Genetic polymorphism of CYP2D6 and CYP2C19 in East- and Southern African populations including psychiatric patients

Abstract Objectives: The study was carried out to investigate the distribution of cytochrome P450 2D6 (CYP2D6) and CYP2C19 genotype frequencies in three African populations and to compare these frequencies between healthy individuals and psychiatric patients. Methods: Three hundred and eighty-four subjects from South Africa (Venda), Tanzania, and Zimbabwe who consented to the study were genotyped for CYP2D6 (CYP2D6*1, *2, *3, *4, *5, and *17) and CYP2C19 (CYP2C19*1, *2, and *3) by PCR-RFLP (polymerase chain reaction restriction fragment length polymorphism) techniques. Results: The genotypes for CYP2D6 predicted a poor metaboliser frequency of 2.3% (2/88) in Tanzanian psychiatric patients, 1.9% (2/106) in Tanzanian healthy controls and 2.6% (2/76) in the South African Venda. The low-activity CYP2D6*17 allele frequency was higher in psychiatric patients (30%, 53/176) than in healthy individuals (20%, 43/212) in Tanzanians. The frequencies for CYP2C19*2 genotypes were predictive of a low prevalence of poor metabolisers (PMs). The CYP2C19*3 allele was absent in the three populations studied. There was no difference in CYP2D6 or CYP2C19 PM genotype frequencies between psychiatric patients and healthy subjects.

Conclusion: The genotype results predict a low prevalence of people with deficient CYP2D6 and CYP2C19 activity among linguistically (Bantu) related populations of East and Southern Africa. The high frequency of the low-activity CYP2D6*17 allele predicts that the Bantu people have a reduced capacity to metabolise drugs that are CYP2D6 substrates.

Key words African populations · Genetic polymorphism

Introduction

Genetic polymorphism of drug-metabolizing enzymes can result in varied interindividual and interethnic pharmacological and toxicological responses upon exposure to therapeutics and environmental pollutants. Polymorphic variants of CYP1A1, CYP2A6, CYP2E1, GSTs, and epoxide hydrolases have been associated with increased susceptibility to developing various cancers. The mechanistic basis for increased susceptibility is either an increased capacity to bioactivate procarcinogens or a diminished capacity to detoxify carcinogens [1]. Conflicting reports have been published associating the CYP2D6 polymorphism with an altered risk of developing cancer and neurological disease [2, 3]. Polymorphisms of CYP2C9, 2C19 and 2D6 have been associated with a reduced capacity to dispose important drugs; this leads to undesirable clinical consequences [4].

CYP2C19 metabolises important therapeutics including omeprazole, proguanil, and diazepam and CYP2D6 metabolises many currently used drugs, which include β-blockers, antidepressants, and neuroleptics [5]. Phenotyping methods using specific marker substrates for CYP2D6 (debrisoquine, metoprolol, dextromethorphan, and sparteine) and for CYP2C19 (mephenytoin) are being extensively used to determine the metabolic status of subjects [6]. Depending on their capacity to metabolise this marker substrate (measured as metabolic ratio), subjects can be classified as extensive metabolisers.
(EM), poor metabolisers (PM), intermediate metabolisers (IM), or ultrarapid metabolisers (UM). The mutations giving rise to these phenotypes for CYP2C19 and CYP2D6 have been characterised, and genotyping methods are now in routine use to establish a person’s genetic status with respect to variants of these enzymes.

The major mutant variants of CYP2D6 which account for most of the phenotypes observed in different populations studied so far are the following: CYP2D6*1 and *2 for EM, CYP2D6*3, *4, and *5 for PM, CYP2D6*10, *17, and *29 for IM, and CYP2D6*2n ≥ 2 for UM. For CYP2C19, the EM variant is CYP2C19*1 and the two major PM variants are CYP2C19*2 and CYP2C19*3 (reviewed by Masimirembwa and Hasler [7]). Interethnic differences in the metabolic polymorphism of CYP2D6 and CYP2C19 have been extensively reported among Caucasians and Orientals and to a lesser extent in Africans [7, 8]. Though there have been a few Chinese or Japanese who have had deficient CYP2D6 function (<1% PM), most of them have an enzyme with reduced activity compared to Caucasians. The molecular genetic basis for more PMs in Caucasians (7–10%) than Orientals is the high frequency of the CYP2D6*4 defect variant (up to 23% allele frequency) compared to its low prevalence in Chinese (less than 1%). The basis of reduced enzyme activity (IM) in Orientals is the high prevalence of the low-activity CYP2D6*10 allele (up to 50% allele frequency), which has a low frequency in Caucasian (less than 5%) [9]. The genetic polymorphism of CYP2C19 has shown the most striking interethnic variation of a CYP so far. The PM frequency ranges from 2–7% in Caucasians, 14–25% in Orientals, and 60% in the Vanuatu [9, 10]. In the Oriental and the Vanuatu, all the PMs can be accounted for by the CYP2C19*2 and *3 alleles. The CYP2C19*3 allele is not found in Caucasians and CYP2C19*2 only accounts for 78% of the PMs. Other rare defective alleles found in Caucasians, *4, *5, and *6, raise the prediction of the PM phenotype to 92% [11].

Phenotyping studies in many African populations have revealed a number of important trends. The frequency of PMs for both CYP2D6 and CYP2C19 has been shown to be dependent on the probe drug used. There is also poor correlation among the EM subjects when phenotyped with the use of different probes drugs. With the exception of a few studies, there is a trend to fewer PM subjects (below 4%), and a high frequency of subjects with a reduced capacity (IM) to metabolise CYP2D6 probe drugs [7]. Ongoing phenotyping and genotyping studies in African populations are revealing new features of the CYP2D6 and CYP2C19 genetic and phenotypic status.

In this study we have used PCR-RFLP based methods to establish the genetic status of CYP2D6 and CYP2C19 in samples from populations of East and Southern Africa. We also wanted to investigate whether there are differences in genotype and allele frequencies between psychiatric patients and healthy subjects. It is hoped that the results will aid in understanding the ethnic diversity of some of the African populations and offer a preliminary basis for more rational usage of drugs that are substrates for CYP2D6 and CYP2C19.

Materials and methods

Permission for carrying out these studies was granted by the following ethics committees: Medical Research Council of Zimbabwe, Muhimbili University College of Health Sciences, and the Ethical Committee of the University of Pretoria, South Africa. Informed consent was obtained from the participants or the next of kin of some of the patients.

Tanzanian subjects

Blood samples were collected from two groups of Tanzanians, psychiatric patients (n = 88) and healthy individuals (n = 106). Patients were recruited from Muhimbili Medical Center, Psychiatric Unit. Patients were on one or a combination of the following drugs: haloperidol, thioridazine, zuclopenthixol, amitriptyline, fluoxetine, chlorpromazine, fluphenazine, and imipramine. The subjects comprised 30 male and 38 female patients with a mean age of 35.8 years (age range 17–61 years). Healthy volunteers consisted of students and staff at the Muhimbili College of Health Sciences. The healthy volunteers were 71 men and 35 women with a mean age of 30.8 years (age range, 16 to 50 years). The blood was frozen at −20 °C and transported to the Department of Biochemistry, University of Zimbabwe, Harare, for genotype analysis.

Venda subjects (South Africa)

Blood samples were collected from people (n = 76) visiting a local clinic in Tshikundamalema, Venda, in South Africa. The frozen blood was transported to the Department of Biochemistry, University of Zimbabwe for genotype analysis.

Zimbabwean subjects

DNA samples came from subjects who had participated in previous studies (n = 114) while additional samples (n = 66) were obtained from new volunteers. All the DNA samples (n = 180) were investigated for the presence of the CYP2C19*3 allele.

Methods

DNA was extracted from whole blood by the phenol/chloroform method [12]. The CYP2C19*1, *2, and *3 alleles were analysed by PCR-RFLP methods described by de Morais et al. [13, 14]. CYP2D6 mutant variants were determined according to the methods of Heim and Meyer [15] (CYP2D6*3 and *4), Steen et al. [16] (CYP2D6*5), and Masimirembwa et al. [17] (CYP2D6*2 and CYP2D6*17).

Results

The genotype and allele frequencies of CYP2D6 variants are shown in Tables 1 and 2. The genotypes predict a poor metaboliser frequency of 2.3% (2/88) in Tanzanian