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Cytochrome P_{450} 2D6 polymorphism in eastern Indian population

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Abstract:

OBJECTIVES: Cytochrome P_{450} 2D6 (CYP2D6) enzyme metabolizes a quarter of prescription drugs. Polymorphisms of CYP2D6 gene and resultant phenotypic variations in metabolic activity have been described in various populations. We assessed the prevalence of CYP2D6 activity phenotypes, employing dextromethorphan (DXM) as probe drug in subjects with at least two parental generations residing in eastern India.

MATERIALS AND METHODS: Unrelated healthy subjects took 60 mg DXM after fasting overnight. Blood samples were collected 3 h after dosing and plasma separated. DXM and its primary metabolite dextrorphan (DXT) were measured by liquid chromatography with tandem mass spectrometry. The DXM-to-DXT metabolic ratio (MR) was obtained for each subject. Histogram of MR values suggested bimodal distribution. A polynomial regression equation derived through probit analysis was solved to identify the antimode of the MR values. Subjects with log(MR) < antimode were extensive metabolizers (EMs). Log(MR) $\geq$ antimode indicated poor metabolizers (PMs).

RESULTS: We evaluated the results from 97 participants. The median MR was 0.209 (interquartile range: 0.090–0.609), while the antimode for MR was 3.055. From these, it was inferred that three subjects were PMs, while the rest were EMs. CYP2D6 polymorphism prevalence is low (3.09%; 95% confidence interval: 0.35%–6.54%) in the population of eastern India and matches the prevalence in other zones of the country.

CONCLUSIONS: Differences in CYP2D6 activity has treatment implications and may lead to adverse events or therapeutic failure. Phenotyping of subjects receiving CYP2D6 metabolized drugs may help clinicians personalize treatment and avert adverse drug-drug interactions. However, the frequency of the PM phenotype is low in India, and routinely phenotyping for CYP2D6 activity will not be cost-effective. We cannot recommend it at this stage.

Keywords:

Cytochrome P_{450} 2D6, dextromethorphan, dextrorphan, genetic polymorphism, India

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Introduction

The cytochrome P_{450} (CYP) family of liver enzymes executes the oxidative clearance of the majority of lipophilic drugs in phase I drug metabolism. Among the various CYP enzymes, CYP P_{450} 2D6 (CYP2D6) metabolizes approximately 25% of drugs used in clinical practice,[1,2] including major groups such as antiarrhythmics, antihistamines, antipsychotics, β-adrenergic blockers, selective serotonin reuptake inhibitors, and tricyclic antidepressants.[2-4] Examples of drugs metabolized to a large extent by CYP2D6 are provided in Table 1.

The enzyme shows genetic polymorphism, and this is a prime cause of variation in CYP2D6 mediated drug metabolism.[5,6] The presence of an allele with reduced metabolic activity leads to the poor metabolizer (PM) phenotype, while the usual extensive metabolizer (EM) phenotype is regarded as

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normal. There may be more than a 20-fold difference in metabolic rates between normal and PMs with clinical implications ranging from subtherapeutic effects to enhanced toxicity.

Globally, genotyping studies of CYP2D6 have established a wide range of mutant alleles – from *1 to *109 at present. The prevalence varies from 0% to 8.4%, depending on the population studied and the genotyping methodology used. Of these alleles, *2 is considered as ultrarapid metabolizer and *3, *4, *6, *9, *10, *14, *17, *29, *41, and *69 are PM types – the remaining are associated with normal drug-metabolizing activity or are yet to be characterized.[7] Inheritance of the PM phenotype is autosomal recessive and up to 10% or still larger proportion of the population can be categorized as PMs.

In a given population, categorizing the extent of PM phenotype of CYP2D6 may help to optimize treatment with relevant drugs. The function of the polymorphic alleles can be established from phenotyping studies using a probe drug,[8] which is a drug primarily metabolized by one specific enzyme. These studies are usually conducted in normal, healthy human participants. Hence, by measuring the extent of metabolic conversion of such a probe drug (the measurement of parent drug and metabolite), the functional/phenotypic activity of that particular enzyme can be delineated. The United States Food and Drug Administration (US FDA) approves dextromethorphan (DXM) as a probe drug for CYP2D6.[9] The effects of DXM and its duration of action can be increased by as much as three times in individuals with the PM phenotype.

Table 2 summarizes population-specific figures of prevalence of CYP2D6 metabolizer phenotypes. Worldwide, phenotyping studies show the incidence of the CYP2D6 PM phenotype to vary widely from 0% to 18.8%.[5,10,11] There is also variation within India, as shown in Table 3.[3,12-16] However, there are no studies from eastern India exploring the metabolizing phenotypes of CYP2D6. We therefore planned the present study with the objective of evaluating the CYP2D6 metabolizer phenotypes in healthy eastern Indian subjects using DXM as a probe drug.

Table 1: Examples of prescription drugs metabolized by CYP2D6

Most TCADs, particularly imipramine, amitriptyline
Most selective SSRI antidepressants, particularly fluoxetine, paroxetine, and fluvoxamine
Most selective SNRI antidepressants, particularly venlafaxine, duloxetine
Other antidepressants, for example, mianserin (tetracyclic antidepressant), mirtazapine, and minaprine (RIMA antidepressant)
Opioids, including codeine (→ morphine), oxycodone, hydrocodone (→ hydromorphone), tramadol (→ O-desmethyltramadol), tapentadol
Antipsychotics, for example, aripiprazole, chlorpromazine, haloperidol, iloperidone, levomepromazine, perphenazine, remoxipride, risperidone, thioridazine, and zuclopenthixol
Beta-blockers, for example, alprenolol, bufuralol, carvedilol, metoprolol, nebivolol, propranolol, and timolol
Class I antiarrhythmics, for example, encainide, flecainide, mexiletine, propafenone, and sparteine
Antiemetics, for example, metoclopramide (dopamine antagonist), ondansetron, and tropisetron
Antihistamines, for example, chlorpheniramine and promethazine
Amphetamine
Atomoxetine
Debrisoquine (antihypertensive)
Dextromethorphan (→ dextrorphan)
Donepezil
Tamoxifen (→ hydroxytamoxifen)

→Implies converted to. TCADs=Tricyclic antidepressants, SSRI=Serotonin reuptake inhibitor, SNRI=Serotonin norepinephrine reuptake inhibitor, RIMA=Reversible inhibitor of monoamine oxidase type A

Table 2: CYP2D6 metabolizer phenotypes worldwide

Metabolizing phenotype	CYP2D6 activity	Ethnic differences
PM	None to low	Caucasians 6%-10%
		Mexican Americans 3%-6%
		African Americans 2%-5%
		Orientals 1%
IM	Low	Not established
EM	Normal	Most subjects are extensive metabolizers
UM	High	Finns and Danes 1%
		North American Whites 4%
		Greeks 10%
		Portuguese 10%
		Saudis 20%
		Ethiopians 30%

PM=Poor metabolizer, IM=Intermediate metabolizer, EM=Extensive metabolizer, UM=Ultrarapid metabolizer

Materials and Methods

We conducted an experimental study in a tertiary care hospital setting. Due approval was obtained from the ethics committee for clinical research of the institute. Written informed consent was obtained from all study volunteers.

Subject selection criteria

Participants were individuals above 18 years of age, of either sex, judged to be healthy based on history and clinical examination, and whose parents were permanently resident in eastern India for at least two consecutive generations. The majority of those volunteering were medical or nursing students or residents working in the hospital. Participants who had a history of smoking or tobacco use, regular alcohol use, any chronic disease, any regular drug use, or any alcohol or drug intake within the past 7 days were excluded from the study. Subjects related by blood to an earlier recruit were also excluded.

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Table 3: Regional differences in poor metabolizer phenotype in Indian studies

Region	Sample size	Prevalence of poor metabolizers Percentage (95% CI)
Western India	149	3.36 (1.4-1.7)
Tamil Nadu	139	3.6 (1.2-8.2)
Northern India	100	3 (0.33-6.33)
Kerala	104	4.8 (1.6-10.9)
Karnataka and Andhra Pradesh	100 + 111	2.8 (Karnataka 4 [1.1-9.93] and Andhra Pradesh 1.8 [1.06-6.08])

CI=Confidence interval

Sample collection

Each participant was asked to report between 9 and 10 AM on the appointed day after overnight fasting. They were asked to avoid consuming alcohol and caffeine 48 h prior to the study. Three hours before reporting, they were asked to consume 60 mg of DXM as DXM hydrobromide orally with 60 mL of water. This was provided to them beforehand as a single bottle of LASTUSS-LA suspension marketed by M/s FDC Limited, Mumbai. All subjects used probe drug medication from the same batch. Age and body mass index (BMI) were recorded. The participants were provided uniform breakfast and observed for 4 h post-dose for any adverse events and then discharged. Telephonic contact was retained subsequently with all participants.

After 3 h following dosing, 5 mL of blood was drawn into a heparinized tube, the plasma immediately separated by centrifugation, and then stored at −20°C. Stored samples were transported to the analytical laboratory of a university-based pharmaceutical institute, in batches, maintaining appropriate cold chain precautions. DXM and its primary metabolite dextrorphan (DXT) were then estimated in the plasma samples through liquid chromatography with tandem mass spectrometry (LC-MS/MS) technique.

Sample processing and analysis

All samples were analyzed with the help of calibration standards that were spiked in human plasma. The methodology followed US-FDA requirements. Preparation of working stock, calibration control, and quality control samples was as follows: 2 mg/mL stock of DXM and DXT was prepared separately in dimethyl sulfoxide, then mixed at 1:1 ratio to get 1 mg/mL final concentration of each analyte. Working stocks were prepared by serial dilutions; 2 µL working stock of cocktail was spiked in 98 µL plasma to prepare 0.098, 0.19, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, and 25 ng/mL calibration control samples of both the analytes. Quality control samples were prepared at 0.29 (low), 10 (medium), and 20 ng/ml (high) concentrations.

Sample processing was performed by protein precipitation technique; 50 µL plasma from calibration and quality control samples were taken in 96-well plate, 150 µL ice-cold acetonitrile incorporating internal standard was added, and the vortex was mixed for 10 min and centrifuged at 15°C and 4000 rpm for 15 min. Then, 100 µL supernatant was collected and diluted with 100 µL water and readied for LC-MS/MS injection. Subject plasma samples were processed following the same procedure after method validation. The metabolic ratio (MR) of DXM to DXT was derived from the concentrations determined in plasma samples.

Sample size and statistical analysis

Being an observational study, we did not go for a formal sample size calculation, but *a priori* planned to recruit at least 100 subjects through purposive sampling. Numerical variables, including the MR, have been summarized by routine descriptive statistics. The normality of MR data was assessed by Kolmogorov–Smirnov goodness-of-fit test. Histogram of the common logarithm (to base 10) of MR of DXM to DXT was plotted which suggested a bimodal distribution. Probit plot was constructed with probit values on the Y-axis and log (MR) on the X-axis. The trend line was fitted to the plot based on a polynomial regression equation. The intercept of this line at the X-axis was considered as the antimode. Subjects with log(MR) value ≥ antimode were regarded as PMs. In contrast, log(MR) value < antimode was treated as characteristic of EMs. The prevalence figures were finally expressed in percentages with 95% confidence intervals (CIs). For statistical analysis, we employed MS-Excel 2007 and MedCalc version 11.6 software.

Results

A total of 104 participants (58 males, 59.79%; rest females) were recruited. The median age was 26 years (range 21–35 years) and mean BMI was 24 kg/m^2 (range: 22–25 kg/m^2, standard deviation: [SD] 2.9 kg/m^2).

The LC-MS/MS analytical conditions and the analyte-dependent parameters of the LC-MS/MS process are summarized in Table 4. Validation parameters were within the acceptable limit as per US-FDA and European Medicines Evaluation Agency guidelines. Regarding the assay quality parameters, the inter- and intra-day coefficients of variation for DXM and DXT assays were, respectively, <10% and 5%. The least quantifiable amount was 0.1 ng/mL for both DXM and DXT. DXM levels

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from seven subjects could not be quantified as they were below the lower limit of detection. For the remaining 97 participants, the descriptive summary of the probe drug-related parameters (DXM level, DXT level, and the MR of the two) is provided in Table 5.

Figure 1 depicts a box and whiskers plot of the MR of DXM to DXT. Clearly, this parameter is not normally distributed, and several outliers can be detected. Figure 2 depicts the histogram of the log (MR) values in 97 subjects. A bimodal pattern can be discerned.

The trend lines from a probit analysis of MR of DXM to DXT in 97 subjects are depicted in Figure 3, on the MR scale as well as log (MR) scale. The polynomial equations of the trend lines are also given.

Considering the equation of the trend line on the log (MR) scale:

$$y = 0.143x^6 + 0.168x^5 - 1.217x^4 - 1.486x^3 + 0.052x^2 + 1.914x + 6.203$$

$$Y = ax^2 + bx + c + 0$$

$$Y\ i = 0.858x^5 + 0.84x^4 - 3.651x^3$$

$$Y\ ii = 4.29x^4 + 3.36x^3 - 10.953x^2$$

$$Y\ iii = 17.16x^3 + 10.08x^2 - 21.906x$$

$$Y\ iv = 51.48x^2 + 20.16x - 21.906$$

Solving for X

$$X = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

Solving $\sqrt{b^2 - 4ac}$

$$= \sqrt{(20.16)^2 - 4\,(51.48)\,(-21.906)}$$

$$= \sqrt{406.4256 + 4510.88352}$$

$$= \sqrt{4917.30912}$$

$$= 70.123$$

$$X = \frac{-b + \sqrt{b^2 - 4ac}}{2a} \text{ or } X = \frac{-b - \sqrt{b^2 - 4ac}}{2a}$$

$$X = \frac{-20.16 + 70.123}{102.96} \text{ or } X = \frac{-20.16 - 70.123}{102.96}$$

$$X = \frac{49.963}{102.96} \text{ or } X = \frac{-90.283}{102.96}$$

$$X = 0.485 \quad \text{or} \quad X = -0.877$$

Antilog X = 3.055 or 7.53

Antilog 3.055 = log (MR) of 0.485 or antilog 7.53 = log (MR) of 0.88

Excluding 0.88 as it does not clearly distinguish PMs from EMs in the histogram, we arrive at the value of 3.055 (antilog, with base 10, of 0.485) as the antimode of MR of DXM to DXT. This is indicated in the histogram in Figure 4.

Therefore, individuals with MR value >3.055 were sorted as PMs and those with MR <3.055 were classified as EMs. Of the 97 participants, 3 (3.09%) were PM with median MR of 5.46 (range 3.92–6.50) and the remaining 94 (96.91%) were EMs with median MR of 0.20 (range: 0.03–2.57). Thus, in our sample, the PM phenotype prevalence was 3.09% (95% CI 0.35%–6.54%).

There was no significant correlation between MR and BMI or age of the subjects. DXM in the single dose of 60 mg was tolerated well by all subjects and no adverse events were encountered.

Table 4: Compound dependent parameters in the liquid chromatography-mass spectrometry/mass spectrometry analysis of CYP2D6 probe drugs

Analyte	Parent ion (Q1)	Production (MS2)	DP	CE
Dextromethorphan	272.2	147.1	90	50
Dextrorphan	258.1	157.1	90	40
Internal standard	687.2	320.1	85	35

LC-MS/MS analytical conditions were System: API4000 (AB-SCIEX) integrated to Shimadzu 20AC LC and CTC-PAL autosampler; Column: TRIAT C18 (2.0×30 mm, 5 μm) YMC; Mobile phase A: 0.1% formic acid in water; Mobile phase B: 0.1% formic acid in 80:20 acetonitrile/water mixture; Injection volume: 20 μL; Elution technique: Gradient; Total run time: 3 min; MS condition (Instrument dependent parameters): GS1:40, GS2:40, IES: 5000, Source temp: 400°C, CAD: 5. LC=Liquid chromatography, MS=Mass spectrometry, DP=Decluster potential, CE=Collision energy

Discussion

CYP2D6 is the most widely probed xenobiotic-metabolizing enzyme that exhibits polymorphism. It is the first such

Table 5: Descriptive summary of the probe drug-related parameters from 97 subjects

Parameter	Range	Mean±SD	95% CI of the mean	Median (IQR)
DXM level (ng/mL)	0.02-15.26	2.08±2.598	1.55-2.60	1.06 (0.55-2.63)
DXT level (ng/mL)	0.10-22.55	5.87±3.818	5.10-6.64	5.42 (2.89-7.79)
Metabolic ratio (DXM/DXT)	0.035-6.500	0.537±0.988	0.338-0.736	0.209 (0.090-0.609)

CI=Confidence interval, IQR=Interquartile range, SD=Standard deviation, DXM=Dextromethorphan, DXT=Dextrorphan

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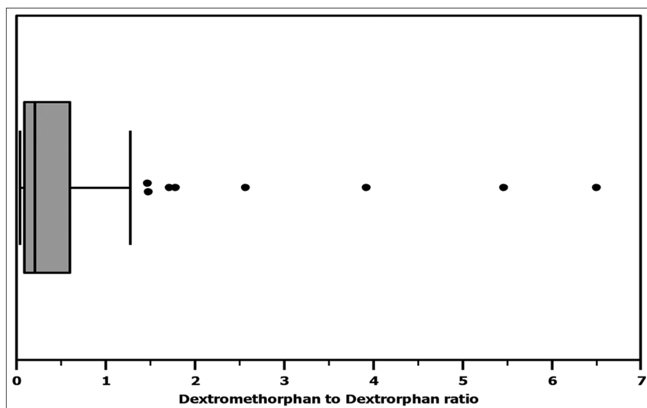

Figure 1: Boxplot of the metabolic ratio of dextromethorphan to dextrorphan in 97 subjects

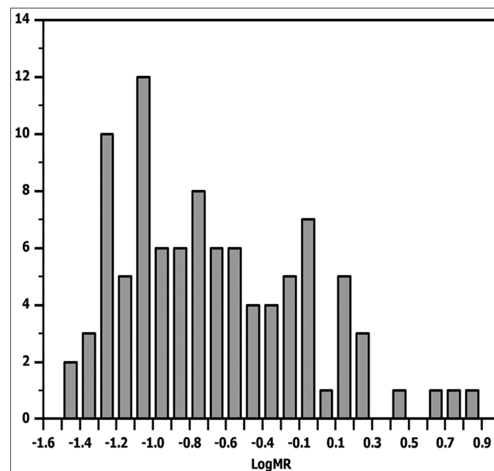

Figure 2: Histogram of the logarithm (to base 10) of metabolic ratio of dextromethorphan to dextrorphan in 97 subjects

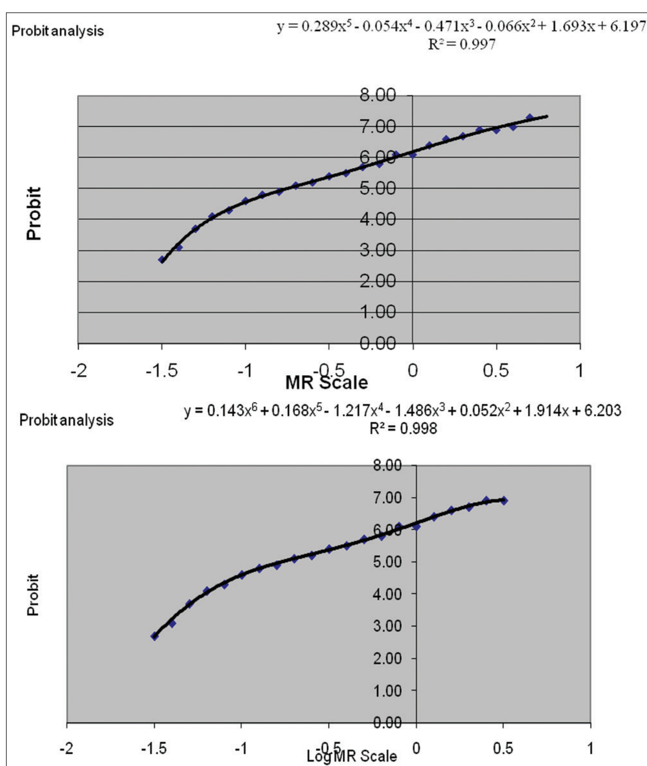

Figure 3: Trend lines from probit analysis of metabolic ratio of dextromethorphan to dextrorphan in 97 subjects. Note that the scales are different for the two panels

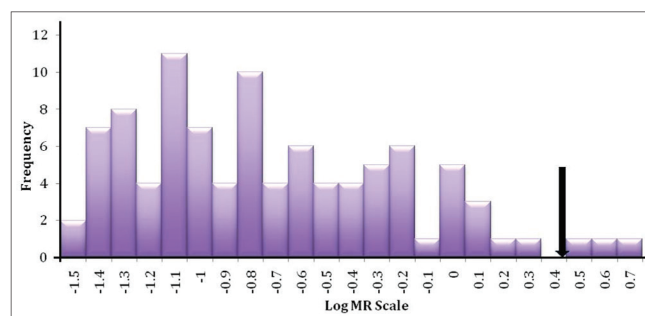

Figure 4: Histogram showing antimode (0.485) of metabolic ratios of dextromethorphan to dextrorphan in 97 subjects

enzyme completely characterized at the molecular level.[17] Three distinct phenotypes have been associated with CYP2D6 polymorphism, namely PM, EM (the normal situation), and ultrarapid metabolizer.[18] Prevalence figures for the PM phenotype in different regions of the world have been summarized in Table 2.

We evaluated the functional activity of CYP2D6 enzyme using MR of DXM to its primary metabolite DXT in healthy unrelated adult participants from eastern India. We found that 3.09% (95% CI: 0.35%–6.54%) were PM and the drug was tolerated well. In our study, we did not encounter the intermediate metabolizer or ultrarapid metabolizer phenotype, unlike in other studies where a proportion of such metabolizers were found.[13,19] This is the first such study from eastern India.

The PM prevalence figure in our eastern Indian population is like that of other Indian populations from West, North, and South India. In the western Indian study,[3] which followed a methodology similar to ours, among 149 subjects, 5 (3.36%; 95% CI: 1.4%–7.6%) were PM with a median MR of 2.80 (range: 2.32–3.01) and the remaining (96.64%; 95% CI: 92.4%–98.6%) were EM with a median MR of 0.25 (range: 0.03–1.29).

In the study from North India,[12] 100 participants received 30 mg DXM at bedtime. DXM and DXT excretion in 8-h urine were quantified using high-performance liquid chromatography (HPLC) analysis. MR (DXM/DXT excretion) was determined from urine samples. Analysis of the data revealed bimodal distribution in the North Indian subjects, and in their case too, 3 of 100 subjects were PM and the rest EM. These two categories excreted 2.67 and 29.82 micromol DXT and 8.82 and 2.59 micromol DXM in 8 h, respectively (mean values). The MR and log (MR) were 197- and 2.2-fold greater in PMs versus EMs.

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The Kerala study[13] was also done with urine samples. After voiding prior to bedtime, volunteers took DXM (30 mg) and urine samples were collected overnight (8 h) for HPLC analysis. Fluorescence detection was used, and the least quantifiable quantity was 20 ng/mL for both DXM and DXT. Phenotype determination was based on the molar urinary ratio (MUR) of DXM to DXT. Population antimode was decided as MUR of 0.3. Subjects with MUR >0.3 were classified as PM. Of the 104 study participants, the MUR of 96 (92.31%) was between 0.005 and 0.192 and they were sorted as EM. Five had MUR between 0.315 and 3.14 and were classified as PM. This led to 4.8% (95% CI: 1.6%–10.9%) prevalence of the PM phenotype. Three subjects who had very low MUR (between 0.0034 and 0.0039) were identified as UM.

The same group also studied other South Indian populations using similar methodology.[14] In the Karnataka population, the prevalence of PM was 4% (95% CI: 1.1%–9.93%), while in the Andhra population, it was 1.8% (85% CI: 1.06%–6.08%). In a later study with 139 Tamil subjects, the prevalence of the PM phenotype was 3.6% (95% CI: 1.2%–1.8%). EM excreted approximately 31.4% of the dose as DXT, but PM eliminated only around 2.8%. PM excreted 38.6 times more DXM than DXT – MR was 0.042 ± 0.049 (mean ± SD) for EM and 2.91 ± 2.64 for PM.

In an earlier paper from South India,[20] 156 healthy unrelated South Indian subjects were phenotyped after administration of 25 mg oral DXM followed by an 8-h urine collection. The analysis was by HPLC with fluorescence detection and this was performed with and without previous deconjugation. The MR was bimodally distributed and the PM phenotype prevalence was estimated at 3.2%. The phenotype assignment remained the same with both the methods of analysis.

Thus, it is clear that the prevalence of PM phenotype in Indian populations varies between 1.8% and 4.8%. This figure is higher compared to other oriental populations in Sri Lanka, mainland China, Japan, and Korea which show prevalence between 0% and 1.2%,[21-24] but lower compared to the prevalence in eastern European and Caucasian populations.[19,25-27] In contrast, research in African countries shows inconsistent results with 0% and 19% prevalence of the PM phenotype.[28] Therefore, there are strong regional variations in CYP2D6 phenotypic expression.

Phenotyping on the basis of MR of a probe drug has certain limitations. Rigid controls have to be imposed (e.g., no concomitant drugs, no caffeinated beverages, and smoking for 48 h) which makes subject recruitment challenging. In addition to enzyme activity, MR is affected by renal drug clearance and also by environmental factors that may lead to inconsistencies in the MR antimode in different ethnic categories.[13] This factor and the relatively small sample size are limitations of our study.

Medicines which are metabolically cleared by CYP2D6 clearly display wide interindividual disparity in metabolic clearance stemming from polymorphisms of the CYP2D6 gene. Fluctuation in the activity of this drug metabolizing has therapeutic implications in that it may underlie therapeutic failure or adverse drug reactions in affected individuals.[29,30] CYP2D6 polymorphism is also important in the context of polypharmacy and over-the-counter procurement of medicines. These are issues particularly of concern in India. Thus, phenotyping of individuals receiving CYP2D6 metabolized drugs may help clinicians personalize treatment and avoid adverse medicinal interactions. However, the frequency of the PM phenotype is low in India and routine phenotyping of CYP2D6 activity will not be cost-effective. We cannot recommend it at this stage.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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