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Original Research

Genetic polymorphism of CYP2D6 in Karnataka and Andhra Pradesh population in India

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KEY WORDS cytochrome P-450 CYP2D6; human; India; high pressure liquid chromatography; dextromethorphan; phenotype; polymorphism(genetics)

ABSTRACT

AIM: To study the prevalence of cytochrome P-450 2D6 (CYP2D6) polymorphism in Karnataka (KA) and Andhra Pradesh (AP) population. **METHODS**: Two hundred and eleven healthy human volunteers participated in the study (100 from KA and 111 from AP). At bed time, after voiding their bladder, the volunteers ingested 30 mg of dextromethorphan hydrobromide (DM). Urine samples were collected for 8 h. DM and its metabolite dextrorphan (DT) were estimated in the urine using HPLC. The metabolic ratio (DM/DT) was used for phenotyping. **RESULTS**: The prevalence of poor metabolisers (PM) in KA is 4 % and AP is 1.8 %. **CONCLUSION**: The frequency of PM phenotype in South Indian population is in between the Western and Oriental population.

INTRODUCTION

Polymorphism in CYP2D6 has been studied intensively in recent years with respect to both effects on drug metabolism and a possible role in susceptibility to certain diseases[1]. The CYP2D6 polymorphism has become one of the most important pharmacogenetic differences involved in clinical drug efficacy and undesirable drug reactions[2].

CYP2D6 is responsible for the metabolism of more than 40 commonly using drugs especially neuroleptics, antidepressants, certain antiarrhythmics and lipophilic β-adrenergic blockers etc[1-4]. Because of the potentially large inter-phenotypic differences in metabolism, determination of this genetic polymorphism may be of clinical value in predicting adverse or inadequate response to certain therapeutic agents and in predicting increased risk of environmental or occupational exposure-linked disease. Thus genotyping/phenotyping of CYP2D6 activity may lead to increased therapeutic efficacy and more cost-effective medication[1,5,6].

Several studies are already reported from different parts of the world regarding the polymorphism of CYP2D6 isoenzyme. The frequency of this polymorphism is dependent on the ethnic origin of the study subjects. The poor metaboliser (PM) phenotype is reported in about 5 % - 10 % of Caucasians[7], less than 1 % of Chinese[8] and Japanese[9], 1 % of Saudi Arabians[10], 0 - 2 % of black population[7], 1.2 % of Thais[11] etc. The studies on the polymorphism of the CYP2D6 from Indian population are very few. A study among subjects residing in Bombay, which is in the west coast of central part of India, reported 2 % PM with respect to CYP2D6[12]. In North Indian subjects, 3 % frequency of PM phenotype has been reported[13]. The study on the debrisoquin oxidation in Sinhalese residing in Sri Lanka observed the PM frequency of about 0 - 2 %[14]. Subjects from Kerala state, which is in the west coastal area of South India showed 4.8 % of PM phenotype[15]. A recent study shows 3.2 % PM in subjects from Hyderabad city (Andhra Pradesh) population[16]. Since Hyderabad is a metropolitan city, this study population is not

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representing the original Andhra population.

In order to get a clear picture of CYP2D6 polymorphism in subjects from Karnataka and Andhra Pradesh states, the present study was undertaken.

Karnataka (KA) is situated on the western edge of the Deccan plateau in South India. Andhra Pradesh (AP), which is in the south central of India along the eastern coast, forms the major link between the north and south of India.

METHODS

Subjects The study was performed in 211 unrelated healthy volunteers (111 from AP and 100 from KA) in the age group of 15 to 45 years. The subjects from KA were mostly students and staff from Krupanidhi College of Pharmacy, Bangalore, KA and the volunteers from AP were mainly students and staff from Rengaraya Medical College, Kakinada, AP. The demographic characteristics of the volunteers are given in Tab 1. All subjects were judged to be in good health as determined by a medical history, physical examination and blood pressure measurement. Individuals were excluded if they were receiving any medications on a chronic basis, or even receiving concomitant therapy with drugs known to induce or inhibit the cytochrome P-450. None of the subjects were regular alcohol users. All subjects gave their informed consent and the study was approved by the Ethics Committee, JIPMER, Pondicherry.

Protocol for phenotyping The dextromethorphan metabolic ratio (MR) was determined from the ratio of the molar recovery of dextromethorphan (DM) to that of dextrorphan (DT) in the urine collected for 8 h. After emptying the bladder, each subject received an oral dose of 30 mg of dextromethorphan hydrobromide (5 mL of Lactuss-LA, cough suspension; FDC Limited, Aurangabad, India) at bed time. Urine was collected overnight for 8 h. A 20-mL aliquot was stored frozen (−20 ℃) until analysis for dextromethorphan and dextrorphan by HPLC method[17]. In brief, 0.5 mL of urine sample was incubated with 0.5 mL of β glucuronidase (8 000 kU/L) at 37 ℃ for 16 h. The samples were then extracted with an organic solvent mixture (20:9:1 of diethyl ether:chloroform:2-propanol). The organic layer was vortexed with 400 μL of HCl 0.2 mol/L. The acid layer was aspirated and 200 μL was injected into the HPLC with cyno column. The mobile phase consisted of methanol, actonitirile and triethylamine (16:3:0.06, vol:vol:vol) in water at pH 2.8. A fluorescence detector was used with excitation wavelength at 230 nm and emission wavelength at 330 nm. The inter and intra-day coefficient of variation for assay of DM and DT (50 − 8000 mg/L) were less than 10 % and 5 % respectively. The least quantifiable amount was 20 mg/L for both DM and DT.

The oxidative phenotype assignment was based on the value of the subject's molar urinary ratio of dextromethorphan to dextrorphan (metabolic ratio, MR) in relation to the population antimode. Dextromethorphan metabolic ratio of 0.3 was considered as the antimode[18]. Subjects with a metabolic ratio greater than or equal to the antimode were classified as poor metabolisers of the CYP2D6 enzyme.

Statistical analysis Statistical analysis were performed using INSTAT computer software. Data are presented as $\bar{x} \pm s$. Analysis of inter-individual variations in CYP2D6 activity to metabolise dextromethorphan was expressed by computing a frequency distribution histogram between log MR on the abscissa and number of subjects on the ordinate. The shift in the frequency

Tab 1. Description of Karnataka (KA) and Andhra Pradesh (AP) study population. $^bP < 0.05$ *vs* AP subjects.

	KA	AP	Total
Total subjects	100	111	211
Male	83[b]	72	155
Female	17[b]	39	56
Age(s)	24.05(4.9)	23.42(6.7)	23.72(5.9)
Body mass index(s)	20.54(3.4)	20.36(3.0)	20.45(3.2)
Metabolic ratio(s)			
EM	0.057(0.03)	0.057(0.04)	0.057(0.04)
PM	3.206(2.34)	0.859(0.28)	2.423(2.18)
Phenotype			
EM(%)	96(96)	109(98.2)	205(97.2)
PM(%)	4(4)	2(1.8)	6(2.8)

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distribution histogram of the study population to that of other ethnic group is analysed by comparing the mean log metabolic ratios (SD) of extensive metabolisers (EM) using unpaired t test. Rest of the data was analysed by two tailed t-test and Fisher's exact test. A P value < 0.05 was considered to be statistically significant.

RESULTS

Out of the 211 study subjects, 6 subjects (2.8 % with a 95 % confidence interval of 1.06 % to 6.09 %) had metabolic ratios exceeding their population antimode and were classified as poor metabolisers of dextromethorphan. The distribution of lgMR in the 0 – 8 h urine of the 100 subjects from Karnataka and 111 subjects from AP are shown in Fig 1 and 2 respectively.

In Karnataka 4 subjects (4 % with a 95 % confidence of interval of 1.1 % – 9.93 %) and in Andhra Pradesh 2 subjects (1.8 % with a 95 % confidence interval of 1.06 % to 6.08 %) were identified as poor metabolisers with respect to CYP2D6. In Karnataka population 96 subjects (96 %) had metabolic ratio between 0.005 and 0.205 and were classified as extensive metabolisers (EM). In AndhraPradesh subjects, the metabolic ratio of 109 EM phenotype (98.2 %) ranged from 0.0053 to 0.194. There was no demographic difference with the PM when compared to the EM. Out of six PM, 4 were males and 2 were females. No side effects or any adverse drug reactions were observed.

The mean age or body mass index was not significantly different between the KA and AP subjects. The mean metabolic ratio of the EM subjects was also not significantly different between the two groups (Tab 1).

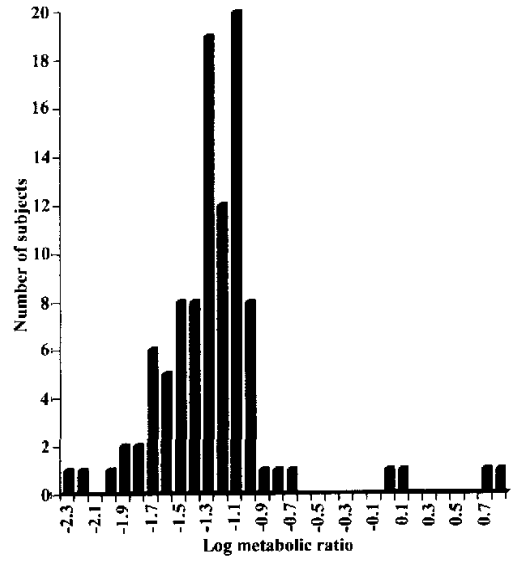

Fig 1. Distribution of the dextromethorphan metabolic ratio among 100 Karnataka population.

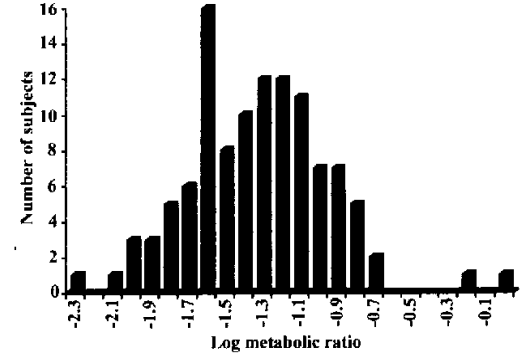

Fig 2. Distribution of the dextromethorphan metabolic ratio among 111 Andhra Pradesh population.

DISCUSSION

In the present study, 4 poor metabolisers were identified among the 100 Karnataka subjects. This represented a PM frequency of about 4 % in this population. The frequency of PM in Andhra subjects (1.8 %) is comparatively less than Karnataka population. The incidence of PM in Karnataka population is more than that observed in other Asian populations (less than 1 % in Chinese[8] and Japanese[9], 1 % in Saudi Arabians[10], 1.2% in Thai population[11], etc). However the low frequency of PM in Andhra Pradesh population is comparable to that of Orientals.

Based upon the genetic distance, the people of India have been broadly classified into four main ethnic groups: Caucasoid Aryans, Caucasoid Dravidians, Australoids, and Mongoloids. The non-tribal population of India consists mainly of Caucasoid Aryans in North India and Caucasoid Dravidians in South India[19]. The population in Karnataka is of Dravidian origin and the present study is the first to be conducted in this population.

Andhra Pradesh consists of three distinct regions. (i) Coastal region generally called Andhra, (ii) the interior region known as Rayalaseema and (iii) Telengana region, consisting of the capital Hyderabad and adjoining districts. The present study population is from Andhra region. The earliest mention of the Andhras is said to be in Aitereya Brahmana (2000 BC). It indicates that

Andhras originally an Aryan race, living in North India, migrated to the south of Vindhyas and later mixed with non-Aryan stocks[20]. The low frequency of PM in Andhra Pradesh population can be compared with the other studies conducted in populations of migrated Indo-Aryan races. The subjects of Bombay and Sinhalese from Sri Lanka also show a low prevalence of PM phenotype (0 – 2 %)[12,14] like Andhra population.

A similar study conducted by the same authors in 104 subjects from Kerala observed a frequency of 4.8 % PM in Keralite population[15]. Kerala is another South Indian state where the population is of Dravidian origin. The studies in Karnataka and Keralite subjects shows that the Dravidian population has a higher frequency of PM phenotype when compared to other Oriental races.

There is a marked rightward shift in the frequency distribution histogram of the metabolic ratio of South Indian population compared to Caucasian population ($P < 0.001$)[21]. This shift is comparatively less when compared to the Chinese population[22]. However the frequency distribution of the present study population is comparable with that reported in North Indian population ($P > 0.05$)[13]. Horai *et al*[23] reported that the frequency distribution curve for metoprolol metabolic phenotype in Chinese population was skewed to the right compared with that in the Japanese population. A similar inter-ethnic difference in the distribution histograms of debrisoquin EMs and metoprolol EMs has also been observed between two non-Oriental (British and Nigerian) populations[24]. Environmental factors may modulate genetic expression, which may give rise to differences in the antimode of the metabolic ratio between ethnic groups[11]. The differences between White subjects and South Indian subjects in life style, dietary habits and/or occupation might have influenced the CYP2D6 isoenzyme in Andhra Pradesh and Karnataka population. This may be the reason for the difference in the mean metabolic ratios of these ethnic groups.

Unlike Western population none of our female participants were smokers or alcoholics. This may be because of the strict social restrictions in this region. In males chronic alcoholism was one of the exclusion criteria, so that only occasional social drinkers participated in the study. Their alcohol consumption was less than 20 units per month.

It has been proven that pregnancy can induce CYP2D6 enzyme activity, so pregnancy was an exclusion criteria while selecting the female subjects[25]. However the menstrual cycle phase has not been considered in female subjects during study because of the lack of influence of menstrual cycle phase on dextromethorphan metabolic ratio[26].

The present study shows that subjects of pure Dravidian origin have a higher frequency of PM compared to the migrated Aryan subjects. However the mean metabolic ratio or frequency distribution histograms of Karnataka and Andhra subjects were not significantly different. Further studies including the genotyping of the subjects may give a more clear picture about the genetic polymorphism in this region.

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CYP2D6在印度卡纳塔克邦和安得拉邦人群中的遗传多态性

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关键词 细胞色素 P-450 CYP2D6; 人类; 印度; 高压液相色谱法; 右美沙芬; 表型; 多态现象(遗传学)

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