

Genotype and Allele Frequency of P450 Oxidoreductase *28 Gene Polymorphism in South Indian Population

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Abstract

Objective: To establish the normative frequency of POR *28 genetic variant in South Indian population. **Methods:** This study is a genetic epidemiological study conducted on healthy volunteers belonging to south India. A total of 108 healthy volunteers who satisfied the eligibility criteria were included in the study. The DNA isolation was done using the QIAGEN Blood DNA isolation kit (Qiagen). The genotyping for POR *28 polymorphism was done using TaqMan genotyping master mix kits (Applied Biosystems) in real-time polymerase chain reaction platform. The data were analyzed using Fisher's exact test. $P < 0.05$ was considered statistically significant. Hardy-Weinberg principle was tested using the Chi-square test. **Results:** The genotyping of the POR *28 showed that the wild-type (homozygous) in the south Indian population was 41.7% and heterozygous and mutant type as 47.2% and 11.1%, respectively. The allele frequency showed that the minor allele frequency (MAF) was 34.7% in the south Indian population. This distribution of allele frequency was found to be significantly different from that of African-Americans 0.191 (95% confidence interval 0.157, 0.231). It was not statistically significant when compared to Caucasians 0.264 (0.228, 0.304); Chinese 0.367 (0.319, 0.419); Mexicans 0.310 (0.265, 0.360); and Japanese 0.40 (0.339, 0.464). **Conclusion:** The frequency distribution of POR *28 genetic variant was established in the south Indian population. The MAF was found to be 34.7% which was significantly different from other major ethnic groups.

Keywords: Genotyping, minor allele frequency, POR *28

INTRODUCTION

Cytochrome P450 (CYP P450) is a major group of the drug metabolizing enzyme. Its activity has been found to be altered by genetic and nongenetic factors. The inter-individual variability in response to drug treatments measured clinically is one of the public health concerns. This variability has been partly due to genetic besides nongenetic factors such as age, gender, and body mass index.^[1] Hence, any alteration in the CYP P450 activity was thought to affect the drug metabolism leading to variability in drug response.

CYP Oxidoreductase (POR) helps in the reactions of microsomal CYP which metabolize various drugs and steroid hormones.^[2-4] The POR is a microsomal protein that contains both flavin adenine dinucleotide and flavin mono-nucleotide (FMN) moieties. The principal function of POR is to transfer electrons from reduced nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) through FMN which then associates with CYP, thereby

permitting catalysis of the reactions leading to drug metabolism process.^[5,6]

Human POR is coded by a single gene POR, which is located on the long arm of chromosome 7 (pter-q22). POR contains 15 protein-coding exons (exons 1–15) and one noncoding exon (exon 1U). Human POR is a 79 kDa protein containing 680 amino acid residues.^[7] This three-dimensional structure of POR was discussed by Wang *et al.*^[8]

With the association of CYP enzymes and POR in relation to hepatic drug metabolism we can expect that a person with POR

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mutation can be associated with impaired drug metabolism that can increase or decrease in drug response. Several studies reported the influence of POR variants on drug metabolizing cytochrome enzymes which are still being explored by many researchers.^[9-12]

It is also found that individuals with POR deficiency present with a broad range of steroidogenic disorders. Congenital adrenal hyperplasia was due to mutations in the gene encoding POR which lead to deficiency of this enzyme causing accumulation of steroid metabolites.^[13,14]

Studies conducted in the sequencing of POR in the different population have showed many polymorphic variants. A study on 842 healthy individuals from different ethnic groups found 140 single-nucleotide polymorphisms (SNPs) of POR and suggested that POR*28 variant (A503V) played an important role in influencing interindividual variation in drug metabolism.^[15] A study conducted in Japanese population discussed the haplotype structure and genetic variation of POR.^[16]

In a study on the pharmacogenomics of POR by Pandey and Sproll in 2014, various aspects of POR mutations were discussed in different population. He also described POR*28 (p. A503V allele) as the most common variant among the polymorphisms which was involved in the various drug metabolizing activity.^[17] POR*28 has been found to be one of the common variants of CYP POR which is known to influence CYP3A4, CYP3A5 and many other cytochrome enzyme activities which are still to be explored.^[18-21]

Although some information is available about POR polymorphism in many ethnic groups on frequency distribution, no data are available in south Indian population. This study will provide the normative frequency of POR*28 in south Indian population which can be used for further clinical pharmacogenetic studies in disease susceptibility and drug response. Hence, the aim of this study was to assess the genotype and allele frequency of POR*28 gene in the south Indian population.

SUBJECTS AND METHODS

This genetic epidemiological study recruited 124 healthy volunteers of which 108 volunteers who satisfied eligibility criteria were included in the study. All the study participants who participated were persons residing in south India for at least three generations. The participants were not related to each other and had any one of the four south Indian languages as their mother tongue. This study was conducted at Mahatma Gandhi Medical College and Research Institute, Pudhucherry during July–August 2017. The study was started after approval from the Institutional Ethics Committee. The participants were explained about the study procedure in detail and written informed consent was obtained.

Inclusion and exclusion criteria

Inclusion criteria were volunteers between 18 and 65 years age group of either gender belonging to south India including

persons residing in Tamil Nadu, Kerala, Karnataka, or Andhra Pradesh for at least three generations. The participants were persons with normal basic clinical examinations and routine blood investigations (complete blood count, random blood sugar, liver function test, renal function test, lipid profile, and urine routine). Separate datasheets were used to know about the status of their smoking, alcohol, drug intake, and lifestyle as well for entry of other related information of each.

Person who had comorbid illness such as hypertension, diabetes, asthma, and pregnant or lactating women were excluded from the study.

Genotyping

A volume of 5 ml of blood was collected from all participants in polypropylene tubes with anticoagulant (100 µl of 10% ethylenediaminetetraacetic acid). The DNA isolation was done after analysis for hematological and biochemical parameters were found to be normal. The DNA was isolated from the blood sample using QIAGEN Blood DNA isolation kit (Qiagen) with instructions followed as per the kit. The genotyping of the DNA samples for POR*28 polymorphism was done using TaqMan genotyping master mix kits (Applied Biosystems) with assay ID C_8890131_30 (Thermo Fisher) in Real Time polymerase chain reaction platform. The results were analysed using Rotor-Gene Q software. The POR*28 was genotyped as wild-type CC, heterozygous CT and mutant TT and minor allele frequency (MAF) in the south Indian population was estimated.

Statistical analysis

Statistical analysis was carried out using Microsoft Excel 2007. Mean, standard deviation (SD), and ratios were used to represent the baseline characteristics of the study participants. The data on genotype and allele frequency were represented as percentage. The data were analyzed using Fisher's exact test. Value of $P < 0.05$ was considered statistically significant. The Hardy–Weinberg principle was tested using the Chi-square test.

RESULTS

The mean age of the participants was 35.4 (SD = 9.37) with a gender ratio of 50.93:49.07 (% Female: Male). The demographic characteristics of the participants are represented in Table 1.

The frequency of allelic variants and genotype of POR*28 in south Indian population is shown in Table 2. The genotyping of the POR*28 showed that the wild-type (homozygous CC) in south Indian population was 41.7% and heterozygous (CT) and mutant type (TT) as 47.2% and 11.1%, respectively. The allele frequency showed that the MAF was 34.7% in our population.

The goodness of fit Chi-square was applied to test the significance between the observed genotype frequency and Hardy–Weinberg expected frequency. The result showed that the observed genotype frequency was not different from Hardy–Weinberg expected frequency and concluded that the genotype frequency in the study population remains

Table 1: Demographic characteristics of the study subjects

Parameters	Subjects (n=108)
Age (years)	35.4±9.37
Sex (%Female: Male)	50.93: 49.07
BMI (kg/m ²)	21.9±2.41
SBP (mmHg)	111.81±8.76
DBP (mmHg)	77.83±5.25
Smokers (Yes: No)	13/95
Alcohol users (Yes: No)	20/88

DBP: Diastolic blood pressure, SBP: Systolic blood pressure, BMI: Body mass index, Values are mean±SD and numbers

Table 2: Genotype and Allele frequency of POR*28 in south Indian Population

Genotype frequency			Allele frequency	
Wild type C/C	Heterozygous C/T	Mutant T/T	C	T
41.70%	47.20%	11.10%	65.30%	34.70%

constant according to the Hardy–Weinberg principle [$P = 0.914$, Table 3].

The results obtained from this study were compared with compiled data reported for different ethnic population [Table 4].^[15,16] The MAF of POR*28 in the south Indian population was 0.347 (95% confidence interval [CI] 0.2599–0.436). The MAF of south Indians was found to be significantly higher than African–Americans 0.191 (95% CI 0.157, 0.231). It was not significant when compared to Caucasians 0.264 (0.228, 0.304); Chinese 0.367 (0.319, 0.419); Mexicans 0.310 (0.265, 0.360); and Japanese 0.40 (0.339, 0.464).

DISCUSSION

Polymorphism of POR*28 gene is found to be associated with endocrinological abnormalities, alteration to various drugs, cholesterol metabolism and many other CYP450 metabolizing enzyme activities.^[10–13] The variations in POR related to metabolism of drugs and xenobiotics are being studied in a variety of populations, and this is yet to be studied in south Indian population.^[15,16]

In a study on NADPH-Cytochrome POR the modulation of POR gene on CYP3A activity via electron transfer to NADPH cytochrome enzymes was discussed. This study explained the variability in CYP3A activity by POR which formed the basis for individual variation of drug metabolism.^[22]

The impact of POR*28 SNP on the metabolism of tacrolimus on CYP3A5 genotype in renal transplantation was tested in a study. They found higher dose requirement of tacrolimus in carriers of CYP3A5*1 allele, but in the individuals with CYP3A5 nonexpressors (CYP3A5*3/*3 allele), the POR*28 had no effect on tacrolimus pharmacokinetics. Hence, this study had shown the influence of POR*28 on CYP3A5 in tacrolimus.^[18]

Table 3: Comparison of observed and expected genotype frequency according to HWP

	Genotype frequency			Total
	Wild type C/C	Heterozygous C/T	Mutant T/T	
Observed	45	51	12	108
Hardy-Weinberg expectation	45.7	49.1	13.2	108

Pearson's Chi square=0.18, $P=0.914$

Table 4: Comparison of Allele frequency of POR*28 in south Indian with other major ethnic groups

Population	Allele frequency	P
South India (n=108)	0.347 (0.2599, 0.436)	
African Americans ^s (n=434)	0.191 ^a (0.157, 0.231)	0.0008
Caucasian ^s (n=518)	0.264 ^b (0.228, 0.304)	0.099
Chinese ^s (n=354)	0.367 ^b (0.319, 0.419)	0.638
Mexican ^s (n=364)	0.310 ^b (0.265, 0.360)	0.529
Japanese ^e (n=235)	0.40 ^b (0.339, 0.464)	0.308

Values given in parentheses are 95% confidence interval. n=number of subjects. ^sCompiled data from reference 15. ^eReference 16. ^aNot significant when compared to south Indian population. ^bSignificant when compared to south Indian population

Similarly, another study showed the influence of POR*28 (variant p. A503V) in CYP3A activity. It was found that POR*28 variant had higher levels of CYP3A activity almost 1.6 fold increase which was measured by midazolam phenotyping test. This study identified the influence of POR*28 on midazolam clearance.^[23]

However, in a study on the effect of POR*28 on *in vitro* and *in vivo* metabolism of sirolimus in kidney transplant recipients found that the POR*28 allele had no significant influence on sirolimus concentrations or dose regardless of the CYP3A5 genotype. They also identified that the absence of influence of POR*28 on sirolimus probably accounts for lack of influence of this polymorphism on adverse effects.^[24]

Studies have been conducted in different populations on POR sequencing to identify the genotype and allele frequency. A study on POR genetic analysis in four ethnic populations detected that the MAF of POR*28 was 19.1% in African–Americans, 26.4% in Caucasian–Americans, 36.7% Chinese–Americans, and 31.0% in Mexican–Americans. The study described 140 SNPs and also found that A503V variant could be responsible for variation in drug response.^[15]

A study conducted in Japanese population showed that the POR*28 (p. A503V variant) frequency was highest at 40%.^[16] This discrepancy in genomic sequence data available at NCBI SNP database was described by Pandey and Sproll.^[17]

Any gene is considered to be polymorphic when the variation in the allele exists more than 1% in the population. These variations can alter the activity of the protein encoded by it

and once this happens, it occurs in relation to the wild-type sequence. In most of the cases, these polymorphisms of the gene with allelic variants were found to be associated with either reduced activity or enhanced activity of the protein coded by it.^[25] With respect to our study POR*28 gene which codes for the CYP enzyme activity, it can bring about alteration in its activity in drug metabolism or steroid metabolism as well as disease susceptibility. The characterization of POR*28 gene in relation to clinical pharmacogenetics studies have to be elucidated, and their clinical importance has to be defined in south Indian population.

With this information from this study, POR*28 gene has to be further explored which can bring about better choices for drug therapies and dose adjustments can be made to improve treatment outcomes and to minimize the adverse reactions which would justify the cost and inconvenience of the test.

CONCLUSION

This study has established the normative frequency distribution of POR*28 gene in south Indian population. The minor allele frequency of POR*28 gene was found to be 34.7% in south Indian population. This frequency was different from other major ethnic groups. Further clinical pharmacogenetics studies will be done which will help to identify the disease susceptibility and drug response in relation to this gene.

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Conflicts of interest

There are no conflicts of interest.

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