Molecular and genetic characterization of a novel P399_E401Dup mutation in P450 oxidoreductase (POR) altering several enzymatic activities in a patient with 46,XX DSD

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Introduction

POR gene mutations can present with disordered sexual development (46,XX virilisation and 46,XY under-masculinisation), perturbed steroidogenesis and mild to severe skeletal malformations. As POR is an obligate electron donating cofactor to many P450s, and as this interaction may vary from partner to partner, the phenotypic spectrum of POR-Deficiency (PORD) is extremely broad. Therefore, to characterize novel POR mutations, specific testing is required.

Case Report

A 46,XX patient, second child of consanguineous Kurdish parents, was born at term with ambiguous genitalia and dysmorphic facial features (Figure 1). In the course of diagnostic work up

- ✓ the newborn sreeening for 21-hydroxylase deficiency and
- ✓ an ACTH-Test were normal.

At age 14 days diagnosis of PORD was made by GC-MS urinary (spot urine) steroid metabolome-analysis showing the pathognomonic pattern of combined impaired activities of 17-hydroxylase and 21-hydroxylase. Genetic analysis revealed a **novel homozygous mutation P399_E401Dup in POR**.

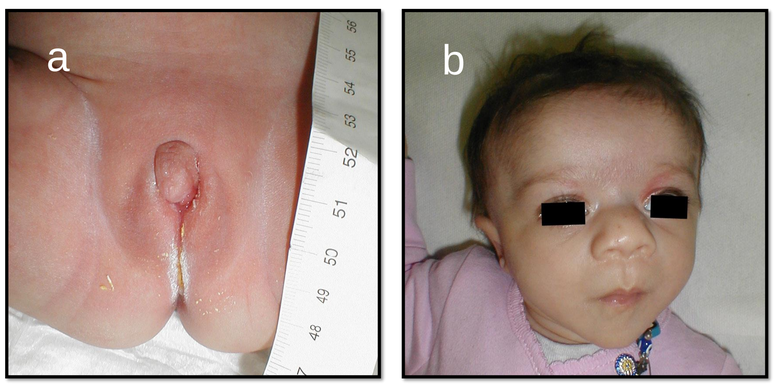

***Figure 1*: Physiological features. (a) Outer genitalia, Prader III and (b) dysmorphic facial features (frontal bossing, low set ears) of the girl**.

Methods

The wild type and mutant human POR and CYP19A1 proteins were expressed in bacteria. The ability of wild type POR and P399_E401Dup variant to reduce ferricyanide, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromid and cytochrome c was monitored by measuring the change in absorbance (420, 610 and 550 nm respectively). The ability of WT and P399_E401Dup variant to support CYP19A1 aromatase activity was determined by tritiated water release assay with radioactive androstenedione as substrate.

Results (I)

Computational analysis showed that P399_E401Dup residues are highly conserved and located in the interface of FAD / NADPH binding domain of POR (Figure 2).

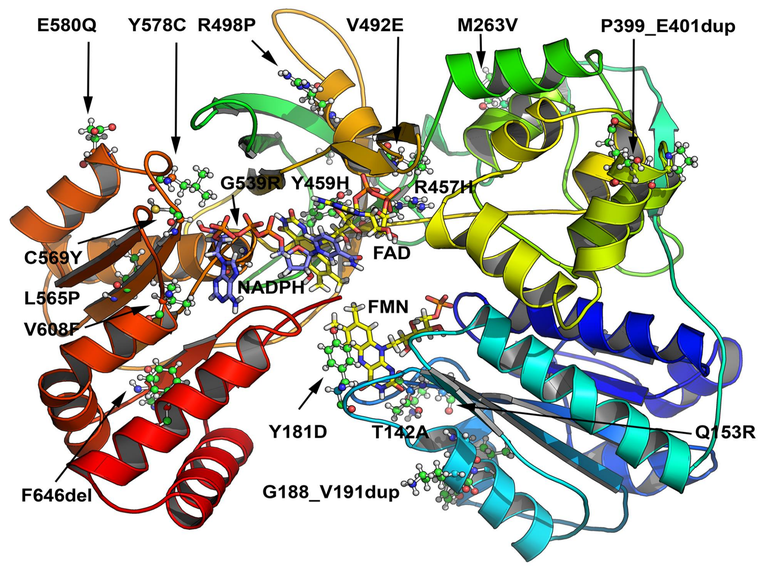

***Figure 2*: Location of P399_E401Dup in POR**.

We found severe effects of the P399_E401Dup mutation on POR activities with different substrates (Table 1 and Figure 3).

Cytochrome c Reduction	POR-WT	POR-399_401 dup
Vmax	873 ± 68.1	355.7 ± 18.9
Km	23.7 ± 3.7	17.9 ±2.2
Vmax/Km	36.8	18.8
%WT	100.0	**51.0**
FeCN Reduction	POR-WT	POR-399_401 dup
Vmax	3890 ± 88.1	2779 ±169.6
Km	27.2 ±2.3	41.5 ±8.7
Vmax/Km	143.0	66.9
%WT	100.0	**46.8**
MTT Reduction	POR-WT	POR-399_401 dup
Vmax	1254 ± 22.7	1477 ±33.2
Km	21.3 ± 1.5	404 ±15.5
Vmax/Km	58.9	3.7
%WT	100.0	6.2

***Table 1*: Kinetic parameters for cytochrome c, ferricyanide and MTT reduction. Vmax in nmol/min/mg**

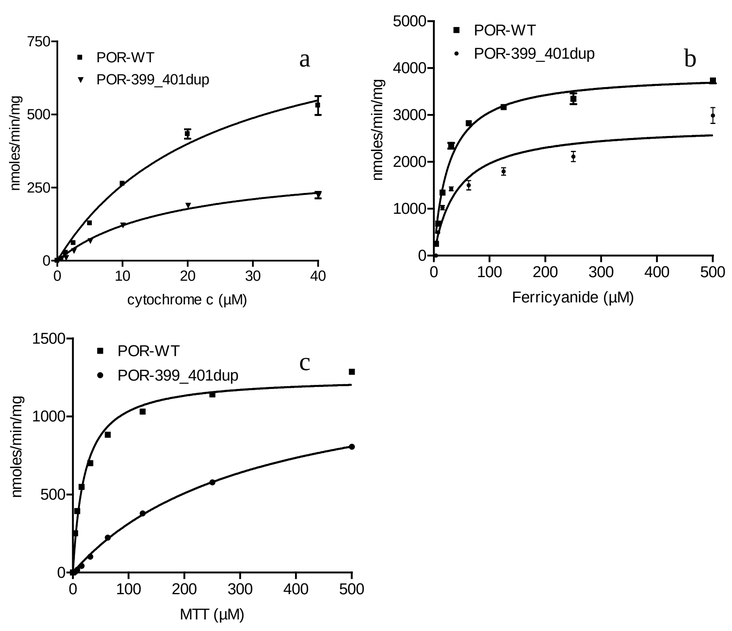

***Figure 3*: Kinetics of (a) cytochrome c, (b) ferricyanide and (c) MTT reduction by WT/P399_E401Dup POR**

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Results (II)

Measurements of co-factor affinity using cytochrome c and MTT as substrates with variable NADPH concentrations showed a severe effect on NADPH binding by P399_E401Dup mutation (Table 2, Figure 4). Figure 5 presents the effect of the variant on CYP19A1 activity.

Cytochrome c Assay	POR-WT	POR-399_401 dup
Vmax	573 ± 31.2	318.5 ± 26.6
Km	5.5 ± 1.1	10.7 ±3.0
Vmax/Km	103.8	29.7
%WT	100.0	**28.6**
MTT Assay	POR-WT	POR-399_401 dup
Vmax	980 ± 61.2	944 ± 69.9
Km	12 ± 2.3	63 ± 8.9
Vmax/Km	84.0	151.1
%WT	100.0	**18.0**

***Table 2*: Kinetic parameters for cytochrome c and MTT (co-factor NADPH affinity). Vmax in nmol/min/mg**

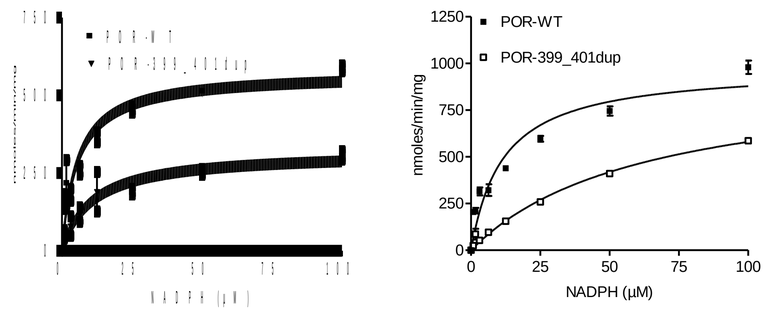

***Figure 4*. Kinetics of co-factor affinity measurements: a) cytochrome c, b) MTT assay**

Conclusions

The results show a clear problem in electron transport mechanism and indicate a structural change by P399_E401Dup mutation in POR affecting protein conformation and stability. A severe effect on aromatase activity and MTT reduction were confirmed. It also affects steroid production as manifested in the steroid metabolome. These results suggest a pathological effect and diagnosis of PORD from the P399_E401Dup mutation. A P399_E401Del mutation in a Turkish child, which had reduced activities of CYP17A1, CYP21A2 and CYP19A1was previously reported. P399_E401 seems a sensitive spot for POR mutations.

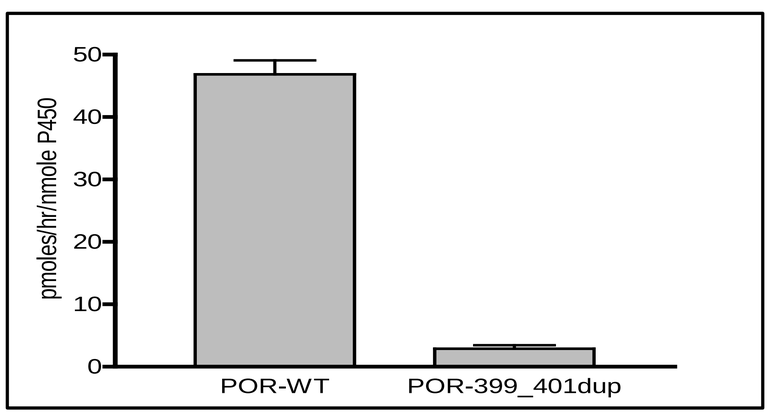

***Figure 5*: Effect on CYP19A1 activity supported by the POR WT and the P399_E401Dup variant.**