

Identification of the Single Nucleotide Polymorphisms and their Frequency Present in the Targeted Region of *POR* Gene in South Indian Population

Rajalakshmi Kumar¹, Adithan Chandrasekaran², Ritushree Kukreti³, Balanehru Subramanian⁴

ABSTRACT

Background and objectives: Cytochrome P450 oxidoreductase (*POR*) gene act as an electron donor to other microsomal cytochrome P450 (CYP) enzymes. This study is attempted to identify the *POR* genetic variants present in the proximal promoter region, hotspot coding regions that affect the function of *POR* gene, and the 3'UTR region encompassing miRNA binding sites among the South Indian population.

Materials and methods: Blood samples were collected from 110 south Indian subjects. Genomic DNA was isolated from whole blood and the target regions of the *POR* gene were amplified using specific primers. The PCR amplicons were sequenced by the Sanger method. The variants present in proximal promoter region, exons (9, 10, 11, and 12) with boundary regions around 100 bp region and 3'UTR region, were analyzed. The microRNA database (miRDB) prediction tool was used to analyze miRNA binding regions in the 3'UTR region.

Results: The SNPs (rs72553971, rs41301394, rs41301400, rs4732514, rs2286822, rs2286823, rs41301427, and rs17685) were observed in the *POR* gene with frequency of 2.7, 31.36, 2.27, 57.27, 23.63, 23.63, 14.54, and 35%, respectively. All these SNPs were previously reported in other populations. No novel variation has been observed in the hotspot coding region of the *POR* gene among South Indian population.

Conclusion: The frequency of *POR* gene polymorphisms is reported for the first time among the South Indian population. This study provides fundamental and useful information on *POR* pharmacogenetics among this population, which can be utilized for future research.

Keywords: Minor allele frequency, Polymorphism, *POR* gene, Single nucleotide polymorphism, South Indian.

SBV Journal of Basic, Clinical and Applied Health Science (2020): 10.5005/jp-journals-10082-02269

INTRODUCTION

The human NADPH cytochrome P450 oxidoreductase (*POR*) gene is present in the chromosome number 7q11.2, which is approximately 72 kb in size and consists of 15 protein-coding exons, with one untranslated exon.¹ The *POR* enzyme regulates multiple metabolic processing enzymes involved in the drug metabolism, steroidogenesis, and also xenobiotics.^{2,3} The *POR* gene is highly polymorphic and exhibits a number of variations among the individuals, which are highly responsible for the altered drug metabolic reactions that are observed in diseases like Antley-Bixler syndrome, polycystic ovarian syndrome, cytochrome P450 oxidoreductase deficiency (PORD), and congenital adrenal hyperplasia.⁴⁻⁷ The *POR* protein consists of 680 amino acids with four domains namely, flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), hinge domain and nicotinamide adenine dinucleotide domain.^{8,9} Flavin moieties act as a cofactor for the catalytic activity of the protein.^{10,11}

The crucial function of the *POR* protein is to donate electrons through a series of reactions to many other CYP enzymes for their proper biological activity.^{12,13} The single nucleotide polymorphisms (SNPs) present in the gene may lead to structural and functional variation in the protein and also alters the drug metabolizing activity among the individuals.¹⁴ Each and every individual shows the differences in the drug response, which can be attributed by the variability in the DNA sequence of the *POR* gene both in exons as well as introns.¹⁵ Variations present in the intronic region have the potential to induce or reduce the splicing mechanism efficiency.¹⁶ The proximal promoter region of the *POR* gene is present immediate to the upstream of exon

^{1,4}Department of Central Inter-disciplinary Research Facility, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, India

²Department of Pharmacology, Mahatma Gandhi Medical College and Research Institute, Puducherry, India

³Department of Functional Genomics and Molecular Medicine Unit, CSIR-IGIB, Delhi, India

Corresponding Author: Rajalakshmi Kumar, Department of Central Inter Disciplinary Research Facility, Sri Balaji Vidyapeeth (Deemed to be University), Puducherry, India, Phone: +91 8148521992, e-mail: raji145.k@gmail.com

How to cite this article: Kumar R, Chandrasekaran A, Kukreti R, Subramanian B. Identification of the Single Nucleotide Polymorphisms and their Frequency Present in the Targeted Region of *POR* Gene in South Indian Population. *J Basic Clin Appl Health Sci* 2020;3(4): 158-163.

Source of support: This study was funded by Dr Vany Adithan Research Fellowship and supported by Sri Balaji Vidyapeeth (Deemed to be University).

Conflict of interest: None

1U, showing various effects of the altering hormone level in case of polymorphisms.^{17,18} The 3'UTR region plays an important role for binding the miRNA, to enhance the gene expression and translation process.¹⁹ Some of the SNPs in this region may inhibit the binding specificity of the miRNA.²⁰

The worldwide study reported that the majority of protein-altering variants and disease-causing variants fall in the hotspot coding regions. The risk variants Ala287Pro and Arg457His were

associated with Antley-Bixler syndrome (ABS) and the breast cancer risk was located within the region of exon 9–12 in other population.^{21,22} This study is attempted to assess the frequency of polymorphisms in selected regions (exon 9–12), promoter region, and 3'UTR region of the *POR* gene among healthy individuals of South Indian population.

MATERIALS AND METHODS

The study protocol was approved by the institutional human ethics committee and a written informed consent was obtained from the participants who satisfied the eligibility criteria.

Inclusion and Exclusion Criteria

The volunteers were of South Indian origin residing in Tamil Nadu, Kerala, Karnataka, Andhra Pradesh, and Puducherry for at least last three generations. The participants were selected with the result showing normal hematological and biochemical parameters like random blood sugar, hemoglobin, lipid profile, renal function, and routine urine test. Severe and complication illnesses like hypertension, diabetes mellitus, and asthma patients were excluded from the study. Pregnancy and lactating mothers were also omitted.

A total of $n = 110$ unrelated healthy volunteers aged between 18 and 65 years were randomly selected and 2 mL of peripheral venous blood was collected in an EDTA (ethylenediaminetetraacetic acid) anticoagulant tubes. Genomic DNA was isolated from whole blood using commercial kits (Qiagen, Germany) and stored at 4°C. Targeted regions in *POR* gene were amplified through polymerase chain reaction using appropriate forward and reverse primers (Table 1) using the following reaction conditions (Table 2). Each 35 μ L

reaction consists of 3 μ L of genomic DNA (50 ng/ μ L), 17.5 μ L of 10 \times PCR Top Taq Master Mix, 1 μ L of each primer (10 pmol/ μ L), and 12.5 μ L of nuclease free water. Amplification was performed using Veriti 96 well Thermal Cycler (Applied Biosystem, USA). The PCR products were visualized in 1% agarose gel with ethidium bromide staining.

Sanger Sequencing

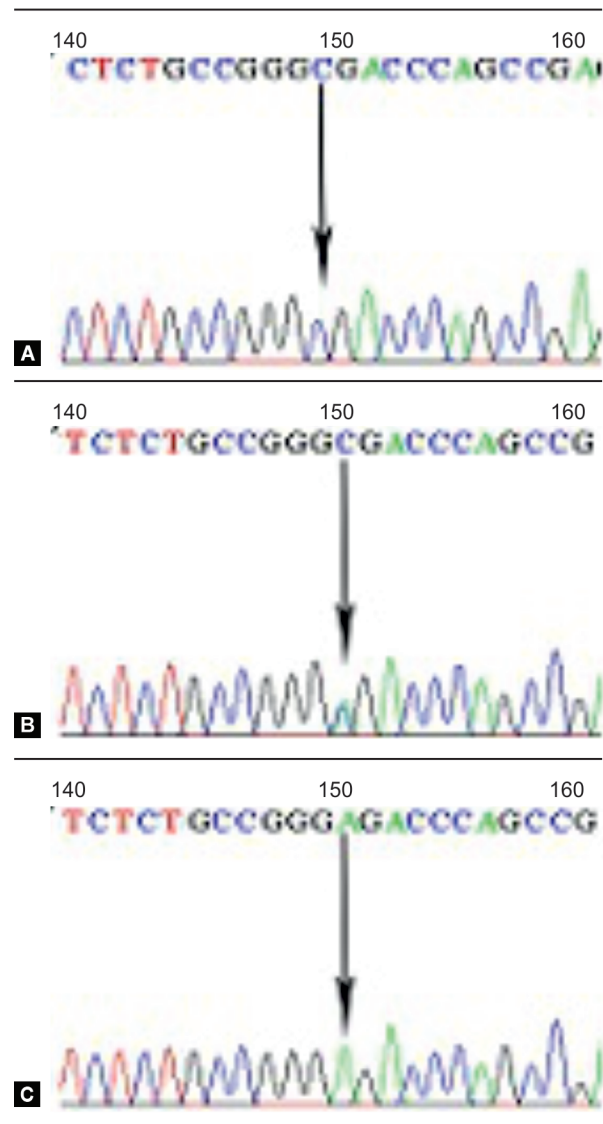
The sequencing of PCR products was performed by MacroGen Inc. (South Korea). The derived electropherogram (Fig. 1) was manually evaluated and confirmed by the sequence with the nucleotide BLAST. The NCBI genomic DNA NC_000007.14: 75914700–75986855 FASTA sequence was used as a reference to analyze variants. The identified intronic variants are numbered according to the NCBI reference sequence, and exons were identified from the initiation codon ATG (A is considered as +1).²³ Gene sequence ID 5447 were used to predict the miRNA targeted region in the 3'UTR region. The miRDB (<http://mirdb.org>) online bioinformatics tool was used to predict the miRNA binding site in the 3'UTR sequences.

Table 1: Primers used to amplify the targeted region of the *POR* gene

Amplified region	Forward primers 5'–3'	Reverse primers 5'–3'	Amplicon size	Ref.
+36_as–325_s	TCAGGC-CACACCACT-GAGG	GCCCGAA-GGAGGAG-GCTAGA	361 bp	17
Exon 9–10	GTAACCG-GTGAGATTTC-CTCAT	ACTATGACAGT-GACGGGGTAGG	692 bp	25
Exon 11–12	AGGGAG-GCATCAGAGA-GCATAG	GGCTGGACA-GATGCTGAGAA	781 bp	25
3'UTR	GGGATGTGCA-GAACACCTTC	GGTTC-CTGGGGTCT-GAGTTA	572 bp	34

Table 2: PCR conditions for amplifying the *POR* gene

PCR conditions	Proximal promoter region	Exon 9–10	Exon 11–12	3'UTR region
Initial denaturation	95°C/3 minutes	95°C/3 minutes	95°C/3 minutes	95°C/3 minutes
Denaturation	95°C/1 minute	95°C/1 minute	95°C/1 minute	95°C/1 minute
Annealing	60°C/30 seconds	59°C/30 seconds	60°C/30 seconds	59°C/30 seconds
Extension	72°C/2 minutes	72°C/1 minute	72°C/2 minutes	72°C/1 minute
Final extension	72°C/7 minutes	72°C/7 minutes	72°C/7 minutes	72°C/7 minutes
Infinity	4°C	4°C	4°C	4°C



Figs 1A to C: Electropherogram shows that the SNP changes in the sequence of the *POR* gene. The black arrow indicates the SNP change: (A) Wildtype alleles CC; (B) Heterozygous alleles CA; (C) Variant alleles

Statistical Analysis

The χ^2 test for goodness of fit was used to determine the genotypic distribution of each SNP following Hardy-Weinberg equilibrium (HWE) calculated using Microsoft Excel. Linkage disequilibrium (LD) was calculated using the Haploview Software version 4.1.²⁴ p values < 0.05 are considered statistically significant.

RESULTS

The proximal promoter region of the *POR* gene sequencing showed SNPs rs72553971 and rs72553972 at the position -173 (C > A) and -152 (C > A) with a frequency of 2.7 and 15.45%, respectively, in the South Indian population (Table 3). We have found 12 SNPs in the noncoding intronic region, which were located nearby the boundary of the hotspot exons and also only one variation, rs17685 (G > A), was found at the 3'UTR region of the *POR* gene with the frequency of 35%, in the 110 human whole blood samples (Table 3). There were three SNPs found in the ninth intronic region rs13223707 (C > G); rs13240147 (G > A); and rs41301394 (C > T) with the variant allele frequency showing 91.36, 90.45, and 31.36%. One SNP was found in the ninth intronic region rs562843415 (G > A) with the minor allele frequency was less than 1%, but their HWE value showed the significant p value > 0.05 of this variant. Four SNPs were found in the eleventh noncoding region of the *POR* gene, rs41301400 (G > C), rs4732514 (C > T), rs4732515 (T > C) and rs4732516 (C > G), with the frequency of variant allele: 2.27; 57.27, 91.81, and 90.45% respectively, whereby three SNPs were consistent with the HWE, except rs4732516. Two SNPs were found in the eleventh intronic region rs2286822 (C > T) and rs2286823 (G > A). Minor allele frequency of 23.63% was obtained for both of the variants, and significant differences in the p value of HWE were observed. Twelfth intronic region has two SNPs rs41301427 (G > A) and rs377357128 (G > A) with the variant allele frequency: 14.54 and 0.90%, and the p value was significant to the equilibrium. From this study, four SNPs were not in HWE (rs72553972, rs13223707, rs13240147, and rs4732516), showing p value < 0.05 . According to miRDB (miRNA database), *POR* gene is predicted to be targeted by 14 miRNAs in miRDB, but no variations were found in the miRNA binding site region in South Indian population. The comparison

of the worldwide population allele frequency of the *POR* gene is shown in Table 4.

The linkage disequilibrium analysis was performed for all 15 SNPs obtained in the South Indian population across the *POR* gene-targeted regions (Fig. 2). The red color indicates the relatively strong linkage among the SNPs. The r^2 value is considered as a guideline for the linkage between a pair of single nucleotide variations and D' is indicated to determine the probability for the previous recombinations. According to the r^2 value, the perfect linkage was found between the SNPs rs2286822 and rs2286823 ($r^2 = 1$; $D' = 1$). The SNPs between rs4732515 and rs4732516 relatively show strong association by the value $r^2 = 0.8$; $D' = 1$. The value $D' = 1$ indicates that the SNPs were strongly linked between both the pairs rs41301394 and rs2286822; rs41301394 and rs2286823, whereas the $r^2 = 0.1$ exhibited weak linkages.

In this study, we have found SNPs only in the intronic region, hence no SNPs were found in the exonic hotspot/protein-coding regions of the *POR* gene.

DISCUSSION

In recent years, there has been a huge pharmacogenomics interest on *POR* genetic variants. The *POR* gene polymorphisms have acquired great attention among researchers, due to their involvement in differential response to cytochrome-mediated drug metabolic activity, steroidogenic metabolism, and they are also indirectly associated with protein expression activity. In this study, we screened the SNPs present in the transcription start site of around 361 bp of untranslated exon ($-325/+36$). *POR* -173 C > A was found in the basal promoter region at a minor allele frequency of 2.7%, compared to other American populations showing that the Mexican Americans 3.1% is closely related to the South Indian population (Table 4).²⁵ The upstream SNP of *POR* -173 C > A has been reported to be negatively associated with the warfarin dose and reduces the gene expression in different ethnic groups.²⁶ The functional polymorphism of the *POR* proximal promoter variant -152 C > A is present at the AP-2 activator protein site, while the effect of the variant remains unclear till today.¹⁷ TFSEARCH (<http://www.cbrc.jp/research/db/TFSEARCH.html>) online tool showed that

Table 3: Allele frequency of *POR* gene in South Indian population

S. no.	Chromosomal position	rsID	<i>POR</i> gene region	Variant	Minor allele frequency (%)	Hardy-Weinberg equilibrium (p value)
1	7:75914929	rs72553971	-173 proximal promoter	C>A	2.7	0.7687
2	7:75914950	rs72553972	-152 proximal promoter	C>A	15.45	<0.05
3	7:75983452	rs13223707	8-intron	C>G	91.36	<0.05
4	7:75983465	rs13240147	8-intron	G>A	90.45	<0.05
5	7:75983485	rs41301394	8-intron	C>T	31.36	0.9366
6	7:75983877	rs562843415	9-intron	G>A	0.90	0.9233
7	7:75983957	rs41301400	10-intron	G>C	2.27	0.8073
8	7:75984680	rs4732514	10-intron	C>T	57.27	0.4548
9	7:75984711	rs4732515	10-intron	T>C	91.81	0.1087
10	7:75984764	rs4732516	10-intron	C>G	90.45	<0.05
11	7:75984970	rs2286822	11-intron	C>T	23.63	0.2570
12	7:75984978	rs2286823	11-intron	G>A	23.63	0.2570
13	7:75985239	rs41301427	12-intron	G>A	14.54	0.6058
14	7:75985331	rs377357128	12-intron	G>A	0.90	0.9618
15	7:75986787	rs17685	3'UTR	G>A	35	0.8422

Table 4: Frequency of POR gene variants in different ethnicities as compared with the South Indian population

Population	Allele frequency															Ref
	rs	rs	rs	rs	rs	rs	rs	rs	rs	rs	rs	rs	rs	rs	rs	
	72553971	72553972	13223707	13240147	41301394	562843415	41301400	4732514	4732515	4732516	2286822	2286823	41301427	377357128	17685	
African American	0.012	0.026	-	-	0.186	-	0.014	-	0.612	0.609	0.208	0.194	0.056	-	-	25
Caucasian American	0.044	0.13	-	-	0.267	-	0.000	-	0.959	0.959	0.319	0.317	0.083	-	-	25
Chinese American	0.015	0.078	-	-	0.368	-	0.000	-	0.867	0.870	0.360	0.360	0.056	-	-	25
Mexican American	0.031	0.077	-	-	0.310	-	0.000	-	0.865	0.869	0.407	0.407	0.058	-	-	25
Japanese	-	0.019	-	0.134	0.434	-	0.098	0.419	0.134	0.132	0.389	0.389	0.011	-	0.385	14
South Indian	0.027	0.1545	0.9136	0.9045	0.3136	0.009	0.0227	0.5727	0.9181	0.9045	0.2363	0.2363	0.1454	0.009	0.35	PS
Moroccan Jewish	-	-	-	-	-	-	-	-	-	0.938	0.393	0.389	-	-	-	14,25
Ashkenazi (T)	-	-	-	-	-	-	-	-	-	0.991	0.385	0.382	-	-	-	14,25
Ashkenazi	-	-	-	-	-	-	-	-	-	0.87	0.36	0.36	-	-	-	14,25
Gomes et al.	-	-	-	-	-	-	-	-	-	-	0.293	0.297	-	-	-	3
Saito et al.	-	-	-	-	-	-	-	-	-	0.132	0.389	0.389	-	-	-	3,14,25
Hart et al.	-	-	-	-	-	-	-	-	-	0.953	0.359	0.374	-	-	0.195	29,34
1000 Ge-nomes	0.022	0.1040	0.811	0.811	0.2749	0.001	0.0251	0.505	0.812	0.812	0.304	0.300	0.072	0.001	0.269	35
TOPMED	0.039	0.1122	0.837	0.837	0.2490	3.981	0.0102	0.569	0.838	0.837	0.295	0.288	0.075	7.964	0.245	35
HapMap projects	-	-	-	-	-	-	-	-	-	0.2701	-	-	-	-	-	36

PS, Present Study

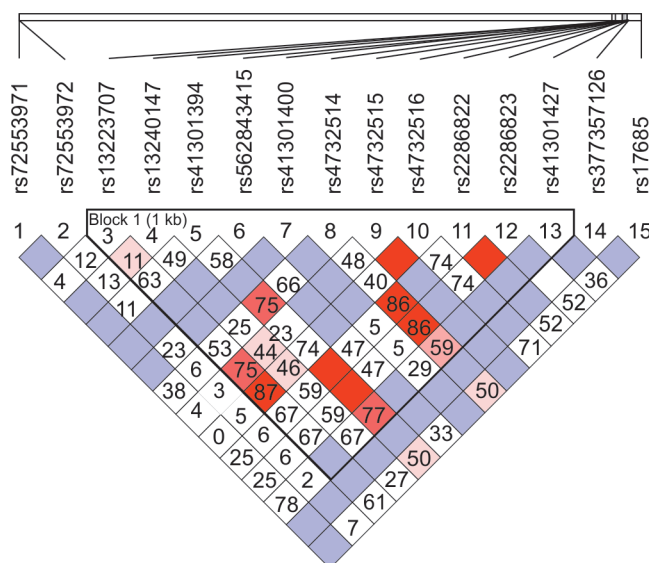


Fig. 2: Linkage disequilibrium pattern of the analyzed polymorphisms across the target regions in *POR* gene in the South Indian population (LOD > 2; bright red: $D' = 1$, shades of red/pink: $D' < 1$; for LOD < 2: blue: $D' = 1$, white $D' < 1$)

the putative binding domain (eg: homeodomain factor NKx2.5) present in the proximal promoter region of the *POR* gene.¹⁸ In connecting that, reports to neither of the two promoter region SNPs had an impact on the transcription of a 325-bp *POR* basal promoter in reporter gene assays.¹⁷ According to the study of Ningwu Huang et al. in the year of 2007, they screened the SNPs present in *POR* gene among 842 individuals of four different ethnic populations. Majority of the protein-altering SNPs identified in the exonic region of 8th to 14th were highlighted in various population.²³ Hence, the 9th to 12th exons were considered as a hotspot region, while the variants Ala287Pro and Arg457His associated with ABS and breast cancer risk variant also fall within this region.^{21,22} The primers were considered for the hotspot region based on the research article by Huang et al. The sequenced results were analyzed with the FASTA sequence from the UCSC genome browser template, recently updated version of *POR* gene human assembly [Dec. 2013 (GRCh38/hg38)]. This showed that the region sequenced was not present in the 8th exon. Our ultimate aim is to find the frequency of *POR* gene variants in South Indian population, while the common variant Ala503Val in exon 13 was already reported in this population with their respective frequency.²⁷ In exon 14, only one variant Tyr607Cys was previously reported in the study with the lower frequency of 0.002 in Caucasian American.²⁵ Hence, the exon 13 and 14 has been excluded from the study. In Chinese population, three intronic variants (rs2286823, rs41301394, and rs2302429) were found to be associated with the altered *CYP1A2*, *CYP2C19*, or *CYP3A4* enzyme activities, whereas in our population, the SNPs (rs41301394 and rs2286823) have been found with the MAF of 31.36 and 23.63%, respectively, which gives a hint for further pharmacogenetic research.²⁸ The polymorphism of the Leu577Pro and rs41301427 (G > A), in other population, was associated with the decreased *POR* protein activity, but not in the other P450 enzyme activities.²⁹ In the present study, we found that MAF of the SNP (rs41301427) exhibited as 14.54% in the South Indian population, which is considered as statistically significant, according to HWE.³⁰ Henceforth, further studies need to be conducted in order to confirm the association of Leu577Pro and rs41301427

(G > A) in the South Indian population, which might bring about decreased *POR* activity. The SNP rs2286822 (C > T) heterozygous sample in Chinese population liver sample shows the decreased level of mRNA expression as compared as homozygous mutant and the rs2286822 and rs2286823 produce higher protein content while comparing with homozygous allele.³¹ The SNP rs2286822 was majorly associated with the congenital adrenal hyperplasia patients caused due to 21 hydroxylase mutations.³² According to our study, four SNPs (rs72553972, rs13223707, rs13240147, and rs4732516) were not following HWE, hence a larger sample pool may be required further to calculate the minor allele frequency. The variant associated with the ABS, Arg457His, was absent in our population. There has been an association of both intron and exon variants, which are involved in drug metabolism, majority of variant association with drug metabolism present in the *POR* gene remains unclear. The therapeutic effects of drugs, influencing the knowledge of *POR* pharmacogenomics, would help both doctors and patients to choose the proper medication and regimen, and provide better guidance for personalized medicine and reference for treatment of disease in clinic.³³

LIMITATIONS OF THE STUDY

In this study, the normal allele frequency is estimated in a targeted region of the *POR* gene in South Indian population. For the frequency estimation in a population, small sample size is required, so we took 110 individual samples. The study can be extended further with greater sample size to estimate normal allele frequency. *POR* gene contains totally 15 coding exon regions, whereby we sequenced only targeted region to estimate the minor allele frequency of SNPs and if we sequence the entire gene there is possibility to get more novel variations.

CONCLUSION

In this study, we identified 15 intronic variants in the *POR* gene-targeted regions. Based on the linkage disequilibrium profile, the analyzed region was mentioned into blocks. This is the first study to provide information on *POR* gene-targeted region polymorphisms and associated LD plot in a South Indian population. The findings of this article will be useful for future pharmacogenetic, epigenetic studies and also to analyze the interindividual differences in drug metabolism of the CYP activity.

ACKNOWLEDGMENTS

We would like to thank Sri Balaji Vidyapeeth (Deemed to be University) for providing basic infrastructure facility. I personally thank Dr Benet Bosco Dhas D for his timely guidance and help to improvise the manuscript.

REFERENCES

1. Shephard EA, Phillips IR, Santisteban I, West LF, Palmer CNA, Ashworth A, et al. Isolation of a human cytochrome P-450 reductase cDNA clone and localization of the corresponding gene to chromosome 7q11.2. *Ann Hum Genet* 1989;53(4):291–301. DOI: 10.1111/j.1469-1809.1989.tb01798.x.
2. Lewis DFW. 57 varieties: the human cytochromes P450. *Pharmacogenomics* 2004;5(3):305–318. DOI: 10.1517/phgs.5.3.305.29827.
3. Gomes AM, Winter S, Klein K, Turpeinen M, Schaeffeler E, Schwab M, et al. Pharmacogenomics of human liver cytochrome P450

- oxidoreductase: multifactorial analysis and impact on microsomal drug oxidation. *Pharmacogenomics* 2009;10(4):579–599. DOI: 10.2217/pgs.09.7.
4. Fukami M, Horikawa R, Nagai T, Tanaka T, Naiki Y, Sato N, et al. Cytochrome P450 oxidoreductase gene mutations and Antley-Bixler syndrome with abnormal genitalia and/or impaired steroidogenesis: molecular and clinical studies in 10 patients. *J Clin Endocrinol Metab* 2005;90(1):414–426. DOI: 10.1210/jc.2004-0810.
5. Huang N, Pandey AV, Agrawal V, Reardon W, Lapunzina PD, Mowat D, et al. Diversity and function of mutations in p450 oxidoreductase in patients with antley-bixler syndrome and disordered steroidogenesis. *Am J Hum Genet* 2005;76(5):729–749. DOI: 10.1086/429417.
6. Homma K, Hasegawa T, Nagai T, Adachi M, Horikawa R, Fujiwara I, et al. Urine steroid hormone profile analysis in cytochrome P450 oxidoreductase deficiency: implication for the backdoor pathway to dihydrotestosterone. *J Clin Endocrinol Metab* 2006;91(7):2643–2649. DOI: 10.1210/jc.2005-2460.
7. Arlt W, Walker EA, Draper N, Ivison HE, Ride JP, Hammer F, et al. Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. *Lancet* 2004;363(9427):2128–2135. DOI: 10.1016/S0140-6736(04)16503-3.
8. Xia C, Panda SP, Marohnic CC, Martásek P, Masters BS, Kim, et al. Structural basis for human NADPH-cytochrome P450 oxidoreductase deficiency. *Proc Natl Acad Sci USA* 2011;108(33):13486–13491. DOI: 10.1073/pnas.1106632108.
9. Campelo D, Esteves F, Palma BB, Gomes BC, Rueff J, Lautier T, et al. Probing the role of the hinge segment of cytochrome P450 oxidoreductase in the interaction with cytochrome P450. *Int J Mol Sci* 2018;19(12):3914. DOI: 10.3390/ijms19123914.
10. Wang M, Roberts DL, Paschke R, Shea TM, Masters BS, Kim JJ. Three-dimensional structure of NADPH-cytochrome P450 reductase: prototype for FMN- and FAD-containing enzymes. *Proc Natl Acad Sci USA* 1997;94(16):8411–8416. DOI: 10.1073/pnas.94.16.8411.
11. Vermilion JL, Ballou DP, Massey V, Coon MJ. Separate roles for FMN and FAD in catalysis by liver microsomal NADPH-cytochrome P-450 reductase. *J Biol Chem* 1981;256(1):266–277.
12. Ellis J, Gutierrez A, Barsukov IL, Huang W-C, Grossmann JG, Roberts GCK. Domain motion in cytochrome P450 reductase: conformational equilibria revealed by NMR and small-angle x-ray scattering. *J Biol Chem* 2009;284(52):36628–36637. DOI: 10.1074/jbc.M109.054304.
13. Montellano PROde. Cytochrome P450: structure, mechanism, and biochemistry Springer; 2015. p. 912.
14. Saito Y, Yamamoto N, Katori N, Maekawa K, Fukushima-Uesaka H, Sugimoto D, et al. Genetic polymorphisms and haplotypes of POR, encoding cytochrome P450 oxidoreductase, in a Japanese population. *Drug Metab Pharmacokinet* 2011;26(1):107–116. DOI: 10.2133/dmpk.dmpk-10-sc-096.
15. Hu L, Zhuo W, He Y-J, Zhou H-H, Fan L. Pharmacogenetics of P450 oxidoreductase: implications in drug metabolism and therapy. *Pharmacogenet Genomics* 2012;22(11):812–819. DOI: 10.1097/FPC.0b013e328358d92b.
16. Seo S, Takayama K, Uno K, Ohi K, Hashimoto R, Nishizawa D, et al. Functional analysis of deep intronic SNP rs13438494 in intron 24 of PCLO gene. *PLoS ONE* 2013;8(10):e76960. DOI: 10.1371/journal.pone.0076960.
17. Tee MK, Huang N, Damm I, Miller WL. Transcriptional regulation of the human P450 oxidoreductase gene: hormonal regulation and influence of promoter polymorphisms. *Mol Endocrinol* 2011;25(5):715–731. DOI: 10.1210/me.2010-0236.
18. Soneda S, Yazawa T, Fukami M, Adachi M, Mizota M, Fujieda K, et al. Proximal promoter of the cytochrome P450 oxidoreductase gene: identification of microdeletions involving the untranslated exon 1 and critical function of the SP1 binding sites. *J Clin Endocrinol Metab* 2011;96(11):E1881–E1887. DOI: 10.1210/jc.2011-1337.
19. Wei R, Yang F, Urban TJ, Li L, Chalasani N, Flockhart DA, et al. Impact of the interaction between 3'-UTR SNPs and microRNA on the expression of human xenobiotic metabolism enzyme and transporter genes. *Front Genet* 2012;3:248. DOI: 10.3389/fgene.2012.00248.
20. Fang Z, Rajewsky N. The impact of miRNA target sites in coding sequences and in 3'UTRs. *PLoS ONE* 2011;6(3):e18067. DOI: 10.1371/journal.pone.0018067.
21. Haiman CA, Setiawan VW, Xia LY, Le Marchand L, Ingles SA, Ursin G, et al. A variant in the cytochrome P450 oxidoreductase gene is associated with breast cancer risk in African Americans. *Cancer Res* 2007;67(8):3565–3568. DOI: 10.1158/0008-5472.CAN-06-4801.
22. McCammon KM, Panda SP, Xia C, Kim J-JP, Moutinho D, Kranendonk M, et al. Instability of the human cytochrome P450 reductase A287P variant is the major contributor to its antley-bixler syndrome-like phenotype. *J Biol Chem* 2016;291(39):20487–20502. DOI: 10.1074/jbc.M116.716019.
23. Antonarakis SE. Recommendations for a nomenclature system for human gene mutations. nomenclature working group. *Hum Mutat* 1998;11(1):1–3. DOI: 10.1002/(SICI)1098-1004(1998)11:13.O.CO;2-O.
24. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21(2):263–265. DOI: 10.1093/bioinformatics/bth457.
25. Huang N, Agrawal V, Giacomini KM, Miller WL. Genetics of P450 oxidoreductase: sequence variation in 842 individuals of four ethnicities and activities of 15 missense mutations. *Proc Natl Acad Sci USA* 2008;105(5):1733–1738. DOI: 10.1073/pnas.0711621105.
26. Zhang X, Li L, Ding X, Kaminsky LS. Identification of cytochrome P450 oxidoreductase gene variants that are significantly associated with the interindividual variations in warfarin maintenance dose. *Drug Metab Dispos* 2011;39(8):1433–1439. DOI: 10.1124/dmd.111.038836.
27. Mirunalini R, Pavithra G, Dhas DBB, Adithan C. Genotype and allele frequency of P450 oxidoreductase *28 gene polymorphism in South Indian population. *J Pharmacol Pharmacotherap* 2019;10(1):7–10. DOI: 10.4103/jpp.JPP_95_18.
28. Liu S, Chen R, Li J, Zhang Y, Wang X, Fu Q, et al. The POR rs1057868-rs2868177 GC-GT diplotypes is associated with high tacrolimus concentrations in early post-renal transplant recipients. *Acta Pharmacol Sin* 2016;37(9):12518. DOI: 10.1038/aps.2016.77.
29. Hart SN, Wang S, Nakamoto K, Wesselman C, Li Y, Zhong X. Genetic polymorphisms in cytochrome P450 oxidoreductase influence microsomal P450-catalyzed drug metabolism. *Pharmacogenet Genomics* 2008;18(1):11–24. DOI: 10.1097/FPC.0b013e3282f2f121.
30. Nakamoto K, Wang S, Jenison RD, Guo GL, Klaassen CD, Wan YJ, et al. Linkage disequilibrium blocks, haplotype structure, and htSNPs of human CYP7A1 gene. *BMC Genet* 2006;7(1):29. DOI: 10.1186/1471-2156-7-29.
31. Zhang H-F, Li Z-H, Liu J-Y, Liu T-T, Wang P, Fang Y, et al. Correlation of cytochrome P450 oxidoreductase expression with the expression of 10 isoforms of cytochrome P450 in human liver. *Drug Metab Dispos* 2016;44(8):1193–1200. DOI: 10.1124/dmd.116.069849.
32. Pecori GF, Verna F, Sesta A, Messina M, Menegatti E, Einanudi S, et al. SAT-346 POR rs2286822 polymorphism is associated with clinical features in patients with congenital adrenal hyperplasia due to 21 hydroxylase deficiency. *J Endocr Soc* 2019;3(Suppl. 1):SAT-346. DOI: 10.1210/js.2019-SAT-346.
33. Gong L, Zhang CM, Lv JF, Zhou HH, Fan L. Polymorphisms in cytochrome P450 oxidoreductase and its effect on drug metabolism and efficacy. *Pharmacogenet Genomics* 2017;27(9):337–346. DOI: 10.1097/FPC.0000000000000297.
34. Hart SN, Li Y, Nakamoto K, Wesselman C, Zhong X. Novel SNPs in cytochrome P450 oxidoreductase. *Drug Metab Pharmacokinet* 2007;22(4):322–326. DOI: 10.2133/dmpk.22.322.
35. Ensembl genome browser 100 [Internet]. [cited 2020 Jun 1]. Available from: <http://asia.ensembl.org/index.html>.
36. Home - SNP - NCBI [Internet]. [cited 2020 May 28]. Available from: <https://www.ncbi.nlm.nih.gov/snp/>.