BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **239,** 298–304 (1997)
ARTICLE NO. RC977466

Phenol Sulfotransferase Pharmacogenetics in Humans: Association of Common *SULT1A1* Alleles with TS PST Phenotype[1]

Rebecca B. Raftogianis, Thomas C. Wood, Diane M. Otterness, Jon A. Van Loon, and Richard M. Weinshilboum[2]

Department of Pharmacology, Mayo Medical School/Mayo Clinic/Mayo Foundation, Rochester, Minnesota 55905

Received August 28, 1995

The phenol sulfotransferases (PSTs) catalyze the sulfation of both small planar phenols and phenolic monoamines. Three highly homologous PST genes, *SULT1A1, SULT1A2,* and *SULT1A3,* are known to exist in humans. The prototypic biochemical phenotype associated with the enzyme encoded by *SULT1A1* is the thermal stable (TS) sulfation of 4 μM 4-nitrophenol (TS PST activity). Biochemical pharmacogenetic studies have demonstrated that individual variation in both TS PST activity and thermal stability in humans are inherited. As a step toward understanding molecular mechanisms responsible for the genetic regulation of PSTs in humans, we report here common *SULT1A1* nucleotide polymorphisms that are associated with phenotypic variation in both platelet TS PST activity and thermal stability. When 905 human subjects were phenotyped for platelet TS PST activity and thermal stability, activity varied more than 50-fold, and thermal stability varied over 10-fold. DNA was isolated from the blood of 33 of these subjects selected on the basis of "extreme" TS PST phenotypes: high activity and high thermal stability; low activity and low thermal stability; or low activity and high thermal stability. These 33 subjects were genotyped for *SULT1A1* by PCR amplification and sequencing of the entire open reading frame (ORF) as well as approximately 1 kb of intron DNA sequence. One common allele, *SULT1A1*2,* was uniformly associated with both very low TS PST activity and low thermal stability. The allele frequency of *SULT1A1*2* in a randomly selected population sample of 150 Caucasian blood donors was 0.31 (31%), indicating that approximately 9% of this population would be homozygous for that allele.

Pharmacogenetics is the study of the role of inheritance in individual variation in drug response or toxicity (1). Pharmacogenetic variation often results from genetic polymorphisms that alter drug metabolism (1,2). Sulfation is an important pathway in the metabolism of many drugs, xenobiotics, neurotransmitters and hormones (3). Sulfate conjugation is catalyzed by cytosolic sulfotransferase enzymes encoded by members of an expanding gene superfamily (4). Attendees at the Second International Sulfotransferase Enzyme Nomenclature Workshop recently agreed that "SULT" should be used as the abbreviation for these enzymes rather than the previously used abbreviation, "ST". Therefore, throughout this manuscript, we will adopt the recently proposed nomenclature (as appropriate) and cross reference SULT names with previously used designations (Table 1).

Six cytosolic SULTs are presently known to be expressed in human tissues. They include three phenol SULTs (PSTs), an estrogen SULT (EST), a hydroxysteroid SULT (DHEA ST), and the human orthologue of a rat N-hydroxy-2-acetylaminofluorene SULT (SULT1C1) (4,5). Previous biochemical pharmacogenetic studies of sulfation were performed at a time when only two human PST isoforms, defined on the basis of thermal stability, substrate specificity and inhibitor sensitivity, were known to exist (6-10). Those enzymes were referred to as the thermostable (TS) or phenol-preferring (P) PST and the thermolabile (TL) or monoamine-preferring (M) PST. TS PST preferentially catalyzed the sulfation of small planar phenols such as 4-nitrophenol at micromolar concentrations and was sensitive to inhibition by 2,6-dichloro-4-nitrophenol (DCNP). TL PST preferentially catalyzed the sulfation of phenolic monoamines such as dopamine at micromolar concentrations and was relatively insensitive to DCNP inhibition. Both of these biochemically defined activities were expressed in a variety of human tissues including liver, lung,

[1] Supported in part by NIH Grants RO1 GM 28157 (R.M.W.) and RO1 GM 35720 (R.M.W.), by a supplement to RO1 GM 35720 supported by the Office of Research on Women's Health (R.M.W.), and by an NRSA (NIH) Individual Postdoctoral Fellowship (R.B.R.).
[2] Correspondence and reprint requests. Fax: (507) 284-9111.

TABLE 1

Human PST Nomenclature

Proposed nomenclature		Previous nomenclature	
Enzyme	Gene	Enzyme	Gene
SULT1A1	*SULT1A1*	TS PST1 (22), P PST (35), ST1A3 (28), HAST1 (36), HAST2 (37)	*STP1*
SULT1A2	*SULT1A2*	TS PST2 (22), ST1A2 (28), HAST4 (29), HAST4V (29)	*STP2*
SULT1A3	*SULT1A3*	TL PST (38), M PST (39,40), HAST3 (37)	*STM*

Note. The proposed nomenclature, as well as previous designations for these enzymes are listed. The previous nomenclature include designations for the same enzyme that were used in reports from different laboratories.

brain, jejunum, kidney and -- of importance for genetic studies -- the blood platelet (6,9-12).

There are large individual variations in platelet TS and TL PST activities (13-15). Platelet TS PST also varies widely in its thermal stability, a property that is often measured to detect differences among proteins in amino acid sequence (16). Segregation analysis of platelet TS PST and TL PST data from family studies demonstrated that both activities, as well as variation in TS PST thermal stability, were controlled by genetic polymorphisms (14,15). Subjects with thermolabile TS PST uniformly exhibited low levels of activity (Fig. 1). Furthermore, the inherited traits of level of platelet TS PST activity and thermal stability have been shown to correlate significantly with those of the enzyme in other organs such as the liver, brain and intestine (17-19), making the platelet both a convenient and relevant tissue in which to study TS PST pharmacogenetics in humans.

cDNAs and genes have been cloned for most of the known human PSTs (4,5). Those studies have revealed that there are actually three PST isoforms encoded by three separate genes, ***SULT1A1, SULT1A2,*** and ***SULT1A3,*** (Table 1)(4,20-25). Those genes, also referred to as ***STP1, STP2,*** and ***STM,*** respectively, are highly homologous and are located within an approximately 120 kb region on the short arm of chromosome 16 (25-27). Both SULT1A1 and SULT1A2 catalyze the sulfation of 4-nitrophenol, but the apparent K_m of SULT1A2 for 4-nitrophenol has been reported to be 10 to 100 times higher than that of SULT1A1 (28,29). Genetic polymorphisms that influence the PSTs could result in variation in the biotransformation, therapeutic efficacy and/or toxicity of many therapeutic agents. Therefore, understanding the molecular basis for these polymorphisms might make it possible to predict individual differences in the efficacy and/or toxicity of drugs that undergo sulfate conjugation. The experiments described subsequently were performed as an attempt to understand the molecular basis for the effects of inheritance on TS PST in humans (13,15), and resulted in the identification of common polymorphisms within the SULT1A1 gene associated with individual variation in both levels of TS PST activity and thermal stability.

MATERIALS AND METHODS

Human platelet samples. Platelet TS PST activity and thermal stability were measured in blood samples from 652 members of 134 randomly selected families and 253 randomly selected unrelated blood donors at the Mayo Clinic in Rochester, MN. Platelets were isolated and platelet homogenates were prepared as described previously (13). Values for platelet TS PST activity and thermal stability in 237 members of 50 of these families and in 218 of the blood donors have been reported previously (13,15). For this study, platelet TS PST data for all 905 of the subjects studied were pooled (Fig. 1). DNA had not been isolated from these subjects originally because no SULT cDNA or gene had been cloned or characterized at the time that these biochemical genetic studies were performed. However, 33 subjects selected from 20 families were located, and they agreed to provide an additional blood sample for DNA isolation. Twenty ml of whole blood was collected from each of these subjects, and leukocytic DNA was isolated using the QIAamp Blood Kit (Qiagen, Chatsworth, CA). Blood samples were also obtained from 150 randomly selected Caucasian blood donors at the Mayo Clinic Blood Bank, and DNA was isolated from those samples to determine ***SULT1A1*** allele frequencies. All of these experiments were reviewed and approved by the Mayo Clinic Institutional Review Board.

PST enzyme activity and thermal stability. TS PST enzyme activity was measured by a modification of the method of Foldes and Meek (30,31). This assay is based on the sulfate conjugation of substrates such as 4-nitrophenol in the presence of [^{35}S]-3′-phosphoadenosine-5′-phosphosulfate (PAPS), the sulfate donor for the reaction. Details of the platelet TS PST assay have been described previously (13). Active samples contained 4 μM 4-nitrophenol and 0.4 μM [^{35}S]-PAPS, conditions shown previously to be optimal for the assay of blood platelet enzyme activity (13). Blank samples did not contain the sulfate acceptor substrate. Activity was expressed as units per 10^8 platelets, and one unit of PST activity represented the formation of 1 nmol of product per hr of incubation. PST thermal stability was measured as described by Reiter and Weinshilboum (32). Specifically, platelet preparations were thawed, diluted, and preincubated for 15 min in a shaking water bath at 44°C, while control aliquots were kept at 4°C. All samples were placed on ice immediately after the preincubation period. PST activity was then measured in both heated and control samples, and thermal stability was expressed as a heated to control (H/C) ratio (16).

SULT1A1 polymerase chain reaction (PCR) amplification and DNA sequencing. ***SULT1A1***-specific PCR primers were designed by comparing the nucleotide sequences of ***SULT1A1, SULT1A2,*** and ***SULT1A3*** (Genbank accession numbers U52852, U34804 and U20499, respectively) and identifying intron sequences that differed

among the three genes (20,22,25). Primers were designed to amplify the entire coding region of ***SULT1A1*** in three genomic segments. The three primer pairs used were I1AF11 (5′-GCTGGGGAACCACCGCATTAGAG-3′) with I4R83 (5′-AACTCCCAACCTCACGTGATCTG-3′); I4F1018 (5′-CCTCAGGTTCCTCCTTTGCCAAT-3′) with I6R93 (5′-TGCCAAGGGAGGGGGCTGGGTGA-3′); and I6F395 (5′-GTTGAGGAGTTGGCTCTGCAGGGTC-3′) paired with DR3296 (5′-CCACTCTGCCTGGCCCACAATCATA-3′).

Oligonucleotide synthesis and automated DNA sequencing were performed in the Mayo Clinic Molecular Core Facility. Single stranded DNA, generated by digesting either the sense or antisense strand of the double stranded PCR amplification product with bacteriophage T7 gene 6 exonuclease (United States Biochemical, Cleveland, OH), was used as the sequencing template to facilitate the detection of heterozygous sequence. Strand-specific digestion was achieved by taking advantage of the fact that this 5′ to 3′ exonuclease will not digest DNA that is protected at the 5′-terminus (33). Therefore, four 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. After digestion with T7 gene 6 exonuclease, the resulting single stranded products were sequenced with an Applied Biosystems Model 377 automated DNA sequencer (Perkin Elmer, Foster City, CA) using dye terminator cycle sequencing chemistry. Digestion of the unphosphorothioated strand involved incubation of 20 μl of the PCR reaction mixture with 20 U of T7 gene 6 exonuclease in 10 mM Tris-HCl, pH 7.5, that contained 20 mM $MgCl_2$, and 50 mM NaCl. This mixture was incubated at 37°C for 4 hr, followed by inactivation of the exonuclease by incubation at 94°C for 15 min. The undigested single stranded DNA was then used as a sequencing template after PCR primers and salts had been removed with a Microcon-100 microconcentrator (Amicon, Beverly, MA). Only the antisense strand was sequenced for samples from 150 randomly selected blood donors that were used to establish ***SULT1A1*** allele frequencies. If sequence ambiguity was observed, both sense and antisense strands were sequenced. To ensure that the PCR had not amplified other members of the PST gene subfamily, all DNA sequences were compared with those of ***SULT1A2*** and ***SULT1A3*** (Table 1).

A slightly different strategy was used when a single round of PCR amplification failed to result in an adequate quantity of product for DNA sequencing. For those samples, the entire gene was amplified using the long PCR, and that product was used as template for the amplification reactions described above. Specifically, the long PCR was performed using the GeneAmp XL PCR Kit (Perkin Elmer) with primer pair 1AF(-119) (5′-CCTGGAGACCTTCACACACCCTGATA-3′), which anneals within the 5′-UTR in exon 1A, and DR3296, which anneals within the 3′-flanking region of the gene. Furthermore, in any situation in which there was ambiguity with regard to ***SULT1A1*** allele assignment, the entire gene was amplified using primers 1AF(-119) and DR3296, subcloned into pCR 2.1 (Invitrogen, San Diego, CA), and sequenced. Allele sequence assignment for the 33 subjects was further facilitated by the fact that family members were included in this group; so segregation analysis could be utilized for these samples.

Data analysis. Statistical comparisons of data were performed by ANOVA with the StatView program, version 4.5 (Abacus Concepts, Berkeley, CA). Sequence analysis was performed using the University of Wisconsin Genetics Computer Group software package, version 9.0.

RESULTS

These studies were performed in an attempt to identify variant alleles for the human SULT1A1 gene that were associated with one of the following three platelet TS PST phenotypes: 1) high activity and high thermal stability, 2) low activity and high thermal stability or 3) low activity and low thermal stability (Fig. 1). The experiments were specifically designed to identify alleles that differed in nucleotide sequence within coding exons or intron sequence which flanked those exons. DNA was isolated from the leukocytes of 33 subjects who had been selected on the basis of phenotype. From this DNA, approximately 2 kb of the SULT1A1 gene was PCR amplified and sequenced for each subject. Those sequences were compared to detect allelic variants, and, the alleles identified were correlated with different platelet TS PST phenotypes. To accomplish these goals, we took advantage of the fact that we had previously phenotyped 905 human subjects for level of platelet TS PST activity and thermal stability (Fig. 1). Subjects with the phenotypes described above were selected from this population sample, and DNA from those subjects was used to identify ***SULT1A1*** alleles. We also took advantage of critical sequence information that we had obtained by cloning and completely sequencing each of the three known human PST genes -- ***SULT1A1, SULT1A2*** and ***SULT1A3*** (20,22,25). By comparing the nucleotide sequences of these three highly homologous genes, areas of sequence divergence were identified, and ***SULT1A1***-specific PCR primers were designed to amplify the entire ORF, as well as some intron sequence, in three separate genomic DNA segments. The locations of PCR primers and the segments amplified within the gene are shown schematically in Fig. 2.

Comparisons of gene sequences for these 33 subjects revealed the presence of six alleles in this selected population sample, five ***SULT1A1*1*** alleles and ***SULT1A1*2*** (Fig. 3). The alleles were named so that different Arabic numerals after the * were assigned to alleles that encoded different amino acids. Alleles that encoded the same amino acid sequence were assigned the same Arabic numeral, but were differentiated with an uppercase Roman letter after the Arabic numeral, i.e., ****1A, *1B,*** etc. This "suballele" assignment was made on the basis of relative allele frequency. The six alleles identified in these 33 subjects included a total of 21 variant nucleotides, seven of which were located within the ORF, and they encoded two different SULT1A1 amino acid sequences. Differences between the amino acid sequences of these two SULT1A1 proteins, *1 and *2, as well as those encoded by two additional less common alleles discovered subsequently when we sequenced ***SULT1A1*** in DNA samples from 150 blood donors, are listed in Table 2. All nucleotide differences among these alleles, including silent variant sequence within exons that did not alter encoded amino acids and variant intron sequence, are listed in Table 3. Table 3 also demonstrates that we observed significant sequence variation for allele ****1,*** but not for allele ****2*** -- both among the 33 selected subjects studied initially and among the 150 randomly selected blood donors described subsequently.

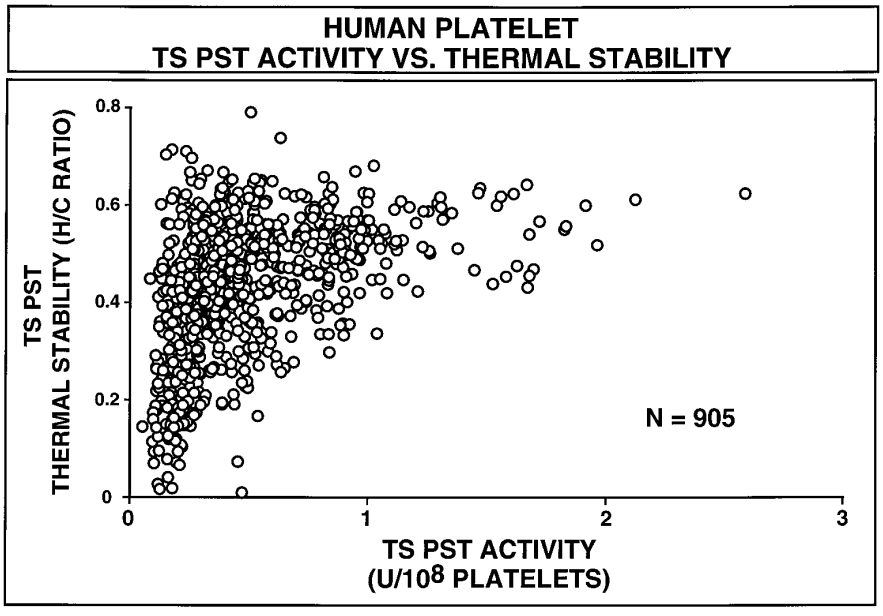

FIG. 1. Human platelet TS PST phenotype. The relationship between TS PST thermal stability (H/C ratio) and enzyme activity in 905 human platelet samples is shown.

Our most important functional observation involved nucleotide 638 within exon 7. That nucleotide was an "A" in ***SULT1A1*2*** and a "G" in all of the other alleles (Table 3). Therefore, His213 in ***SULT1A1*2*** was an Arg in the enzyme encoded by the other alleles. An Arg at this position is conserved among all known PSTs and ESTs. The most striking genotype-phenotype correlation observed in the 33 subjects selected on the basis of platelet TS PST phenotype was the fact that all 13 of the subjects with both low TS PST activity and low TS PST thermal stability were homozygous for the ****2*** allele (Fig. 3). Specifically, subjects homozygous for allele ****2*** uniformly had low thermal stability and low levels of enzyme activity as compared with subjects heterozygous for ****2*** or those homozygous for any of the alleles that encoded Arg213 (Fig. 3A and 3B). A statistical comparison of platelet TS PST thermal stability and level of activity in subjects homozygous for ****2,*** i.e., those homozygous for His213, and all other subjects is shown in Table 4. The relationship between ***SULT1A1*** genotype and level of platelet TS PST activity in subjects with high platelet TS PST thermal stability, i.e., subjects homozygous or heterozygous for any of the ****1*** alleles that encoded Arg213, was less clear (Fig. 3B). We were unable to detect a consistent relationship between ***SULT1A1*** genotype and level of activity in subjects with high thermal stability -- other than the fact that none of them were homozygous for the ****2*** allele that encoded His213. Only 11 of these 33 subjects (all with high thermal stability) did not carry the ****2*** allele (Fig. 3). Those subjects displayed both high and low levels of enzyme activity. However, 5 different ****1*** alleles were included within that group of 11 (Fig. 3B); so a meaningful statistical analysis of allele effect was not possible. In summary, these data demonstrated clearly that, in this selected population sample,

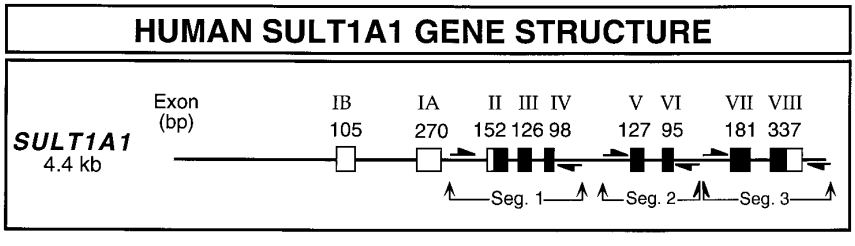

FIG. 2. ***SULT1A1*** gene structure and locations of the primers used to perform PCR amplifications. Black rectangles represent exons that encode cDNA ORF sequence, while open rectangles represent exon sequences that encode cDNA UTR sequence. Roman numerals are exon numbers, and Arabic numerals are exon lengths in bp. The gene length in kb from the initial to final exon is also indicated. Arrows show the locations of ***SULT1A1***-specific primers used to amplify the three ***SULT1A1*** segments that were sequenced to detect different alleles.

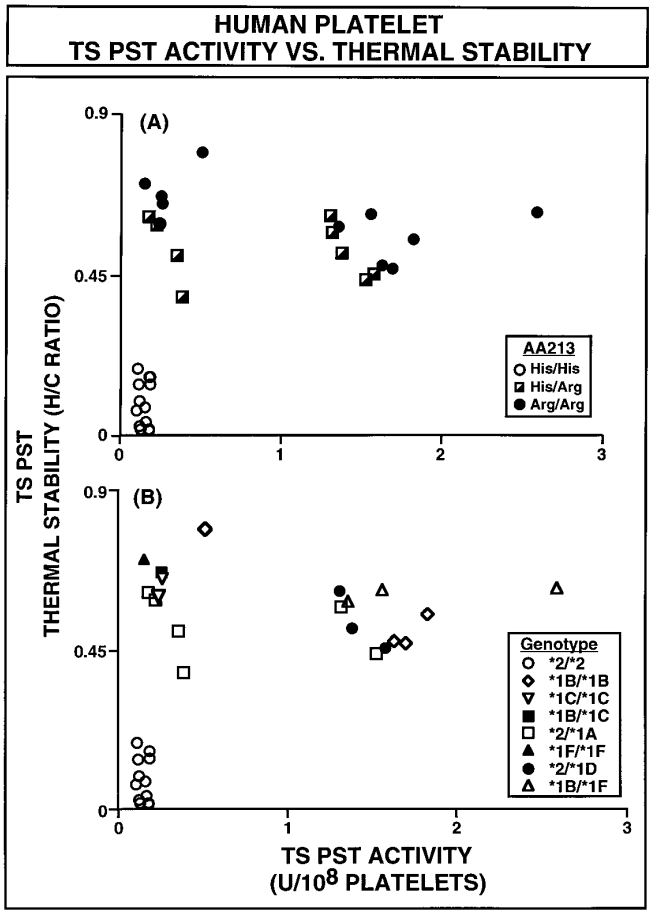

FIG. 3. ***SULT1A1*** genotype correlation with human platelet TS PST phenotype. (A) TS PST phenotypes in 33 of the platelet samples depicted in Fig. 1 are shown with the ***SULT1A1*** allozymes encoded in those subjects. (B) TS PST platelet phenotypes in the same 33 subjects depicted in (A) are shown with the ***SULT1A1*** genotype for that subject. None of the 33 subjects studied carried the ****1E, *1G, *1H, *1I, *3A, *3B*** or ****4*** alleles (Table 2).

subjects homozygous for the ***SULT1A1*2*** allele that encoded His213 uniformly had both low platelet TS PST activity and thermal stability. However, the relationship of ***SULT1A1*1*** alleles to the regulation of level of activity was less clear. The next step in our analysis involved determining allele frequencies for ***SULT1A1*** in a randomly selected population sample to determine how common each allele might be.

SULT1A1 allele frequencies were determined in DNA isolated from 150 randomly selected Caucasian blood donors by completely sequencing all exons and selected introns -- for a total of approximately 300 kb of DNA sequenced. The six alleles that we had identified in the 33 selected subjects were all detected in this population sample, as well as seven additional alleles (Table 3). Three of those seven additional alleles, ****3*** (both ****3A*** and ****3B***) and ****4,*** encoded additional allozymes (Table 2). In the ****3*** alleles, an A → G transition at nucleotide 667 resulted in a Met223 → Val alteration in amino acid sequence. The ****4*** allele encoded a G → A transition at nucleotide 110 resulting in an Arg37 → Gln change. Both the ****3*** and ****4*** alleles encoded Arg at amino acid position 213 (Table 2). Neither of the amino acid changes encoded by allele ****3*** or ****4*** were located within regions known to contain conserved amino acids in other SULT enzymes (3,4,34). Phenotypes associated with the relatively rare allozymes encoded by ****3*** and ****4*** will have to be assessed in the course of future experiments. Frequencies of all alleles identified in samples from the 150 Caucasian blood donors are listed in Table 3. Both the ****3B*** and ****4*** alleles were observed only once. The frequency of allele ****2,*** the allele associated with low TS PST activity and low thermal stability, was 0.31, in remarkable agreement with a value of 0.32 for the phenotypic trait of thermolabile platelet TS PST, a value calculated on the basis of segregation analysis and large population studies (13,15).

DISCUSSION

The PSTs catalyze the sulfate conjugation of many drugs and xenobiotic compounds (3). Previous biochemical pharmacogenetic studies of TS PST in human platelets demonstrated large differences among individuals in both levels of enzyme activity and thermal stability, differences that resulted from common genetic polymorphisms (13,15). The present experiments provide a molecular explanation for inherited variation in TS PST thermal stability, variation that is associated with significant differences in level of enzyme activity in the blood platelet and other human tissues (17-19). We identified thirteen ***SULT1A1*** alleles encoding four allozymes and determined that the ****2*** allele was uniformly associated with decreased level of platelet TS PST activity and thermal stability (Fig. 3, Table 4). Those observations are compatible with the results of segregation analysis of family data for human platelet TS PST which indicated that two alleles could explain inherited differences in platelet TS PST thermal stability (13,15). In those human platelet biochemical

TABLE 2

Human SULT1A1 Allozymes

	Amino acid		
Allozyme	37	213	223
*1	Arg	Arg	Met
*2	Arg	His	Met
*3	Arg	Arg	Val
*4	Gln	Arg	Met

Note. Differences in the amino acid sequences of the four allozymes encoded by SULT1A1*1, *2, *3, and *4 are listed.

TABLE 3

SULT1A1 Allele Nucleotide Sequences

		Exon III		Exon IV												Exon VII							Exon VIII		
Allele	I1A-138	57	*110	153	162	It-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	Allele frequency
*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	0.303
*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	0.237
*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	0.040
*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	0.027
*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	0.020
*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	0.017
*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	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	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	0.007
*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	0.313
*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	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	0.003
*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	0.003

Note. Numbers at the top indicate the nucleotide within the open reading frame, 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, 5′ to 3′, within the 5th intron). Nucleotide numbers preceded by an asterisk indicate polymorphic nucleotides which alter the encoded amino acid (see Table 2). Those nucleotides are shown as white type against a black background. Nucleotides 902 and 973 lie within the 3′-untranslated region of the SULT1A1 mRNA. The values for allele frequencies shown in the right hand column were determined by genotyping DNA from 150 randomly selected Caucasian blood donors.

pharmacogenetic studies, frequencies of the alleles responsible for variation in TS PST thermal stability were 0.32 for the trait of thermolabile TS PST, and 0.68 for thermostable TS PST (13,15). Those frequencies are in striking agreement with the allele frequencies that we obtained by genotyping ***SULT1A1*** in a large randomly selected population sample.

These nucleotide polymorphisms also provided a molecular explanation for a portion of the interindividual variation in TS PST activity, since subjects homozygous for the ****2*** allele consistently displayed low levels of enzyme activity (Fig. 3). However, the various ****1*** alleles detected in this group of 33 subjects were associated with both high and low levels of activity. These observations are also consistent with predictions made on the basis of segregation analysis of biochemical genetic data in which a multiple allele system was proposed as a possible explanation for inherited variation in level of platelet TS PST activity, but only two alleles were required to explain differences in the trait of thermal stability (15). Obviously, additional polymorphisms within regions of the ***SULT1A1*** gene other than the ORF -- e.g., within the promoter or introns -- as well as variation in the regulation of transcription or the occurrence of events such as gene duplication, could also contribute to differences among individuals in levels of TS PST activity. All of those possibilities will have to be explored in the course of future experiments. Finally, the fact that there are two isoforms of TS PST, SULT1A1 and SULT1A2, raises the issue of the relative contributions of these two isoforms to "TS PST phenotype", i.e., enzyme thermal stability and activity measured with the prototypic TS PST substrate, 4-nitrophenol, at a concentration of 4 μM. On the basis of relative apparent K_m values, SULT1A2 is unlikely to be a major contributor to this phenotype since the apparent K_m value of SULT1A2 for 4-nitrophenol is one to two orders of magnitude higher than that of the protein(s) encoded by ***SULT1A1*** (28,29). However, if it were highly expressed, SULT1A2 might contribute to this biochemical phenotype.

TABLE 4

SULT1A1 Allozymes and Human Platelet TS PST Phenotype

Genotype	Amino acid 213	N	H/C ratio	TS PST activity
*1/*1	Arg/Arg	11	$0.62 \pm 0.03^{+}$	1.08 ± 0.25
*1/*2	Arg/His	9	$0.53 \pm 0.03^{+}$	0.90 ± 0.20
*2/*2	His/His	13	$0.09 \pm 0.02^{+}$	$0.14 \pm 0.01^{+}$

Note. Relationship of SULT1A1 alleles to human platelet TS PST phenotype for thermal stability and level of activity. Each value is the mean ± SEM for the 33 selected subjects shown in Fig. 3. TS PST activity is expressed as $U/10^8$ platelets. The ($^{+}$) denotes $p < 0.02$ by ANOVA when compared with values for the other two groups.

In summary, we have identified common nucleotide polymorphisms within the human SULT1A1 gene that encode 4 variant proteins. One of those allozymes, that encoded by allele ****2,*** is consistently associated with both low TS PST activity and low thermal stability. The SULT enzymes play an important role in the biotransformation of many drugs and xenobiotic compounds. Clearly, many additional studies will be required to fully elucidate the molecular genetic regulation of PST activity in humans and to determine the possible clinical consequences of variation in "sulfator

status". However, the ***SULT1A1*** genetic polymorphisms that we have described provide a molecular explanation for inherited variation in the thermal stability of TS PST in humans, and they also explain a portion of the variation among individuals in the level of TS PST activity -- an important step toward the goal of making it possible to predict sulfator status in humans.

ACKNOWLEDGMENTS

We thank Luanne Wussow for her assistance in the preparation of this manuscript. We also thank the many subjects who participated in these experiments.

REFERENCES

1. Kalow, W. (1992) *in* Pharmacogenetics of Drug Metabolism. (Kalow, W., Ed.), pp. 865–878, Pergamon Press, New York.
2. Price Evans, D. A. (1993) *Genetic Factors in Drug Therapy: Clinical and Molecular Pharmacogenetics.* Cambridge University Press, New York.
3. Weinshilboum, R. M., and Otterness, D. M. (1994) *in* Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity (Kauffman, F. C., Ed.), Chap. 22, "Handbook of Experimental Pharmacology" series, Vol. 112, pp. 45–78, Springer-Verlag, Berlin Heidelberg.
4. Weinshilboum, R. M., Otterness, D. M., Aksoy, I. A., Wood, T. C., Her, C., and Raftogianis, R. B. (1997) *FASEB J.* **11,** 3–14.
5. Her, C., Kaur, G. P., Athwal, R. S., and Weinshilboum, R. M. (1997) *Genomics* **41,** 467–470.
6. Rein, G., Glover, V., and Sandler, M. (1982) *in* Phenolsulfotransferase in Mental Health Research. (Sandler, M., and Usdin, E., Eds.), pp. 98–126, MacMillan Publ Ltd, London.
7. Reiter, C., Mwaluko, G., Dunnette, J., Van Loon, J., and Weinshilboum, R. (1983) *Naunyn-Schmied. Archiv. Pharmacol.* **324,** 140–147.
8. Weinshilboum, R. M. (1986) *Fed. Proc.* **45,** 2223–2228.
9. Sundaram, R. S., Szumlanski, C., Otterness, D., Van Loon, J. A., and Weinshilboum, R. M. (1989) *Drug Met. Dispos.* **17,** 255–264.
10. Campbell, N. R. C., Van Loon, J. A., and Weinshilboum, R. M. (1987) *Biochem. Pharmacol.* **36,** 1435–1446.
11. Young, W. F. Jr., Okazaki, H., Laws, E. R. Jr., and Weinshilboum, R. M. (1984) *J. Neurochem.* **43,** 706–715.
12. Hart, R. F., Renskers, K. J., Nelson, E. B., and Roth, J. A. (1979) *Life Sci.* **24,** 125–130.
13. Van Loon, J. A., and Weinshilboum, R. M. (1984) *Biochem. Genet.* **22,** 997–1014.
14. Price, R. A., Cox, N. J., Spielman, R. S., Van Loon, J., Maidak, B. L., and Weinshilboum, R. M. (1988) *Genet. Epidemiol.* **5,** 1–15.
15. Price, R. A., Spielman, R. S., Lucena, A. L., Van Loon, J. A., Maidak, B. L., and Weinshilboum, R. M. (1989) *Genetics* **122,** 905–914.
16. Weinshilboum, R. M. (1981) *in* Genetic Strategies in Psychobiology and Psychiatry. (Gershon, E. S., Matthysse, S., Breakefield, X. O., and Ciaranello, R. D., Eds.), pp. 79–94, Boxwood Press, Pacific Grove, CA.
17. Young, W. F. Jr., Laws, E. R. Jr., Sharbrough, F. W., and Weinshilboum, R. M. (1985) *J. Neurochem.* **44,** 1131–1137.
18. Campbell, N. R. C., and Weinshilboum, R. (1986) *Can. Soc. Clin. Invest.* **9**(Suppl), A14.
19. Sundaram, R. S., Van Loon, J. A., Tucker, R., and Weinshilboum, R. M. (1989) *Clin. Pharmacol. Ther.* **46,** 501–509.
20. Aksoy, I. A., and Weinshilboum, R. M. (1995) *Biochem. Biophys. Res. Commun.* **208,** 786–795.
21. Bernier, F., Leblanc, G., Labrie, F., and Luu-The, V. (1994) *J. Biol. Chem.* **269,** 28200–28205.
22. Her, C., Raftogianis, R., and Weinshilboum, R. M. (1996) *Genomics* **33,** 409–420.
23. Dooley, T. P., Obermoeller, R. D., Leiter, E. H., Chapman, H. D., Falany, C. N., Deng, Z., and Siciliano, M. J. (1993) *Genomics* **18,** 440–443.
24. Dooley, T. P., Probst, P., Monroe, P. B., Mole, S. E., Liu, Z., and Doggett, N. A. (1994) *Biochem. Biophys. Res. Commun.* **205,** 1325–1332.
25. Raftogianis R., Her C., and Weinshilboum R. M. (1996) *Pharmacogenetics* **6,** 473–487.
26. Dooley, T. P., Mitchison, H. M., Munroe, P. B., Probst, P., Neal, M., Siciliano, M. J., Deng, Z., Doggett, N. A., Callen, D. F., Gardiner, R. M., and Mole, S. E. (1994) *Biochem. Biophys. Res. Commun.* **205,** 482–489.
27. Dooley, T. P., and Huang, Z. (1996) *Biochem. Biophys. Res. Commun.* **228,** 134–140.
28. Ozawa, S. H., Nagata, K., Shimada, M., Ueda, M., Tsuzuki, T., Yamazoe, Y., and Kato, R. (1995) *Pharmacogenetics* **5,** S135–S140.
29. Zhu, X., Veronese, M. E., Iocco, P., and McManus, M. E. (1996) *Int. J. Biochem. Cell Biol.* **28,** 565–571.
30. Foldes, A., and Meek, J. L. (1973) *Biochim. Biophys. Acta* **327,** 365–374.
31. Anderson, R. J., and Weinshilboum, R. M. (1980) *Clin. Chim. Acta* **103,** 79–90.
32. Reiter, C., and Weinshilboum, R. (1982) *Clin. Pharmacol. Ther.* **32,** 612–621.
33. Nikiforov, T. T., Rendle, R. B., Kotewicz, M. L., and Rogers, Y.-H. (1994) *PRC Methods & Applicat.* **3,** 285–291.
34. Varin, L., DeLuca, V., Ibrahim, R. K., and Brisson, N. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 1286–1290.
35. Wilborn, T. W., Comer, K. A., Dooley, T. P., Reardon, I. M., Heinrikson, R. L., and Falany, C. N. (1993) *Mol. Pharmacol.* **43,** 70–77.
36. Zhu, X., Veronese, M. E., Sansom, L. N., and McManus, M. E. (1993) *Biochem. Biophys. Res. Commun.* **192,** 671–676.
37. Zhu, X., Veronese, M. E., Bernard, C. C. A., Sansom, L. N., and McManus, M. E. (1993) *Biochem. Biophys. Res. Commun.* **195,** 120–127.
38. Wood, T. C., Aksoy, I. A., Aksoy, S., and Weinshilboum, R. M. (1994) *Biochem. Biophys. Res. Commun.* **198,** 1119–1127.
39. Ganguly, T. C., Krasnykh, V., and Falany, C. N. (1995) *Drug Met. Dispos.* **23,** 945–950.
40. Jones, A. L., Hagen, M., Coughtrie, M. W. H., Roberts, R. C., and Glatt, H. (1995) *Biochem. Biophys. Res. Commun.* **208,** 855–862.