Inhibition of placental CYP19A1 activity remains as a valid hypothesis for 46,XX virilization in P450 oxidoreductase deficiency

Christa E Flück[a,b], **Shaheena Parween**[a,b], **Maria Natalia Rojas Velazquez**[a,b], **and Amit V Pandey**[a,b,1]

[a]Pediatric Endocrinology, Diabetology, and Metabolism, University Children's Hospital, Bern, Switzerland; [b]Department of Biomedical Research, University of Bern, Bern, Switzerland

Cytochrome P450 oxidoreductase deficiency (PORD), caused by mutations in P450 oxidoreductase (POR), is a disorder of steroid metabolism often characterized by disordered sexual development (1–3). POR is required for enzymatic activities of multiple cytochrome P450 enzymes (4). In PNAS, Reich et al. (5) propose "alternative pathway androgen biosynthesis" as the cause of 46,XX virilization in PORD. We are pleased to see the expansion of the role of alternative pathway in sexual development previously demonstrated by us in 46,XY individuals (6), but have some concerns regarding the assumption that virilization of 46,XX individuals in PORD is mainly via alternative pathway. The choice of steroid analysis by Reisch et al. (5) from only 46,XY individuals to propose a hypothesis for 46,XX virilization is baffling. Another recent study found low to undetectable levels of 17-hydroxy-dihydroprogesterone, 17-hydroxy-allopregnanolone, and androsterone, the steroids in alternative pathway produced via CYP17A1, in the 46,XX fetal adrenals and attributed it to a lack of SRD5A1 expression in fetal adrenal (7). We have previously reported that mutations in the key enzymes of the alternative pathway cause 46,XY undervirilization (6). By contrast, mutations in aromatase (CYP19A1) cause genital virilization in 46,XX individuals (8), which prompted us to reexamine the results of Reisch et al. (5).

Mutations in POR reduce the enzymatic activities of CYP17A1 (Fig. 1*A*) (1–4). Reisch et al. (5) reported that an androgen produced via alternative pathway, using CYP17A1 and POR, is not severely impacted by A287P mutation in POR, but enzyme kinetic analysis was not performed. The A287P mutation in POR inhibits CYP17A1 activity but also reduces enzyme turnover/maximum velocity by 40 percent in CYP19A1 assays using androstenedione (Fig. 1*B*) . Therefore, the assumption of Reisch et al. (5) that aromatase activity is unimpacted by A287P mutation in POR is incorrect . Hepatic cytochromes P450, including CYP3A4, can metabolize steroids, and A287P mutation in POR inhibited CYP3A4 activity (Fig. 1*C*). Although the methods are not directly comparable, in our assays, activities of multiple enzymes were adversely affected by A287P mutation in POR (Fig. 1*D*). We have described the role of CYP19A1 in 46,XX virilization with PORD (9). Interestingly, maternal virilization during pregnancy is a specific feature almost exclusively observed in cases with CYP19A1 deficiency (10) or maternal tumor. In fact, maternal virilization during pregnancy is common in PORD but not in 21-hydroxylase deficiency, although both conditions are associated with alternative pathway androgen biosynthesis (2, 4–6, 9). These data strongly suggest that placental aromatase deficiency is the major cause of virilization of 46,XX PORD patients. Therefore, the study by Reisch et al. (5) confirms a role of "alternative pathway androgen production" in 46,XY PORD, but does not negate the role of reduced CYP19A1 activity due to POR mutations. Consequently, both the alternative pathway androgen production and inhibition of aromatase activity in PORD may cause genital virilization of 46,XX patients. 46,XX virilization in PORD requires further exploration, and polymorphisms of POR, cytochromes P450 and related genes may play a role in phenotype variations (4).

ACKNOWLEDGMENTS. This work was supported in part by grants from the Swiss National Science Foundation (31003A-134926) and the Novartis Foundation for Medical-Biological Research (18A053) to A.V.P.

1. Flück CE, et al. (2004) Mutant p450 oxidoreductase causes disordered steroidogenesis with and without antley-bixler syndrome. *Nat Genet* 36(3):228–230.
2. Huang N, et al. (2005) Diversity and function of mutations in p450 oxidoreductase in patients with antley-bixler syndrome and disordered steroidogenesis. *Am J Hum Genet* 76(5):729–49.
3. Huang N, Agrawal V, Giacomini KM, Miller WL (2008) Genetics of p450 oxidoreductase: Sequence variation in 842 individuals of four ethnicities and activities of 15 missense mutations. *Proc. Natl. Acad. Sci. U.S.A.* 105(5):1733–1738.
4. Pandey AV, Flück CE (2013) Nadph p450 oxidoreductase: structure, function, and pathology of diseases. *Pharmacol Ther* 138(2):229–54.
5. Reisch N, et al. (2019) Alternative pathway androgen biosynthesis and human fetal female virilization. *Proc. Natl. Acad. Sci. U.S.A.* 116(44):22294–22299.
6. Flück CE, et al. (2011) Why boys will be boys: two pathways of fetal testicular androgen biosynthesis are needed for male sexual differentiation. *Am J Hum Genet* 89(2):201–18.
7. O'Shaughnessy PJ, et al. (2019) Alternative (backdoor) androgen production and masculinization in the human fetus. *PLoS biology* 17(2):e3000002–e3000002.
8. Ito Y, Fisher CR, Conte FA, Grumbach MM, Simpson ER (1993) Molecular basis of aromatase deficiency in an adult female with sexual infantilism and polycystic ovaries. *Proc. Natl. Acad. Sci. U.S.A.* 90(24):11673–7.
9. Parween S, et al. (2020) Molecular basis of cyp19a1 deficiency in a 46,xx patient with r550w mutation in por: Expanding the pord phenotype. *J Clin Endocrinol Metab* 105(4).
10. Praveen VP, et al. (2020) Novel cyp19a1 mutations extend the genotype-phenotype correlation and reveal the impact on ovarian function. *J Endocr Soc.*

C.E.F. and A.V.P. designed research; C.E.F., S.P., M.N.R.V., and A.V.P. performed research; C.E.F., S.P., M.N.R.V., and A.V.P. analyzed data; and C.E.F., S.P., M.N.R.V., and A.V.P. wrote the paper.

The Authors declare no competing interest.

[1]To whom correspondence may be addressed. Email: amit@pandeylab.org

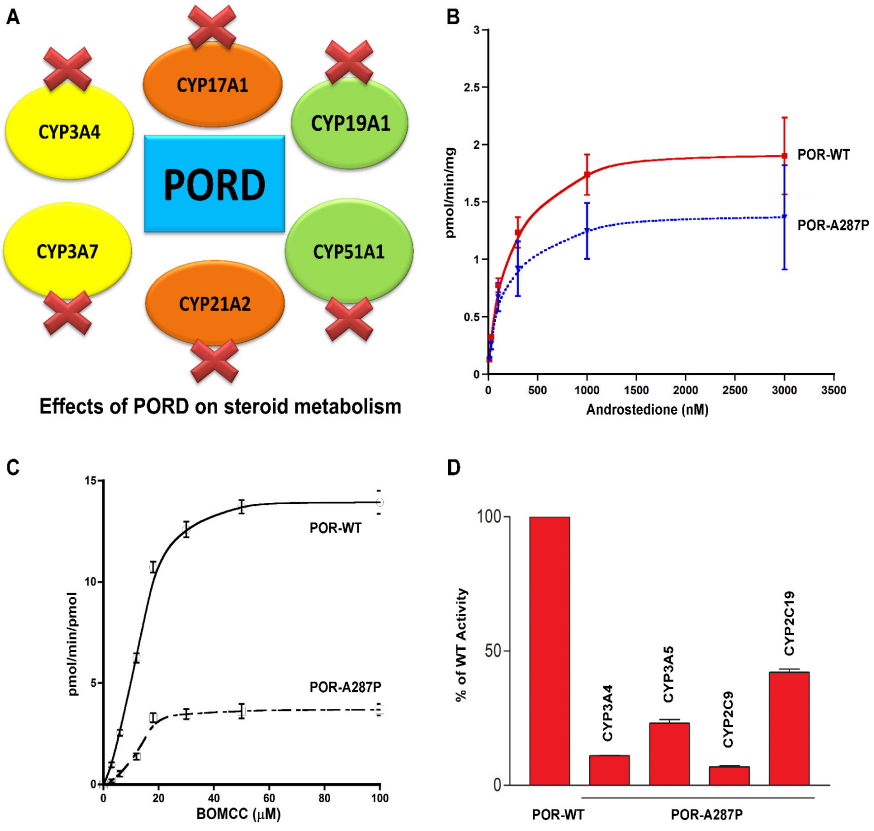

Fig. 1. Impact of mutations in POR on enzymatic activities of cytochrome P450 enzymes. A. Impact of mutations in POR on the activities of steroid metabolizing cytochrome P450 enzymes. POR is necessary for the metabolic activities of cytochrome P450 proteins located in the endoplasmic reticulum (4). These include the steroid metabolizing cytochromes P450 CYP17A1, CYP21A2, CYP19A1, CYP51A1, and CYP26B1 as well as drug-metabolizing cytochromes P450 CYP3A4, CYP3A5, CYP2D6, CYP2C9 and CYP2C19. A reduction in POR activity may lead to loss of both the steroid as well as drug-metabolizing cytochromes P450 enzyme activities. Further considerations might be required for drug-metabolizing enzymes like CYP3A4 that also cause hepatic metabolism of estrogens and androgens. B. Enzymatic activity of CYP19A1 supported by POR-wild type (WT) and POR-A287P. Recombinant CYP19A1 and POR proteins were mixed with lipids, and their activity to convert [3H] androstenedione to estrone was tested by the tritiated water release assay (9). Data were analyzed using the Michaelis-Menten kinetics with GraphPad Prism. C. The activity of cytochrome P450 CYP3A4 supported by POR-WT and POR-A287P. Assay of CYP3A4 activity was performed to compare POR-WT and POR-A287P by using BOMCC as a substrate (9). D. The activity of cytochrome P450 CYP3A4, CYP3A5, CYP2C9, and CYP2C19 supported by POR-WT and POR-A287P. Assay of CYP3A4, CYP3A5 and CYP2C9 activity was performed to compare POR-WT and POR-A287P by using BOMCC as a substrate and assay of CYP2C19 activity was performed to compare POR-WT and POR-A287P by using EOMCC as a substrate (9). Activity with the WT POR was fixed as a hundred percent in all cases, and results are given as a percentage of WT activity. Data are shown as mean ± SEM from triplicate assays.