these metabolites are found in those with increased age, those who are taking acetaminophen, females, and carriers of the (minor) C allele of *SULT2A1* variant rs2547237. Decreased levels of 5alpha-androstan-3beta,17beta-diol disulfate are also found in carriers of the G (minor) allele of *CYP3A7-CYP3A51P* variant rs45446698, but this variant did not impact pregnen-diol disulfate levels. Here, all variables whose confidence intervals do not cross the dotted 0 line have $p < 0.0005$. Also shown are the levels (Panel C) of pregnen-diol disulfate and (Panel D) 5alpha-androstan-3beta,17beta-diol disulfate at visit 1, broken down by rs2547237 and rs45446698 genotype, sex, acetaminophen use, and age.

of the various hormones, and interaction analysis showed these effects to be more additive than multiplicative (Fig. 4C and D). In summary, independent contributions of age, sex, genotype, and acetaminophen use significantly impact the levels of sulfated sex hormones.

3. Discussion

This study represents a metabolomics-informed pharmacogenomics analysis (Neavin et al., 2016) of acetaminophen use. We identified a significant effect of acetaminophen use, age, sex, and *SULT2A1* and *CYP3A7-CYP3A51P* genetic variants on multiple sulfated metabolites of androstenediol, pregnenolone, and DHEA (among others). Each of these factors made strong, independent contributions to the levels of these metabolites. For example, pregnen-diol disulfate levels decreased with age, and the effect of taking acetaminophen on this metabolite was roughly equivalent to the effect of 35 years of aging. These findings have important implications in the context of the current understanding of the mechanisms of action of acetaminophen, the recent literature about the role of sulfated hormones as neurosteroids, and safety concerns regarding adverse effects on androgen exposure during fetal life.

Human cytosolic sulfotransferases (SULTs) are extensively involved in regulating the activities of signaling small-molecules, drugs and xenobiotics (Wang et al., 2014) via transfer of the sulfuryl-moiety (-SO3) from PAPS (3′-phospho- adenosine 5′-phosphosulfate) to the hydroxyls and primary amines of acceptors. The inhibition of SULT activity is caused by trapping PAP in a dead-end complex, which slows the release of nucleotides (Wang et al., 2014). Sulfation is critically involved in the metabolism of acetaminophen *in vivo*; a recent systematic analysis showed that three of the twelve human SULTs, SULT1A1, SULT1A3 and SULT1C4, displayed the strongest sulfating activity towards acetaminophen use. In addition, SULT1E1 and SULT2A1 have also shown activity at a high substrate concentration of acetaminophen (Adjei et al., 2008). On the other hand, five SULTs are associated with steroid sulfation: SULT1A1, SULT1E1, SULT2A1, as well as the two isoforms of the SULT2B1 gene, SULT2B1a and SULT2B1b (reviewed in (Mueller et al., 2015)). There is broad substrate specificity of the sulfotransferase enzymes that is thought to result from highly flexible loops flanking the catalytic binding site that can adapt to various ligands (Mueller et al., 2015). We used a Mendelian randomization approach to zero in on the most likely site of interaction of acetaminophen and the various sex hormones. SULT2A1 (as opposed to SULT1A1 or 2B1) emerged as carrying the most robust genetic evidence for the site of interaction. Importantly, only a precise set of metabolites, all sulfated sex hormones, shared the associations with acetaminophen use and *SULT2A1* variants. In contrast, variants in *CYP3A7-CYP3A51P*, which cause the persistence of enzymatic activity of CYP3A7 during adult life (Smit et al., 2005), resulted in lower circulating levels of a more restricted subset of sulfated sex hormones. However, acetaminophen use does not phenocopy the effect of genetic variation in *CYP3A7-CYP3A51P* – meaning that the set of affected sulfated hormones is different than the acetaminophen use pattern.

The unexpected effect of acetaminophen use on steroid sulfation invites an assessment of existing knowledge on the role of sulfated sex hormones as neurosteroids - endogenous regulators of neuronal excitability (Reddy, 2010). Recent work by Kwon and colleagues (Kwon et al., 2016) has shown that pregnenolone sulfate plays a role in the regulation of allodynia in rats. Pregnenolone sulfate inhibits the GABAA channel and potentiates or activates the NDMA, TRPM1 and TRPM3 receptors. Antagonism of pregnenolone sulfate at the sigma-1 receptor was observed to decrease alphabeta meATP-induced mechanical allodynia (Kwon et al., 2016). Yoon et al. (Yoon et al., 2009; Yoon et al., 2010) indicated that an increase in the spinal DHEAS facilitates nociception via the activation of sigma-1 receptors. Sigma-1 receptor antagonist BD-1047 dose-dependently suppressed DHEAS's facilitatory effect on nociception (Yoon et al., 2009). As shown in the present study, individuals who took acetaminophen had very low levels of neurosteroids such as pregnenolone sulfate and DHEAS, a mechanism that could synergize with acetaminophen's known mode of action in the central nervous system that implicates the COX, vanilloid, and endocannabinoid systems.

Although over half of women take acetaminophen during pregnancy, there are epidemiological, *in vitro*, and animal data that raise questions about the effects of acetaminophen on hormonal homeostasis (Kristensen et al., 2016). Previous studies have discussed inhibition of prostaglandin and INSL3 signalling, and decrease in testosterone levels as possible mechanisms leading to disrupted genital tract formation. The current work identifies the depletion of sulfated sex hormones as a potential mechanism. Specifically, it has been shown that DHEAS is an important carrier for steroids into the placenta (Geyer et al., 2017). In addition to possible risks to the development of the genital track, there are reports of the potential effects on neurocognitive and behavioral development that could be linked to the role of sulfated hormones as neurosteroids (Liew et al., 2014).

In conclusion, the current work showcases the use of pharmacometabolomics to identify unexpected effects of a commonly used drug, acetaminophen, on hormone metabolism, and the mapping of the site of interaction to SULT2A1 by using genomics and Mendelian randomization. Closer scrutiny of this commonly used medication is warranted.

Contributions

AT conceived the experiment(s), IVC. and EC conducted the experiment(s), MWM, TJJ and LG supported metabolome analyses, IVC, EC, JY, NS, JCV and AT analyzed the results. TDS is responsible for the TwinsUK study. All authors reviewed the manuscript.

Declaration of Interests

EC, JY, NS, JCV are employees of Human Longevity, Inc. MWM, TJJ and LG are employees of Metabolome Inc. All other authors have nothing to declare.

Funding

Human Longevity Inc.

Acknowledgements

The Genotype-Tissue Expression (GTEx) Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health, and by NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. The data used for the analyses described in this manuscript were obtained from the GTEx Portal on 10/17/2017.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ebiom.2018.01.033.

References

Adjei, A.A., Gaedigk, A., Simon, S.D., Weinshilboum, R.M., Leeder, J.S., 2008. Interindividual variability in acetaminophen sulfation by human fetal liver: implications for pharmacogenetic investigations of drug-induced birth defects. Birth Defects Res A Clin Mol Teratol 82 (3), 155–165.

Blough, E.R., Wu, M., 2011. Acetaminophen beyond pain and Fever-relieving. Front. Pharmacol. 2, 72.

Dehaven, C.D., Evans, A.M., Dai, H., Lawton, K.A., 2010. Organization of GC/MS and LC/MS metabolomics data into chemical libraries. Aust. J. Chem. 2 (1), 9.

Evans, D.M., Davey Smith, G., 2015. Mendelian randomization: new applications in the coming age of hypothesis-free causality. Annu. Rev. Genomics Hum. Genet. 16, 327–350.
Evans, A.M., DeHaven, C.D., Barrett, T., Mitchell, M., Milgram, E., 2009. Integrated, nontargeted ultrahigh performance liquid chromatography/electrospray ionization tandem mass spectrometry platform for the identification and relative quantification of the small-molecule complement of biological systems. Anal. Chem. 81 (16), 6656–6667.
Gamage, N., Barnett, A., Hempel, N., et al., 2006. Human sulfotransferases and their role in chemical metabolism. Toxicol. Sci. 90 (1), 5–22.
Geyer, J., Bakhaus, K., Bernhardt, R., et al., 2017. The role of sulfated steroid hormones in reproductive processes. J. Steroid Biochem. Mol. Biol. 172, 207–221.
Ghanem, C.I., Perez, M.J., Manautou, J.E., Mottino, A.D., 2016. Acetaminophen from liver to brain: New insights into drug pharmacological action and toxicity. Pharmacol. Res. 109, 119–131.
Hay-Schmidt, A., Finkielman, O.T.E., Jensen, B.A.H., et al., 2017. Prenatal exposure to paracetamol/acetaminophen and precursor aniline impairs masculinisation of male brain and behaviour. Reproduction 154 (2), 145–152.
Kristensen, D.M., Mazaud-Guittot, S., Gaudriault, P., et al., 2016. Analgesic use - prevalence, biomonitoring and endocrine and reproductive effects. Nat. Rev. Endocrinol. 12 (7), 381–393.
Kwon, S.G., Yoon, S.Y., Roh, D.H., et al., 2016. Peripheral neurosteroids enhance P2X receptor-induced mechanical allodynia via a sigma-1 receptor-mediated mechanism. Brain Res. Bull. 121, 227–232.
Liew, Z., Ritz, B., Olsen, J., 2014. Characteristics of acetaminophen users compared with nonusers during pregnancy, behavioral problems, and hyperkinetic disorders—reply. JAMA Pediatr. 168 (9), 865–866.
Lippert, C., Listgarten, J., Liu, Y., Kadie, C.M., Davidson, R.I., Heckerman, D., 2011. FaST linear mixed models for genome-wide association studies. Nat. Methods 8 (10), 833–835.
Long, T., Hicks, M., Yu, H.C., et al., 2017. Whole-genome sequencing identifies common-to-rare variants associated with human blood metabolites. Nat. Genet. 49 (4), 568–578.
Machado, G.C., Maher, C.G., Ferreira, P.H., et al., 2015. Efficacy and safety of paracetamol for spinal pain and osteoarthritis: systematic review and meta-analysis of randomised placebo controlled trials. BMJ 350, h1225.
Mazaleuskaya, L.L., Sangkuhl, K., Thorn, C.F., FitzGerald, G.A., Altman, R.B., Klein, T.E., 2015. PharmGKB summary: pathways of acetaminophen metabolism at the therapeutic versus toxic doses. Pharmacogenet. Genomics 25 (8), 416–426.
Moayyeri, A., Hammond, C.J., Hart, D.J., Spector, T.D., 2013. The UK Adult Twin Registry (TwinsUK Resource). Twin Res Hum Genet 16 (1), 144–149.
Mueller, J.W., Gilligan, L.C., Idkowiak, J., Arlt, W., Foster, P.A., 2015. The regulation of steroid action by sulfation and desulfation. Endocr. Rev. 36 (5), 526–563.
Neavin, D., Kaddurah-Daouk, R., Weinshilboum, R., 2016. Pharmacometabolomics informs pharmacogenomics. Metabolomics 12 (7).
Reddy, D.S., 2010. Neurosteroids: endogenous role in the human brain and therapeutic potentials. Prog. Brain Res. 186, 113–137.
Smit, P., van Schaik, R.H., van der Werf, M., et al., 2005. A common polymorphism in the CYP3A7 gene is associated with a nearly 50% reduction in serum dehydroepiandrosterone sulfate levels. J. Clin. Endocrinol. Metab. 90 (9), 5313–5316.
Telenti, A., Pierce, L.C., Biggs, W.H., et al., 2016. Deep sequencing of 10,000 human genomes. Proc. Natl. Acad. Sci. U. S. A. 113 (42), 11901–11906.
van den Driesche, S., Macdonald, J., Anderson, R.A., et al., 2015. Prolonged exposure to acetaminophen reduces testosterone production by the human fetal testis in a xenograft model. Sci. Transl. Med. 7 (288), 288ra80.
Wagenknecht, L.E., Langefeld, C.D., Scherzinger, A.L., et al., 2003. Insulin sensitivity, insulin secretion, and abdominal fat: the Insulin Resistance Atherosclerosis Study (IRAS) Family Study. Diabetes 52 (10), 2490–2496.
Wang, T., Cook, I., Falany, C.N., Leyh, T.S., 2014. Paradigms of sulfotransferase catalysis: the mechanism of SULT2A1. J. Biol. Chem. 289 (38), 26474–26480.
Yoon, S.Y., Roh, D.H., Seo, H.S., et al., 2009. Intrathecal injection of the neurosteroid, DHEAS, produces mechanical allodynia in mice: involvement of spinal sigma-1 and GABA receptors. Br. J. Pharmacol. 157 (4), 666–673.
Yoon, S.Y., Roh, D.H., Seo, H.S., et al., 2010. An increase in spinal dehydroepiandrosterone sulfate (DHEAS) enhances NMDA-induced pain via phosphorylation of the NR1 subunit in mice: involvement of the sigma-1 receptor. Neuropharmacology 59 (6), 460–467.