Changes in CYP19A1 and CYP3A4 activities due to Population genetic variations in human P450 Oxidoreductase

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Introduction

A broad spectrum of human diseases are caused by mutations in the NADPH cytochrome P450 oxidoreductase (POR)[1]. POR transfers electrons from NADPH to several small molecules, non-P450 redox partners and microsomal cytochrome P450 proteins. Disruption of POR may affect its redox partners with disastrous consequences and POR knock-out mice are embryonically lethal. A number of POR mutations and polymorphisms have been found in patients and from genome sequencing projects. We performed a database search of 1000 genome sequences to identify potentially disease causing variants in POR. Our aim was to check if POR variations from non-clinical samples can be disruptive. P284T variant (rs72557937) has been reported in population studies and found exclusively in africans[2], but was predicted to be likely pathogenic. We performed detailed enzymatic and biochemical characterizations of P284T variant to study its effect on different substrates and redox partners.

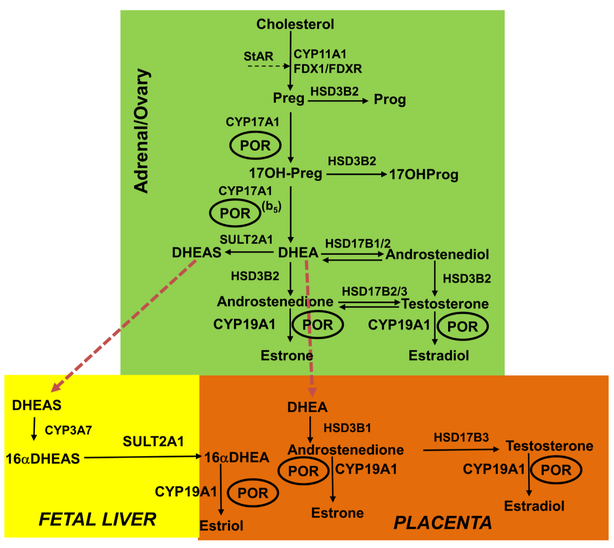

Fig 1: Steroid hormone biosynthesis. The qualitative regulator of steroidogenesis CYP17A1, converts pregnenolone to 17OH-pregnenolone and dehydroepiandrosterone (DHEA). DHEA, the precursor to androgens, is then metabolized in a series of steps involving HSD3B and CYP19A1 to estrogens. Formation of estrogens from androgens requires CYP19A1 activity. CYP19A1 depends on co-factor NADPH and redox partner protein POR for its metabolic activities.

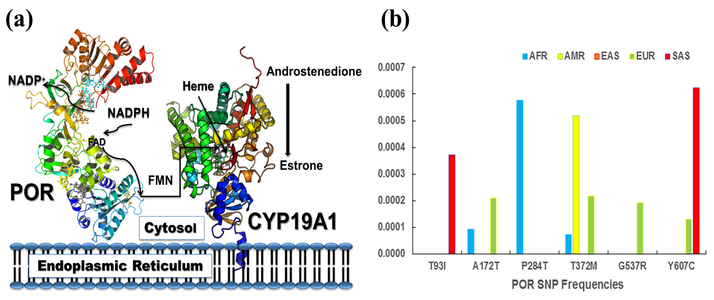

Fig 2: (a) The CYP19A1 activity and role of POR. CYP19A1 interacts with POR in the endoplasmic reticulum to receive electrons used in metabolism of androgens. **(b) POR polymorphisms.** From Burkhardt, Parween, Udhane, Flück & Pandey. J. Steroid Biochem. Mol. Biol. 2017 165: 38-50.

Objective

We performed detailed enzymatic and biochemical characterizations of the P284T variant of POR to study its effect on different redox partner activities.

Methods

The wild type and mutant human POR proteins were expressed in bacteria and membranes were isolated. Human CYP19A1 was produced as His-tag recombinant protein and purified by Metal affinity chromatography. The ability of wild type POR and P284T variant to reduce MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] and ferricyanide was monitored by measuring the change in absorbance at 610 nm and 420 nm respectively. The ability of WT and P284T variant to support CYP19A1 aromatase or CYP3A4 activity was determined using reconstituted liposomes.

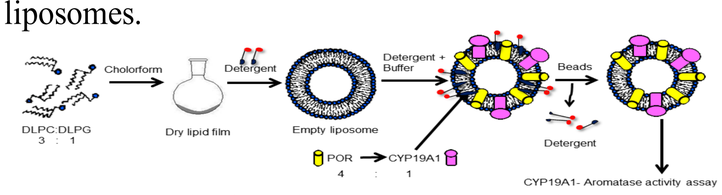

Fig 3: Reconstitution of POR and CYP19A1 in liposomes.

Results

Computational analysis showed that P284 residue is highly conserved and is located in the hinge region of the POR which is required for conformational flexibility.

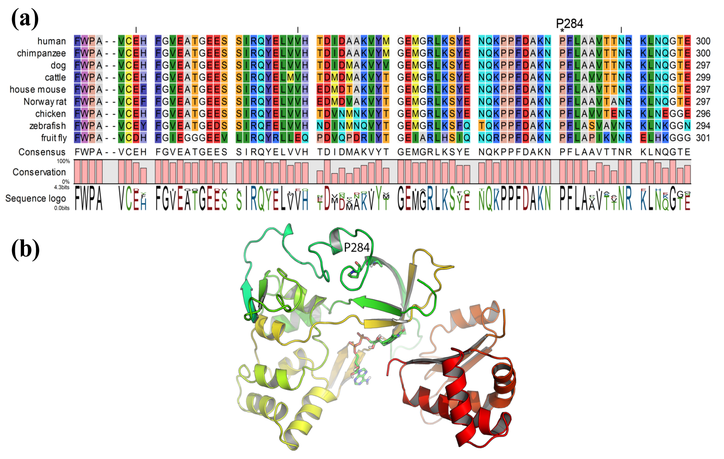

Fig 4: (a) Sequence conservation analysis of P284 residue in POR sequence (b) Location of P284 residue in POR (PDB# 3FJO)

We found variable effects of the P284T mutation on POR activities with different substrates. As compared to WT, P284T variant showed 113% ferricyanide reduction activity but had only 15 % MTT reduction activity.

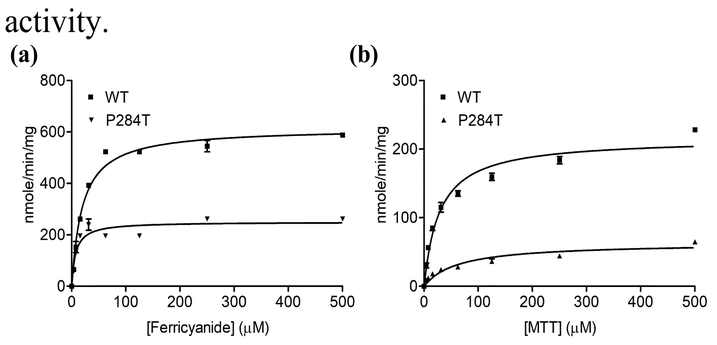

Fig 5: Kinetics of (a) Ferricyanide and (b) MTT reduction by WT and P284T POR. The curves represent the best non-linear fits to Michaelis-Menton equation.

Table 1: Calculated kinetic parameters for Ferricyanide and MTT reduction by WT and P284T variant of POR. All data are Vmax/Km, shown as percentage of wild-type control, set as 100 %.

	Km (μM)	Vmax nmol/min/mg	Vmax/Km	% WT
Ferricyanide reduction				
WT	18.9 ± 3.4	600 ± 19	31.8	100
P284T	6.9 ± 1.6	248.1 ± 11	36	113
MTT reduction				
WT	19.9 ± 1.8	179.5 ± 4	9	100
P284T	48.3 ± 7.5	64.8 ± 3.1	1.3	15

Results

Severe effect on aromatase activity by P284T mutation was observed using androstenedione as substrate.

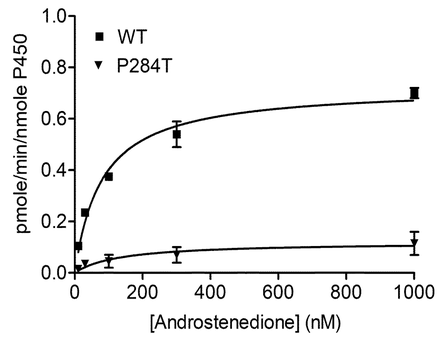

Fig 6: Comparison of aromatase activity supported by POR WT and P284T variant. Bacterially expressed purified recombinant CYP19A1 and POR were mixed to prepare liposome and their activity to convert [^{3}H] labeled androstenedione to estrone was tested by tritiated water release assay. Data fitted with Michaelis-Menten kinetics model using GraphPad Prism.

Table 2: Calculated kinetic parameters for CYP19A1 activity supported by POR WT and P284T variant. All data are Vmax/Km, shown as percentage of wild-type control, set as 100 %.

	Km (nM)	Vmax pmol/min/nmol	Vmax/Km	% WT
WT	80±14.5	0.72±0.03	0.0090	**100**
P284T	165.7±147.9	0.12±0.037	0.0008	**9**

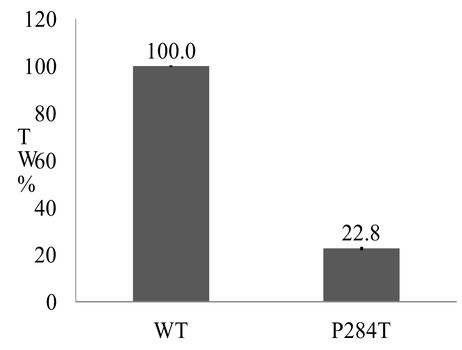

Fig 7: Comparison of CYP3A4 activity supported by POR WT and P284T variant. CYP3A4 and POR were mixed to prepare liposome and CYP3A4 enzyme activity was tested using 20 μM BOMCC as substrate. Activity with WT POR was set as hundred percent and results are shown as percentage of WT activity.

Conclusions

Identification of severe effects of the P284T mutation on both the drug and steroid metabolizing cytochrome P450s indicates that likely pathogenic mutations may be found in apparently normal (non-clinical) population. Their combination as compound heterozygous or as homozygous may lead to severe impact on both the steroid and the drug metabolism by modification of its redox partners activities. Advanced identification of disease causing variants in POR will help in understanding the POR deficiency in patients if the same mutations are later identified.

1. A.V. Pandey and C.E. Flück. NADPH P450 oxidoreductase: Structure, function and pathology of diseases. Pharmacology & Therapeutics. 2013, 138:229-254
2. Burkhard FZ, Parween S, Udhane SS, Flück CE, Pandey AV. P450 Oxidoreductase deficiency: Analysis of mutations and polymorphisms. J Steroid Biochem Mol Biol. (2017) 165:38-50.

The authors declare no conflict of interest.

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