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

Claudia Boettcher1,2, Shaheena Parween1, Eckhard Korsch2, Michella F Hartmann3, Sameer Udhane1, Norio Kagawa4, Christa E Flueck2, Stefan A Wudy3 and Amit V Pandey1

1Paediatric Endocrinology, Diabetology and Metabolism, University Children’s Hospital, and Department of Biomedical Research, University of Bern, Bern Switzerland; 2Paediatric Endocrinology, Children’s Hospital of the City of Cologne, Cologne, Germany; 3Paediatric Endocrinology & Diabetology, Centre of Child & Adolescent Medicine, Justus Liebig University, Giessen, Germany; 4Nagoya University School of Medicine, Nagoya, Japan

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 screening 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.

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).

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

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.

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 CYP19A1 was previously reported. P399_E401 seems a sensitive spot for POR mutations.