

## Metabolic capacity of CYP2D6 within an Iranian population (Mazandaran Province)

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### Abstract

**Background:** CYP2D6 is polymorphically expressed enzyme that show marked interindividual and interethnic variation. Phenotyping of CYP2D6 provides valuable information about real-time activity of this important drug-metabolizing enzymes through the use of specific probe drugs. The aim of this study was to identify the CYP2D6 oxidation phenotype with dextromethorphan (DEX) as a probe drug in Mazandarani ethnic group among Iranian population.

**Methods:** The study included 71 unrelated healthy volunteers. Dextromethorphan hydrobromide (30 mg) was given orally to healthy subjects and peripheral venous blood samples (10 ml) were taken at 3 hr post-dose. Dextromethorphan and the metabolite dextrophan (DOR) were analyzed by the HPLC method. The log DEX/DOR metabolic ratio (MR) at 3 hr plasma sample was used as the index of CYP2D6 activity and a value of 0.3 was used as the antimode separating extensive metabolizers (EM) and poor metabolizers (PM) phenotypes.

**Results:** A 560-fold interindividual variation in dextromethorphan MRs was observed in this study. Considering the antimode 0.3 in log scale, 7.04% (5/71) volunteers were identified as PMs.

**Conclusion:** The result showed that the frequency of CYP2D6 PM phenotypes accounted for 7.04% of subjects in our samples. Despite these findings, we propose a further study in larger samples to provide a wider image and to get more valuable information upon pharmacogenetic basis for individual therapy and personalized medicine.

**Key words:** Phenotype, Polymorphism, CYP2D6, Dextromethorphan, Mazandaran, Iran.

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Variability in patients' responses to medications is partly attributed to variability in individual differences in drug metabolism which include environmental factors and polymorphisms within genes encoding drug-metabolizing enzymes (1). The CYP2D6 has become one of the model traits of pharmacogenetics since it is highly polymorphic and responsible for the metabolism of a wide range of therapeutically used drugs, including antidepressants (mianserine, nortriptyline, and venlafaxine), neuroleptics (levomepromazine, perphenazine, risperidone, and thioridazine), antiarrhythmics (encainide, flecainide, propafenone, and sotalolol), beta-blockers (metoprolol, propranolol, and timolol), anti-hypertensives (debrisoquine, indoramin), dextromethorphan and codeine (2,3). The CYP2D6 polymorphism is due to multiple mutations of the gene, which result in absent, functionally deficient, under-expressed or over-expressed protein (4). Several alleles and allelic variants of the CYP2D6 locus have been identified and frequencies of the alleles widely vary in different populations (4-11). The phenotypic consequences of this variation depend on the combinations of CYP2D6 alleles. Individuals homozygous or heterozygous for deficient CYP2D6 alleles are so-called, poor metabolizers, (PMs) and metabolize drugs at lower rates, which leads to an increased risk of side effects and drug toxicity (1,11).



The incidence of CYP2D6 PMs in healthy Caucasian populations has been estimated from 7-10% (12). However, Asian (Chinese, Japanese, Koreans, and Indian populations) have a much lower incidence of PMs, usually 0-2% (13, 14). The incidence of PMs in Black African population has been estimated from 0-19% (13).

In the present study, we studied the metabolic activity of CYP2D6 in a sample of Mazandarani ethnic group.

## Methods

After approval of the study by the Research Ethics Committee of Mazandaran University of Medical Sciences, of 100 subjects, only 71 healthy unrelated individuals, of either sex (37 female, age range 17-62 year) participated in the study after giving written informed consent. All the subjects were of Mazandarani ethnic origin residing in Sari, Amol, Ghaemshahr, Neka, and Behshahr area but originating from all parts of Mazandaran based on their family history up to two previous generations.

Subjects with known HIV positive serology and who were taking known CYP2D6 inhibitors (e.g. fluoxetine or paroxetine) or inducers (e.g. rifampicin) were excluded from the study.

**Chemicals and drugs:** Dextromethorphan hydrobromide, dextrophan tartrate, and laudanosine were gifts from Dr M.S Lennard from Academic Unit of Clinical Pharmacology, University of Sheffield, UK. Dextromethorphan hydrobromide syrup was supplied by Toliddaru Pharmaceutical Company (Tehran, Iran). Other chemicals were of HPLC or analytical grade and were purchased by commercial suppliers. Ultra-pure water was obtained using a Milli-Q water purification system.

**Determination of CYP2D6 phenotype:** Seventy-one healthy volunteers agreed to participate in the phenotype procedure. They did not have any medical condition that required treatment. After collection of a blank plasma sample, subjects received a single oral dose of 30 mg dextromethorphan hydrobromide syrup with 100 ml water and peripheral venous blood samples (10 ml) and a urine sample (to screen urine pH) were taken at 3h post-dose. After centrifugation for 5 min (4000 rpm), the plasma were transferred to separate sterile propylene tubes and were stored at -20°C pending assay. The plasma concentrations of dextromethorphan (DEX) and its O-demethylated metabolite dextrophan (DOR) were assayed by the method of Chen et

al. (15) with minor modifications. Briefly, thawed plasma (1 ml) was pipetted into a 10-ml screw capped glass tube to which was added 50  $\mu$ l of internal standard solution (laudanosine, 1 $\mu$ g/ml) and then 0.5 ml saturated sodium carbonate solution, 3.4 ml of a mixture of n-heptan: ethylacetate (1:1, v/v) were added. The samples were mixed for 10 minutes on a rotary shaker and centrifuged for 10 minutes at 4000 rpm. The organic phase was transferred to conical plastic tubes containing 300  $\mu$ l of 0.01M hydrochloric acid.

The tubes were placed on a rotary mixer for 7 minutes and the two phases were separated by centrifugation at 4000 rpm for 7 minutes. The upper organic layer was removed by aspiration and an aliquot of the aqueous phase (100  $\mu$ l) was injected onto the column. The chromatographic separation of drugs was performed on a Luna C18 analytical column (3 $\mu$ M particle size, 4.60 mm $\times$ 100 mm I.D.) (Phenomenex, Cheshire, UK) coupled to a security guard C18 precolumn (Phenomenex), using a gradient mobile phase of methanol: water (pH adjusted to 3 by orthophosphoric acid, and containing 0.01% triethylamine).

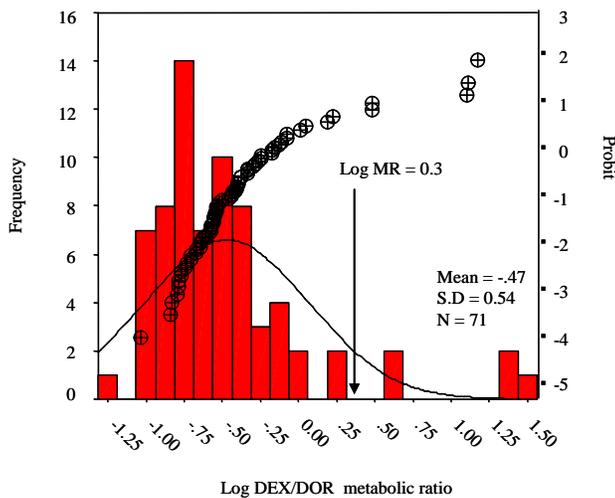
The mobile phase was delivered at a flow-rate of 1 ml/min for 0-14 minutes and then increased to 1.2 ml/min by the end of run (25 min). The limit of quantification was 0.2 and 0.1 (ng/ml) for DEX and DOR, respectively, and the intra- and interday coefficients of variation of the assays were < 18 %.

The log DEX/DOR metabolic ratio at 3 hr was used as the index of CYP2D6 activity and a value of 0.3 in log scale for this ratio was used as the antimode separating EM and PM phenotypes (16).

**Statistical analysis:** Statistical analysis was performed using SPSS for Windows (ver.10; SPSS Inc., Chicago, IL, USA). Multiple linear regression analysis was used to investigate the effects of all available covariables (sex, age, urine pH) on the DEX/DOR log molar metabolic ratio. A pvalue of <0.05 was considered to indicate statistical significance.

## Results

The frequency distribution histogram of log MR values showed a bimodal distribution, indicating the presence of both EMs and poor PMs phenotypes (Figure 1). Subjects with metabolic ratio > 0.3 in log scale were classified as PMs and the remaining subjects with metabolic ratios  $\leq$  0.3 were classified as extensive metabolizers.



**Figure 1.** Frequency distribution and probit plot of dextromethorphan/dextrorphan (DEX/DOR) metabolic ratio as CYP2D6 activity index in the healthy subjects.

The ratio of DEX/DOR at 3 h was used as the index of CYP2D6 activity and a value of 2 (0.3 in log scale) for this ratio was used as the antimode separating EM and PM phenotypes (Yeh, Tao et al. 2003).

A summary of CYP2D6 phenotyping in our healthy subjects is shown in Table 1. From 71 patients phenotyped, 7.04% (5/71) of subjects were PM phenotype. Multiple regression analysis revealed that 4% of the overall variation in the log DEX/DOR MRs was explained by urinary pH. There was no significant contribution from other potential covariates (age and sex).

**Table 1.** A summary of CYP2D6 phenotyping in 71 healthy volunteers.

Phenotype	log DEX/DOR metabolic ratio Mean±SD	95% CI On the Mean	
		Lower limit	Upper limit
<b>All subjects</b>			
Female(n=37)	-0.51±0.54	-0.69	-0.34
Male (n=34)	-0.42±0.54	-0.61	-0.23
<b>EM</b>			
Female(n=35)	-0.62±0.29	-0.72	-0.52
Male(n=31)	-0.55±0.35	-0.68	-0.42
<b>PM</b>			
Female(n=2)	1.37±0.01	1.30	1.44
Male(n=3)	0.87±0.50	-0.36	2.11

log DEX/DOR metabolic ratio = log dextromethorphan/dextrorphan metabolic ratio at 3 hr plasma samples as CYP2D6 activity index in the healthy subjects; PM= poor metabolizer; EM= extensive metabolizer.

## Discussion

CYP2D6 display high levels of genetic polymorphism in human populations. Our results showed that the frequency of PM phenotype (7%) in the Mazandarani ethnic group study was very similar to those documented for Caucasians in Europe and North America (7–10%) (17-22), and Turkish (7-9.4 %) (23, 24), but was higher than those reported for Chinese, Korean, Japanese, Malays, Indians, Arabs, Mexican Americans, Black, Azeri ethnic group among Iranian population, and a non selected population residing in Iran. (6, 13, 14, 17-19, 24-31). A higher proportion of phenotypic poor metabolizers in our study compared with that of previous report (2.5%) (31), may be related to the nonselected subjects who participated in their study or the effect of urinary pH which was not corrected in their study.

In conclusion, the result of present study showed that the frequencies of CYP2D6 PM phenotypes in Mazandarani ethnic group (7.04%) were comparable with those found in Caucasians (7–10%) (17-22) and Turkish (6.7-9.4 %) (23, 24). Despite these findings, we propose further studies in larger samples to provide a wider image and to get more valuable information upon pharmacogenetic basis for individual therapy and personalized medicine. Since CYP2D6 is involved in the metabolism of many clinically important drugs, the observed decrease in metabolic activity within 7% of our subjects may influence the efficacy and toxicity of these agents. Accordingly, clinicians should be aware of the risk of drug toxicity as a result of decreased CYP2D6 activity.

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