Biochemical and Biophysical Research Communications 401 (2010) 149–153

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Reduction in hepatic drug metabolizing CYP3A4 activities caused by P450 oxidoreductase mutations identified in patients with disordered steroid metabolism

Christa E. Flück, Primus E. Mullis, Amit V. Pandey *

Pediatric Endocrinology, Diabetology and Metabolism, Department of Clinical Research, University of Bern, Tiefenaustrasse 120c, CH 3004 Bern, Switzerland

ARTICLE INFO

Article history:
Received 1 September 2010
Available online 16 September 2010

Keywords:
CYP3A4
NADPH P450 oxidoreductase
POR
CPR
Steroid metabolism
Antely-Bixler syndrome
Adrenal hyperplasia
Drug metabolism

ABSTRACT

Cytochrome P450 3A4 (CYP3A4), the major P450 present in human liver metabolizes approximately half the drugs in clinical use and requires electrons supplied from NADPH through NADPH-P450 reductase (POR, CPR). Mutations in human POR cause a rare form of congenital adrenal hyperplasia from diminished activities of steroid metabolizing P450s. In this study we examined the effect of mutations in POR on CYP3A4 activity. We used purified preparations of wild type and mutant human POR and in vitro reconstitution with purified CYP3A4 to perform kinetic studies. We are reporting that mutations in POR identified in patients with disordered steroidogenesis/Antley-Bixler syndrome (ABS) may reduce CYP3A4 activity, potentially affecting drug metabolism in individuals carrying mutant POR alleles. POR mutants Y181D, A457H, Y459H, V492E and R616X had more than 99% loss of CYP3A4 activity, while POR mutations A287P, C569Y and V608F lost 60–85% activity. Loss of CYP3A4 activity may result in increased risk of drug toxicities and adverse drug reactions in patients with POR mutations.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Cytochrome P450 3A4 (CYP3A4) is responsible for the metabolism of a wide range of drugs and xenobiotics, accounting for an estimated 50% of all drugs in clinical use including acetaminophen, diazepam, erythromycin, codeine, cyclosporine A, midazolam, rifampicin and quinidine [1]. In human liver CYP3As are the major P450 enzyme present, accounting for approximately 30% of total P450 content [2]. Several commonly used drugs also act as inhibitors of CYP3A4 including antibiotics (erythromycin), anti-retroviral agents (ritonavir), antidepressants (fluxotine) and calcium channel blockers (verapamil) [3,4]. A complex interplay of inducers and inhibitors of CYP3A4 often causes drug-drug interaction and toxicity problems [3,4]. Cytochrome P450 oxidoreductase (POR, CPR; NM_000941, NP_000932, EC 1.6.2.4) is the obligate electron donor for CYP3A4 and other microsomal P450s (Fig. 1). Characteristics of POR deficiency were long known in the literature as so-called mixed oxidase disease [5] as POR deficiency typically shows a steroid profile suggesting combined deficiencies of steroid 21-hydroxylase (CYP21A2) and 17α-hydroxylase/17,20 lyase (CYP17A1) activities; because, these like other type 2 P450 enzymes, require electrons from NADPH through POR. However, due to essential role of POR in numerous metabolic reactions, mutations in POR itself were deemed unlikely [6]; a theory later supported by embryonic lethality of POR knocks-out mice [7]. In 2004 we and others reported mutations in POR from patients with Antley-Bixler Syndrome (ABS) [8–10]. The POR mutations identified from patients with ABS showed reduced activities of steroid metabolizing P450s CYP17A1 (17, hydroxylase/17,20 lyase) [8,11]. More POR mutations have been identified [9,10,12–18] and allelic variants are listed on CYP/POR database site (www.cypalleles.ki.se/por.htm) [15]. Many POR variants identified from patients and normal population have been tested for enzymatic activities [11,19–22]. While initial studies focused on steroid metabolizing P450s, several recent studies reported effects of POR mutations on drug metabolizing P450s [13,23–26]. The overall physiological role of POR mutations beyond congenital adrenal hyperplasia (e.g. effects on hepatic drug metabolism, heme oxygenase and direct metabolism of small molecules) are still unclear. A perusal of earlier reports of ABS in some children from mothers treated with fluconozole, an antifungal agent which interferes with cholesterol biosynthesis at the level of CYP51 activity, suggests that disordered drug metabolism may result from deficient POR activity [27,28]. Fluconozole is a mild inhibitor of CYP3A4 and aromatase (CYP19A1) [29]. In a clinical study examining in vivo drug metabolism by CYP3A4, variations in CYP3A4 itself did not correlate with changes in enzymatic activity but the POR variant A503 V (POR*28 1508C > T) was associated with higher levels of CYP3A4 activities in a midazolam clearance assay [30].

Since CYP3A4 is the major drug metabolizing enzyme in humans, a change in CYP3A4 activity in patients may have serious impact on

* Corresponding author. Fax: +41 31 308 8028.
E-mail address: amit@pandeylab.org (A.V. Pandey).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.09.035

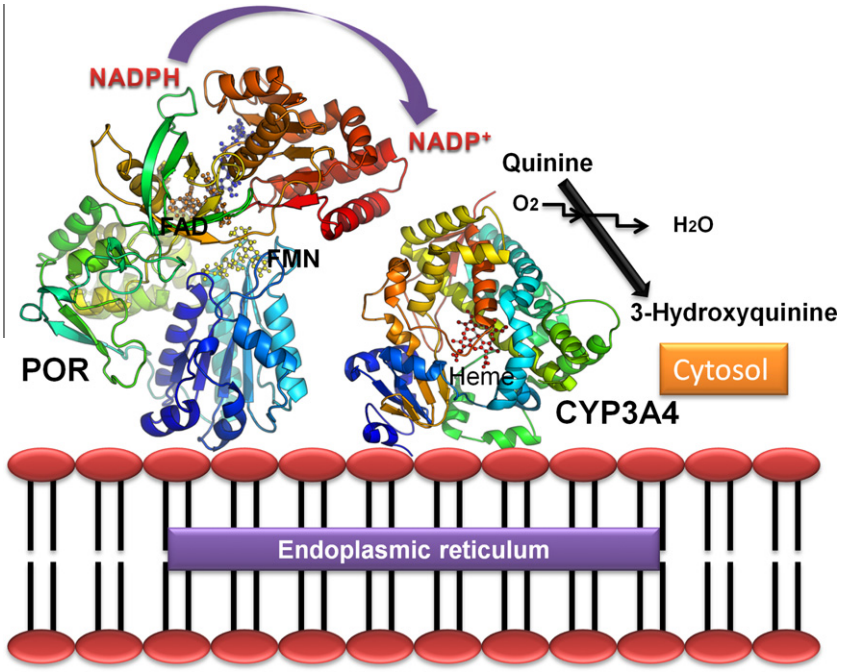

Fig. 1. The CYP3A4 activity supported by NADPH P450 oxidoreductase. In the microsomal membranes, CYP3A4 interacts with POR to receive electrons used in metabolism of drugs and xenobiotics. Heme unit in CYP3A4 is the catalytic center and electrons are transferred through reduced FMN to heme through electrostatic interactions. The POR and CYP3A4 models were drawn with Pymol (www.pymol.org) based on published structures and models.

their drug metabolizing capabilities. Therefore, we sought to evaluate CYP3A4 activities altered by mutations/polymorphisms in POR, especially those identified from patients with the ABS phenotype. We produced WT and mutant human POR and CYP3A4 and used lipid based reconstitution to perform enzymatic assays and detailed kinetic studies. Here we are reporting that mutations in POR found in patients may reduce CYP3A4 activity, potentially leading to altered drug metabolism in individuals with mutant POR alleles.

2. Materials and methods

2.1. Expression and purification of the wild-type and the mutant POR in bacteria

Human POR variants were expressed in bacteria and purified as described previously [23].

2.2. Expression and purification of CYP3A4

The CYP3A4 cDNA [31] in the vector pCWORi+(kind gift of Dr. F. Peter Guengerich, Vanderbilt University, Nashville, TN, USA) was transformed in *E. coli* and recombinant protein was purified by ion-exchange (DEAE-Sepharose) and gel filtration chromatography (Superdex 200 prepgrade, 1.6 × 60 cm, GE Lifesciences) on a BioRad DuoFlow FPLC system (BioRad Laboratories, Hercules, CA, USA) based on methods described previously. The P450 and heme contents were measured as described previously [32,33].

2.3. Expression and purification of cytochrome b_5

Cytochrome b_5 used in CYP3A4 assay was expressed in E. coli and purified to homogeneity as described previously [23,34].

2.4. CYP3A4 assays with WT and variant POR

The in vitro CYP3A4 assays were performed using wild-type or mutant P450 oxidoreductase and cytochrome b_5 at a ratio of 5:1:1 (POR:CYP3A4:b_5) using the fluorogenic substrate BOMCC (Invitrogen Corp, Carlsbad, CA). Reaction mixture consisted of 50 pmol POR, 10 pmol CYP3A4, 10 pmol cytochrome b_5 in 50 mM HEPES buffer containing 25 mM $MgCl_2$, 2.5 μM GSH, 0.5 mM sodium cholate, and 10 μg phospholipid mixture (L-α-dilauroyl-sn-glycero-3-phosphocholine, L-α-dioleoyl-sn-glycero-3-phosphocholine and L-α-phosphatidyl-L-serine 1:1:1) in a final volume of 200 μl. The catalytic reaction was initiated by addition of NADPH to 0.1 mM final concentration and fluorescence was monitored on a Spectramax M2e plate reader (Molecular Devices, Sunnyvale, CA) at an excitation wavelength of 410 nm and emission wavelength of 460 nm. Data were fitted for Michaelis–Menten kinetics using Prism (Graphpad Software, La Jolla, CA USA). Carbon monoxide binding spectra were recorded on a Perkin Elmer Lambda 25 spectrophotometer at a slit width of 1.0 nm and scan speeds of 120 nm/min.

3. Results and discussion

A review of previous enzymatic analysis indicates that effects of specific mutations causing amino acid changes in POR varies with different redox partners. From our first report conflicting results for different target proteins were obtained for different mutation in POR but in general P450 based assays proved more reliable in accessing the effects of mutation in relation to clinical observations [8,11,35,36]. Some general conclusions can be drawn from earlier studies. POR mutations that lead to loss of FAD binding (R457H, Y459H, V492E) cause loss of activity in all assays (Fig. 2) [8,11]. Mutations in NADPH binding domain that seem to cause reduced (C569Y, V608F) or complete loss of NADPH binding also have severe effects in P450 based assays [8,11,14,19,25]. Mutations in other regions of POR molecule have variable effect and show significant variation in activities depending on choice of redox partner in assays. Therefore, no single assay could predict activities of POR mutations for all redox partners and specific effects of many POR variants may vary with different partner proteins. As an example the common POR variant found in European population A287P

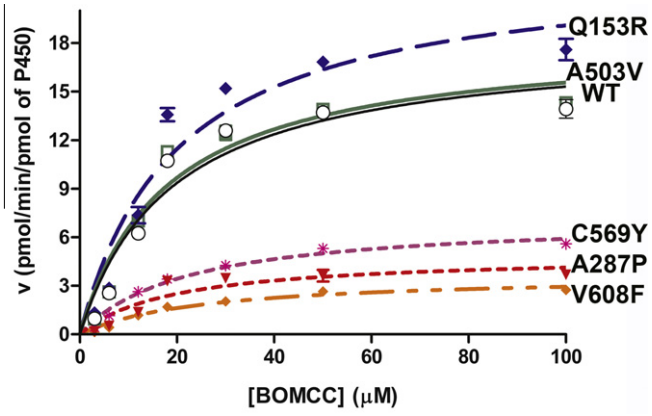

Fig. 2. Kinetics of CYP3A4 supported by WT or mutant POR. Kinetic constants are shown in table 1. Data were fitted with Michaelis–Menten kinetics model using Prism as described in methods.

has only a mild loss of cytochrome c (cyt c) reduction, NADPH oxidation, and 17-OH hydroxylation activities but shows significantly lower activities in supporting 17,20 lyase, CYP1A2 and CYP2C19, and has similar to WT activity for CYP19A1 and CYP21A2. Therefore, each POR variants will require testing with CYP3A4 and other drug metabolizing P450s to assess its effects.

3.1. Activities of POR variants

Purified preparations of CYP3A4 were used to test the activities of the POR variants. A115 V found as a hetrozygote is a conservative change and retained similar to WT activity with CYP3A4 (Table 1), similar to CYP17A1 assays (Table 2). The POR variant Y181D resulted in total loss of CYP3A4 activity while T142A had about 80% of normal activity (Table 1). In human POR residues Y143 and Y181 are directly involved in FMN binding [37]. The POR variant Q153R had Vmax values 25% higher than WT (22.9 vs 18.2 nmol/min/nmol P450) (Table 1, Fig. 2) while variant P228L had close to WT activity. The POR variant A287P which is identified as the major mutation found in "European"/"Caucasian" population showed 75% loss of CYP3A4 activity (Table 1, Fig. 2). The A287 residue is located in the hinge region of POR. The A287P variant has variable effect on various partner proteins of POR; the 17,20 lyase activity of CYP17A1 is significantly reduced while 17 hydroxylase activity of CYP17A1 is affected to lesser extent and activities of CYP19A1 and CYP21 are unaffected [19,38] (Table 2). The POR variants R316 W, G413S identified as polymorphisms also had similar to WT activities. However, the mutations affecting binding of FAD; R457H, Y459H and V492E, resulted in total loss of CYP3A4 activity. Electron transfer from NADPH to FAD is crucial in POR and its disruption also results in loss of CYP17A1, CYP1A2, CYP2C19 and cyt c reduction activities (Table 2). The two POR polymorphs A503 V and G504R found in a loop behind FAD binding site retained close to normal activities. POR mutations C569Y and V608F resulted in 65–85%% loss of CYP3A4 and R616X mutations that results in C-terminally truncated protein lost all activity (Table 1). In the 3D structural model of human POR based on the crystallographic structure of rat POR, C569 interacts with NADPH with distances of 3.05 and 3.42 Å (for human POR) [37]. A change from cysteine to tyrosine will change the binding of NADPH and reduce electron transfer efficiency for the mutated POR. Valine 608 is next to tyrosine 607 and lysine 605 which have multiple contacts with NADPH especially between OH group of tyrosine and first orthophosphate group of NADPH with a distance of 2.96 Å and second orthophosphate group of NADPH is at a distance of 2.49 Å from lysine 605. A change from valine to phenylalaline is likely to disturb the NADPH binding site. CYP3A4 assays both V631I and delF646 had about 90% of WT activities. Although the conservative mutant V631I lies in a highly conserved segment of the NADPH binding domain, it retained normal activity in the cytochrome c assays and 40–50% of activity in the CYP17A1 assays. In-frame deletion of F646 had no effect on 17α-hydroxylase activity but a 54% reduction in 17,20 lyase activity.

Many patients described with apparent combination of defects in enzymatic activities of CYP17A1 and CYP21A2 in the literature revealed no mutations upon analysis of CYP17A1 and CYP21A2 genes and underlying molecular basis of defect in those patients remained unclear till identification of POR mutations by us and other investigators. Initial POR mutations were identified from patients with apparent combined 17α-hydroxylase, 21-hydroxylase deficiency (OMIM 201750) and a broad spectrum of clinical characteristics ranging from severe neonatal skeletal malformations with genital ambiguity (known as Antley Bixler's syndrome; OMIM 207410), to phenotypically minor polycystic ovary syndrome like features [8,11,21,23,37]. Previously we and others have shown that mutations in POR may severely affect activities of several steroid metabolizing enzymes. Abnormal drug metabolism in patients with POR mutations have so far not been investigated clinically but some in vitro studies have been performed [23,25,26,39,40]. However, since POR is the electron donor for major drug metabolizing P450 CYP3A4, we examined the effect of POR variants on CYP3A4 activity to evaluate the effect of POR variants on drug metabolism and predict their consequences in cases of POR deficiency.

The common POR mutations in European population (A287P) as well as common Japanese mutation (R457H) are severely affected by POR mutations in supporting CYP3A4 activity. Further examination of patients carrying these mutations will be required to examine clinical significance of reduced CYP3A4 activity in POR deficiency. Mutation Y181D has shown no activity in all P450 based assays (CYP17A1, CYP2C19, CYP2A1) studied so far apparently due to loss of FMN binding [21,23,37,39] (Table 2). In agreement with results from previous studies POR mutations that destroy FAD binding (R457H, Y459H, V492E) resulted in loss of CYP3A4 activity [8,11]. Mutations in NADPH binding domain (C569Y and V608F) had slightly less severe effects [8,11,14,19,25]. An investigation of redox levels in cellular environment of

Table 1
Activities of CYP3A4 supported by WT or mutant POR. Purified preparations of WT and mutant POR were mixed with phospholipids and CYP3A4 and activities were assayed using fluorogenic substrate BOMCC as described under experimental procedures.

POR	km (μM)	Vmax (nmol/min/nmol)	Vmax/km (% of WT)	Kcat (1/s)
WT	18.9 ± 3.7	18.2 ± 1.3	0.96 (100)	0.30
A115 V	20.7 ± 2.8	16.9 ± 1.6	0.82 (85)	0.28
T142A	19.2 ± 2.5	15.8 ± 5.2	0.82 (85)	0.26
Q153R	20.0 ± 3.9	22.9 ± 1.7	1.14 (119)	0.38
Y181D	–	–	–	–
P228L	18.3 ± 3.4	17.8 ± 2.4	0.97 (101)	0.29
A287P	19.3 ± 5.8	4.9 ± 0.5	0.25 (26)	0.08
R316 W	18.1 ± 3.5	19.3 ± 2.6	1.06 (110)	0.32
G413S	21.3 ± 4.1	20.5 ± 1.8	0.96 (100)	0.34
R457H	–	–	–	–
Y459H	–	–	–	–
V492E	–	–	–	–
A503 V	17.9 ± 3.4	18.4 ± 1.3	1.03 (107)	0.31
G504R	20.2 ± 3.7	18.1 ± 3.0	0.89 (93)	0.30
C569Y	22.99 ± 2.4	7.2 ± 0.3	0.31(32)	0.12
V608F	25.3 ± 3.8	3.7 ± 0.2	0.15 (16)	0.06
R616X	–	–	–	–
V631I	19.7 ± 3.1	16.9 ± 2.4	0.85 (89)	0.28
delF646	18.2 ± 3.8	15.2 ± 1.9	0.84 (88)	0.25

Table 2
WT or mutant POR activities towards CYP3A4 compared with previously reported effects on other redox partners. Data for other studies were compiled from published studies on effects of POR variants [8,11,14,19,25].

POR	**3A4**	Cyt c reduction	NADPH Oxidation	17OH–ase	17,20 lyase	Aromatase	1A2	2C19
WT	**100**	100	100	100	100	100	100	100
A115 V	**85**	63	41	80	71	–		
T142A	**85**	49	52	60	54	–	3	–
Q153R	**119**	9	11	31	27	–	144	284
Y181D	–	–	–	–	–		–	–
P228L	**101**	75	72	100	41	–	20	39
A287P	**26**	93	104	40	21	104	–	–
R316 W	**110**	61	77	94	141	–	109	100
G413S	**100**	76	100	83	110	–	73	104
R457H	–	1	–	3	–	1	–	–
Y459H	–	–	–	11	–	–	–	–
V492E	–	–	–	3	–	<1	–	–
A503 V	**107**	67	56	68	58	–	85	113
G504R	**93**	53	47	91	103	–	82	140
C569Y	**32**	18	7	28	13	51	6	–
V608F	**16**	8	3	80	57	24	5	–
R616X	–	–	6	–	–	–	–	–
V631I	**89**	74	23	51	40	–	6	23
delF646	**88**	36	94	97	46	–	–	–

POR and available NADPH/NADP concentrations will be required to realistically access effects of these variants. Studies in which C569 of human POR was alkylated with iodo-acetic acid eliminated activity, implicating a role for cysteine 569 in binding of NADPH to human POR [41]. The C566S mutagenesis experiment in rat POR (C569 for human) showed a 4.6-fold higher km value for NADPH [42] but alkylation of cysteines in pig POR did not change activity [43]. The mutation C569Y, found in a patient with disordered steroidogenesis but without ABS [8], shows a higher km for NADPH, has 13% of 17,20 lyase activity, 28% of 17α-hydroxylase activity and 50% aromatase activity (Table 2). The C569Y mutation was found as a compound heterozygote with V608F [8] which retained 57% of 17,20 lyase activity and 80% of 17α-hydroxylase activity, thus explaining that individual's hormonal profile resembling isolated 17,20 lyase deficiency. An excess of NADPH may be able to overcome some of the NADPH binding defect as binding does not seem to be completely destroyed. Several earlier experiments were performed under cofactor concentrations optimal for enzymatic activities of P450 enzymes (1 mM NADPH) and probably need repetition with 1–100 μM NADPH.

POR interacts with all 50 human microsomal P450 enzymes, and as the interactions with different P450 enzymes will vary with the geometry of the redox-partner binding site of the P450, no assay based on a single P450 enzyme will reliably forecast all the consequences of a specific POR mutant. Interactions between POR, P450s and their substrates in the microsomal membranes may cause conformational changes in the flavin- and NADPH-binding domains in POR [44,45], as well as inducing conformational binding site changes in the interacting P450 partners. The mechanisms of POR interactions with different cytochromes P450 and other electron acceptors may vary with each enzyme, and further complications may arise by the binding of substrates with P450 that might itself undergo conformational changes after substrate binding.

We have shown that some mutations in POR identified from patients may lead to reduced CYP3A4 activities in vitro. Based on the severity of effect caused by some of the mutations we are recommending that prescription of medication to patients with POR mutations known to reduce CYP3A4 and other drug metabolizing enzyme activities should be monitored carefully for signs of adverse drug reactions and adjustments be made to account for lower levels of enzymatic activities. Further studies to directly measure the in vivo drug metabolism in patients with POR mutations will be required to assess metabolic effects of reduced CYP3A4 activity in affected individuals. Some commonly occurring polymorphisms of POR may also have variable effects on drug metabolizing P450s and will need to be evaluated by in vitro as well as in vivo drug clearance tests.

Acknowledgments

This work was supported by grants to AVP from The Swiss National Science Foundation (3100A3-113719), Novartis Foundation for Biology and Medicine, Roche Research Foundation, Swiss Society for Endocrinology & Diabetology and Bern University Research Foundation.

References

[1] A.P. Li, D.L. Kaminski, A. Rasmussen, Substrates of human hepatic cytochrome P450 3A4, Toxicology 104 (1995) 1–8.
[2] T. Shimada, H. Yamazaki, M. Mimura, Y. Inui, F.P. Guengerich, Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians, J. Pharmacol. Exp. Ther. 270 (1994) 414–423.
[3] G.K. Dresser, J.D. Spence, D.G. Bailey, Pharmacokinetic-pharmacodynamic consequences and clinical relevance of cytochrome P450 3A4 inhibition, Clin. Pharmacokinet. 38 (2000) 41–57.
[4] S. Zhou, S. Yung Chan, B. Cher Goh, E. Chan, W. Duan, M. Huang, H.L. McLeod, Mechanism-based inhibition of cytochrome P450 3A4 by therapeutic drugs, Clin. Pharmacokinet. 44 (2005) 279–304.
[5] R.E. Peterson, J. Imperato-McGinley, T. Gautier, C.H.L. Shackleton, Male pseudohermaphroditism due to multiple defects in steroid-biosynthetic microsomal mixed-function oxidases a new variant of congenital adrenal hyperplasia, N. Engl. J. Med. 313 (1985) 1182–1191.
[6] W.L. Miller, Congenital adrenal hyperplasia, N. Engl. J. Med. 314 (1986) 1321–1322.
[7] A.L. Shen, K.A. O'Leary, C.B. Kasper, Association of multiple developmental defects and embryonic lethality with loss of microsomal NADPH-cytochrome P450 oxidoreductase, J. Biol. Chem. 277 (2002) 6536–6541.
[8] C.E. Flück, T. Tajima, A.V. Pandey, W. Arlt, K. Okuhara, C.F. Verge, E.W. Jabs, B.B. Mendonca, K. Fujieda, W.L. Miller, Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley-Bixler syndrome, Nat. Genet. 36 (2004) 228–230.
[9] W. Arlt, E.A. Walker, N. Draper, H.E. Ivison, J.P. Ride, F. Hammer, S.M. Chalder, M. Borucka-Mankiewicz, B.P. Hauffa, E.M. Malunowicz, P.M. Stewart, C.H. Shackleton, Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study, Lancet 363 (2004) 2128–2135.
[10] M. Adachi, K. Tachibana, Y. Asakura, T. Yamamoto, K. Hanaki, A. Oka, Compound heterozygous mutations of cytochrome P450 oxidoreductase gene (POR) in two patients with Antley-Bixler syndrome, Am. J. Med. Genet. 128A (2004) 333–339.
[11] N. Huang, A.V. Pandey, V. Agrawal, W. Reardon, P.D. Lapunzina, D. Mowat, E.W. Jabs, G. Van Vliet, J. Sack, C.E. Flück, W.L. Miller, Diversity and function of mutations in p450 oxidoreductase in patients with Antley-Bixler syndrome and disordered steroidogenesis, Am. J. Hum. Genet. 76 (2005) 729–749.

[12] M. Fukami, R. Horikawa, T. Nagai, T. Tanaka, Y. Naiki, N. Sato, T. Okuyama, H. Nakai, S. Soneda, K. Tachibana, N. Matsuo, S. Sato, K. Homma, G. Nishimura, T. Hasegawa, T. Ogata, Cytochrome P450 oxidoreductase gene mutations and Antley-Bixler syndrome with abnormal genitalia and/or impaired steroidogenesis: molecular and clinical studies in 10 patients, J. Clin. Endocrinol. Metab. 90 (2005) 414–426.
[13] S.N. Hart, S. Wang, K. Nakamoto, C. Wesselman, Y. Li, X.B. Zhong, Genetic polymorphisms in cytochrome P450 oxidoreductase influence microsomal P450-catalyzed drug metabolism, Pharmacogenet. Genomics 18 (2008) 11–24.
[14] N. Huang, V. Agrawal, K.M. Giacomini, W.L. Miller, Genetics of P450 oxidoreductase: sequence variation in 842 individuals of four ethnicities and activities of 15 missense mutations, Proc. Natl. Acad. Sci. USA 105 (2008) 1733–1738.
[15] S.C. Sim, W.L. Miller, X.B. Zhong, W. Arlt, T. Ogata, X. Ding, C.R. Wolf, C.E. Flück, A.V. Pandey, C.J. Henderson, T.D. Porter, A.K. Daly, D.W. Nebert, M. Ingelman-Sundberg, Nomenclature for alleles of the cytochrome P450 oxidoreductase gene, Pharmacogenet. Genomics 19 (2009) 565–566.
[16] K. Homma, T. Hasegawa, T. Nagai, M. Adachi, R. Horikawa, I. Fujiwara, T. Tajima, R. Takeda, M. Fukami, T. Ogata, Urine steroid hormone profile analysis in cytochrome P450 oxidoreductase deficiency: implication for the backdoor pathway to dihydrotestosterone, J. Clin. Endocrinol. Metab. 91 (2006) 2643–2649.
[17] M. Fukami, T. Hasegawa, R. Horikawa, T. Ohashi, G. Nishimura, K. Homma, T. Ogata, Cytochrome P450 oxidoreductase deficiency in three patients initially regarded as having 21-hydroxylase deficiency and/or aromatase deficiency: diagnostic value of urine steroid hormone analysis, Pediatr. Res. 59 (2006) 276–280.
[18] M. Adachi, Y. Asakura, M. Matsuo, T. Yamamoto, K. Hanaki, W. Arlt, POR R457H is a global founder mutation causing Antley-Bixler syndrome with autosomal recessive trait, Am. J. Med. Genet. A 140 (2006) 633–635.
[19] A.V. Pandey, P. Kempna, G. Hofer, P.E. Mullis, C.E. Flück, Modulation of human CYP19A1 activity by mutant NADPH P450 oxidoreductase, Mol. Endocrinol. 21 (2007) 2579–2595.
[20] C.E. Flück, A.V. Pandey, N. Huang, V. Agrawal, W.L. Miller, P450 oxidoreductase deficiency - a new form of congenital adrenal hyperplasia, Endocr. Dev. 13 (2008) 67–81.
[21] C.E. Flück, C. Nicolo, A.V. Pandey, Clinical, structural and functional implications of mutations and polymorphisms in human NADPH P450 oxidoreductase, Fundam. Clin. Pharmacol. 21 (2007) 399–410.
[22] A.V. Pandey, C.E. Flück, P.E. Mullis, Altered heme catabolism by heme oxygenase-1 caused by mutations in human NADPH cytochrome P450 reductase, Biochem. Biophys. Res. Commun. 400 (2010) 374–378.
[23] C. Nicolo, C.E. Flück, P.E. Mullis, A.V. Pandey, Restoration of mutant cytochrome P450 reductase activity by external flavin, Mol. Cell. Endocrinol. 321 (2010) 245–252.
[24] M. Kranendonk, C.C. Marohnic, S.P. Panda, M.P. Duarte, J.S. Oliveira, B.S. Masters, J. Rueff, Impairment of human CYP1A2-mediated xenobiotic metabolism by Antley-Bixler syndrome variants of cytochrome P450 oxidoreductase, Arch. Biochem. Biophys. 475 (2008) 93–99.
[25] V. Agrawal, N. Huang, W.L. Miller, Pharmacogenetics of P450 oxidoreductase: effect of sequence variants on activities of CYP1A2 and CYP2C19, Pharmacogenet. Genomics 18 (2008) 569–576.
[26] A.M. Gomes, S. Winter, K. Klein, M. Turpeinen, E. Schaeffeler, M. Schwab, U.M. Zanger, Pharmacogenomics of human liver cytochrome P450 oxidoreductase: multifactorial analysis and impact on microsomal drug oxidation, Pharmacogenomics 10 (2009) 579–599.
[27] T.J. Pursley, I.K. Blomquist, J. Abraham, H.F. Andersen, J.A. Bartley, Fluconazole-induced congenital anomalies in three infants, Clin. Infect. Dis. 22 (1996) 336–340.
[28] K.A. Aleck, D.L. Bartley, Multiple malformation syndrome following fluconazole use in pregnancy: report of an additional patient, Am. J. Med. Genet. 72 (1997) 253–256.
[29] L. Kragie, S.D. Turner, C.J. Patten, C.L. Crespi, D.M. Stresser, Assessing pregnancy risks of azole antifungals using a high throughput aromatase inhibition assay, Endocr. Res. 28 (2002) 129–140.
[30] B. Oneda, S. Crettol, E. Jaquenoud Sirot, M. Bochud, N. Ansermot, C.B. Eap, The P450 oxidoreductase genotype is associated with CYP3A activity in vivo as measured by the midazolam phenotyping test, Pharmacogenet. Genomics 19 (2009) 877–883.
[31] E.M. Gillam, T. Baba, B.R. Kim, S. Ohmori, F.P. Guengerich, Expression of modified human cytochrome P450 3A4 in Escherichia coli and purification and reconstitution of the enzyme, Arch. Biochem. Biophys. 305 (1993) 123–131.
[32] A.V. Pandey, S.K. Joshi, B.L. Tekwani, V.S. Chauhan, A colorimetric assay for heme in biological samples using 96-well plates, Anal. Biochem. 268 (1999) 159–161.
[33] T. Omura, R. Sato, The carbon monoxide-binding pigment of liver microsomes I. evidence for its hemoprotein nature, J. Biol. Chem. 239 (1964) 2370–2378.
[34] A.V. Pandey, W.L. Miller, Regulation of 17, 20 lyase activity by cytochrome b5 and by serine phosphorylation of P450c17, J. Biol. Chem. 280 (2005) 13265–13271.
[35] A.V. Pandey, C.E. Flück, N. Huang, T. Tajima, K. Fujieda, W.L. Miller, P450 oxidoreductase deficiency: A new disorder of steroidogenesis affecting all microsomal P450 enzymes, Endocr. Res. 30 (2004) 881–888.
[36] W.L. Miller, N. Huang, C.E. Flück, A.V. Pandey, P450 oxidoreductase deficiency, Lancet 364 (2004) 1663.
[37] C.E. Flück, P.E. Mullis, A.V. Pandey, Modeling Of Human P450 Oxidoreductase Structure By In Silico Mutagenesis And Md Simulation, Mol. Cell. Endocrinol. 313 (2009) 17–22.
[38] V. Dhir, H.E. Ivison, N. Krone, C.H. Shackleton, A.J. Doherty, P.M. Stewart, W. Arlt, Differential inhibition of CYP17A1 and CYP21A2 activities by the P450 oxidoreductase mutant A287P, Mol. Endocrinol. 21 (2007) 1958–1968.
[39] C.C. Marohnic, S.P. Panda, K. McCammon, J. Rueff, B.S. Masters, M. Kranendonk, Human cytochrome P450 oxidoreductase deficiency caused by the Y181D mutation: molecular consequences and rescue of defect, Drug Metab. Dispos. 38 (2010) 332–340.
[40] W.L. Miller, N. Huang, V. Agrawal, K.M. Giacomini, Genetic variation in human P450 oxidoreductase, Mol. Cell. Endocrinol. 300 (2009) 180–184.
[41] M. Haniu, M.E. McManus, D.J. Birkett, T.D. Lee, J.E. Shively, Structural and functional analysis of NADPH-cytochrome P-450 reductase from human liver: complete sequence of human enzyme and NADPH-binding sites, Biochemistry 28 (1989) 8639–8645.
[42] A.L. Shen, M.J. Christensen, C.B. Kasper, NADPH-cytochrome P-450 oxidoreductase. The role of cysteine 566 in catalysis and cofactor binding, J. Biol. Chem. 266 (1991) 19976–19980.
[43] M. Haniu, T. Iyanagi, K. Legesse, J.E. Shively, Structural analysis of NADPH-cytochrome P-450 reductase from porcine hepatic microsomes sequences of proteolytic fragments, cysteine-containing peptides, and a NADPH-protected cysteine peptide, J. Biol. Chem. 259 (1984) 13703–13711.
[44] J. Ellis, A. Gutierrez, I.L. Barsukov, W.C. Huang, J.G. Grossmann, G.C. Roberts, Domain motion in cytochrome P450 reductase: conformational equilibria revealed by NMR and small-angle x-ray scattering, J. Biol. Chem. 284 (2009) 36628–36637.
[45] D. Hamdane, C. Xia, S.C. Im, H. Zhang, J.J. Kim, L. Waskell, Structure and function of an NADPH-cytochrome P450 oxidoreductase in an open conformation capable of reducing cytochrome P450, J. Biol. Chem. 284 (2009) 11374–11384.