

Gene–environment interaction: the role of *SULT1A1* and *CYP3A5* polymorphisms as risk modifiers for squamous cell carcinoma of the oesophagus

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An imbalance in the activities of enzymes involved in the metabolism, conjugation and transport of xenobiotics may account for the variability in susceptibility to the development of complex diseases such as cancer between different population groups. In this study we investigated a functional polymorphism in the *SULT1A1* gene in 245 patients and 288 controls. Previous studies have shown that the 638G→A polymorphism that results in the substitution of arginine by histidine at codon 213 (*SULT1A12) results in decreased *SULT1A1* activity. The same group of samples used in this study had been previously genotyped for *CYP3A5* genetic polymorphisms. Among Black subjects the burning of wood or charcoal for cooking and keeping warm was significantly associated with increased risk for oesophageal cancer (OC) (AOR, 15.2; $P = 0.001$) as was the consumption of home-brewed beer (AOR, 6.97; $P = 0.0001$). Among the Mixed Ancestry group, tobacco smoking combined with alcohol consumption were significantly associated with higher risk for OC (AOR, 5.18; $P = 0.0005$). In both Blacks and Mixed Ancestry subjects, starting to smoke below the age of 20 years was associated with significantly increased risk for OC (AOR, 3.5 among the Blacks and AOR, 12 among the Mixed Ancestry). The homozygous *SULT1A1**2/*2 genotype was associated with increased risk for OC among smokers. The *SULT1A1**2/*2 genotype in combination with the *CYP3A5* heterozygous genotypes was associated with significantly increased risk for OC (AOR, 3.60; $P = 0.001$) with the risk being even higher among smokers compared with non-smokers. The above findings confirm the association between alcohol consumption and tobacco smoking with increased risk for OC. The genotype results show that *SULT1A1**2/*2 genotype is associated with increased risk for OC among subjects exposed to tobacco-smoke-related carcinogens.**

Abbreviations: AOR, adjusted odds ratios; CB, commercial beer; HB, home brewed beer; WS, wine and spirits; OC, oesophageal cancer; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SCC, squamous cell carcinoma.

Introduction

Oesophageal cancer (OC) is ranked as the eighth highest cancer in the world, but it is the second highest malignancy among Black South African males (1,2). The incidence of squamous cell carcinoma (SCC) of the oesophagus in some regions of South Africa is among the highest in the world (3). It has been observed that SCC of the oesophagus is more prevalent in the so-called maize consuming belt, which runs through Southern, Central and Eastern Africa, parts of the Middle East, south of China and parts of Brazil (4–6). Incidentally, these are also all ‘developing countries’. The other type of OC, adenocarcinoma is commonly found in the developed world. A variety of risk factors for SCC of the oesophagus have been proposed, these include environmental factors such as tobacco smoking, alcohol consumption (7–9), exposure to aflatoxin-contaminated and fumonisin-contaminated maize (10–13), and genetic factors such as somatic mutations in several genes, polymorphism in the androgen receptor gene (14,15), polymorphisms in *CYP2A6*, *CYP2E1* and *CYP3A5* (5,16,17), HPV infection (18) and the use of emetics. Mutations in the *p53* gene, which have been reported in most cancers, have been observed to be rare among South African breast cancer (19) and OC patients (20).

Sulfate conjugation is a key pathway in the metabolism of a broad range of compounds such as phenolic xenobiotics, catecholamines, hydroxylated aromatic amines, certain classes of drugs, neurotransmitters and oestrogenic hormones. Sulfation results in either inactivation or metabolic activation of compounds (21–23). Sulfation is catalysed by sulfotransferases encoded by members of the *SULT* gene superfamily (24,25). Data obtained in studies using recombinant proteins indicated that *SULT1A1* is probably responsible for the bulk of *SULT* activity in tissues (26).

A known single nucleotide polymorphism in *SULT1A1*, 638G→A, results in the substitution of an arginine at position 213 with histidine giving rise to *SULT1A1**2. The *SULT1A1**2 variant has reduced enzyme activity and stability compared with the wild-type enzyme (24,26). These biological properties are particularly relevant to chemically-induced diseases such as cancer, where there is a strong association with cigarette smoking and alcohol consumption. The role of the *SULT1A1**2 allele in cancers is not well understood, but the few studies done to date show different results for different malignancies. For example, while *SULT1A1**2 allele was not associated with risk for colorectal cancer (27), Zheng *et al.* (28) and Han *et al.* (29) observed an association between the *SULT1A1**2/*2 genotype and increased risk for breast cancer among Caucasian and Chinese women. The same genotype (*SULT1A1**2/*2) was reported to be associated with reduced risk of bladder cancer (30).

The role of *SULT1A1**2 in OC was investigated because *SULT1A1* enzyme is important in the second step of carcinogen metabolism in living organisms. The *SULT1A1* genotype

data was combined with those of CYP3A5 genotypes on the same patients and controls (17) because both *SULT1A1* (21,26) and CYP3A5 (31,32) are expressed in the oesophagus. We report a strong association between increased risk of SCC of the oesophagus and the *SULT1A1*2*2* genotype. In addition, there was a stronger risk of SCC of the oesophagus with the *SULT1A1*2*2* genotype combined with the CYP3A5 heterozygous genotypes especially among tobacco smokers.

Materials and methods

Patients were recruited from those admitted to the Cardio–Thoracic Division at Groote Schuur Hospital for dysphagia and only those patients with histologically confirmed SCC were included in the study ($n = 245$). Age-matched healthy controls ($n = 288$) were recruited from the same geographical location as the patients and not from the hospital. The patients and controls were recruited between 1997 and 2003. Written consent was obtained before volunteers were enrolled into the study. All subjects completed a questionnaire that contained questions on demographics (age, sex and race), smoking habits, alcohol consumption and family history of cancer. The Black subjects were mainly Xhosa-speaking South Africans who originally came from either the Transkei area of the Eastern Cape or Western Cape. The Mixed Ancestry individuals (sometimes referred to as ‘coloureds’) are a mixture of nationalities resulting from gene flow between Black South Africans, Western Europeans, the San, the Khoikhoi, Indonesians and Malaysians who settled in the Cape from the 17th century. Information on cooking and heating sources during the last 20 years was recorded and smokers were classified as individuals who smoked at least one cigarette per day for at least 1 year. Subjects were also defined as alcohol consumers if they took alcohol regularly (at least once every week). The quantities of alcohol consumed and tobacco smoked were not quantified because the subjects consumed different types of brews with varying and sometimes unknown concentrations of alcohol. The same applied to tobacco since many patients, especially among the Black subjects, smoked home-rolled cigarettes, which may vary with respect to amount of tobacco. After the interview, 10 ml of blood was withdrawn from each patient and stored at -20°C until isolation of DNA. Genotyping for *SULT1A1* was possible in 503 subjects (237 patients and 266 controls). This study was approved by the University of Cape Town Human Ethics Committee.

Genotyping the *SULT1A1* alleles

The *SULT1A1* polymorphism, 638G→A, in exon 7 was determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis according to the method of Coughtrie *et al.* (33) with minor modifications. The primers 5′-GTT GGC TCT GCA GGGTTT CTA GGA-3′ and 5′-CCC AAA CCC CCT GCT GGC CAG CAC CC-3′ were used to amplify a 333 bp fragment using the following conditions; initial denaturation at 94°C for 5 min, followed by 40 cycles at 94°C for 30 s, 63°C for 30 s and 72°C for 30 s. A final extension step at 72°C for 5 min completed the reaction. Each PCR reaction contained 100 ng of genomic DNA in $1\times$ PCR buffer, 1.5 mM MgCl_2 , 0.2 mM dNTP mixture, 0.2 μM of *SULT1A1* primers and 1 U *Taq* DNA polymerase (Biotaq™) in a final volume of 50 μl . The amplified products were digested with *Hae*II to generate 168 and 165 bp fragments in the wild-type and a single undigested 333 bp fragment in the mutant and visualized by electrophoresis in ethidium bromide-stained 2% agarose gels. The heterozygous genotype was identified by the presence of the 333, 168 and 165 bp fragments. The same subjects genotyped for *SULT1A1* were also previously genotyped for CYP3A5 and CYP2E1 (16,17).

Statistical analysis

Genotype frequencies and Hardy–Weinberg analyses were performed using the χ^2 -test. The χ^2 -test and logistical regression analysis were used to compare the distribution of *SULT1A1* genotypes and allele frequencies between patients and controls, to calculate the adjusted odds ratios (AOR) and the 95% confidence interval (95% CI) and to estimate the associations between OC risks in the various genotypes. Multivariate logistic regression analysis eliminated the effects of potential confounders such as age, sex, alcohol consumption and tobacco smoking. Analysis was also done on the effects of exposure to what the subjects used for cooking and keeping warm in the last 20 years (electricity, paraffin, wood, gas and charcoal). The use of electricity was treated as the reference whereas wood and charcoal were combined. Alcohol consumption was classified into the different types of alcohol consumed as follows; commercial beer (CB), home brewed beer (HB), wine (W) and spirits (S). The wines and spirits categories were combined into one, WS, because of the low number of consumers. The patients and controls from the Black and Mixed Ancestry subjects were only pooled for the combined

analysis of the *SULT1A1* genotypes and the CYP3A5 genotypes and only subjects who were genotyped for both were included in the calculations. The contribution of the different *SULT1A1* and CYP3A5 genotype combinations to OC risk was also calculated. The risk of OC with respect to each genotype combination was calculated as follows:

$$\text{Risk} = \frac{\text{Frequency of patients with genotype combination A}}{\text{Frequency of controls with genotype combination A}}$$

For all statistical analyses, P -value < 0.05 was considered statistically significant.

Results

Table I shows the distribution of the demographic characteristics of the two groups in the South African population. There was no difference in the average age of patients and controls among the Black subjects but the controls among the Mixed Ancestry subjects were on average older than the patients. The exposures associated with increased risk for SCC of the oesophagus among the Mixed Ancestry included sex (AOR, 2.71; $P = 0.007$), tobacco smoking (AOR, 3.59; $P = 0.006$), alcohol consumption (AOR, 2.37; $P = 0.01$) and both smoking and alcohol consumption (OR, 5.18; $P = 0.0005$). Among the Black subjects, the use of wood or charcoal for cooking and keeping warm was associated with significantly increased risk for OC (AOR, 15.2; $P = 0.0001$). Most of the Mixed Ancestry patients were tobacco smokers (93%) compared with 65% among Black patients.

The risks of OC associated with tobacco smoking and alcohol consumption are shown in Table II. Starting to smoke at an early age (20 years of age or less), which implies longer exposure time was associated with increased risk for OC among both the Blacks (AOR, 3.5; $P = 0.001$) and the Mixed Ancestry (AOR, 12; $P = 0.002$). Generally, smoking >10 cigarettes was associated with increased risk for SCC of the oesophagus, which was significant among the Mixed Ancestry group (AOR, 3.49; $P = 0.021$). The use of pipes was associated with increased risk of OC among both the Black subjects (AOR, 2.81; $P = 0.004$) and the Mixed Ancestry subjects (AOR, 6.93; $P = 0.038$). Consuming commercial beer was associated with increased risk among the Mixed Ancestry group whereas home brew was associated with increased risk among the Black subjects (AOR, 6.97; $P = 0.0001$). There were few people who used snuff, thus the corresponding statistical analyses were not significant.

The distributions of *SULT1A1* genotypes among the Blacks and the Mixed Ancestry in the different risk categories are shown in Tables III and IV. As calculated from these two Tables, *SULT1A1*2* allelic variant occurred at frequencies of 42 and 40% in patients and 37 and 29% in controls among Blacks and the Mixed Ancestry, respectively. There was a higher frequency of *SULT1A1*2*2* genotype in the patients compared with controls among the Black subjects (32 versus 20%) (Table III) and among the mixed ancestry (31 versus 14%) (Table IV). The *SULT1A1*2*2* genotype was associated with increased risk for OC among tobacco smoking Black subjects (AOR, 2.12; $P = 0.046$), among the Mixed Ancestry (AOR, 2.28; $P = 0.045$) and among males of Mixed Ancestry origin (AOR, 3.39; $P = 0.032$). There were more patients with the homozygous *SULT1A1* genotype among alcohol consumers, tobacco smokers and males in the two groups (Table III and IV).

Table V shows the various combinations of genotypes observed between *SULT1A1* and CYP3A5 with the two

Table I. Distribution of demographic characteristics and potential confounding factors among OC patients and controls in two South African populations

Characteristics	Black subjects		Mixed ancestry	
	Patients <i>n</i> (freq)	Controls <i>n</i> (freq)	Patient <i>n</i> (freq)	Controls <i>n</i> (freq)
Age (in years)				
Mean ± SEM	61.23 ± 0.86	61.85 ± 0.71	61.49 ± 1.22	69.53 ± 0.95
≤60	70 (0.52)	106 (0.55)	51 (0.51)	11 (0.12)
>60	75 (0.48)	88 (0.45)	49 (0.49)	83 (0.88)
AOR ^a (95% CI; <i>P</i> -value)	1.34 (0.86–2.10; 0.192)		0.15 (0.07–0.33; 0.043)	
Sex				
Females	60 (0.41)	83 (0.43)	22 (0.22)	49 (0.52)
Males	85 (0.59)	111 (0.57)	78 (0.78)	45 (0.48)
AOR ^a (95% CI; <i>P</i> -value)	1.02 (0.62–1.66; 0.947)		2.71 (1.32–5.55; 0.007)	
Energy source for cooking and heating in the last 20 years				
Electricity	36 (0.36)	174 (0.90)	52 (0.65)	69 (0.73)
Wood and charcoal	63 (0.64)	20 (0.10)	28 (0.35)	25 (0.27)
AOR ^a (95% CI; <i>P</i> -value)	15.2 (8.15–28.2; 0.001)		1.19 (0.60–2.34; 0.617)	
Tobacco smoking				
None	50 (0.35)	71 (0.37)	7 (0.07)	20 (0.21)
Yes	95 (0.65)	123 (0.63)	93 (0.93)	74 (0.79)
AOR ^b (95% CI; <i>P</i> -value)	1.06 (0.63–1.79; 0.822)		3.59 (1.44–8.95; 0.006)	
Alcohol consumption				
None	47 (0.32)	67 (0.35)	27 (0.27)	49 (0.52)
Yes	98 (0.68)	127 (0.65)	73 (0.73)	45 (0.48)
AOR ^c (95% CI; <i>P</i> -value)	1.07 (0.63–1.81; 0.873)		2.37 (1.23–4.57; 0.01)	
Cigarette smoking/alcohol consumption				
No/No	36 (0.25)	44 (0.23)	6 (0.06)	19 (0.20)
Yes/No	11 (0.08)	23 (0.12)	21 (0.21)	30 (0.32)
No/Yes	14 (0.10)	27 (0.14)	1 (0.01)	1 (0.01)
Yes/Yes	84 (0.58)	100 (0.52)	72 (0.72)	44 (0.47)
OR ^d	0.58 (0.25–1.34; 0.210)		2.22 (0.77–6.30; 0.142)	
OR ^e	0.63 (0.29–1.38; 0.251)		-	
OR ^f	1.03 (0.61–1.74; 0.922)		5.18 (1.97–13.6; 0.0005)	

SEM, standard error of mean. AOR, adjusted odd ratio; OR, odds ratio; The odds ratios for the combined smoking and alcohol consumption groups (cigarette smoking/alcohol consumption).

^aAdjusted for alcohol consumption and tobacco smoking.

^bAdjusted for alcohol consumption only.

^cAdjusted for tobacco smoking only.

^dOR (Yes/No versus No/No).

^eOR (No/Yes versus No/No).

^fOR (Yes/Yes versus No/No).

groups combined. The analysis was done on all the subjects combined (Blacks and Mixed Ancestry) because individual analysis had no statistical power. The various genotype combinations were grouped according to expected enzyme activity. The heterozygous CYP3A5 genotype, *CYP3A5**1/*X (which refers to the sum of the following CYP3A5 genotypes; *CYP3A5**1/*2, *1/*3, *1/*4, *1/*5, *1/*6 and *1, *7) in combination with the homozygous *SULT1A1**2/*2 genotype was associated with increased risk of SCC of the oesophagus (OR, 3.60; *P* = 0.001). This CYP3A5/SULT1A1 genotype combination is associated with normal CYP3A5 activity and severely reduced SULT1A1 activity.

The homozygous wild-type and heterozygous genotypes were combined for each of the genes (CYP3A5 and SULT1A1) and classified as normal activity (Table V), whereas the homozygous mutant genotypes were classified as abolished for CYP3A5 or reduced for SULT1A1. The combination having genotypes coding for normal CYP3A5 normal activity and reduced SULT1A1 activity was associated with increased risk of SCC of the oesophagus (AOR, 2.21; *P* = 0.0009). The risk associated with the above combination (normal CYP3A5 activity/reduced SULT1A1 activity) was greater among smokers (OR, 3.17; *P* = 0.003) compared

with non-smokers (OR, 1.52; *P* = 0.295). Figure 1 shows a comparison of the risk conferred by each of the CYP3A5/SULT1A1 haplotype combinations among tobacco smokers and non-smokers. The highest risk for SCC of the oesophagus was conferred by the genotype combination B (which represents normal CYP3A5 activity in combination with reduced SULT1A1 activity) (Risk = 2.78) whereas the lowest risk was associated with combination C (which represents absence of CYP3A5 activity and normal SULT1A1 activity).

Discussion

The cause of the high incidence of SCC of the oesophagus among Black South Africans is not known. Several risk factors such as exposure to aflatoxin/fumonisin contaminated maize, the use of iron pots in cooking and brewing of home beers, the practice of induced vomiting as a cleansing ritual have been proposed (11–13), but the effects of genetic polymorphisms in xenobiotic metabolizing enzymes have not been extensively studied in this population (16,17,34).

Our results show that after classification of subjects according to type of alcohol consumed or type of tobacco smoked, smoking of pipes, burning of wood or charcoal for cooking or

Table II. Risk of OC associated with alcohol consumption and tobacco smoking in the two South African groups

Groups	Tobacco smoking and alcohol consumption							
	Age at start of smoking (years)*				Amount smoked (cigarettes or pipes)*			
	None n (freq)	≤20 n (freq)	>20 and ≤50 n (freq)	>50 n (freq)	None n (freq)	<10/day n (freq)	≥10/day n (freq)	
Black subjects								
Patients	29 (0.31)	36 (0.39)	27 (0.30)	0	50 (0.35)	58 (0.40)	36 (0.25)	
Controls	71 (0.39)	23 (0.13)	84 (0.46)	6 (0.03)	71 (0.37)	85 (0.44)	38 (0.19)	
AOR; <i>P</i> -value	1.0	3.5; 0.001	0.7; 0.333	—	1.0	0.94; 0.819	1.29; 0.447	
Mixed ancestry								
Patients	6 (0.07)	64 (0.78)	12 (0.15)	0	7 (0.07)	33 (0.33)	60 (0.60)	
Controls	20 (0.22)	46 (0.51)	24 (0.26)	1 (0.01)	20 (0.21)	44 (0.47)	30 (0.32)	
AOR; <i>P</i> -value	1.0	12; 0.002	2.8; 0.066	—	1.0	1.43; 0.500	3.49; 0.021	
	Type of tobacco smoked*				Type of beer*			
	None n (freq)	Cigarettes n (freq)	Snuff n (freq)	Pipes n (freq)	None n (freq)	CB n (freq)	HB n (freq)	WS n (freq)
Black subjects								
Patients	50 (0.35)	50 (0.35)	3 (0.02)	41 (0.28)	47 (0.32)	24 (0.17)	70 (0.48)	4 (0.03)
Controls	71 (0.37)	92 (0.47)	11 (0.06)	20 (0.10)	67 (0.33)	65 (0.34)	16 (0.08)	46 (0.24)
AOR; <i>P</i> -value	1.00	0.74; 0.316	0.38; 0.157	2.81; 0.004	1.00	0.58; 0.105	6.97; 0.0001	0.14; 0.0001
Mixed ancestry								
Patients	7 (0.07)	82 (0.82)	3 (0.03)	8 (0.08)	27 (0.27)	29 (0.29)	4 (0.04)	40 (0.40)
Controls	20 (0.21)	72 (0.77)	0	2 (0.02)	49 (0.52)	12 (0.13)	4 (0.04)	29 (0.31)
AOR; <i>P</i> -value	1.00	3.25; 0.012	—	6.93; 0.038	1.00	3.55; 0.004	1.44; 0.633	2.01; 0.059

OR, odds ratio; AOR, adjusted odds ratio. Groups classified according to alcohol consumption characteristics had their odds ratios adjusted for tobacco smoking whereas the groups classified according to tobacco smoking parameters had their odds ratios adjusted for alcohol consumption. CB, commercial brew; HB, home brew and WS, wine and or spirits.

*The total number of patients in groups in this table are less than those in Table I because some subjects did not complete some of the relevant sections thus they were excluded from these analyses.

heating, and consumption of home brewed beer were associated with increased risk of SCC of the oesophagus among the Black subjects. The finding of significant associations between home brewed beer and exposure to burning wood or charcoal among the Black subjects could partially explain the high incidence of SCC of the oesophagus among this group compared with the Mixed Ancestry who are not exposed to the above. It will be important to identify any causative agent either in the home brew or burnt wood in order make definitive statements concerning these two risk factors. If indeed the causative agent is found in home brew then this would be supported by the observation that among the Blacks, beer is mostly consumed by males who are also at higher risk of SCC of the oesophagus. SCC of the oesophagus, however, has been reported mostly among the least developed countries with predominance over the maize consuming populations. The general association between the risk for developing SCC of the oesophagus and both commercial and home-brewed beer confirms a possible role for alcohol especially its carcinogenic metabolite, acetaldehyde (9). It is, therefore, important to also investigate the role of polymorphisms in the alcohol metabolizing genes such as alcohol dehydrogenases 2 and 3 (ADH2 and ADH3) and aldehyde dehydrogenase 2 (ALDH2) in this population.

Our observation of different types of tobacco or alcoholic beverages conferring different levels of risk to SCC of the oesophagus is comparable with the findings of others (35–38). In general, our results show that tobacco smoking, alcohol consumption and burning of wood or charcoal for

cooking or heating are associated with increased risk of developing SCC of the oesophagus. The risk for SCC of the oesophagus was more dependent on length of exposure as subjects who started smoking at an early age had the highest risks and this is comparable with the findings of Launoy *et al.* (39) in a French study.

In this study, the *SULT1A1**2 allele was investigated in two South African populations. Sulfation plays an important role in the detoxification and bioactivation of many dietary and environmental mutagens including heterocyclic amines and polycyclic aromatic hydrocarbons, which are components of tobacco smoke, overcooked meat, burning of wood or charcoal and environmental pollution (29). The frequency of *SULT1A1**2 variant of 37% among the South African Black controls is higher than that reported among Nigerians (25%) (33), African Americans (25%) (40) and Egyptians (14%) (41). The frequency of the *SULT1A1**2 variant of 29% among Mixed Ancestry is comparable with those in published studies but much higher than that of the Chinese (8%) (40). There are conflicting reports on the role of *SULT1A1**2/*2 in different malignancies, with some studies associating it with reduced or no risk for colorectal, oesophageal and breast cancer (27, 42–44), whereas others have reported increased risk of breast cancer among post-menopausal women (28). In this study we report a significant association between *SULT1A1**2/*2 genotype with increased risk for SCC of the oesophagus among males compared with females. The results of this study support the finding by Wu *et al.* (44) of a significantly increased risk of OC among carriers of the *SULT1A1**2 variant

Table III. Distribution of SULT1A1 genotypes in OC patients and controls amongst the Black South African group

SULT1A1 genotype	Patients <i>n</i> (freq)	Controls <i>n</i> (freq)	AOR (<i>P</i> -value)
All subjects			
<i>SULT1A1</i> *1/*1	67 (0.47)	80 (0.47)	1.00
<i>SULT1A1</i> *1/*2	30 (0.21)	57 (0.33)	0.63 (0.102) ^a
<i>SULT1A1</i> *2/*2	45 (0.32)	35 (0.20)	1.60 (0.100) ^a
Tobacco smoking			
No			
<i>SULT1A1</i> *1/*1	24 (0.49)	26 (0.38)	1.00
<i>SULT1A1</i> *1/*2	7 (0.14)	23 (0.34)	0.29 (0.020) ^b
<i>SULT1A1</i> *2/*2	18 (0.37)	19 (0.28)	1.00 (0.990) ^b
Yes			
<i>SULT1A1</i> *1/*1	43 (0.46)	54 (0.52)	1.00
<i>SULT1A1</i> *1/*2	23 (0.25)	34 (0.33)	0.85 (0.4–1.7; 0.63)
<i>SULT1A1</i> *2/*2	27 (0.29)	16 (0.15)	2.12 (1.0–4.4; 0.046)
Alcohol consumption			
No			
<i>SULT1A1</i> *1/*1	20 (0.44)	24 (0.39)	1.00
<i>SULT1A1</i> *1/*2	9 (0.20)	24 (0.39)	0.44 (0.102) ^c
<i>SULT1A1</i> *2/*2	16 (0.36)	14 (0.22)	1.30 (0.583) ^c
Yes			
<i>SULT1A1</i> *1/*1	47 (0.48)	56 (0.51)	1.00
<i>SULT1A1</i> *1/*2	21 (0.22)	33 (0.30)	0.72 (0.347) ^c
<i>SULT1A1</i> *2/*2	29 (0.30)	21 (0.19)	1.74 (0.166) ^c
Both smoking and alcohol consumption			
No to both			
<i>SULT1A1</i> *1/*1	15 (0.43)	15 (0.36)	1.00
<i>SULT1A1</i> *1/*2	7 (0.20)	16 (0.38)	0.44 (0.1–1.4; 0.155)
<i>SULT1A1</i> *2/*2	13 (0.37)	11 (0.26)	1.18 (0.4–3.5; 0.761)
Yes to both			
<i>SULT1A1</i> *1/*1	38 (0.46)	45 (0.54)	1.00
<i>SULT1A1</i> *1/*2	21 (0.25)	26 (0.31)	0.96 (0.5–2.0; 0.903)
<i>SULT1A1</i> *2/*2	24 (0.29)	13 (0.15)	2.19 (1.0–4.9; 0.056)
Sex			
Females			
<i>SULT1A1</i> *1/*1	26 (0.46)	36 (0.46)	1.00
<i>SULT1A1</i> *1/*2	13 (0.23)	23 (0.29)	0.85 (0.705) ^a
<i>SULT1A1</i> *2/*2	18 (0.31)	20 (0.25)	1.40 (0.430) ^a
Males			
<i>SULT1A1</i> *1/*1	41 (0.48)	44 (0.47)	1.00
<i>SULT1A1</i> *1/*2	17 (0.20)	34 (0.37)	0.53 (0.087) ^a
<i>SULT1A1</i> *2/*2	27 (0.32)	15 (0.16)	1.94 (0.088) ^a

All odds ratios are calculated with the homozygous wild-type as the reference.

^aAOR adjusted for tobacco smoking and alcohol consumption.

^bAOR adjusted for alcohol consumption.

^cAOR adjusted for tobacco smoking.

in Taiwanese men. The observation of increased risk of OC among male carriers of the *SULT1A1**2/*2 genotype compared with females with the same genotype could be due to males having a higher exposure to certain carcinogens possibly through lifestyle (e.g. consuming home brewed beer) and failing to carry out detoxification. In the two South African populations studied in this report, the *SULT1A1**2/*2 homozygous genotype was more prevalent among patients compared with controls. This could reflect a generally defective sulfation pathway being associated with increased risk of OC.

The SULT1A1 genotype data need to be explained together with other polymorphisms in xenobiotic metabolizing enzymes to get a better understanding of their interaction. The SULT1A1 genotype data were combined with that of CYP3A5 (17) because both analyses were done on the same subjects. The following combinations (i) heterozygous *CYP3A5**1/**X* (which refers to the sum of the following

Table IV. Distribution of SULT1A1 genotypes in OC patients and controls amongst the Mixed Ancestry South African group

SULT1A1 genotype	Patients <i>n</i> (freq)	Controls <i>n</i> (freq)	AOR (<i>P</i> -value)
All subjects			
<i>SULT1A1</i> *1/*1	48 (0.51)	52 (0.55)	1.00
<i>SULT1A1</i> *1/*2	17 (0.18)	29 (0.31)	0.50 (0.073) ^a
<i>SULT1A1</i> *2/*2	29 (0.31)	13 (0.14)	2.28 (0.045) ^a
Tobacco smoking			
No			
<i>SULT1A1</i> *1/*1	3 (0.50)	15 (0.75)	1.00
<i>SULT1A1</i> *1/*2	1 (0.17)	2 (0.10)	—
<i>SULT1A1</i> *2/*2	2 (0.33)	3 (0.15)	—
Yes			
<i>SULT1A1</i> *1/*1	45 (0.51)	37 (0.50)	1.00
<i>SULT1A1</i> *1/*2	16 (0.18)	27 (0.37)	0.45 (0.045) ^b
<i>SULT1A1</i> *2/*2	27 (0.31)	10 (0.13)	2.20 (0.075) ^b
Alcohol consumption			
No			
<i>SULT1A1</i> *1/*1	12 (0.52)	31 (0.63)	1.00
<i>SULT1A1</i> *1/*2	2 (0.09)	13 (0.27)	0.33 (0.190) ^c
<i>SULT1A1</i> *2/*2	9 (0.39)	5 (0.10)	—
Yes			
<i>SULT1A1</i> *1/*1	36 (0.51)	21 (0.47)	1.00
<i>SULT1A1</i> *1/*2	15 (0.21)	16 (0.35)	0.54 (0.173) ^c
<i>SULT1A1</i> *2/*2	20 (0.28)	8 (0.18)	1.48 (0.434) ^c
Both smoking and alcohol consumption			
No to both			
<i>SULT1A1</i> *1/*1	2 (0.40)	15 (0.79)	1.00
<i>SULT1A1</i> *1/*2	1 (0.20)	2 (0.10)	—
<i>SULT1A1</i> *2/*2	2 (0.40)	2 (0.11)	—
Yes to both			
<i>SULT1A1</i> *1/*1	35 (0.50)	21 (0.48)	1.00
<i>SULT1A1</i> *1/*2	15 (0.21)	16 (0.36)	0.56 (0.2–1.4; 0.204)
<i>SULT1A1</i> *2/*2	20 (0.29)	7 (0.16)	1.71 (0.6–4.7; 0.299)
Sex			
Females			
<i>SULT1A1</i> *1/*1	14 (0.74)	29 (0.59)	1.00
<i>SULT1A1</i> *1/*2	0 (0.00)	12 (0.25)	—
<i>SULT1A1</i> *2/*2	5 (0.26)	8 (0.16)	—
Males			
<i>SULT1A1</i> *1/*1	34 (0.45)	23 (0.51)	1.00
<i>SULT1A1</i> *1/*2	17 (0.23)	17 (0.38)	0.66 (0.345) ^a
<i>SULT1A1</i> *2/*2	24 (0.32)	5 (0.11)	3.39 (0.032) ^a

All odds ratios are calculated with the homozygous wild-type as the reference.

^aAOR adjusted for tobacco smoking and alcohol consumption.

^bAOR adjusted for alcohol consumption.

^cAOR adjusted for tobacco smoking.

CYP3A5 genotypes, *1/*2, *1/*3, *1/*4, *1/*5, *1/*6, and *1/*7) and homozygous *SULT1A1**2/*2 and (ii) homozygous *CYP3A5**1/*1 and *SULT1A1**2/*2 were associated with significantly increased risk of SCC of the oesophagus, confirming a possible role of CYP3A5 mediated bioactivation of carcinogens to reactive intermediates that may accumulate in the absence of SULT1A1 activity (*SULT1A1**2/*2). The risks associated with the above CYP3A5/SULT1A1 genotype combinations are greater among tobacco smokers compared with non-smokers, further confirming that some of the carcinogens could be components of tobacco smoke. This conclusion is strengthened by the observation that haplotypes that result in the absence of CYP3A5 activity in combination with reduced or normal SULT1A1 activity are associated with lower risks of OC compared with haplotype coding for normal CYP3A5 and reduced SULT1A1 activity (Table V and Figure 1). Being homozygous for mutant CYP3A5 alleles (i.e. *2/*2, *2/*3,

Table V. The distribution of the combinations of SULT1A1 and CYP3A5 genotypes in the combined South African population

Genotype combination	Patients <i>n</i> (freq)	Controls <i>n</i> (freq)	OR (95% CI) <i>P</i> -value
CYP3A5/ SULT1A1			
3A5*1/*1/1A1*1/*1	35 (0.15)	45 (0.17)	1.00
3A5*1/*1/1A1*1/*2	16 (0.07)	30 (0.12)	0.69 (0.33–1.44) 0.324
3A5*1/*1/1A1*2/*2	20 (0.09)	19 (0.07)	1.35 (0.63–2.89) 0.439
3A5*1/*X/1A1*1/*1	62 (0.26)	50 (0.19)	1.59 (0.90–2.84) 0.113
3A5*1/*X/1A1*1/*2	23 (0.10)	40 (0.15)	0.74 (0.38–1.45) 0.381
3A5*1/*X/1A1*2/*2	42 (0.18)	15 (0.06)	3.60 (1.73–7.47) 0.001
3A5*X/*X/1A1*1/*1	18 (0.08)	36 (0.14)	0.64 (0.32–1.31) 0.226
3A5*X/*X/1A1*1/*2	6 (0.02)	16 (0.06)	0.48 (0.18–1.33) 0.163
3A5*X/*X/1A1*2/*2	12 (0.05)	11 (0.04)	1.40 (0.56–3.50) 0.475
Expected enzyme activity CYP3A5/SULT1A1			
<i>In all samples</i>			
Normal/normal	136 (0.58)	165 (0.63)	1.00
Abolished/ normal	24 (0.10)	52 (0.20)	0.56 (0.33–0.95) 0.032
Normal/reduced	62 (0.27)	34 (0.13)	2.21 (1.38–3.55) 0.0009
Abolished/reduced	12 (0.05)	11 (0.04)	1.32 (0.58–3.03) 0.512
<i>Among smokers</i>			
Normal/normal	103 (0.57)	109 (0.62)	1.00
Abolished/normal	22 (0.12)	42 (0.24)	0.55 (0.31–0.99) 0.045
Normal/reduced	45 (0.25)	15 (0.09)	3.17 (1.68–6.00) 0.003
Abolished/reduced	10 (0.09)	9 (0.05)	1.18 (0.47–2.94) 0.735
<i>Among non-smokers</i>			
Normal/normal	33 (0.61)	56 (0.64)	1.00
Abolished/normal	2 (0.04)	10 (0.12)	0.34 (0.00–1.48) 0.163
Normal/reduced	17 (0.31)	19 (0.22)	1.52 (0.70–3.30) 0.295
Abolished/reduced	2 (0.04)	2 (0.02)	1.70 (0.29–10.1) 0.602

CYP3A5 normal activity estimated from the genotypes *CYP3A5*1/*1* combined with the heterozygous genotype e.g. *CYP3A5*1/*X* (where X is any of the CYP3A5 alleles *2, *3, *4, *5, *6 or *7) as determined by Dandara et al. (17) while the CYP3A5 reduced activity is estimated from the sum of the *CYP3A5*X/*X* genotypes. SULT1A1 normal phenotype is estimated from SULT1A1*1/*1 and SULT1A1*1/*2 genotypes while the SULT1A1 reduced phenotype is estimated from SULT1A1*2/*2 genotype.

*2/*4, *2/*5, *2/*6, *2/*7, *3/*3, *3/*4, *3/*5, *3/*6, *3/*7, *4/*4, *4/*5, *4/*6, *4/*7, *5/*5, *5/*6, *5/*7, *6/*6, *6/*7 and *7/*7), which is referred to in Table V as *CYP3A5*X/*X* is associated with the inability to bioactivate carcinogens and, therefore, is associated with reduced risk of OC in combination with any of the SULT1A1 haplotypes.

When smoking was considered together with the genotype combinations, genotype combination B (representing normal CYP3A5 activity and reduced SULT1A1 activity) was associated with increased risk for SCC of the oesophagus and the risk was much higher among smokers (2.78) compared with non-smokers (1.44). This observation further supports a role for CYP3A5 in the bioactivation of some components of tobacco-smoke and a possible role for SULT1A1 in the detoxification of products of CYP3A5 metabolism. Although there is evidence that carcinogens can exert their effects through mutations in the *p53* gene, the commonly reported mutations in *p53* are rare among South Africans (19,20). The presence of human papillomavirus (HPV) E6 protein may, however, provide an alternative mechanism for the loss of *p53* activity. It has been shown that HPV is present in ~40% of oesophageal tumours in South Africa (18).

It should also be noted that other phase I and II enzymes involved in the biotransformation of xenobiotics such as CYP1A1, CYP1A2, NAT2 and microsomal epoxide hydrolases could play important roles in this interaction between

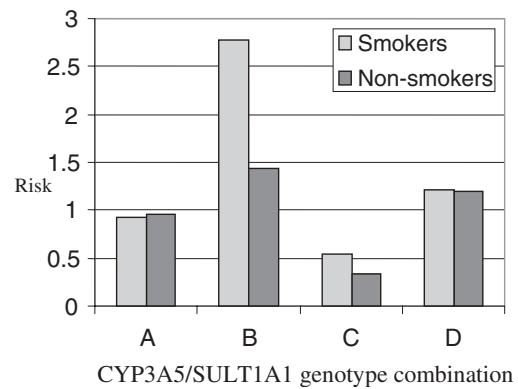


Fig. 1. Distribution of CYP3A5 and SULT1A1 genotype combinations in the South African populations. SULT1A1 genotypes were analysed as described in Materials and methods, whereas the CYP3A5 genotype data was taken from Dandara et al. (17) as described in Table V. The risks of developing SCC for combinations of the two genotypes are indicated. A represents normal CYP3A5 and normal SULT1A1 activity; B, normal CYP3A5 and reduced SULT1A1 activity; C, absence of CYP3A5 activity and normal SULT1A1 activity; and D, absence of CYP3A5 activity and reduced SULT1A1 activity. The risk of developing OC for each genotype is calculated as shown in Materials and methods.

tobacco smoke and combinations of CYP3A5 and SULT1A1 genotypes. Therefore, there is a need to investigate other phase II enzymes especially those with a predominant oesophageal expression. Some of the xenobiotic metabolizing enzymes that have been shown to be polymorphic in the African populations and whose roles in OC have not been investigated include CYP1A2 (46), *N*-acetyltransferase 2 (34,47) and microsomal epoxide hydrolase (48).

In conclusion, the *SULT1A1*2/*2* genotype has been shown to be associated with increased risk for OC with the odds ratios being higher in individuals exposed to smoking and alcohol consumption. *SULT1A1*2* in combination with *CYP3A5*1* could be potential markers to identify individuals at high risk of developing OC.

Acknowledgements

This study was supported by grants from the Medical Research Council (MRC) of South Africa, the Cancer Association of South Africa (CANSA) and the University of Cape Town. C.D. is a recipient of the National Research Foundation (NRF) Postdoctoral fellowship and University Research Committee (URC) fellowship.

Conflict of Interest Statement: None declared.

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Received July 26, 2005; revised October 24, 2005; accepted October 26, 2005