Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical Pharmacology 65 (2003) 1787–1796
Biochemical Pharmacology
www.elsevier.com/locate/biochempharm

Pharmacogenetics of human 3′-phosphoadenosine 5′-phosphosulfate synthetase 1 (PAPSS1): gene resequencing, sequence variation, and functional genomics☆

Zhen-Hua Xu[a,1], Bianca A. Thomae[a], Bruce W. Eckloff[b], Eric D. Wieben[b], Richard M. Weinshilboum[a,*]

[a]*Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Medical School-Mayo Clinic-Mayo Foundation, Rochester, MN 55905, USA*
[b]*Department of Biochemistry and Molecular Biology, Mayo Medical School-Mayo Clinic-Mayo Foundation, Rochester, MN 55905, USA*

Received 22 May 2002; accepted 30 September 2002

Abstract

3′-Phosphoadenosine 5′-phosphosulfate (PAPS) is the high-energy ''sulfate donor'' for reactions catalyzed by sulfotransferase (SULT) enzymes. The strict requirement of SULTs for PAPS suggests that PAPS synthesis might influence the rate of sulfate conjugation. In humans, PAPS is synthesized from ATP and SO_4^{2-} by two isoforms of PAPS synthetase (PAPSS): PAPSS1 and PAPSS2. As a step toward pharmacogenetic studies, we have resequenced the entire coding sequence of the human *PAPSS1* gene, including exon–intron splice junctions, using DNA samples from 60 Caucasian-American and 58 African-American subjects. Twenty-one genetic polymorphisms were observed—1 insertion–deletion event and 20 single nucleotide polymorphisms (SNPs)—including two non-synonymous coding SNPs (cSNPs) that altered the following amino acids: Arg333Cys and Glu531Gln. Twelve pairs of these polymorphisms were tightly linked, and a total of twelve unequivocal haplotypes could be identified—two that were common to both ethnic groups and ten that were ethnic-specific. The Arg333Cys polymorphism, with an allele frequency of 2.5%, was observed only in DNA samples from Caucasian subjects. The Glu531Gln polymorphism was rare, with only a single copy of that allele in a DNA sample from an African-American subject. Transient expression in mammalian cells showed that neither of the non-synonymous cSNPs resulted in a change in the basal level of enzyme activity measured under optimal assay conditions. However, the Glu531Gln polymorphism altered the substrate kinetic properties of the enzyme. The Gln531 variant allozyme had a 5-fold higher K_m value for SO_4^{2-} than did the wild-type allozyme and displayed monophasic kinetics for Na_2SO_4. The wild-type allozyme (Glu531) showed biphasic kinetics for that substrate. These observations represent a step toward testing the hypothesis that genetic variation in PAPS synthesis catalyzed by PAPSS1 might alter *in vivo* sulfate conjugation.

Keywords: 3′-Phosphoadenosine 5′-phosphosulfate (PAPS); PAPS synthetase (PAPSS); Sulfation; Sulfate conjugation; Genetic polymorphisms; Pharmacogenetics; Haplotype

1. Introduction

Common genetic polymorphisms for genes that encode drug transporters, drug-metabolizing enzymes, and drug targets can contribute to individual differences in drug efficacy and in the occurrence of adverse drug reactions [1,2]. Sulfation, or more accurately sulfonation, is an important reaction in the biotransformation of drugs and other xenobiotics as well as endogenous compounds such as catecholamines, steroid hormones, and bile acids

☆The DNA sequencing data described in this manuscript have been deposited in the NIH-sponsored Pharmacogenetics Research Network database PharmGKB with Accession Number PKB_K00000464.

* Corresponding author. Tel.: +1-507-284-2246; fax: +1-507-284-9111.
E-mail address: weinshilboum.richard@mayo.edu (R.M. Weinshilboum).
[1] Present address: PK-Clinical Drug Evaluation, Johnson and Johnson Pharmaceutical Research & Development, LLC, 920 Route 202, POB 300, Raritan, NJ 08869, USA.

Abbreviations: PAPS, 3′-phosphoadenosine 5′-phosphosulfate; PAPSS, PAPS synthetase; PCR, polymerase chain reaction; pUni, pUni/V5-His-TOPO; SNPs, single nucleotide polymorphisms; cSNPs, coding SNPs; SULT, sulfotransferase; and UTR, untranslated region.

0006-2952/03/$ – see front matter
doi:10.1016/S0006-2952(03)00104-7

[3,4]. Sulfate conjugation is catalyzed by a superfamily of SULTs [5]. A series of genetic polymorphisms in SULT genes have been described, including many functionally significant non-synonymous cSNPs [6–9]. PAPS is a cosubstrate for all sulfation reactions in mammals, and PAPS depletion can influence sulfate conjugation *in vivo* [10]. Therefore, ''pharmacogenetic'' variation in the rate of PAPS synthesis could potentially influence sulfation.

The synthesis of PAPS from ATP and inorganic sulfate (SO_4^{2-}) involves two sequential reactions. In lower organisms, ATP sulfurylase initially catalyzes the formation of adenosine 5′-phosphosulfate (APS) from ATP and SO_4^{2-}. APS is then phosphorylated by APS kinase to yield PAPS [11,12]. However, in higher organisms, these two reactions are catalyzed by a single bifunctional enzyme, PAPSS [13,14]. Two PAPSS isoforms, PAPSS1 and PAPSS2, are expressed in both humans and mice [15–19]. Even though the synthesis of PAPS has generally been thought to occur in the cytosol, recent evidence indicates that PAPSS1 may be nuclear in its subcellular localization, as is PAPSS2 when coexpressed with PAPSS1 [20]. Rare inactivating mutations within the *PAPSS2* gene have been shown to cause the inherited skeletal disorders spondyloepimetaphyseal dysplasia in humans and brachymorphism in mice [17,18]. PAPSS2, but not PAPSS1, was inactivated in patients and mice with these disorders. Those observations suggest that, even though PAPSS1 and PAPSS2 catalyze the same reaction, they have non-redundant physiological roles, perhaps as a result of tissue-specific expression.

We set out several years ago to apply a genotype-to-phenotype strategy to study the pharmacogenetics of PAPSS1 and PAPSS2. As an initial step, we cloned the genes encoding both PAPSS isoforms in humans [21]. *PAPSS1* and *PAPSS2* were 106 and 85 kb in length, respectively, and had very similar structures that included 12 exons with virtually identical exon–intron splice junctions [21,22]. *PAPSS1*, on the basis of Northern blot analysis, was expressed in virtually all human tissues [21], suggesting that this enzyme might be essential for sulfation in many organs. In the present studies, we have now resequenced all 12 *PAPSS1* exons using DNA from 60 Caucasian-American and 58 African-American subjects. Twenty-one genetic polymorphisms were observed, including 2 non-synonymous cSNPs and 1 insertion–deletion event. Expression constructs were then created for the two non-synonymous cSNPs, and functional genomic studies of the allozymes encoded by these alleles were performed after transient expression in mammalian cells. One of those allozymes, Gln531, displayed a significant difference from the wild-type allozyme in its substrate kinetics. These studies represent an important step toward testing the hypothesis that inherited variation in PAPS synthesis might contribute to the regulation of sulfate conjugation.

2. Materials and methods

2.1. DNA samples and PAPSS1 resequencing

DNA samples from 60 Caucasian-American and 60 African-American subjects were obtained from the Coriell Institute Cell Repository. These samples had been anonymized, and written informed consent had been obtained from all donors for the use of their DNA for this purpose. Our experiments were reviewed and approved by the Mayo Clinic Institutional Review Board. Twelve PCR reactions were performed with each DNA sample to amplify all *PAPSS1* exons and splice junctions. The amplicons were then sequenced using dye-primer sequencing chemistry to facilitate the identification of heterozygous bases [23]. To make that possible, universal M13 sequencing tags were added to the 5′-ends of each forward and reverse primer. The M13 sequence tag for forward primers was 5′-TGTAAAACGACGGCCAGT-3′. For reverse primers, the M13 sequence tag was 5′-CAGGAAACAGCTATGACC-3′. The locations of primers within the gene were chosen to avoid repetitive sequence as well as regions of known homology between the two *PAPSS* genes. Primer sequences and locations, as well as PCR cycling conditions for the amplification reactions, are listed in Table 1. Amplifications were performed with AmpliTaq Gold DNA polymerase (Perkin Elmer) using a ''hot start'' to help ensure amplification specificity. Amplicons were sequenced in the Mayo Molecular Biology Core Facility with an ABI 377 DNA sequencer using BigDye™ (Perkin Elmer) dye-primer sequencing chemistry. Both DNA strands were sequenced in all cases. Since the 5′-flanking region of *PAPSS1* near the site of transcription initiation is very GC rich, multiple attempts to obtain high quality sequencing chromatograms for that region of the gene were unsuccessful. Finally, to exclude PCR-induced artifacts, independent amplification, followed by DNA sequencing, was performed for all samples in which a SNP was only observed once among the samples resequenced. DNA sequence chromatograms were analyzed using the PolyPhred 3.0 [24] and Consed 8.0 [25] programs developed by the University of Washington. The University of Wisconsin GCG software package, Version 10, was also used to analyze the nucleotide sequence. GenBank Accession Numbers for the *PAPSS1* reference sequences were AF097710 to AF097721 [21].

2.2. PAPSS1 expression constructs and recombinant allozymes

PAPSS1 cDNA sequences for the two non-synonymous cSNPs that we observed during the resequencing experiments were created by using the QuickChange Site-Directed Mutagenesis kit (Stratagene), with the wild-type PAPSS1 cDNA open reading frame (ORF) in the pUni vector (Invitrogen) as template. Specifically, the full-length wild-type ORF (GenBank Accession Number AF105227)

Table 1
Primers and amplification conditions used for resequencing human *PAPSS1*

Primer name	Primer location	Primer sequence (5′ → 3′)	PCR cycling conditions
UF (−83) M13	5′-FR	AGCCCCGCCCCGCTCGCTGGCCTG	35 cycles: 30 sec at 94°, min at 72°
I1R152 M13	Intron 1	GCCCCAGCCGGGAGGCGCCG	
I1F (−103) M13	Intron 1	GCTTTTGGCATGTTACATAG	35 cycles: 30 sec at 94°, 30 sec at 65°, 2 min at 72°
I2R116 M13	Intron 2	TCGTGATGCTCCAAATACAAG	
I2F (−67) M13	Intron 2	AAAGTATTACTACATAGTTATCC	35 cycles: 30 sec at 94°, 30 sec at 60°, 2 min at 72°
I3R119 M13	Intron 3	AGCTGGGGAGGAGTAGAGTTA	
I3F (−102) M13	Intron 3	TTTCCCACTAAATTGGATGA	35 cycles: 30 sec at 94°, 30 sec at 60°, 2 min at 72°
I4R231 M13	Intron 4	CTCCCGAGCCCCAA	
I4F (−159) M13	Intron 4	TAATTAGAAATCTCCCAAGAA	35 cycles: 30 sec at 94°, 20 sec at 64°, 1 min at 72°
I5R179 M13	Intron 5	ACGGTGCTCCCCACAACA	
I5F (−280) M13	Intron 5	TGAGGCCACCTCTCATTTGT	35 cycles: 30 sec at 94°, 20 sec at 60°, 2 min at 72°
I6R192 M13	Intron 6	ATGGTAACTTGGGAACATGGTTG	
I6F (−143) M13	Intron 6	TCTTTGTTAGTTTGGTATA	20 cycles: 30 sec at 94°, 30 sec at 55°, 45 sec at 72° 20 cycles: 30 sec at 94°, 30 sec at 70°, 45 sec at 72°
I7R155 M13	Intron 7	CTTAAATAAAGTGTTCGGTA	
I7F (−109) M13	Intron 7	TACAGCCTTTTATTATTTG	20 cycles: 30 sec at 94°, 30 sec at 55°, 45 sec at 72° 20 cycles: 30 sec at 94°, 30 sec at 70°, 45 sec at 72°
I8R167 M13	Intron 8	CCAAAATGACAAGAG	
I8F (−93) M13	Intron 8	AGCTTACAACGACTGTATTTAGC	35 cycles: 30 sec at 94°, 30 sec at 65°, 2 min at 72°
I9R155 M13	Intron 9	ACCCAGGCTAGTTTTGATTG	
I9F (−68) M13	Intron 9	TTGCGTATCCTTTGGAAAG	35 cycles: 30 sec at 94°, 30 sec at 65°, 2 min at 72°
I10R70 M13	Intron 10	TGCCCCTAGCATCCA	
I10F (−148) M13	Intron 10	CTGGCTTCCCAGGATGATA	35 cycles: 30 sec at 94°, 30 sec at 65°, 2 min at 72°
I11R146 M13	Intron 11	GGGAAATTACTTTCTGGGTTTACC	
I11F (−91) M13	Intron 11	TTTGTCTAATATGAACAGAAGG	35 cycles: 30 sec at 94°, 30 sec at 60°, 2 min at 72°
R2005 M13	3′-UTR	AAGTTAAGGAAAATGGTCTG	

A universal M13 forward primer (5′-TGTAAACGACGGCCAGT-3′) was added to the 5′-ends of all forward primers, and a universal M13 reverse primer (5′-CAGGAAACAGCTATGACC-3′) was added to the 5′-termini of all reverse primers. Brackets associated with primer names represent primer pairs used to perform specific amplifications, and the brackets under "PCR cycling conditions" indicate two sequential sets of cycling conditions that were used to perform the indicated amplification. "F" represents forward; "R", reverse; "U", upstream; "I", intron; "FR", flanking region; and "UTR", untranslated region. The numbering scheme for primers located in exons and in the 5′-FR was based on the cDNA sequence, with the "A" in the translation initiation codon designated as +1. Positions 5′ to that location were assigned negative numbers, and nucleotides 3′ to that position were assigned positive numbers. Intron-based primers were numbered on the basis of nucleotide distance from splice junctions, with +1 as the first nucleotide at the 5′-end, and with (−1) as the first nucleotide at the 3′-end of the region.

was amplified using human brain Marathon-Ready cDNA (Clontech) as template with primer pair F1 (5′-GAGGAGGAATTCATGGAGATCCCCGGGAGCTTG-3′) and R1982 (5′-GATAAGGAATTCTTAGGAAGCATGTCCAGACAGACAC). The resultant PAPSS1 cDNA was subcloned into pUni, a vector that is only 2.3 kb in length, so it is well suited for performing "circular PCR" during site-directed mutagenesis. Site-directed mutagenesis was then performed using internal primers that contained the variant nucleotide sequences. Since both F1 and R1982 contain *Eco*RI sites (underlined in the sequences), the PAPSS1 cDNA inserts in pUni could be easily excised and re-ligated into the eukaryotic expression vector p91023(b) [26]. The sequences of inserts in p91023(b) were confirmed by completely sequencing both strands. Expression constructs for the wild-type and variant PAPSS1 sequences were then used to transfect COS-1 and HEK293 cells using the TransFast™ reagent (Promega), with a 1:1 charge ratio. pSV-β-Galactosidase (Promega) was cotransfected as an internal control to make it possible to correct for transfection efficiency. The COS-1 and HEK293 cells were harvested after 48 hr and were homogenized with a Polytron homogenizer (Brinkmann Instruments) in 25 mM potassium phosphate buffer, pH 7.8, that contained 1 mM dithiothreitol (DTT) and 1 mM EDTA. Cell homogenates were centrifuged at 15,000 *g* for 15 min at 4°, and the resultant supernatant preparations were used to perform enzyme assays and substrate kinetic studies.

2.3. Protein assay

Protein concentrations were measured with the dye-binding method of Bradford [27] using bovine serum albumin as a standard.

2.4. PAPSS1 enzyme assays and substrate kinetic studies

PAPSS activity was measured with the coupled radiochemical assay described by Xu *et al.* [28]. The first step in that assay involves the generation of PAPS from ATP and Na_2SO_4. The PAPS formed during this initial step is then used as a substrate for the sulfate conjugation of [2,4,6,7-^{3}H]estradiol in a reaction catalyzed by recombinant human SULT1E1. Advantages of this assay include: the resistance of SULT1E1 to inhibition by ATP (a substrate for the PAPSS-catalyzed step), the low K_m values of SULT1E1 for both cosubstrates, and the commercial availability of high specific activity radioactively labeled estradiol [28]. All of these features serve to enhance assay sensitivity. Specifically, PAPS synthesis was catalyzed by recombinant PAPSS1 in the presence of 1 mM ATP, 4 mM Na_2SO_4, 1 mM $MgCl_2$, and 2 mM DTT in 60 mM glycine–NaOH buffer, pH 8.6. "Blank" samples included the same quantity of 15,000 *g* supernatant from COS-1 or HEK293 cells that had been transfected with "empty" p91023(b) to make it possible to correct for endogenous activity. The endogenous COS-1 cell activity was at most 10% of that assayed for the recombinant enzyme under optimal assay conditions. Reaction mixtures were incubated at 37° for 20 min, and this "stage 1" reaction was terminated by heating for 1 min at 100°. An aliquot from the initial PAPS generating step of the assay was then used to initiate the second, coupled reaction. That reaction included 27 nM [2,4,6,7-^{3}H]estradiol, 8 mM DTT, and 1.25 mM $MgCl_2$ in 10 mM potassium phosphate buffer, pH 6.5. The second, SULT1E1-catalyzed step was terminated by the addition of KOH, followed by organic solvent extraction performed with chloroform. The radioactivity of the sulfate conjugated [2,4,6,7-^{3}H]estradiol that remained in the aqueous phase after organic solvent extraction was then measured in a liquid scintillation counter. PAPSS activities for recombinant PAPSS1 allozymes were compared after correction for transfection efficiency by measuring the activity of cotransfected β-galactosidase. β-Galactosidase activity in the COS-1 and HEK293 cell preparations was measured with the β-Galactosidase Assay System (Promega) as described by the manufacturer.

To estimate apparent K_m values of allozymes for the two cosubstrates for the reaction, a series of eight ATP (0.125 to 4 mM) and nine Na_2SO_4 (0.03125 to 8 mM) concentrations were tested. When ATP was the varied substrate, the concentration of Na_2SO_4 was 4 mM, and when Na_2SO_4 was the varied substrate, the concentration of ATP was 1 mM. Blanks for each concentration of substrate were included by assaying COS-1 cell preparations obtained after transfection with empty p91023(b) vector. We chose COS-1 expressed recombinant enzyme for use in the substrate kinetic studies because we had observed higher levels of PAPSS1 expression in COS-1 than in HEK293 cells. Substrate kinetic data were fitted to a series of kinetic models, and the most appropriate model was selected on the basis of the dispersion of residuals and a determination of whether the *F*-test showed a significant reduction ($P < 0.05$) in the residual sums of squares.

2.5. Data analysis

Levels of enzyme activity and kinetic parameters for recombinant PAPSS1 allozymes were compared statistically by ANOVA performed with the StatView program, version 4.5 (Abacus Concepts). Linkage analysis was performed after all DNA samples had been genotyped at each of the 21 polymorphic sites observed. Then, D' values, a quantitative method for reporting linkage data that is independent of allele frequency [29,30], were calculated. The genotype data were also used to assign inferred haplotypes using a program based on the E-M algorithm [31,32]. Unambiguous haplotype assignment was also possible on the basis of genotype for samples that contained no more than one heterozygous polymorphism.

3. Results

3.1. PAPSS1 resequencing and polymorphisms

Twelve separate PCR amplifications were performed for each of the 120 DNA samples studied. However, two of the samples from African-American subjects consistently failed to amplify with any of the primer pairs. Therefore, the subsequent data include only 58 samples from African-Americans. As a result, the DNA resequencing experiments involved the analysis, in total, of approximately 1.1 million bp of sequence. All PCR amplicons were sequenced on both strands, making it possible to verify the presence of polymorphisms by the use of data from the complementary strand. We observed a total of 21 polymorphisms, including 20 SNPs and 1 insertion–deletion event (Fig. 1 and Table 2). Variant allele frequencies ranged from 0.8 to 53.6%, with striking differences between the African-American and Caucasian-American subjects (Table 2). Nineteen polymorphisms were observed in 58 DNA samples from African-American subjects, while only 13 were found in the 60 samples from Caucasian-American subjects. The overall number of *PAPSS1* polymorphisms per kb of sequence in the 118 samples studied (4.3 polymorphisms/kb) was close to that (4.6/kb) observed in similar studies of other human genes [33]. Seven of the SNPs were present in the coding region (cSNPs), and two of those cSNPs—located in exons 8 and 11—were non-synonymous, resulting in alterations in the following encoded amino acids: Arg333Cys and Glu531Gln. The Arg333Cys polymorphism had a

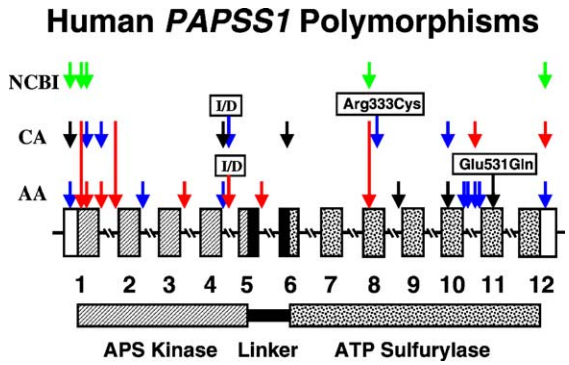

Fig. 1. Human *PAPSS1* polymorphisms. Locations of the 21 polymorphisms observed during the resequencing of *PAPSS1* are depicted schematically. The gene has three major "domains": an adenosine 5′-phosphosulfate (APS) kinase domain (hatched lines), a "linker" sequence (black), and an ATP sulfurylase domain (dotted). These domains within the encoded protein are depicted schematically at the bottom of the figure. Rectangles in the gene structure represent exons, with white rectangles representing exons encoding 5′- and 3′-untranslated regions. The lengths of exons and introns are not drawn to scale. Colors of arrows indicate the frequencies of polymorphisms. Red represents frequencies of greater than 10%, blue represents frequencies from 1 to 10%, and black arrows represent frequencies of less than 1%. Green arrows are used to indicate those polymorphisms available in either the NCBI SNP database or the EST database. "I/D" represents an insertion–deletion event. Changes in encoded amino acids as a result of non-synonymous cSNPs are also indicated.

Table 2
Human *PAPSSI* polymorphisms

Location in gene	Polymorphism position	Nucleotide change	Amino acid change	Frequency of variant allele	
				African-American	Caucasian-American
5′-UTR*	−44	C → T		0.091	0.008
Exon 1*	19	C → T		0.254	0.583
Exon 1*	36	G → A		0.254	0.058
Intron 1	I1 (107)	G → G		0.536	0.067
Intron 1	I1 (−34)	G → A		0.272	0.593
Intron 2	I2 (55)	C → T		0.017	0.000
Intron 3	I3 (36)	A → G		0.138	0.000
Intron 4	I4 (18)	C → T		0.094	0.008
Intron 4	I4 (86)	9-bp Ins		0.215	0.008
Intron 5	I5 (144)	G → C		0.103	0.000
Exon 6	675	T → C		0.000	0.008
Exon 8*	963	C → T		0.211	0.246
Exon 8	997	C → T	Arg333Cys	0.000	0.025
Intron 8	I8 (−14)	G → C		0.008	0.000
Exon 10	1260	A → G		0.008	0.017
Intron 10	I10 (12)	G → T		0.017	0.000
Intron 10	I10 (125)	A → G		0.017	0.000
Intron 10	I10 (−32)	C → G		0.052	0.254
Intron 10	I10 (−7)	A → G		0.017	0.000
Exon 11	1591	G → C	Glu531Gln	0.008	0.000
3′-UTR*	1945	G → A		0.043	0.254

Locations of polymorphisms within the gene, nucleotide sequence alterations, encoded amino acid sequence changes, and observed frequencies of the variant allele in the 58 African-American and 60 Caucasian-American DNA samples studied are listed. In a few instances, individual amplifications failed. Therefore, all allele frequencies are based on the actual number of samples studied. The rules for numbering polymorphisms are the same as those used for primers (see Table 1). "Ins" refers to an insertion of 5′-ACTCTTACA-3′. Polymorphisms located within exons have been boxed. An asterisk (*) indicates that the polymorphism is already publicly available from either the NCBI dbSNP or by the analysis of the human EST database (see text for details).

frequency of 2.5% in Caucasians but was not observed in DNA from African-American subjects. The Glu531Gln polymorphism was rare, with only one copy of the variant allele in a single African-American DNA sample. Of the 21 polymorphisms, 3 were observed only once in the 236 alleles that we resequenced successfully. As mentioned previously, to exclude artifacts introduced by PCR-dependent misincorporation, we performed independent amplifications and sequenced the amplicons in all cases in which a polymorphism was observed only once among the DNA samples studied. The proximal 5′-flanking region of *PAPSS1* is very GC rich, so we were unable to resequence that region of the gene using either dye primer or dye terminator sequencing chemistry.

We also compared the SNPs that we observed with those present in publicly accessible databases. As of April 2002, a total of 31 human *PAPSS1* sequence variations had been deposited in the SNP database (www.ncbi.nlm.nih.gov/SNP), 22 located in introns, 1 in the 5′-UTR, 2 in the 5′-flanking region, 3 in the 3′-UTR, and 3 in the coding region—including 1 synonymous and 2 non-synonymous cSNPs. Of those 31 database SNPs, only 3 were observed during our resequencing studies. They included a C → T transition at nucleotide (−44) in the 5′-UTR, a G → A transition in the 3′-UTR, and a synonymous A → G cSNP at nucleotide 36 in exon 1. The two non-synonymous cSNPs that had been deposited in the SNP database differed from those that we had observed and resulted in amino acid changes of Leu270Phe and Gln355Arg. During our studies, we also observed a 9 bp insertion at nucleotides 86–94 of intron 4. However, the SNP database showed 5 consecutive SNPs at that same location, an obvious error that involved the mistaken designation of this insertion as a series of SNPs. The remaining 21 database SNPs located in introns and the 5′-flanking region were not located in regions of the gene that we had resequenced. We also used the human PAPSS1 cDNA (GenBank Accession Number AF105227) to search the EST database. We then analyzed the 124 PAPSS1 EST sequences that had been deposited for the presence of polymorphisms. Only the initial 400 bp of each EST sequence was used to perform this analysis to ensure high sequence quality. Three cSNPs, all synonymous, out of the seven cSNPs that we observed in our studies, were present in these EST sequences. In summary, 5 of the 21 polymorphisms that we had observed by resequencing the gene were available in public databases (indicated by ∗ in Table 2).

3.2. PAPSS1 linkage analysis and haplotype prediction

We also performed linkage analysis for all possible pairwise combinations of the *PAPSS1* polymorphisms that we had observed. To do that, D' values were calculated. D' values reflect the degree of linkage between two loci and can range from (+1.0), when two polymorphisms are maximally positively associated, to (−1.0), when two polymorphisms never occur together [29,30]. *PAPSS1* polymorphisms with variant allele frequencies lower than 2.5% were excluded from this analysis because of lack of statistical power. The linkage analysis showed that 11 pairs of polymorphisms were in tight positive linkage, with D' values greater than 0.7 (Table 3). Furthermore, 12 unequivocal haplotypes could be identified (Table 4), but a total of 29 and 7 additional haplotypes could be inferred for the African-American and Caucasian-American samples, respectively, by use of the E-M algorithm [31,32]. Data with regard to the inferred haplotypes can be obtained from the authors upon request. As shown in Table 4, we were able to account for 59 and 86% of all samples based on these unequivocal haplotypes for DNA samples from African-American and Caucasian-American subjects, respectively. The unequivocal haplotypes included 2 that were common to both ethnic groups, and 5 each that were ethnic-specific for African-American and Caucasian-American subjects (Table 4). Initial haplotype designations were made on the basis of encoded amino acid sequence, with all "wild-type" sequences designated **1*, those with the Cys333 variant designated **2*, and although we are presently unable to determine these haplotypes unequivocally, those with the Gln531 variant designated **3*. We then assigned letter designations based on descending allele frequencies, starting with the African-American samples.

Table 3
PAPSS1 polymorphism linkage analysis

Nucleotide positions of polymorphism pair		D' value	
		African-American	Caucasian-American
−44	I1 (107)	1.00	–
19	I1 (−34)	1.00	0.93
36	I1 (107)	0.76	1.00
36	I3 (36)	1.00	–
36	1945	1.00	–
I1 (107)	14 (18)	1.00	–
I3 (36)	I10 (−32)	0.78	–
I3 (36)	1945	1.00	–
966	I10 (−32)	1.00	1.00
963	1945	1.00	1.00
I10 (−32)	1945	1.00	1.00

Pairwise analyses were performed for all *PAPSS1* polymorphisms observed during the gene resequencing studies. Only polymorphisms with allele frequencies greater than 2.5% and with absolute D' values greater than 0.7 are listed. Dashes indicate either that pairs of polymorphisms were not in tight positive linkage in that ethnic group ($D' < 0.7$) or that one of the polymorphisms did not have an allele frequency >2.5% in that ethnic group. See text for details.

3.3. Recombinant PAPSS1 allozyme activity

Expression constructs were created for the two *PAPSS1* non-synonymous cSNPs that we had observed during the gene resequencing studies, and those constructs were used to transiently transfect COS-1 and HEK293 cells to perform

Table 4
Human PAPSS1 haplotype analysis

Allele	Frequency		Exon 1					Exon 8		Exon 10	
designation	AA	CA	19	36	I1(107)	I1(-34)	I3(36)	963	997	1260	I10(-32)
*1A	0.231	0.371	**T**	G	C	**A**	A	C	C	A	C
*1B	0.053	0.267	C	G	C	G	A	C	C	A	C
*1C	0.169		C	G	**G**	G	A	C	C	A	C
*1D	0.081		C	**A**	**G**	G	A	C	C	A	C
*1E	0.031		C	G	**G**	G	A	**T**	C	A	C
*1F	0.014		C	G	C	G	A	**T**	C	A	C
*1G	0.008		C	**A**	**G**	G	**G**	C	C	A	C
*1H		0.192		G	C	**A**	A	**T**	C	A	**G**
*1I		0.008	**T**	G	C	**A**	A	C	C	**G**	C
*1J		0.008	**T**	G	C	G	A	C	C	A	C
*1K		0.008	C	G	C	**A**	A	C	C	A	C
*2A		0.008	**T**	G	C	**A**	A	C	**T**	A	C

Twelve unambiguous haplotypes were observed in the 118 DNA samples resequenced. Variant sequence within each of the alleles is highlighted as a white letter against black background. Positions not listed in the table had the consensus *PAPSS1* nucleotide sequence. "AA" refers to African-American, and "CA" refers to Caucasian-American samples. Initial haplotype designations were made on the basis of the encoded amino acid sequence with all "wild-type" sequences being designated *1, those with the Cys333 variant designated *2, and, although we are presently unable to determine these haplotypes unequivocally, those with the Gln531 variant designated *3. We then assigned "letter" designations based on descending allele frequencies, starting with unequivocal haplotypes present in both ethnic groups, and, when present in only one ethnic group, making initial assignment for the DNA samples obtained from African-American subjects.

functional genomic studies. We chose mammalian cells for these experiments to ensure both possible post-translational modification and the presence of mammalian protein degradation systems. After transfection, the COS-1 and HEK293 cell preparations were assayed for PAPSS activity under optimal conditions for the wild-type enzyme [28]. Under those assay conditions (i.e. in the presence of 1 mM ATP and 4 mM Na_2SO_4), neither of the variant PAPSS1 allozymes displayed a significant difference in the level of PAPSS activity when compared with the wild-type allozyme after expression in either COS-1 or HEK293 cells. Specifically, in COS-1 cells, the Cys333 allozyme had $95.8 \pm 7.4\%$ (mean $\pm$ SEM, N = 3) of the wild-type activity, while the Gln531 allozyme had $96.4 \pm 8.6\%$ of the wild-type activity. All of these data were corrected for transfection efficiency. The next step in the functional genomic studies involved performing experiments designed to determine whether substrate kinetic properties might differ among these PAPSS1 allozymes.

3.4. Recombinant PAPSS1 substrate kinetic studies

Although we had not observed significant differences in basal levels of PAPSS1 activity for either of the variant allozymes, it was still possible that alterations in amino acid sequence might change substrate kinetics. Therefore, a series of eight ATP (0.125 to 4 mM) and nine Na_2SO_4 (0.03125 to 8 mM) concentrations were used to study the recombinant wild-type, the Cys333, and the Gln531 variant PAPSS1 allozymes. All three allozymes exhibited allosteric kinetics for ATP, as reported previously [19,34], with very similar apparent S_{50} values (Table 5). However, when Na_2SO_4 was the varied cosubstrate, substrate kinetics for the Gln531 allozyme differed from those of the wild-type and Cys333 allozymes. The Gln531 allozyme displayed monophasic substrate kinetics for Na_2SO_4, while the wild-type and Cys333 allozyme had, as described previously [28], biphasic kinetics for that substrate (Fig. 2 and Table 5). In addition, the Gln531 variant allozyme had

Table 5
PAPSS1 allozyme substrate kinetics

Substrate	Kinetic parameter	Wild-type	Cys333	Gln531
ATP	Kinetic model	Allosteric	Allosteric	Allosteric
	S_{50} (mM)	0.566 ± 0.002	0.496 ± 0.001	0.499 ± 0.000
	Hill coefficient	5.50 ± 0.10	5.99 ± 0.23	6.25 ± 0.03
	V_{max} (nmol/hr/mg)	163 ± 1.0	145 ± 0.8	151 ± 0.6
Na_2SO_4	Kinetic properties	Biphasic	Biphasic	Monophasic
	K_{m1} (mM)	0.081 ± 0.004	0.075 ± 0.001	0.496 ± 0.004
	$V_{max\ 1}$ (nmol/hr/mg)	139 ± 3.9	121 ± 0.8	126 ± 0.3
	K_{m2} (mM)	5.8 ± 0.9	4.4 ± 0.2	NA
	$V_{max\ 2}$ (nmol/hr/mg)	832 ± 2.8	73.1 ± 1.2	NA

Values are expressed as means $\pm$ SEM (N = 3). NA is "not applicable". See text for details.

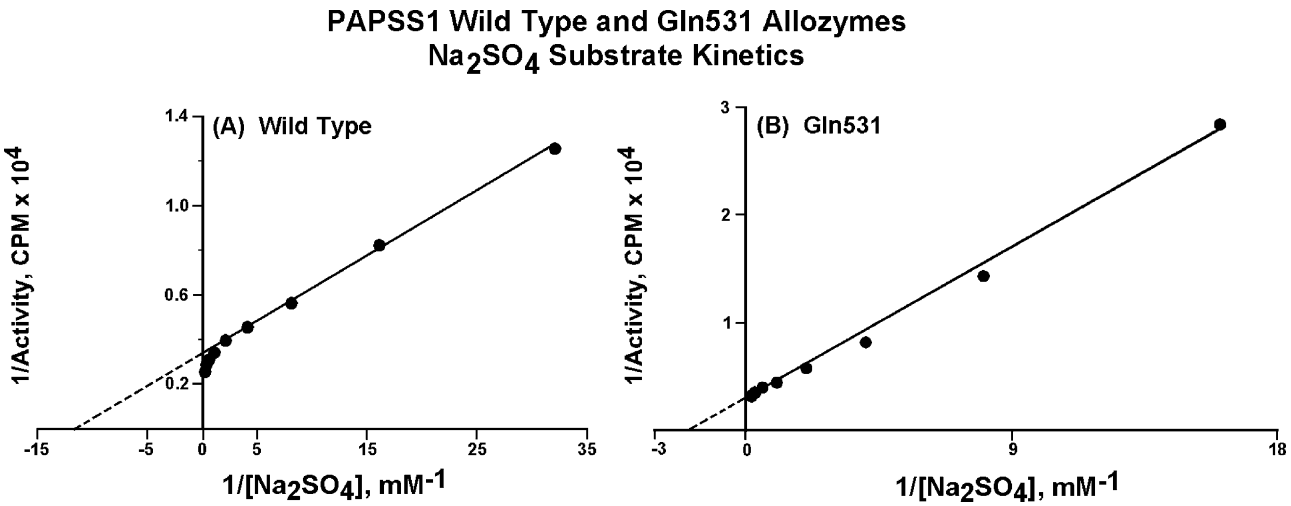

Fig. 2. Wild-type and Gln531 PAPSS1 SO_4^{2-} substrate kinetic studies. Double inverse plots of the effect of SO_4^{2-} concentration on the formation of PAPS are shown for recombinant (A) wild-type and (B) Gln531 PAPSS1 allozymes. A series of nine Na_2SO_4 concentrations (0.03125 to 8 mM) was used to measure PAPSS activity in COS-1 cell preparations transfected with wild-type and with Gln531 PAPSS1 allozyme expression constructs. The concentration of ATP used in all assays was 1 mM. See text for details.

an apparent K_m value for SO_4^{2-} that was more than 5-fold higher than that of either the wild-type or the Cys333 allozymes (Fig. 2 and Table 5).

4. Discussion

Sulfation is a major pathway in the biotransformation of drugs and other xenobiotics [4,5]. Sulfate conjugation is also involved in the metabolism of many neurotransmitters and hormones and is essential for the normal function of many macromolecules such as glycosaminoglycans and proteoglycans [35]. Sulfation involves three indispensable steps: transport of inorganic sulfate into cells; synthesis of the high-energy sulfate donor, PAPS; and the transfer by SULTs of SO_3^- from PAPS to ''acceptor'' molecules. Alterations in any of these steps might result in impaired sulfation. It is known that rare mutations in the gene encoding the human sulfate transporter SLC26A2 and in the *PAPSS2* gene can cause dwarfism and severe joint deformity [18,36]. Several years ago we set out, using a genotype-to-phenotype strategy, to test the hypothesis that common genetic variation that does not inactivate PAPSS1 and PAPSS2 completely might contribute to individual differences in sulfate conjugation. The present studies have focussed on PAPSS1. The fact that PAPSS1 is expressed in many tissues suggests that it might contribute to a large number of sulfation reactions in humans [21]. Furthermore, PAPSS1 is the major isoform catalyzing PAPS formation in the brain [21] where neurotransmitters undergo sulfate conjugation and where a decrease in keratan sulfation has been reported to be associated with Alzheimer's disease [37].

In the present studies, we resequenced all *PAPSS1* exons, including exon–intron splice junctions, using DNA samples from two ethnic groups, and identified 21 sequence variations in the 236 alleles resequenced, including 20 SNPs and 1 insertion–deletion event (Fig. 1 and Table 2). Nineteen polymorphisms were observed in DNA samples from 58 African-American subjects and 13 polymorphisms were present in DNA samples from 60 Caucasian-American subjects. These observations are compatible with previous observations which indicated greater DNA sequence diversity among African populations than other ethnic groups [9,38]. Many of the *PAPSS1* polymorphisms were tightly linked (Table 3), and a series of unequivocal haplotypes were observed in both population groups (Table 4).

After identifying common sequence variation in the human *PAPSS1* gene, we performed functional genomic experiments to study the possible functional consequences of the two non-synonymous cSNPs that we had observed. Neither of those polymorphisms was located within known conserved PAPSS sequence motifs such as the ATP-binding P-loop, FISP, HXXH, PP-loop, or the N-terminal nuclear localization sequence [19,20]. Nor did either of these two cSNPs alter the level of expression of the encoded allozyme after transient expression in COS-1 and HEK293 cells. However, the Gln531 variant allozyme did display altered substrate kinetics. Specifically, wild-type human PAPSS1 had allosteric substrate kinetics for ATP, and biphasic kinetics for SO_4^{2-}. Those observations were in agreement with previous studies in which allosteric ATP substrate kinetics were observed with both recombinant human PAPSS1 and PAPSS2 after expression in COS-1 cells [19,34]. PAPSS1 and PAPSS2 have similar affinities for ATP (apparent S_{50} values of 0.57 mM for PAPSS1 and 0.70 mM for PAPSS2) [34]. However, PAPSS2 had a 10-fold higher K_m value for SO_4^{2-} (0.87 mM) [34] than did PAPSS1 (0.08 mM), as determined in the present study.

The Cys333 PAPSS1 variant allozyme in the present study had substrate kinetic characteristics that were virtually identical with those of the wild-type protein (Table 5), and both variant allozymes displayed allosteric substrate kinetics for ATP. However, the Gln531 *PAPSS1* polymorphism altered the substrate kinetic characteristics of the enzyme with respect to SO_4^{2-}. It is of interest that this polymorphism is located within the ATP sulfurylase domain of the enzyme (Fig. 1). The Gln531 allozyme had a 5-fold higher apparent K_m value for SO_4^{2-} than did the wild-type or the Cys333 allozyme (Table 5). Furthermore, the Gln531 allozyme exhibited monophasic substrate kinetics, while the wild-type and Cys333 allozymes had biphasic kinetics for SO_4^{2-} (Fig. 2). We had subtracted endogenous PAPSS activity present in the COS-1 cells by inclusion of "blanks" for each substrate concentration used in the kinetic studies, so we were able to exclude the possibility that the biphasic kinetics resulted from endogenous PAPSS activity in COS-1 cells. Since previous studies have demonstrated that *in vivo* sulfate conjugation decreases when concentrations of SO_4^{2-} are limiting [10], these observations suggest that subjects with the Gln531 variant might be at risk for impaired PAPS generation and, thus, for impaired ability to catalyze sulfation in situations in which the concentration of SO_4^{2-} is decreased. However, the mechanism by which a change in amino acid from Glu to Gln at codon 531 might alter the substrate kinetic characteristics of the enzyme remains unclear. We will be in a much better position to evaluate the functional effects of the Glu531Gln polymorphism when the crystal structure of PAPSS1 is solved.

In summary, we have identified a series of genetic polymorphisms and haplotypes for the human *PAPSS1* gene, several of which are quite common (Tables 2 and 4). Functional genomic studies of the two non-synonymous cSNPs that we observed demonstrated kinetic changes as a result of the Glu531Gln polymorphism that could have physiological and/or pharmacological significance. It should also be pointed out that we observed a number of common polymorphisms within introns. Since polymorphisms located in regions other than coding sequence can affect transcription, RNA splicing, mRNA stability, or translation, future studies will be required to examine the possible functional implication of polymorphisms present in those regions of the gene. Finally, it is becoming increasingly clear that haplotype may be important for predicting the functional significance of genetic polymorphisms [39,40]. Therefore, the linkage and haplotype data that we have observed will also have to be studied for their possible functional implications.

PAPS-dependent sulfation plays an important role in the biotransformation of many drugs, hormones, and neurotransmitters [3,4], and it is also involved in a variety of important physiological processes such as cartilage development [18], platelet aggregation [41], blood clotting [42], and response to viral infections [43]. The present studies represent a significant step toward testing the hypothesis that pharmacogenetic variation in *PAPSS1* might contribute to interindividual differences in the biotransformation of drugs and in risk for the development of disease.

Acknowledgments

We thank Mrs. Luanne Wussow for her assistance with the preparation of this manuscript, and Dr. Daniel Schaid for his assistance with the linkage and haplotype analysis. This work was supported, in part, by NIH Grants RO1 GM28157 (R.M.W.), RO1 GM35720 (R.M.W.), and UO1 GM61388 (R.M.W. and E.D.W.) and by a Merck Sharp & Dohme International Clinical Pharmacology Fellowship (Z.-H.X.).

References

[1] Ingelman-Sundberg M, Oscarson M, McLellan RA. Polymorphic human cytochrome P450 enzymes: an opportunity for individualized drug treatment. Trends Pharmacol Sci 1999;20:342–9.
[2] Weinshilboum RM. Thiopurine pharmacogenetics: clinical and molecular studies of thiopurine methyltransferase. Drug Metab Dispos 2001;29:601–5.
[3] Weinshilboum RM, Otterness DM. Sulfotransferase enzymes. In: Kaufmann FC, editor. Conjugation-deconjugation reactions in drug metabolism and toxicity. Handbook of experimental pharmacology series, vol. 112. Berlin: Springer; 1994. p. 45–78 [chapter 22].
[4] Falany CN. Enzymology of human cytosolic sulfotransferases. FASEB J 1997;11:206–16.
[5] Weinshilboum RM, Otterness DM, Aksoy IA, Wood TC, Her C, Raftogianis RB. Sulfotransferase molecular biology: cDNAs and genes. FASEB J 1997;11:3–14.
[6] Raftogianis RB, Wood TC, Otterness DM, Van Loon JA, Weinshilboum RM. Phenol sulfotransferase pharmacogenetics in humans: association of common *SULT1A1* alleles with TS PST phenotype. Biochem Biophys Res Commun 1997;239:298–304.
[7] Raftogianis RB, Wood TC, Weinshilboum RM. Human phenol sulfotransferases *SULT1A2* and *SULT1A1*: genetic polymorphisms, allozyme properties, and human liver genotype-phenotype correlations. Biochem Pharmacol 1999;58:605–16.
[8] Freimuth RR, Eckloff B, Wieben ED, Weinshilboum RM. Human sulfotransferase *SULT1C1* pharmacogenetics: gene resequencing and functional genomic studies. Pharmacogenetics 2001;11:747–56.
[9] Thomae BA, Eckloff BW, Freimuth RR, Wieben ED, Weinshilboum RM. Human sulfotransferase SULT2A1 pharmacogenetics: genotype to phenotype studies. Pharmacogenomics J 2002;2:48–56.
[10] Levy G. Sulfate conjugation in drug metabolism: role of inorganic sulfate. Fed Proc 1986;45:2235–40.
[11] Farooqui AA. 3′-Phosphoadenosine-5′-phosphosulfate metabolism in mammalian tissues. Int J Biochem 1980;12:529–36.
[12] Klaassen CD, Boles JW. The importance of 3′-phosphoadenosine-5′-phosphosulfate (PAPS) in the regulation of sulfation. FASEB J 1997;11:404–18.
[13] Geller DH, Henry JG, Belch J, Schwartz NB. Co-purification and characterization of ATP-sulfurylase and adenosine-5'-phosphosulfate kinase from rat chondrosarcoma. J Biol Chem 1987;262:7374–82.
[14] Lyle S, Stanczak J, Ng K, Schwartz NB. Rat chondrosarcoma ATP sulfurylase and adenosine 5′-phosphosulfate kinase reside on a single bifunctional protein. Biochemistry 1994;33:5920–5.
[15] Li H, Deyrup A, Mensch Jr JR, Domowicz M, Konstantinidis AK, Schwartz NB. The isolation and characterization of cDNA encoding

the mouse bifunctional ATP sulfurylase-adenosine 5′-phosphosulfate kinase. J Biol Chem 1995;270:29453–9.
[16] Girard J-P, Baekkevold ES, Amalric F. Sulfation in high endothelial venules: cloning and expression of the human PAPS synthetase. FASEB J 1998;12:603–12.
[17] Kurima K, Warman ML, Krishnan S, Domowicz M, Krueger Jr RC, Deyrup A, Schwartz NB. A member of a family of sulfate-activating enzymes causes murine brachymorphism. Proc Natl Acad Sci USA 1998;95:8681–5.
[18] ul Haque MF, King LM, Krakow D, Cantor RM, Rusiniak ME, Swank RT, Superti-Furga A, Haque S, Abbas H, Ahmad W, Ahmad M, Cohn DH. Mutations in orthologous genes in human spondyloepimetaphyseal dysplasia and the brachymorphic mouse. Nat Genet 1998;20: 157–62.
[19] Venkatachalam KV, Akita H, Strott CA. Molecular cloning, expression and characterization of human bifunctional 3'-phosphoadenosine 5'-phosphosulfate synthase and its functional domains. J Biol Chem 1998;273:19311–20.
[20] Besset S, Vincourt JB, Amalric F, Girard JP. Nuclear localization of PAPS synthetase 1: a sulfate activation pathway in the nucleus of eukaryotic cells. FASEB J 2000;14:345–54.
[21] Xu ZH, Otterness DM, Freimuth RR, Carlini EJ, Wood TC, Mitchell S, Moon E, Kim UJ, Xu JP, Siciliano MJ, Weinshilboum RM. Human 3′-phosphoadenosine 5′-phosphosulfate synthetase 1 (PAPSS1) and PAPSS2: gene cloning, characterization and chromosomal localization. Biochem Biophys Res Commun 2000;268:437–44.
[22] Kurima K, Singh B, Schwartz NB. Genomic organization of the mouse and human genes encoding the ATP sulfurylase/adenosine 5′-phosphosulfate kinase isoform SK2. J Biol Chem 1999;274:33306–12.
[23] Chadwick RB, Conrad MP, McGinnis MD, Johnston-Dow L, Spurgeon SL, Kronick MN. Heterozygote and mutation detection by direct automated fluorescent DNA sequencing using a mutant *Taq* DNA polymerase. Biotechniques 1996;20:676–83.
[24] Nickerson DA, Tobe VO, Taylor SL. PolyPhred: automating the detection and genotyping of single nucleotide substitutions using fluorescence-based resequencing. Nucleic Acids Res 1997;25: 2745–51.
[25] Gordon D, Abajian C, Green P. *Consed*: a graphical tool for sequencing finishing. Genome Res 1998;8:195–202.
[26] Wong GG, Witek JS, Temple PA, Wilkens KM, Leary AC, Luxenberg DP, Jones SS, Brown EL, Kay RM, Orr EC, Shoemaker C, Golde DW, Kaufman RJ, Heurick RM, Wang EA, Clark SC. Human GM-CSF: molecular cloning of the complementary DNA and purification of the natural and recombinant proteins. Science 1985;228:810–5.
[27] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72:248–54.
[28] Xu ZH, Wood TC, Adjei A, Weinshilboum RM. Human 3′-phosphoadenosine 5′-phosphosulfate synthetase (PAPSS): radiochemical enzymatic assay, biochemical properties and hepatic variation. Drug Metab Dispos 2001;29:172–8.
[29] Hartl DL, Clark AG. Principles of population genetics, 3rd ed. Sunderland, MA: Sinauer Associates; 1997. p. 96–106 [chapter 3].
[30] Hedrick PW. Genetics of populations, 2nd ed. Sudbury, MA: Jones & Bartlett; 2000. p. 396–405.
[31] Long JC, Williams RC, Urbanek M. An E-M algorithm and testing strategy for multiple locus haplotypes. Am J Hum Genet 1995;56: 799–810.
[32] Excoffier L, Slatkin M. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evol 1995; 12:921–7.
[33] Halushka MK, Fan JB, Bentley K, Hsie L, Shen N, Weder A, Cooper R, Lipshutz R, Chakravarti A. Patterns of single-nucleotide polymorphisms in candidate genes for blood-pressure homeostasis. Nat Genet 1999;22:239–47.
[34] Xu ZH, Freimuth RR, Eckloff B, Wieben E, Weinshilboum RM. Human 3′-phosphoadenosine 5′-phosphosulfate synthetase 2 (PAPSS2) pharmacogenetics: gene resequencing, genetic polymorphisms and functional characterization of variant allozymes. Pharmacogenetics 2002;12:11–21.
[35] Huttner WB. Sulphation of tyrosine residues—a widespread modification of proteins. Nature 1982;299:273–6.
[36] Hästbacka J, de la Chapelle A, Mahtani MM, Clines G, Reeve-Daly MP, Daly M, Hamilton BA, Kusumi K, Trivedi B, Weaver A. The diastrophic dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage disequilibrium mapping. Cell 1994;78:1073–87.
[37] Lindahl B, Eriksson L, Spillmann D, Caterson B, Lindahl U. Selective loss of cerebral keratan sulfate in Alzheimer's disease. J Biol Chem 1996;271:16991–4.
[38] Kaessmann H, Heissig F, von Haeseler A, Pääbo S. DNA sequence variation in a non-coding region of low recombination on the human X chromosome. Nat Genet 1999;22:78–81.
[39] Drysdale CM, McGraw DW, Stack CB, Stephens JC, Judson RS, Nandabalan K, Arnold K, Ruano G, Liggett SB. Complex promoter and coding region β_2-adrenergic receptor haplotypes alter receptor expression and predict *in vivo* responsiveness. Proc Natl Acad Sci USA 2000;97:10483–8.
[40] Judson R, Stephens JC, Windemuth A. The predictive power of haplotypes in clinical response. Pharmacogenomics 2000;1:15–26.
[41] Dong JF, Hyun W, López JA. Aggregation of mammalian cells expressing the platelet glycoprotein (GP) Ib-IX complex and the requirement for tyrosine sulfation of GP Ibα. Blood 1995;86:4175–83.
[42] Leyh TS. The physical biochemistry and molecular genetics of sulfate activation. Crit Rev Biochem Mol Biol 1993;28:515–42.
[43] Shukla D, Liu J, Blaiklock P, Shworak NW, Bai X, Esko JD, Cohen GH, Eisenberg RJ, Rosenberg RD, Spear PG. A novel role for 3-*O*-sulfated heparan sulfate in herpes simplex virus 1 entry. Cell 1999;99:13–22.