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# On the role of genetic polymorphisms in the sulfation of cholesterol by human cytosolic sulphotransferase SULT2B1b

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**Sulphated cholesterol, like its unsulphated counterpart, is known to be biologically active and serves a myriad of biochemical/physiological functions. Of the 13 human cytosolic sulphotransferases (SULTs), SULT2B1b has been reported as the main enzyme responsible for the sulphation of cholesterol. As such, SULT2B1b may play the role as a key regulator of cholesterol metabolism. Variations in the sulphating activity of SULT2B1b may affect the sulphation of cholesterol and, consequently, the related physiological events. This study was designed to evaluate the impact of the genetic polymorphisms on the sulphation of cholesterol by SULT2B1b. Ten recombinant SULT2B1b allozymes were generated, expressed, and purified. Purified SULT2B1b allozymes were shown to display differential cholesterol-sulphating activities, compared with the wild-type enzyme. Kinetic studies revealed further their distinct substrate affinity and catalytic efficiency toward cholesterol. These findings showed clearly the impact of genetic polymorphisms on the cholesterol-sulphating activity of SULT2B1b allozymes, which may underscore the differential metabolism of cholesterol in individuals with different *SULT2B1b* genotypes.**

*Keywords*: cytosolic sulphotransferase; cholesterol; SULT; SULT2B1b; sulphation.

*Abbreviations*: ANOVA, analysis of variance; ATP, adenosine 5′-triphosphate; cSNP, coding SNP; DHEA, dehydroepiandrosterone; DTT, dithiothreitol; HEPES, N-2-hydroxylpiperazine-N′-2-ethanesulfonic acid; PAPS, 3'-phosphoadenosine 5'-phosphosulphate; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SULT, cytosolic sulphotransferase; TLC, thin-layer chromatography; UniProt, Universal Protein Resource; UTR, untranslated region.

Cholesterol is known to be involved in a variety of critical physiological functions such as the biosynthesis of steroid hormones and the maintenance of cell membrane integrity (*1*, *2*). Studies have demonstrated the presence of sulfoconjugated form of cholesterol, cholesterol sulphate, in human tissues and body fluids, with plasma concentrations ranging 253–690 μM (*3–6*). Importantly, cholesterol sulphate has been shown to be biologically active, being involved in the increase of cell membrane stability, the regulation of the activity of serine proteases involved in blood clotting, the support for platelet adhesion, and the regulation of cholesterol synthesis (*3*). Studies have shown that cholesterol sulphate is able to inhibit cholesterol synthesis by inhibiting 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, the rate limiting enzyme in cholesterol synthesis pathway (*7*, *8*). Moreover, cholesterol sulphate appears to play a role in keratinocyte differentiation. Studies have shown that the ratio of cholesterol sulphate to cholesterol is much higher in the skin (1:10–1:5) than in the blood (1:500) (*3*, *9*). Like cholesterol, cholesterol sulphate can also be used as a biosynthetic precursor for steroid hormones without prior removal of sulphate moiety, particularly in foetal adrenal mitochondria (*10*, *11*). Moreover, cholesterol sulphate has been reported to play a regulatory role in steroidogenesis by regulating the rate of conversion of cholesterol to pregnenolone (*10*). It is noted that cholesterol sulphate deficiency has been reported to be associated with pathological conditions such as autosomal recessive congenital ichthyosis and atherosclerosis (*12*, *13*).

Sulphation, a major conjugation reaction in humans and other mammals, is involved in the homeostasis of key endogenous compounds and the inactivation and removal of xenobiotics (*14*). Sulphation occurs under the action of the cytosolic sulphotransferase (SULT) enzymes that catalyse the transfer of a sulphonate group from the sulphonate donor, 3′-phosphoadenosine 5′-phosphosulphate (PAPS), to the hydroxyl or amino group of the substrate compound (*15*, *16*). Of the known SULT enzymes, members of the SULT2 family have been shown to be responsible for the sulphation of steroids and sterols (*17–19*). In humans, the SULT2 family consists of three members, SULT2Al, SULT2Bla, and SULT2Blb (*20*, *21*). Interestingly, SULT2Bla and SULT2B1b have been shown to be coded by the same gene (designated *SULT2B1*) and are generated as a result of alternative transcription initiation and alternative splicing (*22*). Consequently, SULT2Bla and SULT2B1b differ only in their N-terminal regions, with 8- and 23-amino acid extensions, respectively (*23*). SULT2A1 is commonly known as the dehydroepiandrosterone

(DHEA) SULT; whereas SULT2Bla and SULT2B1b are known as pregnenolone and cholesterol SULT, respectively (*20*, *21*). SULT2B1b mRNA was first reported to be present in human placenta, prostate, trachea, lung and small intestine tissues, and has since been shown to be also expressed in the skin, ovary, uterus, brain, liver, colon and platelet (*22*, *24–26*). The genetic polymorphisms of the *SULT2B1* gene have been reported in (*27–33*). An important question is whether the genetic polymorphisms of the gene encoding SULT2Blb may influence its cholesterol-sulphating activity and thus the multitude of physiological functions related to unsulphated and sulphated cholesterol in different individuals.

In this study, we performed a systematic search of *SULT2B1* single nucleotide polymorphisms (SNPs) deposited in several SNP databases. Ten SULT2B1b allozymes coded by missense SNPs, selected based on predicted importance of the amino acid variations, were generated, expressed and purified. The sulphating activity of the purified SULT2B1b allozymes toward cholesterol was examined. Kinetic studies were performed to analyse their differential substrate affinity and catalytic efficiency with cholesterol as a substrate.

## Materials and Methods

### Materials

Cholesterol, hydroxypropyl-β-cyclodextrin, adenosine 5′-triphosphate (ATP), N-2-hydroxylpiperazine-N′-2-ethanesulfonic acid (HEPES), dimethyl sulfoxide, Trizma base, and dithiothreitol (DTT) were products of Sigma-Aldrich (St Louis, MO, USA). Silica gel thin-layer chromatography (TLC) plates and Ultrafree-MC 5000 NMWL filter units were from EMD Millipore (Billerica, MA, USA). Carrier-free sodium [$^{35}$S]sulphate was from American Radiolabeled Chemicals (St Louis, MO, USA). Ecolume scintillation cocktail was purchased from MP Biomedicals, LLC. (Irvine, CA, USA). Recombinant human bifunctional ATP sulfurylase/adenosine 5′-phosphosulfate kinase was prepared as previously described in (*34*). PrimeSTAR Max DNA polymerase was a product of Takara Bio (Mountain View, CA, USA). Protein molecular weight markers were from Bioland Scientific LLC. (Paramount, CA, USA). Oligonucleotide primers were synthesized by Eurofins Genomics (Louisville, KY, USA). PAP[$^{35}$S] was synthesized using recombinant human bifunctional PAPS synthase as described previously in (*34*). All other chemicals were of the highest grade commercially available.

### Identification and analysis of human SULT2B1 SNPs

Since SULT2B1b is coded by the *SULT2B1* gene, three online databases, located at the websites of US National Centre for Biotechnology Information (NCBI), the Ensembl Variation database, and the Universal Protein Resource (UniProt), were systematically searched using the keyword 'human SULT2B1'. The human *SULT2B1* SNPs identified were analysed and categorized based on the locations of the nucleotide variations in the region specifically encoding SULT2B1b.

### Generation, expression and purification of SULT2B1b allozymes

PrimeSTARMax DNA polymerase was used to generate cDNAs encoding SULT2B1b allozymes via PCR. Briefly, the wild-type SULT2B1b cDNA packaged in pGEX-4T-2 prokaryotic expression vector was used as a template in conjunction with specific mutagenic primers (see for the mutagenic primer sets). The PCR amplification conditions were 12 cycles of 30 s at 95°C, 1 min at 55°C, and 6 min at 72°C. At the end of PCR, the reaction mixtures were treated with *Dpn* I to degrade the wild-type SULT2B1b cDNA/pGEX-4T-2. The 'mutated' SULT2B1b cDNA/pGEX-4T-2 plasmids present in *Dpn* I-treated reaction mixtures were individually introduced to competent DH5α *Escherichia coli* cells for the amplification and purification of the plasmids. Specific 'mutations' in individual SULT2B1b cDNA/pGEX-4T-2 plasmids prepared were verified by nucleotide sequencing. pGEX-4T-2 vector harbouring individual 'mutated' SULT2B1b cDNAs were introduced to competent BL21 *E. coli* cells for the expression of recombinant SULT2B1b allozymes. Transformed cells were grown in 1 l of LB medium containing 100 μg/ml ampicillin to $A_{600\ nm} = \sim 0.5$, and then induced with 0.1 mM IPTG overnight at 25°C. The IPTG-treated cells collected by centrifugation and resuspended in 20 ml aliquots of an ice-cold lysis buffer (10 mM Tris-HCl, pH 8.0, 150 mm NaCl and 1 mM ethylenediaminetetraacetic acid). The resuspended cells were homogenized using an Aminco French press. The crude homogenates were subjected to centrifugation at 10,000 × g for 20 min at 4°C, and the supernatants collected were individually fractionated using 1 ml aliquots of glutathione-Sepharose. For each recombinant SULT2B1b allozyme clone, the bound protein fusion was treated with 2 ml of a thrombin digestion buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl and 2.5 mM $CaCl_2$) containing 3.5 U/ml bovine thrombin. The preparation was incubated for 20 min at room temperature with constant agitation. Afterwards, the preparation was subjected to centrifugation. The recombinant SULT2B1b allozyme present in the supernatant was collected and analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE).

### Sulphotransferase assay

The sulphating activity of the recombinant SULT2B1b allozymes was assayed using PAP[$^{35}$S] as the sulphate donor. The standard assay mixture, with a final volume of 20 μl, consists of 0.5 μg of a SULT2B1b allozyme, 50 mM HEPES buffer (pH 7.4), 1 mM DTT, 14 μM PAP[$^{35}$S], and cholesterol dissolved in hydroxypropyl-β-cyclodextrin. A control with hydroxypropyl-β-cyclodextrin alone was assayed in parallel. The reaction mixture was incubated at 37°C for 10 min and then terminated by heating at 100°C for 3 min. The analysis of the [$^{35}$S]sulphated cholesterol was carried out by spotting 4 μl of the final reaction mixture on a silica gel TLC plate. The spotted TLC plate was subjected to TLC analysis using a solvent system containing acetic acid: n-butanol in a ratio of 2:1 (by volume). The [$^{35}$S]sulphated cholesterol spot identified by autoradiography was cut from the TLC plate and eluted with 0.5 ml water. Afterwards, the [$^{35}$S]radioactivity was counted using a liquid scintillation counter as previously described in (*35*). For the kinetic studies on the sulphation of cholesterol by individual SULT2B1b allozymes, varying concentrations of cholesterol ranging 0.5–50 μM were used with 50 mM HEPES (pH 7.4), according to the assay procedure described earlier. To determine the $k_m$ of SULT2B1b allozymes for PAPS, varying concentrations of PAPS ranging 0.1–50 μM were used with 50 mM HEPES (pH 7.4), according to the assay say procedure described earlier.

### Statistical analysis

To calculate the kinetic constants, data were processed based on Michaelis-Menten kinetics using non-linear regression curve generated by GraphPad Prism v 6.0 software. One-way analysis of variance (ANOVA) was used for inter-group comparison followed by Dunnett's test to calculate statistical differences between SULT2B1b-wt (SULT2B1b wild-type) and individual allozymes. $P$-values $< 0.05$ were considered statistically significant.

## Results and Discussion

As a first step toward clarifying the mechanisms underlying the phenotypic consequences of *SULT2B1* genetic polymorphisms, this study was carried out to examine the differential cholesterol-sulphating activity of SULT2B1b allozymes resulting from missense coding SNPs (cSNPs) of the *SULT2B1* gene.

### Identification and categorization of SNPs of human SULT2B1 gene

A systematic search of three databases, including the NCBI SNP database, the Ensembl Variation database, and the UniProt database, yielded a total of 6, 393 *SULT2B1* SNPs, which were grouped into coding (synonymous, non-synonymous (missense) and non-sense) SNPs and non-coding (introns, 5'-untranslated region

**Table I. Primer sets used in the site-directed mutagenesis of the cDNA-encoding human SULT2B1b Allozymes**

| SULT2B1b allozyme and corresponding amino acid substitution | MAF[a] | Mutagenic primer set |
|---|---|---|
| SULT2B1b-Pro69Ala | 0.000008 | 5'-ATCTTTATCATCACCTACGCCAAGTCAGGCACGACC-3'<br>5'-GGTCGTGCCTGACTTGGCGTAGGTGATGATAAAGAT-3' |
| SULT2B1b-Gly72Val | 0.000009 | 5'-ATCACCTACCCCAAGTCAGTCACGACCTGGATGATC-3'<br>5'-GATCATCCAGGTCGTGACTGACTTGGGGTAGGTGAT-3' |
| SULT2B1b-Thr73Met | 0.00002 | 5'-ACCTACCCCAAGTCAGGCATGACCTGGATGATCGAG-3'<br>5'-CTCGATCATCCAGGTCATGCCTGACTTGGGGTAGGT-3' |
| SULT2B1b-Arg147His | 0.000008 | 5'-AAGGTGATCTACATGGGCCACAACCCCCGGGACGTT-3'<br>5'-AACGTCCCGGGGGTTGTGGCCCATGTAGATCACCTT-3' |
| SULT2B1b-Asp191Asn | 0.008 AA | 5'-CAGTTTGGCTCCTGGTTCAACCACATTAAGGGCTGG-3'<br>5'-CCAGCCCTTAATGTGGTTGAACCAGGAGCCAAACTG-3' |
| SULT2B1b-Arg230His | 0.008 AA | 5'-ATCTGTGGGTTCCTGGGCCATCCGCTGGGCAAGGAG-3'<br>5'-CTCCTTGCCCAGCGGATGGCCCAGGAACCCACAGAT-3' |
| SULT2B1b-Ser244Thr | 0.0001 | 5'-GGCTCCGTCGTGGCACACACAACCTTCAGCGCCATG-3'<br>5'-CATGGCGCTGAAGGTTGTGTGTGCCACGACGGAGCC-3' |
| SULT2B1b-Arg274Gln | 0.00002 | 5'-CGTCGCGGGGCCTTCCTCCAGAAAGGGGTCTGCGGC-3'<br>5-GCCGCAGACCCCTTTCTGGAGGAAGGCCCCGCGACG-3' |
| SULT2B1b-Gly276Val | NA | 5'-GGGGCCTTCCTCCGGAAAGTGGTCTGCGGCGACTGG-3'<br>5'-CCAGTCGCCGCAGACCACTTTCCGGAGGAAGGCCCC-3' |
| SULT2B1b-Pro345Leu | 0.025 CA | 5'-CTGGAGCGTGAGCCCAGACTCAACTCCAGCCCCAGC-3'<br>5'-GCTGGGGCTGGAGTTGAGTCTGGGCTCACGCTCCAG-3' |

[a]Minor allele frequency.
AA, African American population; CA, Caucasian American population; NA, not available.

(5'UTR), and 3'UTR) SNPs. Of the 3, 370 *SULT2B1* SNPs, 168 were found to be *SULT2B1b* missense cSNPs that resulted in amino acid changes in the protein products. These *SULT2B1b* cSNPs were further scrutinized for the location (in the substrate binding site, PAPS-binding sites, and dimerization motif) of the amino acid changes, as well as the chemical nature of the amino acid changes (acidic to/or from basic, charged to/from uncharged, and turn-inducing to/from non-turn-inducing residues) (*23*, *36*, *37*). Based on these criteria, ten missense SULT2B1b cSNPs were selected for further studies as described below. Table I shows the amino acid changes and the locations, as well as the sense and antisense mutagenic primers designed for use in the PCR-amplification of the corresponding SULT2B1b cDNAs.

### *Effects of* SULT2B1 *genetic polymorphism on the cholesterol-sulphating activity of SULT2B1b allozymes*

cDNAs encoding different SULT2B1b allozymes packaged in pGEX-4T-2 prokaryotic expression vector, generated as described in the Materials and Methods section , were individually introduced to BL21 *E. coli* cells for expressing the recombinant enzymes. The recombinant SULT2B1b allozymes were fractionated from the *E. coli* cell homogenates using glutathione-Sepharose, followed by thrombin digestion to release the untagged recombinant SULT2B1b allozymes. Recombinant SULT2B1b allozymes thus prepared were analysed by SDS-polyacrylamide gel electrophoresis and found to be highly homogeneous (Fig. 1). The apparent molecular weights of the ten SULT2B1b allozymes were similar to the predicted molecular weight (41, 307) of the wild-type SULT2B1b (Lane 2 in Fig. 1).

### *Characterization of the cholesterol-sulphating activity of human SULT2B1b allozymes*

The sulphating activity of purified SULT2B1b allozymes was analysed using cholesterol as a substrate. As shown in Table II, 3 of the 10 SULT2B1b allozymes, SULT2B1b-Gly72Val, SULT2B1b-Arg147His and SULT2B1b-Gly276Val, showed no detectable activity, while the other seven SULT2B1b allozymes exhibited differential sulphating activity toward cholesterol. It was noted that while four (SULT2B1b-Asp191Asn, SULT2B1b-Arg230His, SULT2B1b-Ser244Thr and SULT2B1b-Pro345Leu) of these seven allozymes displayed a considerable (2-fold or greater) decrease in cholesterol-sulphating activity, the other three (SULT2B1b-Pro69Ala, SULT2B1b-Thr73Met and SULT2B1b-Arg274Gln) exhibited a much greater (more than 20-fold) decrease in cholesterol-sulphating activity compared with the wild-type enzyme (SULT2B1b-wt). Among them, SULT2B1b-Arg274Gln showed the lowest cholesterol-sulphating activity (0.04 nmol/min/mg) which is only 0.50% of the SULT2B1b-wt. Among the SULT2B1b allozymes tested, SULT2B1b-Arg274Gln had previously implicated in autosomal recessive congenital ichthyosis caused by elevated cholesterol level and absence of cholesterol sulphate in the skin (*12*). It should be pointed out also that the sulphating activity of SULT2B1b-Asp191Asn, SULT2B1b-Arg230His and SULT2B1b-Pro345Leu are considerably lower than the sulphating activity of the same allozymes previously characterized using DHEA as a substrate (*33*). This discrepancy could be due to the use of different substrates (DHEA versus cholesterol) and/or the use of different enzyme preparation (enzymes expressed in monkey kidney cells (COS-1) cells versus purified recombinant enzymes) (*33*).

To investigate further the effects of genetic polymorphisms on the cholesterol-sulphating activity of SULT2B1b

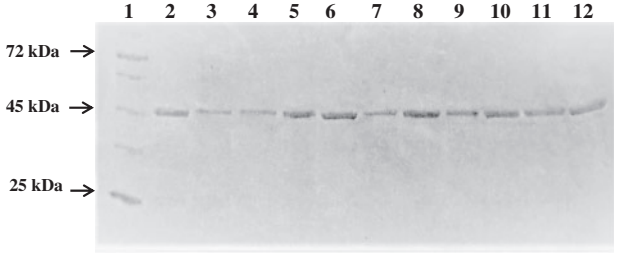


**Fig. 1 SDS gel electrophoretic pattern of the purified SULT2B1b allozymes.** SDS-PAGE was performed on a 12% gel, followed by Coomassie blue staining. Lane 1 indicates the migrating positions of protein molecular weight markers co-electrophoresed. Samples analysed in Lanes 2 through 12 correspond to SULT2B1b-wt, SULT2B1bPro69Ala, SULT2B1b-Gly72Val, SULT2B1b-Thr73Met, SULT2B1b-Arg147His, SULT2B1b-Asp191Asn, SULT2B1b-Arg230His, SULT2B1b-Ser244Thr, SULT2B1b-Arg274Gln, SULT2B1b-Gly276Val and SULT2B1b-Pro345Leu.

**Table II. Specific activities of the human SULT2B1b allozymes with cholesterol as substrate**

| Enzyme | Specific activity (nmol/min/mg) | Relative activity (% of wild type) |
|---|---|---|
| SULT2B1b-wt | 8.03 ± 0.2 | 100% |
| SULT2B1b-Pro69Ala | 0.36 ± 0.01*** | 4.48% |
| SULT2B1b-Gly72Val | N.D. | N.D. |
| SULT2B1b-Thr73Met | 0.28 ± 0.03*** | 3.49% |
| SULT2B1b-Arg147His | N.D. | N.D. |
| SULT2B1b-Asp191Asn | 4.38 ± 0.04*** | 54.55% |
| SULT2B1b-Arg230His | 3.94 ± 0.36*** | 49.07% |
| SULT2B1b-Ser244Thr | 2.32 ± 0.18*** | 28.89% |
| SULT2B1b-Arg274Gln | 0.04 ± 0.01*** | 0.50% |
| SULT2B1b-Gly276Val | N.D. | N.D. |
| SULT2B1b-Pro345Leu | 2.99 ± 0.06*** | 37.24% |

Concentration of cholesterol used in the enzymatic assay was 50 μM. Results shown represent mean ± SD derived from three independent analyses. N.D. refers to no detected activity. ***Statistical significance from SULT2B1b-wt ($P$-value $< 0.0001$) using one-way ANOVA followed by Dunnett's *post hoc* analysis.

allozymes, kinetic experiments were performed using varying concentrations (ranging from 0.5 to 50 μM) of cholesterol as a substrate and HEPES buffer at pH 7.4. As shown in Fig. 2, the sulphation of cholesterol appeared to follow the Michaelis-Menten kinetics. Table III shows the kinetic constants, $K_m$, $V_{max}$, $K_{cat}$ and $K_{cat}/K_m$, determined for the wild-type and SULT2B1b allozymes. When compared with SULT2B1b-wt, the $K_m$ values were found to be at least 3 times higher for most of the variants (SULT2B1b-Pro69Ala, SULT2B1b-Thr73Met, SULT2B1b-Arg230His, SULT2B1b-Ser244Thr and SULT2B1b-Pro345Leu), indicating that these amino acid changes may have led to a decrease in cholesterol binding affinity. All SULT2B1b allozymes examined displayed lower $V_{max}$ compared with that of the wild-type enzyme. Three allozymes (SULT2B1b-Asp191Asn, SULT2B1b-Ser244Thr and SULT2B1b-Pro345Leu) exhibited a more than 33% decrease in $V_{max}$, while the other two (SULT2B1b-Pro69Ala and SULT2B1b-Thr73Met) displayed a >90% reduction in $V_{max}$ compared with the wild-type. Consequently, the catalytic efficiency as reflected by calculated $k_{cat}/k_m$ was lower for all SULT2B1b allozymes analysed. Four of them (SULT2B1b-Asp191Asn, SULT2B1b-Arg230His, SULT2B1b-Ser244Thr and SULT2B1b-Pro345Leu) showed a >45% decrease than the wild-type (SUL T2B1b-wt), while two (SULT2B1b-Pro69Ala and SUL T2B1b-Thr73Met) showed a much more dramatic decrease (>95%) compared with the wild-type.

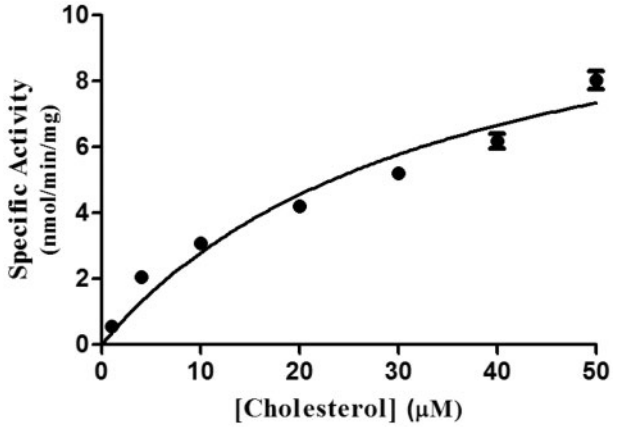


**Fig. 2 Kinetic analysis of the sulphation of cholesterol by wild-type SULT2B1b.** The figure shows the saturation curve analysis of the sulphation of cholesterol. The fitting curve was generated based on Michaelis-Menten kinetics. Data shown represent calculated mean ± SD derived from three experiments.

To investigate the effects of genetic polymorphisms on the PAPS-binding affinity, the $k_m$ values of SULT2B1b allozymes for PAPS were determined using varying concentrations (ranging 0.1–50 μM) of PAPS in the assays. As shown in Table III, compared with the wild-type SULT2B1b, three of the six allozymes analysed showed significant changes in the $k_m$ for PAPS. Of the three, two (SULT2B1b-Pro69Ala and SULT2B1b-Thr73Met) showed, respectively, 1.8- and 2.5-fold increase, whereas the other allozyme (SULT2B1b-Asp191Asn) showed a 2-fold decrease in $k_m$ for PAPS, compared with SULT2B1b-wt.

The crystal structure of human SULT2B1b has been solved (*23*). In view of the reported crystal structure, the above-mentioned results are not surprising since most of the SULT2B1b allozymes examined have amino acid changes either within or close to the PAPS-binding pocket (Fig. 3) (*23*). Moreover, most of the amino acid changes in these allozymes were dramatic from the biochemistry point of view, with potential for affecting substrate affinity or catalytic activity (*38*). For example, the change from proline (a turn-inducing amino acid residue) to alanine (a non-turn-inducing residue) in SULT2B1b-Pro69Ala could be the reason for the significant increase in the $K_m$ for PAPS, indicating a lowered affinity toward the PAPS molecules. As a result, the catalytic efficiency (with a $k_{cat}$ of 7.96 $s^{-1}$ $M^{-1}$) of SULT2B1b-Pro69Ala became dramatically lower than that (324.20 $s^{-1}$ $M^{-1}$) of the wild-type enzyme (*38*). In the case of SULT2B1b-Thr73Met, the replacement of threonine (a polar amino acid residue) with methionine (a non-polar residue) might have affected the hydrogen-bonding between the hydroxyl group of threonine with the oxygen atom O4P of 5'-phosphate in the PAPS, which resulted in a lower affinity for PAPS as judged by an increase in the $K_m$ value (9.4 μM), compared

**Table III. Kinetic constants of the human SULT2B1b allozymes in catalysing the sulphation of cholesterol**

| Enzyme | Cholesterol | | | | PAPS |
|---|---|---|---|---|---|
| | $K_m$ (μM) | $V_{max}$ (nmol/min/mg) | $K_{cat}$ ($s^{-1}$) $\times 10^{-3}$ | $K_{cat}/K_m$ ($s^{-1}$ $M^{-1}$) | $K_m$ (μM) |
| SULT2B1b-wt | 21.9 ± 3.6 | 10.2 ± 0.8 | 7.0 ± 0.5 | 324.2 ± 27.2 | 3.5 ± 0.7 |
| SULT2B1b-Pro69Ala | 69.6 ± 7.7*** | 0.8 ± 0.1*** | 0.6 ± 0.0*** | 8.0 ± 0.7*** | 6.5 ± 0.9** |
| SULT2B1b-Thr73Met | 79.7 ± 8.5*** | 0.7 ± 0.0*** | 0.5 ± 0.0*** | 5.8 ± 1.0*** | 9.4 ± 1.6*** |
| SULT2B1b-Asp191Asn | 23.6 ± 2.2 | 6.0 ± 0.2*** | 4.2 ± 0.1*** | 175.2 ± 12.8*** | 1.5 ± 0.2* |
| SULT2B1b-Arg230His | 69.6 ± 6.1*** | 8.7 ± 0.5* | 6.0 ± 0.3* | 86.4 ± 12.0*** | 3.5 ± 0.6 |
| SULT2B1b-Ser244Thr | 84.9 ± 21.1*** | 5.9 ± 1.0*** | 4.1 ± 0.7*** | 51.4 ± 21.3*** | 1.8 ± 0.2 |
| SULT2B1b-Pro345Leu | 66.7 ± 10.6*** | 6.8 ± 0.7*** | 4.6 ± 0.5*** | 69.3 ± 4.3*** | 3.9 ± 0.3 |

Results shown represent mean ± SD derived from three independent determinants.
*Statistical significance from SULT2B1b-wt (*$p < 0.05$; **$p < 0.001$; ***$p < 0.0001$) using one-way ANOVA followed by Dunnett's *post hoc* analysis. SULT2B1b-Arg274Gln the kinetic constants could not be determined accurately due to the weak activity of this allozyme toward cholesterol.

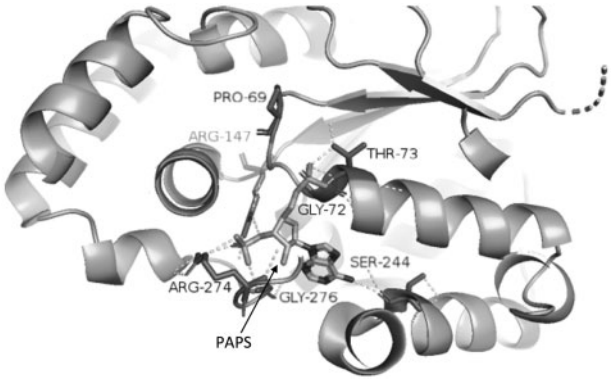


**Fig. 3 Locations of the *SULT2B1* cSNP-associated amino acid exchanges in the PAPS-binding region of the SULT2B1b molecule**. The figure shows a close view of the PAPS-binding region in complex with the PAPS molecule and the locations of the amino acid residues associated with the cSNPs analysed in this study. The dashed lines represent the hydrogen-bonding between the amino acid side chains and the PAPS molecule. This figure was generated using the PyMOL software and the reported crystal structure of SULT2B1b (*18*) with the PDB ID:1q22.

with that (3.5 μM) of the wild-type enzyme (*23*, *38*). Moreover, the replacement of arginine by glutamine in SULT2B1b-Arg274Gln, which showed a dramatically lower specific activity compared with the wild-type enzyme; (Table II) might have weakened the polar interaction between the positively charged nitrogen atom of arginine with O3P phosphate oxygen of the PAPS molecule (*23*). On the other hand, the amino acids substitutions that occur in SULT2B1b-Gly72Val, SULT2B1b-Arg147His and SULT2B1b-Gly276Val were found to completely abolish the cholesterol-sulphating activities. The substitution of glycine (which presumably provides conformation flexibility) to valine (which may cause a restriction in the conformation) in SULT2B1b-Gly72Val and SULT2B1b-Gly276Val might have disrupted the hydrogen-bonding with the O4P and O2P phosphate oxygens, respectively, of the PAPS (*23*, *38*). In the case of SULT2B1b-Arg147His, the replacement of the arginine residue, an amino acid which is necessary to form the hydrogen bond with the oxygen atom O3P of the 3'phosphate of the PAPS, with histidine might be the reason for the loss of the sulphating activity (*23*). In general, the activity data obtained for the SULT2B1b allozymes analysed indicated clearly the importance of the amino acid residues in the proper functioning of the SULT2B1b enzyme.

## Conclusions

This study represented the first attempt to gather information concerning the effects of *SULT2B1b* genetic polymorphisms on the cholesterol-sulphating activity of SULT2B1b allozymes. Activity data obtained indicated that seven of the ten SULT2B1b allozymes examined displayed lower and differential sulphating activity toward cholesterol in comparison to the wild-type enzyme, with the other three showing no detectable activity. Although the information concerning the allelic frequencies of the tested *SULT2B1b* genotypes in the population is incomplete and remains to be clarified, this study provided convincing evidence that the coded SULT2B1b allozymes exhibited significant and sometimes dramatic differences in their enzymatic activities. These results imply that individuals with different *SULT2B1b* genotype may have differential capacity in sulphating cholesterol. Pending additional studies, such information may have significance in predicting risk for diseases, as well as aiding in the formulation of personalized regimens for drugs that may be metabolized by SULT2B1b for individuals with distinct *SULT2B1b* genotypes.

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### Conflict of Interest
None declared.

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