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The frequency of Cytochrome *P*450 2*E*1 polymorphisms in Black South Africans

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Abstract. Polymorphisms in the promoter region of the Cytochrome P4502E1 (*CYP2E1*) gene reportedly modify the metabolic activity of CYP2E1 enzyme, and have been associated with increased susceptibility to squamous cell carcinoma (SCC) of the oesophagus in high prevalence areas such as China. To assess the frequency of these polymorphisms in Black South Africans, a population with a high incidence of oesophageal SCC, this study examined genomic DNA from 331 subjects for restriction fragment length polymorphisms in the *CYP2E1* (*RsaI* and *PstI* digestion). The frequency of the *CYP2E1* c1/c1 and c1/c3 genotypes was 95% and 5% respectively. The frequency of the *CYP2E1* allele distribution was found to be markedly different between Chinese and South African populations; hence it is important to place racial differences into consideration when proposing allelic variants as genetic markers for cancer.

Keywords: Cytochrome P450, alleles, squamous cell carcinoma, polymorphism, oesophagus

1. Introduction

Cytochrome P450 (CYP450) proteins are haem-containing enzymes, which metabolize exogenous compounds such as drugs, environmental pollutants and dietary chemicals, as well as endogenous compounds including steroids, fatty acids and prostaglandins [12]. The CYP2E1 enzymes are known to activate N-nitrosamines, which are implicated in the aetiology of squamous cell carcinoma (SCC) of the oesophagus [9,11], oral cancer [2] and lung cancer [13]. It has been reported that susceptibility to N-nitrosamine-linked carcinogenesis may be influenced by polymorphisms in the 5'-flanking region of the *CYP2E1* gene [6, 14]. These polymorphisms have been shown to modify CYP2E1 transcriptional and catalytic activity [3, 15], and may influence the observed inter-individual susceptibility to N-nitrosamine carcinogenesis [1,14].

Several different *CYP2E1* alleles have been described, some of which are known to affect gene expression [12]. Hayashi et al. [3] described two polymorphisms in the 5'-flanking region of *CYP2E1* (−1053C → T and −1293G → C). The alleles for these polymorphisms were designated c1 (*Rsa*I+, *Pst*I−) and c2 (*Rsa*I−, *Pst*I+) depending on the presence or absence of restriction sites for the endonucleases, *Rsa*I and *Pst*I. These alleles were initially thought to be in complete linkage disequilibrium but some non-concordant genotypes have been reported by Kato et al. [5]. A recent report by the committee on standardization of the nomenclature recommended the naming of human cytochrome P450 alleles by numeral and letter suffixes [4]. Thus, following this new nomenclature, the c1 allele has been renamed as *CYP2E1*1* and the c2 al-

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lele as *CYP2E1*5* [4]. Subsequently, alleles c3 (*Rsa*I+, *Pst*I+) and c4 (*Rsa*I−, *Pst*I−) have been reported in African Americans and Caucasians [8]. These alleles have not yet been integrated into the new system of nomenclature and for that reason, allelic naming in this study will be as reported by Liu et al. [8].

It has been observed in the Chinese population that subjects with the c1 variant allele had a 3.2-fold higher risk of developing oesophageal SCC compared to those with the c2 allele [14]. The possible role of c3 and c4 alleles in cancer susceptibility is unclear as they do not occur in this population. The distribution of *CYP2E1* alleles reportedly varies globally among different ethnic groups [5,13]. The high incidence of SCC of oesophagus in the Black population of South Africa, particularly in certain rural areas [10], is of concern. In endemic areas, numerous factors have been associated with the high incidence of this disease including N-nitrosamines, which commonly contaminate food [7]. No reports on N-nitrosamine analysis in South African food and environment are available, but their possible role in the risk of oesophageal SCC and the relationship with polymorphic variants in the CYP2E1 gene cannot be overlooked. A recent report by Li et al. [6] showed that the c2 (*CYP2E1*5*) allele was not associated with a higher risk of SCC in South Africa. However, these findings were based on a population of a mixed ancestry. No studies have been conducted, specifically targeting the Black population of South Africa. Since the role of CYP2E1 *Rsa*I/*Pst*I polymorphisms in the aetiology of SCC is still inconclusive, the current study was carried out to establish the frequency of the 5'-flanking *CYP2E1* polymorphisms (c1, c2, c3, c4 alleles) in the Black South African population from the province of KwaZulu-Natal, South Africa.

2. Materials and methods

2.1. Study subjects

The study group comprised Black South Africans (n = 331). Some of these study subjects (n = 70) had histologically proven SCC of oesophagus and the remainder (n = 261) were healthy individuals. The age range of the study group was 18–74 years. Alcohol consumption and smoking habits were not recorded. Informed written consent was obtained from all subjects before blood samples were taken. Ethical approval for the study was granted by the Ethics Committee of the Nelson R. Mandela School of Medicine, University of Natal, Durban (Ref. H072/00).

2.2. Genotyping

Genomic DNA was extracted from blood using phenol/chloroform/isoamyl alcohol (25:24:1 v/v/v, pH 6.7). DNA was precipitated from solution using absolute ethanol and 5 mol/l sodium acetate, pelleted and the dry pellet re-suspended in 200 μl distilled sterile water and stored at -20°C. Detection of the *CYP2E1 RsaI and PstI* polymorphisms was performed using the primers and PCR protocols described previously in literature [3]. Sequencing of PCR products to confirm amplification of the specific regions was carried out using the BigDye Terminator cycle sequencing kit from Applied Biosystems (Foster City, CA, USA). The PCR products were restricted with the appropriate enzymes and separated using 10% polyacrylamide gel electrophoresis. The following restriction products were sought: the *CYP2E1* product (410bp) on digestion yielded 360 and 50 bp fragments (*Rsa*I), and 290 and 120 base pair fragments (*Pst*I).

2.3. Statistical analysis

Chi-square test was used to analyze differences in the genotype and allelic frequencies observed between the two subject groups. A p-value less or equal to 0.05 was considered significant.

3. Results

RFLP polymorphism analysis showed that c1 allele was the most frequently occurring in this population. The homozygous c1/c1 genotype was detected in 313 (95%) of all subjects (Table 1). The c3 allele (*Rsa*I+, *Pst*I+) was detected in 18 subjects (5%) as a heterozygous genotype (c1/c3). The c1 and c3 allelic frequency in all subjects was 0.97 and 0.03 respectively. There was no difference in the distribution of these alleles between the individuals with oesophageal SCC and healthy subjects ($p > 0.05$). The observed genotype frequency among the control subjects was in agreement with the Hardy-Weinberg equilibrium ($p^2 + 2pq + q^2 = 1$) (Chi square = 0.002; p = 0.762). The c2 and c4 alleles were not detected in any of the tested subjects.

4. Discussion

Exposure to dietary nitrosamines and the ability of the host to activate them has been linked to increased risk of SCC in China [16]. The CYP2E1 enzymes, key activators of nitrosamines, are influenced by allelic variants c1, c2, c3 and c4, found in the 5'-flanking region of *CYP2E1* gene [8]. The present study describes the prevalence of two of these alleles, c1 and c3 in black South Africans, a population with a high incidence of oesophageal cancer. The c1 (*CYP2E1*1*) allele was found to occur far more frequently (95%) than the c3 allele (5%) in this cohort. No difference was found in the distribution of these alleles between the subjects who had oesophageal SCC and those who did not. Patient numbers were, however, small and a larger study is recommended in the future for further validation of our findings. Nevertheless, our findings are similar to those reported in a South African population of mixed ancestry in which the c1 prevalence was 97% [6]. However, our findings differ markedly from a Chinese study in which, not only was the c1 allele less common in the non-cancer groups (44%), but the overall frequency of the c1 allele was only 57% [14]. Based on the results of our study, it is unlikely that *CYP2E1* allelic variants play any role in the aetiology of oesophageal SCC in the South African population. Environmental factors including N-nitrosamines, in concert with genetic polymorphisms, reportedly increase the chances of developing oesophageal SCC [7,11]. Exposure to N-nitrosamine in our population has not yet been investigated, but this may play a role.

The c2 allele, which was not detected in our study, has been shown to be less frequent (2–8%) in both Caucasian and African Americans than in the Asian population (24–27%) [5]. This allele is thought to code for a poor CYP2E1 metabolizer phenotype [15]. In our study, the rare c3 allele was detected in both SCC and healthy subjects. The 5% frequency of this allele is similar to that reported in African Americans [8]. The frequency of the c3 allele has previously been shown to vary in American populations, with African-Americans exhibiting a higher prevalence than their Caucasian counterparts [8]. It has been reported that this allele is rare in Asians and has been detected in the Japanese population [5]. Reports in the literature suggest that the c2 and c3 alleles do not confer any risk for oral cancer [8] as they are believed to code for a poor metabolizer enzyme. It has also been suggested that *Rsa*I restriction site and not that of *Pst*I may modulate the expression of CYP2E1 [15].

Table 1
Distribution of the *CYP2E1* genotype frequencies in Black South Africans

		Genotype	
	n	Wild type* c1/c1	Variant** c1/c3
Healthy subjects	261	246 (94%)†	15 (6%)
SCC subjects	70	67 (96%)†	3 (4%)
Total	331	313 (95%)	18 (5%)

*"Wild type" genotype = *Rsa*I++/*Pst*− −.
**Variant genotype = *Rsa*I++/*Pst*I+−.
†No significant difference observed ($p > 0.05$).

Studies in which the *CYP2E1* genotype was assessed using only the *Pst*I and not the *Rsa*I restriction cannot distinguish between the c2 and c3 alleles because the c3 allele has restriction sites for both endonucleases (*Rsa*I+/*Pst*I+). Since Li et al. [6] did not report the *Rsa*I+/*Pst*I+ polymorphisms in c1 to c4 format, it is possible that the subjects in their study genotyped as c2, in fact belong to the c3 allele category. The nomenclature committees have not yet addressed the complexities of multiple nucleotide changes that can result in alternative enzyme-restriction profiles that do not fit into the current nomenclature system. In our current study we did not identify any individual with the c2 allele. In the report by Li et al. [6], the prevalence of c1 and c2 alleles was 94–97% and 3–6% respectively for patients and controls. Thus, the reporting of *CYP2E1* alleles may need to be reviewed in the future.

In conclusion, this study has demonstrated that c1 is the most prevalent *CYP2E1*-allele in the Black South African population. Further studies are recommended to determine whether this allele plays any role in cancer development when subjects are exposed N-nitrosamines. The distribution of *CYP2E1* alleles in South African populations is markedly different from that of the Chinese and Caucasian populations and it is therefore important that racial differences be taken into consideration when proposing allelic variants as genetic markers for cancer.

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