Aromatase activity is disrupted by mutations in P450 oxidoreductase(POR)

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Introduction

The steroidogenic cytochrome P450 aromatase (CYP19A1) is an enzyme located in endoplasmic reticulum (ER) that catalyzes the conversion of androgens to estrogens. Both deficiency and excess of aromatase activity lead to disease states implicating its role in human biology. Cytochrome P450 (CYP) enzymes in ER use reduced nicotinamide adenine dinucleotide phosphate through cytochrome P450 oxidoreductase (POR) for their metabolic activities. Mutations in POR cause disorders of sexual development due to the deficiencies in several steroid metabolizing enzymes like CYP17A1, CYP21A2 and CYP19A1. The effect of POR mutations on different P450 activities depends on individual partner proteins. So each P450-POR mutant combination should be studied individually.

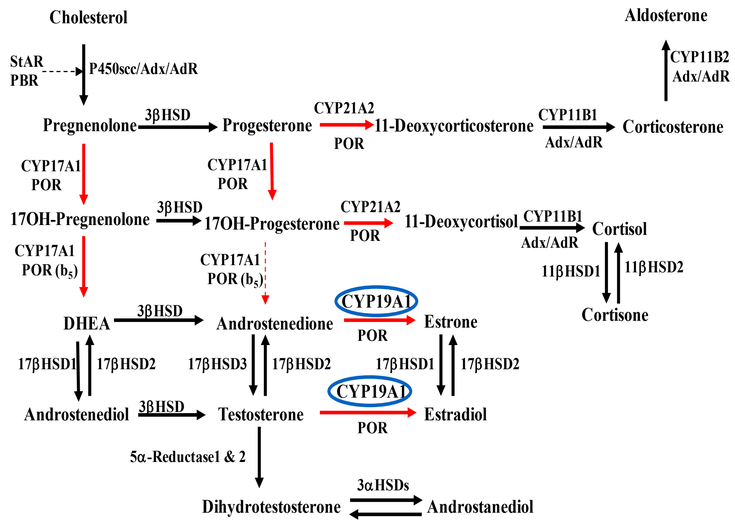

Fig 1: Steroid hormone biosynthesis. The qualitative regulator of steroidogenesis CYP17A1, converts pregnenolone to 17OH-pregnenolone and dehydroepiandrosterone (DHEA). DHEA, the precursor to androgens, is then metabolized in a series of steps involving HSD3B and CYP19A1 to estrogens either directly in the placenta or through intermediates formed in the fetal liver and then sent to the placenta. Formation of estrogens from androgens requires CYP19A1 activity. CYP19A1 depends on co-factor NADPH and redox partner protein POR for its metabolic activities. (Figure adapted from jsbmb.2016.03.031)

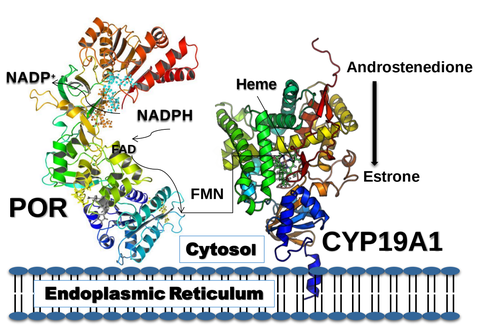

Fig 2: The CYP19A1 activity and role of POR. CYP19A1 interacts with POR in the endoplasmic reticulum to receive electrons used in metabolism of androgens. (Figure adapted from jsbmb.2016.03.031)

Objective

Study the impact of mutations in the flavin binding domain of POR (A115V, T142A, P284L, P284T and Q153R) on CYP19A1 activity, which can potentially influence the estrogen metabolism.

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 Ni^{2+} metal chelate chromatography. POR variants were characterized by standard cytochrome *c* reduction assay and flavin content of proteins was analyzed. Bacterial membranes containing WT or mutant POR along with CYP19A1 were reconstituted into liposomes and aromatase activity was determined by tritiated water release assay.

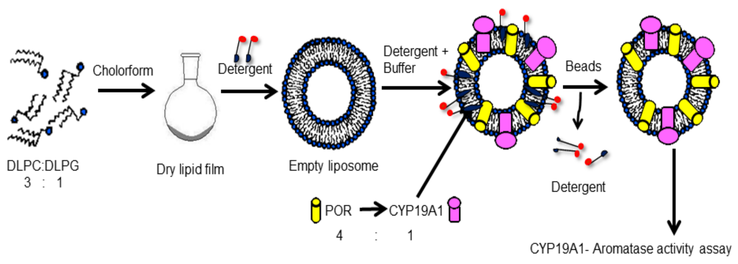

Fig 3: Reconstitution of POR and CYP19A1 into liposomes.

Results

Mutations in the flavin binding domain of POR alter the cytochrome c reduction rate. We found severe effect of POR mutation on CYP19A1 enzyme activity. POR mutants A115V, T142A, P284L and P284T showed less than 20% activity in supporting CYP19A1 aromatase reactions. Interestingly, the POR variant Q153R showed 50% higher activity than wild type.

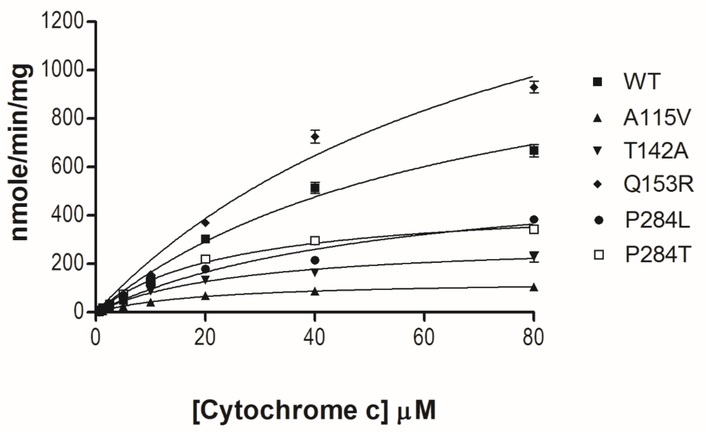

Fig 4: Steady-state kinetics of cytochrome c reduction by WT and POR variants. The curves represent the best non-linear fits to Michaelis-Menton equation.

Table 1: Calculated kinetic parameters for cytochrome c reduction by POR variants. All data are Vmax/Km, shown as percentage of wild-type control, set as 100 %.

	Km (µM)	Vmax nmol/min/mg	Vmax/Km	% WT
WT	67.4±11.2	1279±123.7	19	100
A115V	20.9±11.1	133.4±2.8	6.4	34
T142A	27.0±13.4	298.1±15.4	11	58
Q153R	81.5±18.2	1968±270.2	24.2	127
P284L	53.7±10.5	610±63.5	11.4	60
P284T	24.7±1.8	461.8±14.5	18.6	98

Results

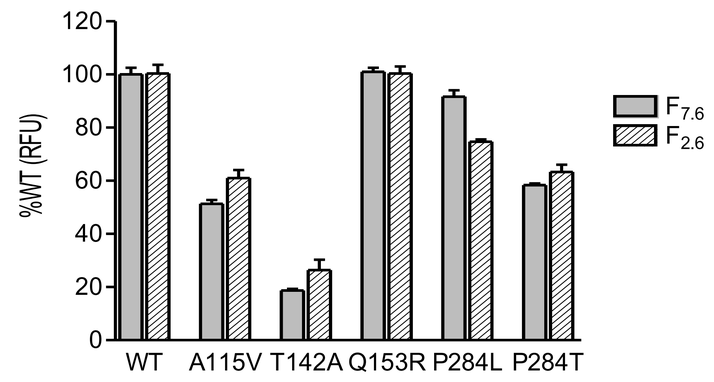

Fig 5: Comparative flavin content of POR variants. Flavins were released by boiling proteins and total fluorescence with excitation at 450 nm and emission at 535 nm was measured. Relative fluorescence unit (RFU) of POR variants obtained at pH 7.6 ($F_{7.6}$) and pH 2.6 ($F_{2.6}$) is plotted; RFU of WT set as 100%.

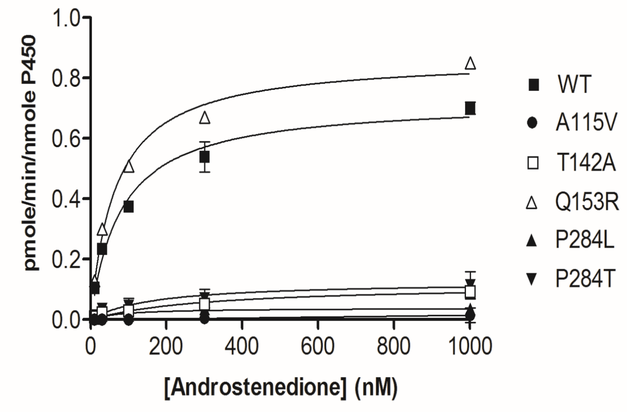

Fig 6: Comparison of aromatase activity supported by POR variants. Bacterially expressed purified recombinant CYP19A1 and POR were mixed to prepare liposome and their activity to convert [^{3}H] labeled androstenedione to estrone was tested by tritiated water release assay. Data fitted with Michaelis-Menten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

Table 2: Calculated kinetic parameters for CYP19A1 activity supported by POR variants. 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
A115V	nd	nd	nd	nd
T142A	308.4±121.5	0.11±0.01	0.0004	5
Q153R	65.62±11.4	0.86±0.03	0.0132	147
P284L	82.4±62.5	0.039±0.008	0.0005	5
P284T	165.7±147.9	0.12±0.037	0.0008	9

Conclusions

Many POR variants found in patients as well as in normal population affects CYP19A1 activity *in-vitro*. Lower aromatase activities due to POR mutation might affect the fetal androgen metabolism, especially in pregnant women with a male child.

Reference:
C.E. Flück, A.V. Pandey, Impact on CYP19A1 activity by mutations in NADPH cytochrome P450 oxidoreductase J. Steroid Biochem. Mol. Biol. (2016) http://dx.doi.org/10.1016/j.jsbmb.2016.03.031

The authors declare no conflict of interest.

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