Phenol sulphotransferase *SULT1A1* polymorphism: molecular diagnosis and allele frequencies in Caucasian and African populations

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Sulphation, catalysed by members of the sulphotransferase (SULT) enzyme family, is an important component of the body's chemical defence mechanism, but also acts to bioactivate mutagens such as hydroxylated aryl and heterocyclic amines. A major human sulphotransferase, SULT1A1 (P-PST), metabolizes and/or bioactivates many drugs, iodothyronines and hydroxylated aromatic amines. The enzyme activity varies widely within the population and is under genetic control. We have developed an assay detecting a G → A transition in *SULT1A1* that causes an Arg^{213} → His substitution associated with low SULT activity and altered enzyme properties, and have used it to assess the *SULT1A1* genotype in Caucasian ($n = 293$) and African (Nigerian, $n = 52$) populations. We show that the mutant *SULT1A1*2* allele is present at frequencies of 0.321 and 0.269 in the Caucasian and African populations respectively. We also demonstrate a significant age-related difference in *SULT1A1* genotype within our Caucasian population, with increasing incidence of *SULT1A1*1* homozygosity and decreasing incidence of *SULT1A1*2* homozygosity with increasing age, indicating a potential association of SULT1A1*1 allozyme(s) with protection against cell and/or tissue damage during aging.

INTRODUCTION

Sulphation has evolved as a key step in xenobiotic metabolism and chemical defence, but also has important roles in modulating the biological activity of numerous potent endogenous chemicals including iodothyronines, steroids and catecholamines [1–4]. Sulphation is catalysed by members of the sulphotransferase (SULT) enzyme family, and in humans there are at least seven different SULT isoforms. On the basis of amino acid sequence identity and substrate specificity, the SULT enzyme family can be divided into two subfamilies, commonly called phenol SULTs (family 1) and hydroxysteroid SULTs (family 2) [1,2,4]. Within the human phenol SULT family, there are six characterized isoforms that sulphate a range of xenobiotics, iodothyronines, catecholamines and oestrogens. Probably the major form of phenol SULT in adult human liver is called variously P-PST, TS-PST, HAST1 and now SULT1A1 (at a recent International Sulphotransferase Nomenclature Workshop a new nomenclature was proposed under which this enzyme is renamed SULT1A1; the proposed new nomenclature will be used throughout this paper). This enzyme has a broad substrate specificity, including phenolic xenobiotics and iodothyronines, and we and others have demonstrated that it is capable of the metabolism and bioactivation of promutagenic and/or procarcinogenic hydroxyaryl amines, hydroxyheterocyclic amines and arylhydroxamic acids [5–8]. The SULT1A1 enzyme is encoded by a gene, *SULT1A1* (also called *STP1*), which maps to chromosome 16p12.1–p11.2 and whose sequence has been determined [9–12].

Although usually considered a detoxification reaction, sulphation is an essential step in the bioactivation of many dietary and environmental procarcinogens and promutagens [8,13]. The sulphate esters of, for example, hydroxyarylamines, arylhydroxamic acids and benzylic alcohols of polycyclic aromatic hydrocarbons are normally highly unstable, and decompose spontaneously to form extremely reactive species that adduct to DNA [8,13]. The requirement for sulphation in the activation of such chemicals can be demonstrated *in vivo* and *in vitro* [8,14–18].

Tissue sulphotransferase enzyme activities vary considerably within the human population, and there is good evidence for genetic polymorphisms associated with a number of different SULTs [2,19]. Population and heritability studies indicate a strong genetic influence on the activity and thermal stability of platelet sulphotransferase (normally measured with 4-nitrophenol as substrate) [19–21]. On the basis of the strong correlation between the level of 4-nitrophenol sulphotransferase activity and enzyme protein expression in platelets [22], it is reasonable to assume that most, if not all, of this platelet activity is catalysed by SULT1A1. More recently a number of cDNA clones (from various human tissues) coding for SULT1A1 have been isolated [23–27]; analysis of the amino acid sequences derived from these cDNA species suggests that different SULT1A1 allozymes exist within the population. *SULT1A1* gene sequencing studies by Weinshilboum and co-workers [28] demonstrated that a number of allelic variants of the gene do indeed exist in humans. These workers also showed that platelet homogenates from individuals homozygous for an allele provisionally named *SULT1A1*2* had significantly lower 4-nitrophenol sulphotransferase activity (and thermal stability) than those from individuals displaying other genotypes [28]. The SULT1A1 allozyme coded for by this variant has an arginine → histidine substitution at amino acid 213

Abbreviations used: RFLP, restriction fragment length polymorphism; SULT, sulphotransferase.

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resulting from a G → A transition at nucleotide 638 in the coding region of *SULT1A1* [23,25,28]. This change alters the recognition site for the restriction enzyme *Hae*II. We have recently found that different SULT1A1 allozymes have significantly different kinetic and physical properties (A. M. Hood, R. Dajani and M. W. H. Coughtrie, unpublished work). Taken together, these results suggest that mutations in the gene coding for the SULT1A1 enzyme might affect an individual's capacity and/or efficiency for the sulphation of important endogenous compounds and xenobiotics. This might be particularly relevant for susceptibility to chemical-induced diseases such as adverse drug reactions, and to cancers in which exposure to chemicals activated by sulphation is implicated, including those of the gastrointestinal tract and bladder.

To facilitate investigation of the molecular epidemiology of this enzyme, we have designed a PCR–restriction fragment length polymorphism (PCR–RFLP) assay to detect the G → A transition that results in the $Arg^{213} \rightarrow His$ replacement in SULT1A1 and report here the incidence of *SULT1A1* alleles in Caucasian and African populations.

EXPERIMENTAL

Materials

Oligonucleotide primers were purchased from Cruachem (Glasgow, Scotland, U.K.) and deoxynucleotide triphosphates (dNTPs) were obtained from Pharmacia Biotech (Milton Keynes, Bucks., U.K.). Restriction enzymes and *Taq* polymerase were purchased from Promega (Southampton, Hants., U.K.). Agarose was from Biogene Ltd. (Bolnhurst, Beds., U.K.) and all other reagents were of analytical grade and purchased from either Sigma (Poole, Dorset, U.K.) or Merck Ltd. (Glasgow, Scotland, U.K.).

Subjects and sample preparation

Ethical approval was obtained from the Tayside Committee on Medical Research Ethics and from the North Staffordshire Hospital Ethics Committee. Subjects were either (1) Caucasians resident in the Tayside area of Scotland or the North Staffordshire area of England, both of which are recognized as having a stable population base, or (2) Nigerians, without evidence of disease, from a Yoruba rural community near Ibadan in Oyo State [29]. The Tayside group comprised 187 randomly selected unrelated individuals undergoing routine laboratory haematological analysis in Ninewells Hospital, Dundee [mean age (±S.D.) 53.4±20.9 years, range 12–99; 70.6% male, 29.4% female]. The North Staffordshire group comprised 106 unrelated individuals without evidence of malignancy or inflammatory pathology and comprised in-patients and out-patients undergoing investigation and/or treatment for varicose veins, hernias, haemorrhoids, mild iron-deficiency anaemia, benign breast lumps and tension headaches [mean age (±S.D.) 53.8±17.9 years, range 17–90; 57.6% male, 42.4% female]. Information on age and gender was not available for the 52 unrelated Nigerian individuals.

DNA isolated from white cell lysates from the Tayside population was used in the development of the PCR–RFLP assay and for the initial assessment of allele frequencies. TE [10 mM Tris/HCl (pH 7.6)/1 mM EDTA] (750 μl) was added to 100 μl of whole blood (collected into EDTA), and the mixture was centrifuged at 12000 ***g*** for 1 min. The resulting pellet was washed twice further with 500 μl of TE. White cells were then lysed by the addition of 100 μl of a solution containing 20 mM Tris/HCl, pH 8.0, 50 mM KCl, 2.5 mM $MgCl_2$, 0.45% (v/v) Tween 20, 0.45% (v/v) Nonidet P40 and 200 μg/ml proteinase K, and heating at 55 °C for 20 min. Distilled water (100 μl) was then added, and proteinase K was inactivated by being boiled for 10 min. Lysates were stored at −20 °C until use. For all other samples, genomic DNA was prepared from whole blood (approx. 5 ml, collected into EDTA) by standard procedures [30], resuspended in TE and stored at −20 °C until use.

PCR–RFLP assay for *SULT1A1* genotype analysis

The *SULT1A1* gene is one of three very closely related sulphotransferase genes located on chromosome 16 [2,11]. The open reading frames derived from *SULT1A1* (*STP1*) and its neighbours *SULT1A2* (*STP2*) and *SULT1A3* (*STM*) share more than 94% identity, and thus to design a primer pair specifically to amplify the appropriate region from *SULT1A1* we used intron sequences flanking exon VII. The sequences of the oligonucleotide primers used were 5′-GTTGGCTCTGCAGGGTTTCTAGGA-3′ (1A1VIIF) and 5′-CCCAAACCCCCTGCTGGCCAGC-ACCC-3′ (1A1VIIR). Amplification of template DNA [10 μl of lysates; 1 μl (approx. 200 ng) of genomic DNA] was performed in an incubation mixture (total volume 50 μl) comprising 25 pmol each of 1A1VIIF and 1A1VIIR, 200 μM dNTPs, 2 mM $MgCl_2$ and 0.5 units of *Taq* polymerase in buffer containing 10 mM Tris/HCl, pH 9.0, 50 mM KCl and 0.1% (w/v) Triton X-100. The PCR conditions were: initial denaturation (94 °C for 5 min) followed by 40 cycles of denaturation (94 °C for 30 s), annealing (63 °C for 30 s) and extension (72 °C for 30 s). PCR performed under these conditions resulted in the specific amplification of a 333 bp fragment of DNA including exon VII of the *SULT1A1* gene. PCR reaction products (10 μl) were then incubated with 1 unit of *Hae*II for 60 min in a reaction mixture (total volume 20 μl) containing the appropriate enzyme reaction buffer supplied by the manufacturer (Promega). After digestion, fragments were resolved on 3% (w/v) agarose gels containing ethidium bromide, and detected under UV. The 333 bp fragment containing exon VII yields two fragments of 168 and 165 bp after digestion with *Hae*II, and although these two fragments cannot be resolved on 3% (w/v) agarose gels, homozygotes for both alleles and heterozygotes can be readily identified by this method. Digestion does not take place with DNA fragments amplified from *SULT1A1* alleles harbouring the CGC → CAC change at codon 213, because this alters the restriction site recognition sequence for *Hae*II.

Statistical analysis

Statistical analysis was performed with the StatXact Turbo software package to obtain exact *P* values. Yates's corrected χ^2 and Fisher's exact tests were used, as appropriate, to examine for homogeneity between control groups. The Armitage trend test was used to examine associations between allele frequencies and age group within the Caucasian population.

RESULTS

PCR–RFLP genotyping assay for *SULT1A1* alleles

Careful analysis of the nucleotide sequences of the genes *SULT1A1*, *SULT1A2* and *SULT1A3* allowed the design of oligonucleotide primers that would specifically amplify exon VII of *SULT1A1*, where a single base substitution (G → A) at codon 213 results in the $Arg^{213} \rightarrow His$ amino acid substitution and abolishes the *Hae*II recognition sequence. Incubation of the products from these PCR amplifications with the restriction enzyme, followed by separation of the fragments on agarose gels, facilitated the identification of the genotype for this mutation.

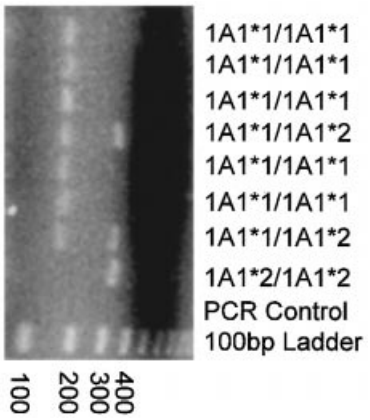

Figure 1 Agarose-gel electrophoresis showing representative results of PCR–RFLP assay for the mutation in exons VII of *SULT1A1*, with five individuals homozygous for the *SULT1A1*1* genotype, one individual homozygous for the *SULT1A1*2* genotype, and two heterozygotes

The control lane shows results from a PCR reaction performed in the absence of template.

Figure 1 shows a representative agarose gel electrophoretogram indicating the fragment migration patterns of individuals homozygous and heterozygous for the *SULT1A1* alleles resulting from the base change in codon 213, within exon VII.

SULT1A1 allele frequencies in Caucasian and Nigerian populations

To assess the frequencies of the *SULT1A1*1* and *SULT1A1*2* alleles within Caucasians, we tested 187 unrelated individuals from the Tayside area of Scotland, and 106 unrelated individuals from the North Staffordshire region of England (Table 1). Additionally, to determine whether there might be ethnic differences in the *SULT1A1* allele frequencies, we genotyped DNA samples from 52 unrelated Nigerians (Table 1). There was no significant difference between the *SULT1A1* allele distributions in the two Caucasian populations; by using the data obtained from a total of 293 individuals we determined the allele frequencies to be 0.679 and 0.321 for *SULT1A1*1* and *SULT1A1*2* respectively. These results are remarkably similar to those from Weinshilboum and co-workers [28], which showed a frequency of 0.313 for the *SULT1A1*2* allele and 0.674 for the *SULT1A1*1* alleles, determined by sequencing the *SULT1A1* genes from a population of 150 Caucasians. The frequency of the *SULT1A1*2* allele was slightly lower in the Nigerian population (0.269) but this was not significantly different from the Caucasian frequency (Fisher's exact test, $P = 0.626$). All allele distributions conformed to the Hardy–Weinberg equilibrium.

Association between *SULT1A1* genotype and age

During analysis of the data from the Caucasian control populations, we identified a strong association between *SULT1A1* genotype and age. Stratification of the data by age showed

Table 1 *SULT1A1* genotype and allele frequencies within the sample Caucasian and Nigerian populations

Correlations: North Staffs. versus Tayside, Pearson $\chi^2_2 = 0.506$, $P = 0.776$; Nigerian versus total Caucasian, Pearson $\chi^2_2 = 1.06$, $P = 0.588$.

	SULT1A1 genotype frequency								
	*1A1*1/1A1*1*		*1A1*1/1A1*2*		*1A1*2/1A1*2*		Allele frequency		
Population source	Number	Percentage	Number	Percentage	Number	Percentage	*1A1*1*	*1A1*2*	Chromosomes tested
Tayside	88	47.0	77	41.2	22	11.8	0.676	0.324	374
North Staffs.	49	46.2	47	44.4	10	9.4	0.684	0.316	212
Total Caucasian	137	46.8	124	42.3	32	10.9	0.679	0.321	586
Nigerian	28	53.8	20	38.5	4	7.7	0.731	0.269	104

Table 2 Age-related variation in *SULT1A1* allele frequencies in Caucasian subjects

Analysis of the allele frequency data revealed a statistically significant increase in incidence of *1A1*1* and decrease in *1A1*2* alleles with increasing age (Armitage Trend Test, $P = 0.017$).

			SULT1A1 genotype frequency								
			*1A1*1/1A1*1*		*1A1*1/1A1*2*		*1A1*2/1A1*2*		Allele frequency		
Age group	Age range (years)	*n*	Number	Percentage	Number	Percentage	Number	Percentage	*1A1*1*	*1A1*2*	Chromosomes tested
1	12–39	82	32	39.0	41	50.0	9	11.0	0.640	0.360	164
2	40–49	54	21	38.9	25	46.3	8	14.8	0.620	0.380	108
3	50–59	40	19	47.5	16	40.0	5	12.5	0.675	0.325	80
4	60–69	49	25	51.0	20	40.8	4	8.2	0.714	0.286	98
5	70–99	68	40	58.8	22	32.4	6	8.8	0.750	0.250	136
Total	12–99	293	137	46.8	124	42.3	32	10.9	0.679	0.321	586

that incidence of the *SULT1A1*1* allele increased, whereas the incidence of the *SULT1A1*2* allele decreased (Table 2). Application of trend analysis (Armitage trend test) confirmed that the trend was statistically significant ($P = 0.017$). A χ^2 analysis also showed that the allele distribution was significantly different between the age groups below and above 60 years of age ($\chi^2_2 = 8.642$, $P = 0.013$). These results suggest that homozygosity for the *SULT1A1*1* allele might predispose to longevity, a finding that deserves further investigation.

DISCUSSION

Genetic polymorphisms in xenobiotic-metabolizing enzymes such as N-acetyltransferases, glutathione S-transferases and cytochromes *P*-450 have been widely implicated in susceptibility to cancer and other diseases. However, little is known of the role of SULT polymorphism in disease susceptibility. To facilitate such investigations we have devised a simple PCR–RFLP assay for determining two major *SULT1A1* genotypes by using lymphocyte DNA as template. The test detects a G → A transition within exon VII of the *SULT1A1* gene that causes an $Arg^{213} \rightarrow His$ substitution in the SULT1A1 enzyme protein. During the preparation of this paper, a similar procedure for genotyping this *SULT1A1* mutation was reported [31]; however, a different pair of primers was used for PCR amplification. These authors found similar allele frequencies (*SULT1A1*1* = 0.682; *SULT1A1*2* = 0.317) to those reported here, although no information on ethnic origin, age or sex for the 52 liver samples genotyped was provided [31]. Studies *in vitro* have shown that SULT1A1 variants with the His^{213} substitution have altered functions (thermolability, decreased substrate affinity and decreased enzyme activity) (see, for example, [28]) and it is therefore possible that this genetic polymorphism might influence individual susceptibility to certain chemical-induced diseases.

Arg^{213} is within a region that is highly conserved between orthologous SULT1A family members from different species (human, monkey, dog, cow, rat and mouse). Examination of the X-ray crystal structure of the mouse oestrogen sulphotransferase mEST (SULT1E1) and comparison with the amino acid sequence of human SULT1A1 show that Arg^{213} is conserved between the two proteins; in the mouse SULT1E1 it is located in the loop joining the α-10 and α-11 helices [32]. Although the structure of human SULT1A1 has not yet been solved, the protein fold is predicted to have a strong resemblance to that of mouse SULT1E1 and it is reasonable to assume that Arg^{213} will be similarly located in the structure. Substitution of a histidine at this position might either alter the conformation of this region of the molecule, including the α-12 helix, which contains a residue (Phe^{229}) shown to be involved in PAPS binding [32], or have an effect on the local chemistry in the vicinity of the enzyme's active site, either of which could explain the observed effects of the $Arg^{213} \rightarrow His$ mutation on enzyme function and thermostability. Confirmation of this hypothesis will require detailed enzymological characterization of the SULT1A1*1 and SULT1A1*2 allozymes coupled with three-dimensional structure information from the human SULT1A family; these experiments are under way in our laboratory.

Using the PCR–RFLP assay we established *SULT1A1*1* and *SULT1A1*2* allele frequencies in control populations from two different ethnic groups, Caucasian and African (Nigerian), and found no differences in allele distribution between the groups. This is in contrast with other xenobiotic-metabolizing enzymes: substantial ethnic differences in genotype (and phenotype) have been observed for members of the *GST* and *CYP* families [29,33–36]. This suggests that the *SULT1A1*1* and *SULT1A1*2* alleles have an ancient evolutionary origin. The allele frequencies that we determined (0.321 for *SULT1A1*2* and 0.679 for *SULT1A1*1*) were identical with those obtained from segregation analysis of family data obtained from biochemical studies conducted with platelet SULT1A1 enzyme thermostability (assessed with 4-nitrophenol as substrate) [20,21]. It is worth stressing that, although biochemical and genetic analyses separated by more than 10 years and conducted in different laboratories have produced exactly the same result, platelets do not necessarily reflect accurately the overall sulphation capacity for individual compounds. This is principally because (1) platelets express a different complement of SULT enzymes from other tissues, with evidence so far for only the expression of the SULT1A1 and SULT1A3 (M-PST) enzymes, and (2) the capacity of platelets for sulphation *in vivo* has not been conclusively demonstrated or quantified.

A particularly interesting observation was that there was a significant age effect associated with the *SULT1A1* genotype within the Caucasian population. The proportion of *SULT1A1*1* homozygotes rose from 39 % in the age group under 40 years to 59 % in the age group over 70 years, with a corresponding decrease in the proportion of individuals homozygous for *SULT1A1*2*. The results suggest that the SULT1A1*1 allozyme might exert a protective effect during aging and that this might reflect a greater potential of this form for detoxification of unknown dietary, environmental and/or endogenous chemicals that contribute towards cell and tissue damage. Information about subject age was unfortunately not available for the Nigerian samples (and the population size was too small for meaningful age stratification), so it is not possible to say whether this phenomenon also occurs in populations other than the Caucasian one studied here. It is, however, advisable that age be considered in future case-control studies of *SULT1A1* genotype. Our results certainly support the need for further investigation of this phenomenon in a larger healthy aging population to determine whether 'rapid sulphator' status contributes substantially to longevity.

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