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Human Phenol Sulfotransferases *SULT1A2* and *SULT1A1*

GENETIC POLYMORPHISMS, ALLOZYME PROPERTIES, AND HUMAN LIVER GENOTYPE–PHENOTYPE CORRELATIONS

Rebecca B. Raftogianis, Thomas C. Wood and Richard M. Weinshilboum†*

DEPARTMENT OF PHARMACOLOGY, MAYO MEDICAL SCHOOL/MAYO CLINIC/MAYO FOUNDATION, ROCHESTER, MN 55905, U.S.A.

ABSTRACT. Phenol sulfotransferases (PSTs or phenol SULTs) catalyze the sulfate conjugation of phenolic drugs, xenobiotics, and monoamines. Two human PST isoforms have been defined biochemically, a thermostable (TS), or phenol-preferring, and a thermolabile (TL), or monoamine-preferring form. Pharmacogenetic studies showed that levels of both TS PST activity and TS PST thermal stability (an indirect measure of variation in amino acid sequence) in the platelet were regulated by genetic polymorphisms. Subsequent molecular genetic experiments revealed the existence of three human PST genes, two of which, *SULT1A1* and *SULT1A2*, encode proteins with "TS PST-like" activity. We recently reported common nucleotide polymorphisms for *SULT1A1* that are associated with variations in platelet TS PST activity and thermal stability. In the present experiments, we set out to determine whether functionally significant DNA polymorphisms also might exist for *SULT1A2*, to compare the biochemical properties of all common allozymes encoded by *SULT1A2* and *SULT1A1*, and to study phenol SULT genotype–phenotype correlations in the human liver. We phenotyped 61 human liver biopsy samples for TS PST thermal stability and activity. The open reading frames of *SULT1A2* and *SULT1A1* then were amplified with the polymerase chain reaction and sequenced for each of these hepatic tissue samples. We observed 13 *SULT1A2* alleles that encoded 6 allozymes. These alleles were in linkage disequilibrium with alleles for *SULT1A1*. Biochemical characterization of common allozymes encoded by both genes suggested that *SULT1A1* was primarily responsible for "TS PST phenotype" in the human liver. In summary, both *SULT1A2* and *SULT1A1* have a series of common alleles encoding enzymes that differ functionally and are associated with individual differences in phenol SULT properties in the liver. BIOCHEM PHARMACOL **58**;4:605–616, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. sulfotransferase; SULT1A1; SULT1A2; genetic polymorphism; pharmacogenetics

Pharmacogenetics is the study of the role of inheritance in variation in drug response—variation that often results from individual differences in drug metabolism [1, 2]. Sulfation is an important pathway in the metabolism of many drugs, other xenobiotics, neurotransmitters, and hormones [3]. Sulfate conjugation is catalyzed by members of a gene superfamily of cytosolic sulfotransferase enzymes [4]. Attendees at the 3rd International Sulfation Workshop held in Drymen, Scotland, agreed that "SULT" will be used as an abbreviation for these enzymes [5]. Therefore, throughout this manuscript we will adopt that nomenclature whenever appropriate. Selected examples of previously used names for these enzymes in humans as well as their proposed SULT‡ designations are listed in Table 1. Included among the nine cytosolic SULTs presently known to be expressed in human tissues are three phenol SULTs, SULT1A1, 1A2, and 1A3 (Table 1), which catalyze the sulfate conjugation of many phenolic drugs and other xenobiotics.

Biochemical studies of human phenol SULTs (previously "PSTs") led to the identification of two isoforms, which were defined on the basis of their substrate specificities, inhibitor sensitivities, and thermal stabilities—a TS, or phenol-preferring form, and a TL, or monoamine-preferring form [6–10]. "TS PST" preferentially catalyzed the sulfation of micromolar concentrations of small planar phenols such as 4-nitrophenol and was sensitive to inhibition by DCNP. "TL PST" preferentially catalyzed the sulfation of micromolar concentrations of phenolic monoamines such as dopamine and was relatively insensitive to DCNP inhibition [6–10]. Both of these biochemically defined activities were expressed in a variety of human tissues including liver, brain, jejunum, and, of importance for pharmacogenetic studies, the blood platelet [6, 9–12]. Human platelet TS PST displayed wide individual variations, not only in level of activity, but also in thermal

* Present address: Department of Pharmacology, Fox Chase Cancer Center, Philadelphia, PA 19111.

† Corresponding author. Tel. (507) 284-2246; FAX (507) 284-9111; E-mail: weinshilboum.richard@mayo.edu

‡ *Abbreviations*: SULT, sulfotransferase; TL, thermolabile; TS, thermostable; PST, phenol sulfotransferase; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; H/C, heated/control; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; DCNP, 2,6-dichloro-4-nitrophenol; and ORF, open reading frame.

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TABLE 1. Human cytosolic SULT enzymes and genes

Proposed gene/enzyme nomenclature	Selected previous enzyme nomenclature	Previous gene nomenclature	Gene chromosomal localization
SULT1A1	TS PST1	*STP1*	16p11.2–12.1
SULT1A2	TS PST2	*STP2*	16p11.2–12.1
SULT1A3	TL PST	*STM*	16p11.2
SULT1B1	ST1B2		4q13
SULT1C1	SULT1C1		2q11.1–11.2
SULT1E1	EST	*STE*	4q13.1
SULT2A1	DHEA ST	*STD*	19q13.3
SULT2B1	{ SULT2B1a SULT2B1b }		19q13.3

The proposed gene nomenclature, selected examples of the previous nomenclature used to refer to these enzymes, the previous gene nomenclature and chromosomal locations of genes are listed. SULT1A1, 1A2, and 1A3 are phenol SULTs, 1B1 catalyzes the sulfation of thyroid hormone, 1E1 is an estrogen SULT, 1C1 is a possible orthologue of a rat enzyme that catalyzes the metabolic activation of procarcinogens, and 2A1, 2B1a and 2B1b are hydroxysteroid SULTs.

stability [13, 14], a sensitive indirect indicator of differences among proteins in amino acid sequence [15]. Segregation analysis of data from family studies of human platelet TS PST showed that levels of this activity as well as individual variations in its thermal stability were controlled by genetic polymorphisms [14]. The correlation of level of TS PST activity with TS PST thermal stability in 905 individual platelet samples is shown in Fig. 1A [16]. The figure shows that samples with the inherited trait of low thermal stability (low heated over control or H/C ratios) uniformly had low basal TS PST activity.

Molecular genetic experiments performed several years after these biochemical pharmacogenetic studies showed that there are three "PST genes" in the human genome, two of which, *SULT1A1* and *SULT1A2*, encode proteins with TS PST-like activity [17–24]. The remaining gene, *SULT1A3*, encodes TL PST [18–20]. DNA sequences and structures of the genes for these enzymes are highly homologous, and all three map to a phenol SULT gene complex on the short arm of human chromosome 16 [17, 23, 24]. The present challenge is to merge this molecular genetic information with the results of previous biochemical pharmacogenetic experiments. As an initial step, we recently reported the existence of a series of *SULT1A1* alleles, one of which, *SULT1A1*2*, was associated consistently with the inherited traits of low TS PST activity and thermal stability in the human platelet (Fig. 1B) [16]. The experiments described subsequently were performed to determine whether *SULT1A2*, like *SULT1A1*, might also have common genetic polymorphisms, to express and determine the properties of allozymes encoded by common polymorphic alleles for both of these genes, and, finally, to study phenol SULT genotype–phenotype correlations in an important human drug-metabolizing organ, the liver.

MATERIALS AND METHODS

Human Liver Biopsy Samples

Human hepatic "surgical waste" tissue was obtained from 61 Caucasian patients undergoing clinically indicated hepatectomies or open hepatic biopsies and was stored at −80°. All tissue samples were obtained under guidelines approved by the Mayo Clinic Institutional Review Board. These frozen hepatic tissue samples were homogenized and centrifuged at 100,000 *g* for 1 hr as described previously [9] to obtain high-speed supernatant (HSS) preparations.

PST Enzyme Activity, Thermal Stability, and Inhibitor Sensitivity

TS PST enzyme activity was measured by the method of Foldes and Meek [25] as modified by Anderson and Weinshilboum [26] and by Campbell *et al.* [9] to provide optimal conditions for the assay of TS PST activity in human hepatic cytosol preparations. The assay involves the sulfate conjugation of substrate, in this case 4-nitrophenol, in the presence of [^{35}S]PAPS, the sulfate donor for the reaction. Blanks were samples that did not contain sulfate acceptor substrate. When assays of recombinant SULTs were performed, an additional control involved assays performed with COS-1 cells transfected with vector that did not contain an insert. Unless otherwise stated, concentrations of 4-nitrophenol and PAPS were 4 and 0.4 μM, respectively [9]. Substrate kinetic experiments were conducted in the presence of a series of concentrations of 4-nitrophenol and PAPS to make it possible to calculate apparent K_m values. Enzyme activity was expressed as nanomoles of sulfate-conjugated product formed per hour of incubation. Protein concentrations were measured by the dye-binding method of Bradford [27] with BSA as a standard.

Thermal stability was determined as described by Reiter *et al.* [7, 28]. Specifically, hepatic high-speed supernatant (HSS) preparations were thawed, diluted, and then either subjected to thermal inactivation for 15 min at 44° or kept on ice as a control. These conditions had been shown to be optimal for the measurement of TS PST thermal stability with human liver preparations [9]. H/C ratios were used as a measure of thermal stability in these experiments. The thermal stability of recombinant proteins was measured by incubating diluted, transfected COS-1 cell HSS for 15 min

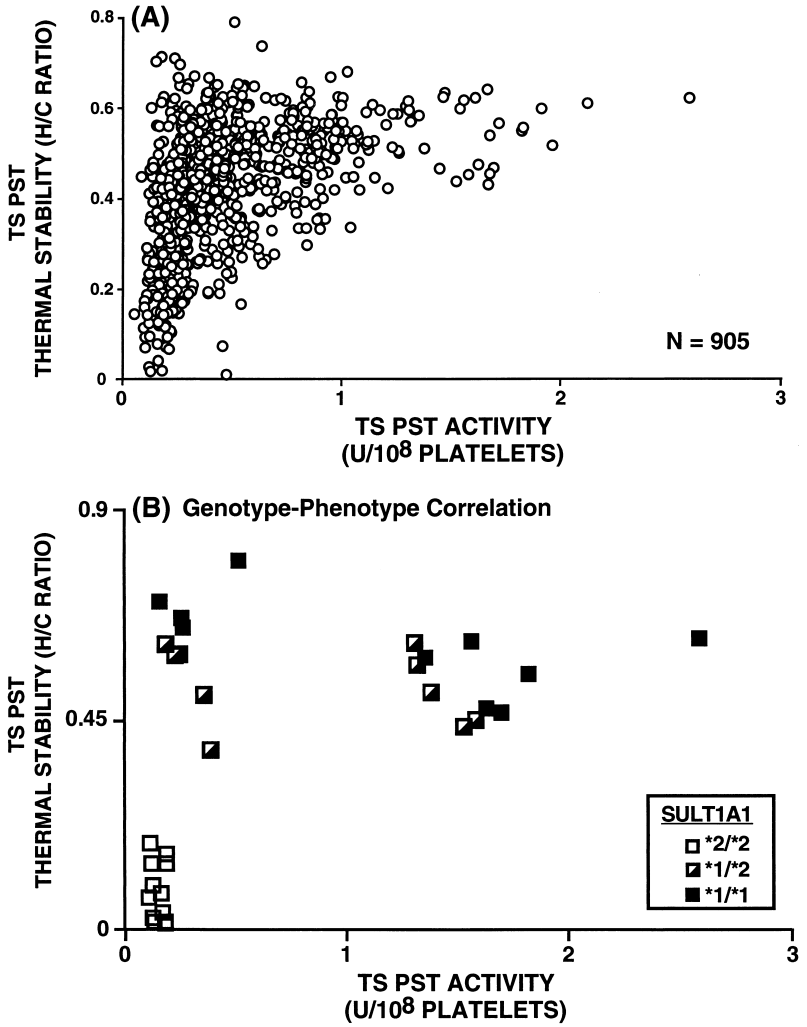

FIG. 1. (A) Human platelet TS PST phenotypes. The relationship between TS PST enzymatic activity and thermal stability measured as a heated/control (H/C) ratio in 905 human platelet samples is shown. (B) Human platelet *SULT1A1* genotype–TS PST phenotype correlation. DNA from subjects selected for extreme platelet TS PST phenotypes were genotyped for *SULT1A1* alleles. The allozymes encoded by those *SULT1A1* alleles are shown superimposed on phenotype. Modified from Ref. 16 and reproduced courtesy of Academic Press.

in a Perkin–Elmer 2400 thermal cycler at a series of temperatures. All samples were placed on ice immediately after the thermal inactivation step, and PST activity was measured in both heated and control unheated samples. Thermal inactivation curves then were constructed for each recombinant protein by plotting SULT activity expressed as a percentage of the control value. The concentration of 4-nitrophenol used to assay each of the recombinant proteins was determined on the basis of the results of the substrate kinetic experiments during which apparent K_m values had been determined. Those concentrations were SULT1A1 (*1, *2, *3), 4 μM; SULT1A2*1, 100 μM; SULT1A2*2, 3 mM; SULT1A2*3, 50 μM; and SULT1A3, 3 mM.

DCNP inhibition was determined by measuring enzyme activity in the presence of a series of concentrations of this SULT inhibitor dissolved in dimethyl sulfoxide. Blank samples for those experiments contained the appropriate concentration of DCNP, but no sulfate acceptor substrate. The concentration of 4-nitrophenol used to study each recombinant protein was the same as that used in the thermal stability experiments. All assays for the determination of apparent K_m values, thermal stability, or DCNP inhibition were performed in triplicate, and all experiments were performed at least three times, i.e. each of the data points shown subsequently represents the average of at least nine separate assays.

PCR Amplification and DNA Sequencing

Total genomic DNA was isolated from the human liver biopsy samples with the QIAamp Tissue Kit (Qiagen, Inc.). Gene-specific primers for the PCR were designed by comparing the sequences of *SULT1A1*, *SULT1A2*, and *SULT1A3* (Genbank accession numbers U52852, U34804, and U20499, respectively) and identifying intron sequences

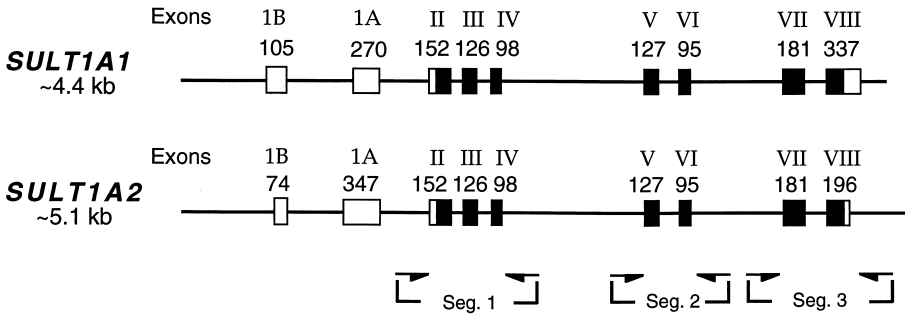

FIG. 2. Human *SULT1A1* and *SULT1A2* gene structures and the PCR strategy used to amplify the ORF of each gene in three segments. Black rectangles represent exons that encode cDNA ORF sequence, while open rectangles represent exons or portions of exons that encode cDNA untranslated region (UTR) sequence. Roman numerals are exon numbers, and Arabic numerals are exon lengths in bp. Gene lengths in kb from initial to final exons are also indicated. Forward and reverse arrows indicate the placement within introns of the PCR primers used to amplify, in three separate reactions, the ORFs of *SULT1A1* or *SULT1A2*.

that differed among the three genes. These gene-specific primers were then used to amplify, in three separate segments for each gene, the coding regions of either *SULT1A1* or *SULT1A2* (Fig. 2). To assure specificity, an initial long PCR amplification was performed using oligonucleotide primers that annealed to unique sequences present in the 5′- and 3′-flanking regions of each gene. Those long PCR products were then used as templates for the subsequent PCR reactions to amplify coding regions of the genes. Sequences of the PCR primers used to perform these experiments are listed in Table 2. DNA sequencing was performed with single-stranded DNA as template to help assure the detection of heterozygous samples. To make that possible, single-stranded DNA was generated by exonuclease digestion of either the sense or antisense strand of the double-stranded PCR amplification products. Phosphorothioate groups were conjugated to the 5′-end of either the forward or reverse PCR primer, depending on which of the two strands was to be protected from exonuclease digestion. Specifically, the PCR amplification of gene segments was performed in a 50-μL reaction mixture using AmpliTaq Gold DNA polymerase (Perkin–Elmer). Digestion of the non-phosphorothioated strand involved incubation of 16 μL of the post-amplification reaction mixture with 20 U of T7 gene 6 exonuclease (United States Biochemical) in 10 mM Tris–HCl buffer, pH 7.5, that contained 200 μM DTT and 20 μg/mL of BSA [29]. This mixture was incubated at 37° for 4 hr, followed by inactivation of the exonuclease by incubation at 80° for 15 min. The resulting single-stranded DNA was used as a sequencing template after PCR primers and salts had been removed with a Microcon-100 microconcentrator (Amicon). DNA sequencing was performed in the Mayo Clinic Molecular Biology Core Facility with an ABI model 377 sequencer (Perkin–Elmer) using dye terminator cycle sequencing chemistry. In those few instances in which haplotype assignment was ambiguous, the initial long PCR amplification product containing the entire gene was subcloned into pCR2.1 (Invitrogen) prior

TABLE 2. Sequences of primers used to perform PCR amplifications

PCR amplification	Primer designation	Primer sequence
SULT1A1 Gene-specific amplifications		
Long PCR	1AF (−119)	5′-CCTGGAGACCTTCACACACCCTGATA-3′
	DR3296	5′-CCACTCTGCCTGGCCCACAATCATA-3′
Segment 1	I1AF11	5′-GCTGGGGAACCACCGCATTAGAG-3′
	I4R83	5′-AACTCCCAACCTCACGTGATCTG-3′
Segment 2	I4F1018	5′-CCTCAGGTTCCTCCTTTGCCAAT-3′
	I6R93	5′-TGCCAAGGGAGGGGGCTGGGTGA-3′
Segment 3	I6F395	5′-GTTGAGGAGTTGGCTCTGCAGGGTC-3′
	DR3296	5′-CCACTCTGCCTGGCCCACAATCATA-3′
SULT1A2 Gene-specific amplifications		
Long PCR	1AF (−90)	5′-GGGCCCCGTTCCACGAGGGTGCTTTCAC-3′
	DR4590	5′-TGACCCCACTAGGAAGGGAGTCAGCACCCCTACT-3′
Segment 1	I1AF16	5′-GGAACCACCACATTAGAAC-3′
	I4R86	5′-TGGAACTTCTGGCTTCAAGGGATCT-3′
Segment 2	I4F1117	5′-CCTCAGCTTCCTCCTTTGCCAAA-3′
	I6R81	5′-TGGCTGGGTGGCCTTGGC-3′
Segment 3	I6F688	5′-GCTGGCTCTATGGGTTTTGAAGT-3′
	DR4094	5′-CTGGAGCGGGGAGGTGGCCGTATT-3′

"I" represents "intron," "F" represents "forward," "R" represents "reverse," and "D" ("downstream") represents the 3′-flanking region of the gene.

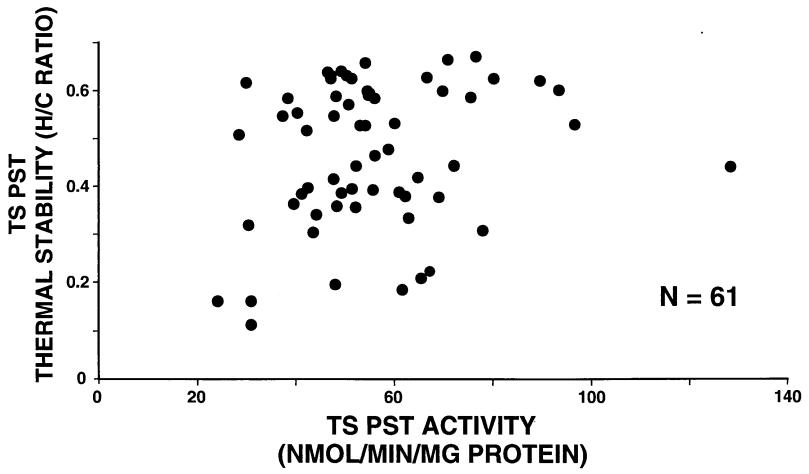

FIG. 3. Human liver TS PST phenotypes. The relationship between TS PST enzymatic activity and thermal stability measured as a heated/control (H/C) ratio in 61 human liver biopsy samples is shown.

to DNA sequencing. In the case of polymorphisms detected in only a single sample, the validity of the polymorphisms was confirmed by performing a separate, independent PCR analysis of that sample.

COS-1 Cell Expression

Seven different SULT expression constructs were used to transfect COS-1 cells. These constructs included cDNA sequences for all of the common SULT1A1 and 1A2 allozymes observed during the present experiments, 1A1*1, 1A1*2, 1A1*3, 1A2*1, 1A2*2, and 1A2*3, as well as SULT1A3. As a control, transfection was also performed with expression vector that lacked an insert. All SULT cDNA sequences used to create the expression constructs either had been cloned in our laboratory (SULT1A1*2, SULT1A2*2, SULT1A3), were obtained from the Expressed Sequence Tag (EST) database and the American Type Culture Collection (SULT1A1*3, SULT1A2*1) [30], or were created by site-directed mutagenesis (SULT1A1*1, SULT1A2*3). Each SULT cDNA then was amplified with the PCR and was subcloned into the eukaryotic expression vector pCR3.1 (Invitrogen). All inserts were sequenced after subcloning to assure that no variant sequence had been introduced during the PCR amplifications. COS-1 cells were then transfected with these expression constructs by use of the DEAE-dextran method [31, 32]. After 48 hr in culture, the transfected cells were harvested, and cytosols were prepared as described previously [31]. Aliquots of these cytosol preparations were stored at -80° prior to assay.

Data Analysis

Apparent K_m values were calculated by using the method of Wilkinson [33] with a computer program written by Cleland [34]. The IC_{50} values and 50% thermal inactivation (T_{50}) values were calculated with the GraphPAD InPlot program (GraphPAD InPlot Software). Statistical comparisons of data were performed by ANOVA with the StatView program, version 4.5 (Abacus Concepts, Inc.) Linkage analysis was performed using the EH program developed by Terwilliger and Ott [35].

RESULTS

Human Liver TS PST Phenotype

Population and family studies had demonstrated previously that levels of TS PST thermal stability and activity in the platelet, measured under optimal conditions with 4 μM 4-nitrophenol as substrate, were controlled by common genetic polymorphisms [13, 14] (Fig. 1). Because human platelet TS PST activity and thermal stability were reported to be correlated with hepatic TS PST activity and thermal stability [36], our initial experiment involved the measurement of both of these variables under optimal assay conditions [9] in 61 human liver biopsy samples. A scatter-diagram of those data is shown in Fig. 3. We then set out to sequence all exons encoding protein for both *SULT1A2* and *SULT1A1*, using DNA from these 61 hepatic tissue samples to detect nucleotide polymorphisms, and to determine whether there were significant correlations between genotypes for *SULT1A2* and/or *SULT1A1* and TS PST phenotype in the liver.

SULT1A2 and SULT1A1 Genetic Polymorphisms

All exons encoding protein for both *SULT1A2* and *SULT1A1* were PCR amplified in three segments (Fig. 2), and then were sequenced on both strands. Approximately 2 kb of DNA was sequenced for each gene. Therefore, a total of approximately 250 kb of sequence was analyzed for these 61 hepatic biopsy samples. We observed 13 different *SULT1A2* alleles among the 122 alleles sequenced in the 61 biopsy samples. These alleles resulted from various combinations of ten different SNPs (Table 3B). Four of

TABLE 3. SULT1A2 allozymes and *SULT1A2* alleles

(A) SULT1A2 allozymes

Allozyme	Amino acid 7	19	184	235	Allozyme frequency in 61 hepatic biopsy samples
*1	I1e	Pro	Arg	Asn	0.508
*2	Thr	Pro	Arg	Thr	0.287
*3	I1e	Leu	Arg	Asn	0.180
*4	Thr	Pro	Cys	Thr	0.008
*5	Thr	Pro	Arg	Asn	0.008
*6	I1e	Pro	Arg	Thr	0.008

(B) *SULT1A2* alleles

Allele	Exon II 20	24	56	I2–34	I5–78	Exon VI 550	Exon VII 704	I7–9	Exon VIII 895	902	Allele frequency in 61 hepatic biopsy samples
*1A	T	T	C	T	T	C	A	C	T	A	0.467
*1B	T	T	C	T	C	C	A	C	T	A	0.025
*1C	T	T	C	C	C	C	A	C	T	A	0.008
*1D	T	T	C	T	C	C	A	C	C	A	0.008
*2A	C	C	C	C	C	C	C	C	C	G	0.262
*2B	C	C	C	T	C	C	C	C	C	G	0.016
*2C	C	C	C	C	C	C	C	T	C	G	0.008
*3A	T	T	T	T	C	C	A	C	T	A	0.156
*3B	T	T	T	T	T	C	A	C	T	A	0.016
*3C	T	T	T	T	C	C	A	T	T	A	0.008
*4	C	C	C	C	C	T	C	C	C	G	0.008
*5	C	C	C	C	C	C	A	C	C	G	0.008
*6	T	T	C	T	T	C	C	C	C	G	0.008

(A) SULT1A2 allozymes. Numbers at the top indicate amino acid position from the N-terminus. The right-hand column indicates allozyme frequencies in the 61 hepatic biopsy samples studied. (B) *SULT1A2* alleles. Numbers st the top indicate the nucleotide position within the ORF, in which 1 = the "A" in the "ATG" start codon; or introns, in which an "I" followed by a numeral indicates the location of the nucleotide within the intron (i.e. I2–34 is the 34th nucleotide from the 5′-end of intron 2). Nucleotides shown against a shaded background alter the encoded amino acid. Nucleotides 895 and 902 lie within the 3′-UTR of the SULT1A2 mRNA. The values shown in the right-hand column indicate allele frequencies in the 61 hepatic biopsy samples.

those SNPs altered the encoded amino acid, resulting in six different SULT1A2 allozymes (proteins with different amino acid sequences), three of which appeared to be "common" (frequency $\geq$ 1%, Table 3A). Four alleles were observed only once (Table 3B), but their existence was confirmed by independent PCR amplifications and sequencing reactions. The allele nomenclature used here assigns different numerals after the asterisk to alleles that encode different allozymes, with a subsequent alphabetic designation for alleles that also differ with regard to "silent" SNPs. Since we had the advantage of population data, the numeric assignments were not made randomly, but rather could be assigned on the basis of relative allele frequency in the population sample studied, i.e. **1* was observed more often than **2*, **2* was more common than **3*, etc.

We had reported previously the existence of 13 different *SULT1A1* alleles in a population sample of 150 randomly selected Caucasian blood donors [16]. Those alleles encoded four different allozymes for *SULT1A1* (Table 4A). We identified 10 of the 13 previously identified *SULT1A1* alleles in these 61 liver samples (Table 4B). The alleles in the liver samples encoded three of the four SULT1A1 allozymes reported previously. Alleles *SULT1A1*1*G, **1H*, **1I*, **3A*, and **4* were not present in these liver samples, but two novel *SULT1A1* alleles, **1J* and **1K*, were detected, bringing the total number of *SULT1A1* alleles that have been identified to 15 (Table 4B). Those 15 alleles involve various permutations of 24 individual SNPs located within the approximately 2 kb of *SULT1A1* DNA sequenced (Table 4B).

The newly discovered alleles for *SULT1A2* appeared to be in linkage disequilibrium with alleles for *SULT1A1*. *SULT1A1*1* and **3* were linked to *SULT1A2*1* and **3*, while *SULT1A1*2* was linked to *SULT1A2*2*. Specifically, the hypothesis of no association between the polymorphisms for these two genes was rejected by linkage analysis [35], but the hypothesis of association was supported with $\chi^2 = 53.83$ ($P < 0.0001$). Of the 122 sets of *1A1/1A2*

TABLE 4. SULT1A1 allozymes and *SULT1A1* alleles

(A) SULT1A1 allozymes

Allozyme	Amino acid 37	Amino acid 213	Amino acid 223	Allozyme frequency in 61 hepatic biopsy samples	Allozyme frequency in 150 random blood donors
*1	Arg	Arg	Met	0.671	0.674
*2	Arg	His	Met	0.311	0.313
*3	Arg	Arg	Val	0.016	0.010
*4	Gln	Arg	Met	N.D.	0.003

(B) *SULT1A1* alleles

		Exon II		Exon III												Exon VII							Exon VIII		Allele frequency in 61 hepatic biopsy samples	Allele frequency in 150 random blood donors
Allele	I1A–138	57	110	153	162	I5–34	I5–35	I6–11	I6–14	I6–17	I6–35	I6–45	I6–64	I6–488	I6–509	600	638	645	667	I7–16	I7–69	I7–120	902	973		
*1A	T	G	G	T	A	C	G	C	T	A	A	C	A	T	G	G	G	G	A	C	T	C	A	C	0.328	0.303
*1B	T	G	G	T	A	C	A	G	C	T	T	A	G	T	G	G	G	G	A	C	T	C	A	C	0.221	0.237
*1C	T	A	G	C	G	C	G	C	T	A	A	C	A	C	A	C	G	A	A	C	C	C	A	T	0.041	0.040
*1D	C	G	G	T	A	C	G	C	T	A	A	C	A	T	G	G	G	G	A	C	T	C	A	C	0.016	0.027
*1E	T	G	G	T	A	C	A	G	C	T	T	A	G	T	G	G	G	G	A	C	T	G	A	C	0.016	0.020
*1F	C	G	G	T	A	C	A	G	C	T	T	A	G	T	G	G	G	G	A	C	T	C	A	C	0.033	0.017
*1G	T	G	G	T	A	C	G	G	C	T	T	A	G	T	G	G	G	G	A	C	T	C	A	C	N.D.	0.013
*1H	T	A	G	C	G	C	G	C	T	A	A	C	A	T	G	G	G	G	A	C	C	C	A	C	N.D.	0.010
*1I	T	A	G	T	A	C	G	C	T	A	A	C	A	T	G	G	G	G	A	C	T	C	A	C	N.D.	0.007
*1J	T	A	G	C	G	C	G	C	T	A	A	C	A	C	A	C	G	G	A	C	C	C	G	T	0.008	N.D.
*1K	T	G	G	C	G	C	G	C	T	A	A	C	A	C	A	C	G	A	A	C	C	C	A	C	0.008	N.D.
*2	T	G	G	C	G	C	G	C	T	A	A	C	A	C	A	C	A	G	A	T	C	C	G	T	0.311	0.313
*3A	T	G	G	T	A	C	G	C	T	A	A	C	A	T	G	G	G	G	G	C	T	C	A	C	N.D.	0.007
*3B	T	G	G	T	A	T	A	G	C	T	T	A	G	T	G	G	G	G	G	C	T	C	A	C	0.016	0.003
*4	T	A	A	T	A	C	A	G	C	T	T	A	G	T	G	G	G	G	A	C	T	C	A	C	N.D.	0.003

(A) *SULT1A1* allozymes. Numbers at the top indicate amino acid position from the N-terminus. The right-hand columns indicate allozyme frequencies in the 61 hepatic biopsy samples studied and in 150 randomly selected Caucasian blood donors [16]. (B) *SULT1A1* alleles. Numbers at the top indicate the nucleotide position within the ORF, in which 1 = the "A" in the "ATG" start codon; or introns, in which an "I" followed by a numeral indicates the intron number, and the number after the dash indicates the location of the nucleotide within the intron (i.e. I5–34 is the 34th nucleotide from the 5′-end of the 5th intron). Nucleotides shown against a shaded background alter the encoded amino acid. Nucleotides 902 and 973 lie within the 3′-UTR of the SULT1A1 mRNA. The values in the right-hand columns indicate allele frequencies in the 61 hepatic biopsy samples studied or in DNA from 150 randomly selected Caucasian blood donors [16]. N.D. indicates "not detected".

TABLE 5. Recombinant human SULT biochemical and physical properties

Allozyme	Apparent K_m (μM)		Thermal stability	DCNP inhibition
	4-Nitrophenol	PAPS	T_{50} (°C)	IC_{50} (μM)
SULT1A1				
*1	0.88 ± 0.07	1.21 ± 0.02	39.3 ± 0.64	1.44 ± 0.11
*2	0.78 ± 0.08	0.98 ± 0.03	37.2 ± 0.43	1.38 ± 0.28
*3	0.31 ± 0.01	0.17 ± 0.02	38.9 ± 0.03	1.32 ± 0.27
SULT1A2				
*1	8.70 ± 1.10	0.05 ± 0.001	43.6 ± 0.15	6.94 ± 0.55
*2	373 ± 33	0.50 ± 0.001	46.3 ± 0.09	44.4 ± 1.50
*3	5.65 ± 1.14	0.28 ± 0.006	38.8 ± 0.19	0.97 ± 0.001
SULT1A3	4960 ± 810	0.28 ± 0.001	32.6 ± 0.19	86.9 ± 6.00

Properties of common SULT1A1 and SULT1A2 allozymes as well as SULT1A3 were measured as described in the text. Each value represents the mean ± SEM of nine separate determinations.

alleles sequenced for each gene, only 10 displayed discordance. As discussed subsequently, the existence of linkage disequilibrium complicated attempts to determine which of these two gene products might be responsible for the phenol SULT phenotype. Therefore, to help clarify possible genotype–phenotype correlations for these enzymes, we next determined the biochemical and physical properties of the proteins encoded by all common alleles for *SULT1A1* and *SULT1A2*.

COS-1 Cell Expression of SULT1A1 and SULT1A2 Allozymes

Expression constructs for each of the common (frequency ≥ 1%) alleles for *SULT1A1* and *SULT1A2* were used to transfect COS-1 cells. Then selected biochemical and physical properties of the expressed enzymes were determined. Those properties included apparent K_m values for 4-nitrophenol and PAPS, the two cosubstrates for the enzyme reaction; thermal stability; and sensitivity to inhibition by DCNP. The substrate kinetic experiments were performed in two steps. Initially a wide range of concentrations of 4-nitrophenol that varied over at least three orders of magnitude was tested, followed by a detailed study of concentrations close to the apparent K_m value for that allozyme. This "two-stage" approach was used because the SULTs display profound substrate inhibition [3]. Concentrations of 4-nitrophenol that were used to calculate apparent K_m values ranged from 0.02 to 5.0 μM for SULT1A1*1, 1A1*2, and 1A1*3; 0.08 to 10.0 μM for SULT1A2*1 and 1A2*3; 1.0 to 1000 μM for SULT1A2*2; and 4.0 to 3000 μM for SULT1A3. Data from these experiments then were used to construct double inverse plots that were used to calculate apparent K_m values (Table 5). The results of the substrate kinetic studies suggested that the TS PST phenotype in human liver might be due primarily to the expression of *SULT1A1*, since optimal conditions for the assay of TS PST activity in the human liver involved the use of 4 μM 4-nitrophenol as a substrate [9]. That concentration would be optimal for assay of the activities of allozymes encoded by alleles for *SULT1A1*, but it was below the apparent K_m values for all of the SULT1A2 allozymes (Table 5). Of particular importance for the genotype–phenotype correlation analysis described subsequently is the fact that SULT1A2*2 had a very high apparent K_m value for 4-nitrophenol (Table 5).

Apparent K_m values of the recombinant SULTs for PAPS also were determined. In those studies, as well as in the thermal stability and DCNP inhibition experiments, the concentrations of 4-nitrophenol used to perform the assays were 4 μM for SULT1A1*1, *2, and *3; 100 μM for SULT1A2*1; 50 μM for 1A2*3; and 3000 μM for SULT1A2*2 and SULT1A3. These concentrations were based on results of the 4-nitrophenol substrate kinetic experiments and represented the concentration at which maximal activity had been observed for that particular allozyme. Apparent K_m values of the recombinant SULT proteins for PAPS are also listed in Table 5. With one exception, those values varied from approximately 0.2 to 1.2 μM—similar to apparent K_m values for PAPS reported during previous studies of human liver and platelet "TS PST" activity [9, 13]. The single exception was SULT1A2*1, with an apparent K_m value approximately an order of magnitude lower than those of the other allozymes studied (Table 5).

The thermal stabilities of the seven expressed proteins also were determined, and they varied widely. The rank order of the thermal stabilities was 1A2*2 > 1A2*1 ≫ 1A1*1 ~ 1A1*3 ~ 1A2*3 > 1A1*2 ≫ 1A3 (Table 5). These observations were consistent with our previous report that *SULT1A1*2* was associated with a "thermolabile" phenotype in the platelet (Fig. 1) [16], since that allozyme had the lowest T_{50} value of the recombinant "TS-PST-like" allozymes studied (Table 5). They also indicated that it would be unlikely that allozyme SULT1A2*2 could explain a "thermolabile" phenotype, since it was the most "thermostable" of the allozymes studied. These issues also will be addressed subsequently during the discussion of genotype–phenotype correlations for the human liver.

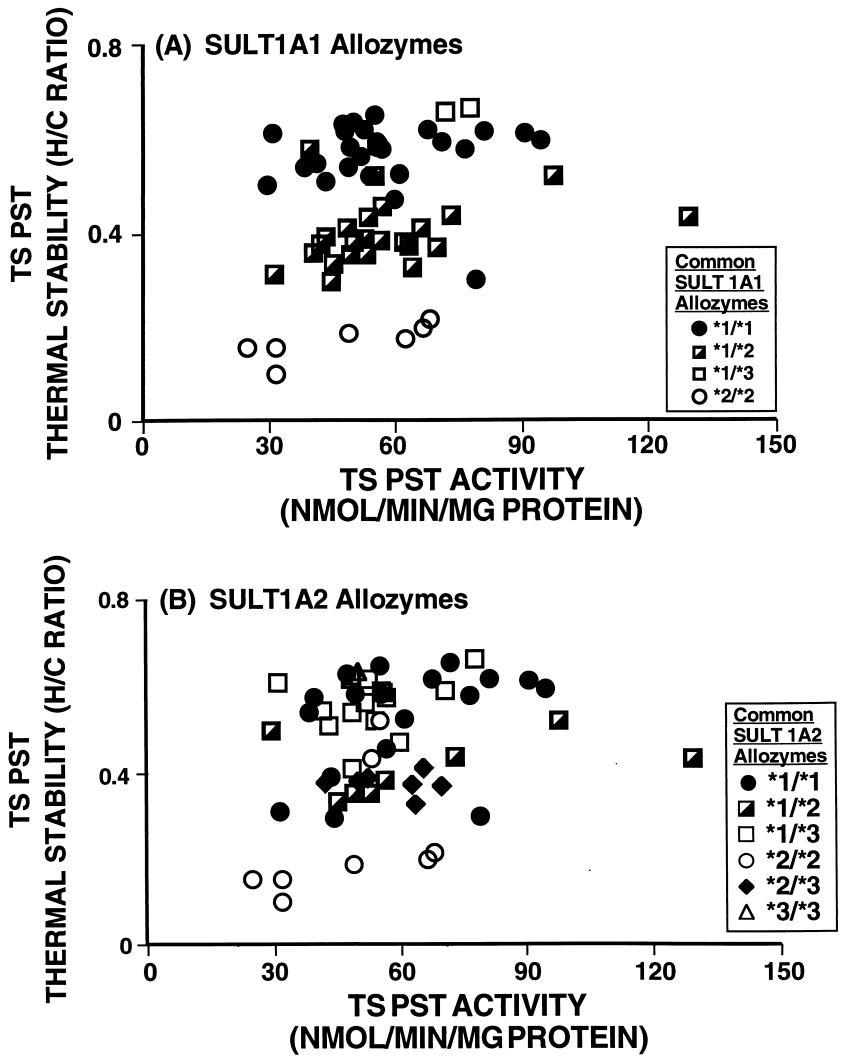

FIG. 4. Correlation of *SULT1A1* and *SULT1A2* genotypes with human liver TS PST phenotypes. TS PST phenotypes in the human liver samples depicted as in Fig. 3 are shown with (A) common SULT1A1 allozymes or (B) common SULT1A2 allozymes superimposed. In (B), three samples are not shown because those SULT1A2 allozymes were observed only once in this population sample.

Finally, sensitivity of the recombinant proteins to inhibition by DCNP was determined. Sixteen different concentrations of this SULT inhibitor, ranging from 0.01 to 1000 μM, were tested with each recombinant allozyme. The IC_{50} values for DCNP also varied widely, with SULT1A2*3 being most sensitive, and SULT1A3 least sensitive to inhibition (Table 5). After all of these data had been obtained, the final step in this series of experiments was an attempt to correlate human liver TS PST phenotype with *SULT1A1* and/or *SULT1A2* genotype.

Human Liver Genotype–Phenotype Correlation

We had demonstrated previously that subjects homozygous for the allele *SULT1A1*2* uniformly had low levels of both TS PST activity and thermal stability in their platelets (Fig. 1B) [16]. The genotype–phenotype correlation for *SULT1A1* in the 61 liver samples studied in the present experiments is shown in Fig. 4A. Similar data for *SULT1A2* are plotted in Fig. 4B. The figure demonstrates clearly that the *SULT1A1*2* allele appeared to be associated with low TS PST thermal stability in the liver—just as it was in the human blood platelet (Fig. 1B). For example, the average H/C ratio for samples homozygous for *SULT1A1*1* was 0.57 ± 0.01 (N = 28, mean ± SEM), while that for heterozygous *1A1*1/1A1*2* samples was 0.40 ± 0.01 (N = 24), and samples homozygous for *SULT1A1*2* had an average H/C ratio of 0.18 ± 0.01 (N = 7, $P < 0.001$ by ANOVA).

Although the *SULT1A1*2* allele was highly correlated with low TS PST thermal stability in the liver, unlike the situation in the platelet, low thermal stability was not correlated significantly with low levels of TS PST activity (compare Figs. 1B and 4A). Of possible importance is the fact that, when the data were stratified on the basis of diagnosis, of the seven samples homozygous for

*SULT1A1*2*, the three obtained from patients with benign hepatic disease had the lowest levels of TS PST activity, while the four samples from patients with malignant disease had the highest activity (28.5 ± 2.3 vs 59.8 ± 4.0, mean ± SEM, respectively, $P < 0.002$). Obviously, these observations for a very small number of samples can serve only to suggest possible directions for future studies.

The results of the substrate kinetic experiments (Table 5) as well as the results of the thermal stability studies suggested that TS PST phenotype in the liver was most likely a measure of *SULT1A1* expression. As pointed out previously, that was true because both K_m values for 4-nitrophenol and T_{50} values for recombinant SULT1A2 allozymes were above those found to be optimal for the determination of TS PST phenotype in human liver cytosol preparations (Table 5). Testing that hypothesis directly is complicated by the fact that SULT1A1 and 1A2 share 95% or greater identity for both protein amino acid and mRNA nucleotide sequences [37–42], so neither western nor northern blots can distinguish easily between them. However, our biochemical studies of recombinant SULT allozymes suggested that the sulfation of 100 μM 4-nitrophenol might represent a relatively specific measure of SULT1A2 activity (Table 5). That is true because, as a result of the profound substrate inhibition that these enzymes display [3], SULT1A1 allozymes would show little or no activity at that concentration, and SULT1A3 would not contribute significantly to activity measured at that concentration because of its very high K_m value for 4-nitrophenol (Table 5). Therefore, we used 100 μM 4-nitrophenol as a substrate with pooled cytosol from six individual liver samples in an attempt to measure SULT1A2 activity. However, no activity was detected at five different pooled cytosol protein concentrations, suggesting that SULT1A2 is not highly expressed in the human liver—as also has been reported by other groups [43].

In summary, we observed common genetic polymorphisms for both *SULT1A1* and *SULT1A2* in humans. However, the proteins encoded by these alleles differed in their biochemical and physical properties. The allele *SULT1A1*2* was associated with decreased TS PST thermal stability in the liver—just as it is in the blood platelet [16], but, unlike the situation in the platelet, none of the *SULT1A1* or *SULT1A2* alleles appeared to be systematically associated with the level of TS PST activity in these hepatic tissue samples.

DISCUSSION

We recently reported a series of alleles for *SULT1A1*, one of which, *SULT1A1*2*, was associated consistently with significantly decreased levels of both TS PST activity and thermal stability in the blood platelet [16] (Fig. 1). The present experiments were conducted to determine whether *SULT1A2*, like *SULT1A1*, might also have a series of alleles and allozymes, to compare the properties of all common SULT1A1 and 1A2 allozymes and to study phenol SULT genotype–phenotype correlations in an important drug-metabolizing organ, the liver.

We found that *SULT1A2*, like *SULT1A1*, had several common alleles and allozymes (Table 3). Furthermore, those alleles were in linkage disequilibrium with the alleles for *SULT1A1*. Both *SULT1A1*2* and *SULT1A2*2* were associated with decreased TS PST thermal stability in the human liver (Fig. 4), but the biochemical and physical properties of recombinant SULT allozymes indicated that "TS PST phenotype" in the liver was most likely due to the expression of SULT1A1 (Table 5). For example, as discussed subsequently, based both on its apparent K_m value for 4-nitrophenol and its T_{50} value, it is unlikely that SULT1A2*2 would contribute to this phenotype (Table 5). Unlike the situation in the blood platelet, *SULT1A1*2* was not consistently associated with a low level of TS PST activity in the liver. However, data from a small number of subjects raised the possibility that *SULT1A1*2* might be associated with lower levels of TS PST activity in tissue from subjects with benign rather than neoplastic disease. That possibility will have to be pursued in the course of larger studies in the future. Obviously, polymorphisms located in regions of these genes other than their ORFs, e.g. within promoters or introns, could also contribute to individual differences in levels of TS PST activity, as could factors involved in the regulation of transcription or in mRNA and protein stability. Each of those possibilities will have to be explored in the course of future experiments.

The existence of two SULT isoforms, 1A1 and 1A2, that encode proteins with "TS PST-like" activity raises the broader issue of the relative contributions of these two isoforms to PST phenotype. As noted previously, the biochemical characteristics of recombinant SULT proteins indicate that the sulfation of 4 μM 4-nitrophenol would be expected to be catalyzed predominantly by SULT1A1 rather than SULT1A2 (Table 5). It is particularly unlikely that SULT1A2*2 would contribute to the TS PST phenotype under these assay conditions, since its apparent K_m value for 4-nitrophenol is two orders of magnitude higher than those of the proteins encoded by the *SULT1A1* alleles. Furthermore, our failure to detect TS PST activity in liver cytosol preparations under optimal assay conditions for SULT1A2 suggests that allozymes for SULT1A2 probably are not highly expressed in human hepatic tissue, a conclusion also reached by others [43]. Obviously, our observations do not provide any information with regard to the possible expression of SULT1A2 in other human tissues or organs.

The relatively large number of *SULT1A1* and *SULT1A2* alleles and allozymes, with three "common" allozymes for each gene—at least among Caucasian subjects—indicates the potential complexity of human SULT pharmacogenetics. These observations also serve to emphasize the need for simple techniques that can be used to determine complete haplotypes, rather than allele-specific techniques designed merely to detect SNPs. We found that alleles for *SULT1A1* were in linkage disequilibrium with alleles for *SULT1A2*.

That situation does serve to restrict the collective *SULT1A1–SULT1A2* genotype somewhat. The gene for *SULT1A3* (TL PST) also is located on chromosome 16, close to *SULT1A1* and *SULT1A2* [21, 24]. If functional polymorphisms for *SULT1A3* exist, as has been reported on the basis of biochemical pharmacogenetic experiments [44], those alleles also might be in linkage disequilibrium with alleles for the other two human PST isoforms. Therefore, it is possible that the number of relatively common human "phenol SULT genotypes" might be less extensive in practical terms than might be anticipated purely on the basis of allele numbers. In summary, we have identified a series of polymorphisms for the human *SULT1A2* gene. We then determined the properties of recombinant SULT1A1 and SULT1A2 allozymes and studied phenol SULT genotype–phenotype correlations in the liver. These observations represent a step toward merging SULT genomic and biochemical pharmacogenetic data, with the ultimate goal of understanding the possible contribution of inheritance to individual differences in the sulfate conjugation of drugs and other xenobiotics in humans.

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