

Impaired hepatic drug and steroid metabolism in congenital adrenal hyperplasia due to P450 oxidoreductase deficiency

Tomalik-Scharte, D; Maiter, D; Kirchheiner, J; Ivison, HE; Fuhr, U; Arlt, Wiebke

DOI:
[10.1530/EJE-10-0764](https://doi.org/10.1530/EJE-10-0764)

Citation for published version (Harvard):

Tomalik-Scharte, D, Maiter, D, Kirchheiner, J, Ivison, HE, Fuhr, U & Arlt, W 2010, 'Impaired hepatic drug and steroid metabolism in congenital adrenal hyperplasia due to P450 oxidoreductase deficiency', *European Journal of Endocrinology*, vol. 163, no. 6, pp. 919-24. <https://doi.org/10.1530/EJE-10-0764>

[Link to publication on Research at Birmingham portal](#)

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

CLINICAL STUDY

Impaired hepatic drug and steroid metabolism in congenital adrenal hyperplasia due to P450 oxidoreductase deficiency

Dorota Tomalik-Scharte¹, Dominique Maiter², Julia Kirchheiner³, Hannah E Ivison, Uwe Fuhr¹ and Wiebke Arlt
School of Clinical and Experimental Medicine, Centre for Endocrinology, Diabetes and Metabolism (CEDAM), University of Birmingham, Birmingham B15 2TT, UK, ¹Department of Pharmacology, University Hospital, University of Cologne, 50931 Cologne, Germany, ²Department of Endocrinology, University Hospital Saint Luc, 1200 Brussels, Belgium and ³Department of Pharmacology of Natural Products and Clinical Pharmacology, University of Ulm, 89019 Ulm, Germany

(Correspondence should be addressed to W Arlt; Email: w.arlt@bham.ac.uk)

Abstract

Objective: Patients with congenital adrenal hyperplasia due to P450 oxidoreductase (POR) deficiency (ORD) present with disordered sex development and glucocorticoid deficiency. This is due to disruption of electron transfer from mutant POR to microsomal cytochrome P450 (CYP) enzymes that play a key role in glucocorticoid and sex steroid synthesis. POR also transfers electrons to all major drug-metabolizing CYP enzymes, including CYP3A4 that inactivates glucocorticoid and oestrogens. However, whether ORD results in impairment of *in vivo* drug metabolism has never been studied.

Design: We studied an adult patient with ORD due to homozygous POR A287P, the most frequent POR mutation in Caucasians, and her clinically unaffected, heterozygous mother. The patient had received standard dose oestrogen replacement from 17 until 37 years of age when it was stopped after she developed breast cancer.

Methods: Both subjects underwent *in vivo* cocktail phenotyping comprising the oral administration of caffeine, tolbutamide, omeprazole, dextromethorphan hydrobromide and midazolam to assess the five major drug-metabolizing CYP enzymes. We also performed genotyping for variant CYP alleles known to affect drug metabolism.

Results: Though CYP enzyme genotyping predicted normal or high enzymatic activities in both subjects, *in vivo* assessment showed subnormal activities of CYP1A2, CYP2C9, CYP2D6 and CYP3A4 in the patient and of CYP1A2 and CYP2C9 in her mother.

Conclusions: Our results provide *in vivo* evidence for an important role of POR in regulating drug metabolism and detoxification. In patients with ORD, *in vivo* assessment of drug-metabolizing activities with subsequent tailoring of drug therapy and steroid replacement should be considered.

European Journal of Endocrinology 163 919–924

Introduction

P450 oxidoreductase (POR) deficiency (ORD) is a variant of congenital adrenal hyperplasia that is not caused by mutations in a steroidogenic enzyme itself, but in the co-factor enzyme POR (1–3). POR facilitates electron transfer from NADPH to key enzymes involved in glucocorticoid and sex steroid synthesis, 17 α -hydroxylase/17,20 lyase (CYP17A1), CYP21A2 (21-hydroxylase) and CYP19A1 (aromatase). Therefore, mutant POR results in clinical presentation with disordered sex development (DSD) and glucocorticoid deficiency (1, 2). Intriguingly, individuals of both sexes may present with DSD, i.e. 46,XX DSD and 46,XY DSD. While undervirilization of affected male individuals is readily explained by insufficient sex steroid synthesis, virilization in girls is thought to be consequent to the exaggerated activity of an alternative pathway towards androgen synthesis that is active in fetal life and ceases

activity shortly after birth (1). Individuals affected by ORD also often present with skeletal malformations, most likely consequent to impaired synthesis of cholesterol (4, 5) and retinoic acid metabolism (6), both of which play a crucial role in the regulation of fetal bone development and growth (5, 7).

In addition to facilitating steroid and sterol synthesis, POR provides electrons to all major drug-metabolizing cytochrome P450 (CYP) enzymes, and thus it is conceivable that mutant POR will also disrupt drug and xenobiotic metabolism (Fig. 1). Five CYP enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4) are responsible for the metabolism of more than 80% of all drugs (8). CYP3A4 represents the major player involved in the metabolism of more than half of existing drugs, notably also in the inactivation of steroid hormones including glucocorticoids and sex steroids (9).

A functional impact of sequence variants in the POR gene on hepatic drug metabolism by POR sequence has

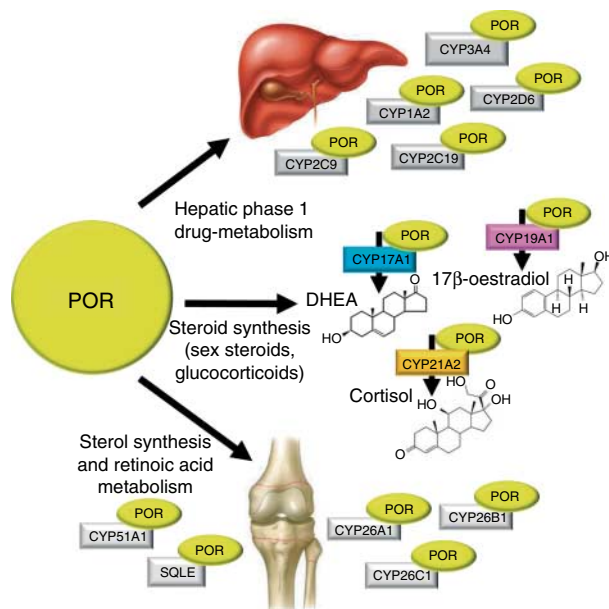


Figure 1 P450 oxidoreductase (POR) transfers electrons to all microsomal cytochrome P450 (CYP) enzymes. POR thereby impacts on the function of three major metabolic systems involving microsomal CYP enzymes: steroid synthesis in adrenal glands and gonads, cholesterol synthesis (e.g. in developing bone) and metabolism of steroids, drugs and other xenobiotics, mainly in the liver; drug-metabolizing microsomal CYP enzymes: CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4; steroidogenic microsomal CYP enzymes: CYP17A1 (17 α -hydroxylase/17,20 lyase), CYP21A2 (21-hydroxylase) and CYP19A1 (aromatase); microsomal CYP enzymes involved in sterol synthesis and metabolism: CYP51A1 (14 α -lanosterol demethylase) and SQLE (squalene epoxidase); microsomal CYP enzymes involved in retinoic acid metabolism: CYP26A1, CYP26B1 and CYP26C1.

been suggested by a number of *in vitro* studies (10–13) and a recent *in vivo* study in healthy individuals (14). However, the significance of mutant POR for human drug metabolism has not been studied yet in the clinical context of ORD. Here, we have studied the activities of the five major drug-metabolizing CYP enzymes in two individuals carrying the POR variant A287P, the most frequently observed disease-causing POR mutation in Caucasians.

Subjects and methods

Subjects

A 48-year-old patient with congenital adrenal hyperplasia due to ORD and her clinically unaffected 72-year-old mother participated in the study. The neonatal and pubertal presentation of this patient has been reported elsewhere (J Idkowiak, unpublished observations); in brief, she presented at birth with severely virilized external genitalia, i.e. 46,XX DSD, and was wrongly assigned to the male gender. At the age of 5 years, sex reassignment was prompted by the birth of

a virilized sister. Owing to lack of spontaneous pubertal development, puberty had been induced at the age of 17 years by equine oestrogens and progesterone. Detailed assessment at the age of 24 years revealed elevated serum progesterone and 17OH-progesterone, low serum androgens, partial adrenal insufficiency with an impaired cortisol response to ACTH stimulation and clinical and radiological evidence of mild dysmorphic features (low set ears, clinodactyly, camptodactyly and brachydactyly). Blood pressure was elevated on repeated measurements (systolic 140–160 mmHg and diastolic 90–95 mmHg).

Oestrogen/progestin replacement was continued until the age of 37 years, when she was diagnosed with breast cancer, despite a negative family history. The tumour was 3 cm in diameter, with a mixed histological picture featuring foci of ductal adenocarcinoma as well as stromal myxoid changes; tumour tissue was negative for oestrogen and progesterone receptor staining and Her-2/neu amplification. Oestrogen/progestin replacement was stopped post surgical follow-up until her current age of 49 years did not reveal a recurrence.

Methods

POR and CYP enzyme genotyping To ascertain the diagnosis of ORD, genetic analyses were performed after obtaining written informed consent and with approval of the South Birmingham Research Ethics Committee. The coding region of the POR gene (GeneID 5447) was amplified as previously described (1), employing genomic DNA from the patient and her mother.

In addition to POR sequencing, we performed genotyping for common variant alleles of the genes encoding the major drug-metabolizing CYP enzymes since such sequence variants have been shown to contribute to an individual's drug response. Analyses included the determination of variant alleles *2 and *3 of CYP2C9 (15), allele *2 of CYP2C19 (16) and alleles *3, *4, *5, *6 and the gene duplication of CYP2D6 (17). **n* denotes the individual allele as decided by the Human CYP Allele Nomenclature Committee (<http://www.cypalleles.ki.se/>).

In vivo low dose cocktail phenotyping Both subjects underwent *in vivo* phenotyping to assess the activities of the major hepatic drug-metabolizing CYP enzymes carried out after obtaining informed consent and with approval of the ethics committee of the University Hospital Cologne. Low doses of validated substrates selective for each of the five major drug-metabolizing CYP enzymes were orally administered simultaneously as a 'cocktail' (Table 1), thereby allowing for estimation of several metabolic pathways in a single assessment (18). Phenotyping was performed under in-patient conditions. After an overnight fast, blood samples were drawn before and 1, 3, 6 and 24 h after the oral

Table 1 *In vivo* low dose drug cocktail phenotyping for assessment of drug-metabolizing cytochrome P450 (CYP) enzymes including selective substrates employed, respective doses and phenotyping parameters.

Drug-metabolizing CYP enzymes	Drug	Dose (mg)	Phenotyping parameter (unit)
CYP1A2	Caffeine	150	Oral caffeine clearance (ml/min per kg body weight)
CYP2C9	Tolbutamide	125	Plasma concentration of tolbutamide 24 h post dose (µg/ml)
CYP2C19	Omeprazole	20	Ratio of the molar plasma concentrations of omeprazole over 5-hydroxyomeprazole 3 h post dose (–)
CYP2D6	Dextromethorphan hydrobromide	30	Ratio of the molar plasma concentrations of dextromethorphan over dextrorphan 3 h post dose (–)
CYP3A4	Midazolam	2	Oral midazolam clearance (L/hl)

administration of 150 mg caffeine, 125 mg tolbutamide, 20 mg omeprazole, 30 mg dextromethorphan hydrobromide and 2 mg midazolam at 0900 h (Table 1). Plasma concentrations of the probe drugs and their metabolites were determined by liquid chromatography/tandem mass spectrometry as previously described (19). The results of the cocktail phenotyping were projected on to the reference range obtained in normal controls (CYP1A2: *n*=768 (20); CYP2C9: *n*=26 (21); CYP2C19: *n*=153, estimated according to (22); CYP2D6: *n*=51, unpublished in-house data; CYP3A4: *n*=66 (23)).

Results

ORD was confirmed by *POR* sequencing, which revealed subjects 1 and 2 as homozygous and heterozygous carriers respectively of the c.852G→C (g.29,556G→C) mutation in exon 8 of the *POR* gene, encoding for an amino acid change from alanine to proline in position 287 of the *POR* protein, p.A287P.

Both subjects had *CYP2C19* genotypes indicative of a rapid metabolizer phenotype, i.e. high enzymatic activity, and their *CYP2C9* genotypes were predictive of normal activity (Table 2). *CYP2D6* genotyping found *CYP2D6* gene duplication in subject 1 (patient), consistent with an ultrarapid metabolizer phenotype, while variant alleles identified in subject 2 (mother) predicted a rapid metabolizer phenotype (Table 2).

However, despite *CYP* genotyping results predictive of normal to high enzymatic activities, *in vivo* drug cocktail phenotyping revealed low activities of all tested CYP enzymes in both subjects (Fig. 2). The patient and her mother had similarly decreased activities of CYP1A2 (oral caffeine clearance (ml/min per kg body weight); subject 1, 0.25; subject 2, 0.35) and CYP2C9 (plasma tolbutamide concentration 24 h post dose (µg/ml); subject 1, 13.9; subject 2, 7.8), clearly below the 5th percentile of a normal reference population (Fig. 2). Also CYP2C19 activity was similarly low in both subjects (plasma omeprazole/5-hydroxyomeprazole 4 h

post dose; subject 1, 2.79; subject 2, 2.66), albeit still within the lowest quartile of the normal reference range (Fig. 2). However, with regards to CYP2D6 and CYP3A4, we observed lower metabolic capacities for subject 1 who was homozygous for *POR* A287P than for subject 2 who carried only one mutant *POR* A287P allele. Subject 1 showed clearly subnormal conversion of the probe drugs, whereas observed metabolic capacities of CYP2D6 and CYP3A4 for subject 2 still varied within the lowest normal range (CYP2D6: plasma dextromethorphan/dextrorphan 3 h post dose; subject 1, 5.05; subject 2, 0.68; CYP3A4: oral midazolam clearance (L/hl); subject 1, 10.7; subject 2, 39.7) (Fig. 2).

Discussion

Here, we have provided *in vivo* evidence for impaired drug metabolism as a consequence of mutant *POR*. *In vivo* cocktail phenotyping revealed grossly diminished activities of drug-metabolizing CYP enzymes in the two subjects carrying the *POR* A287P mutation, a disease-causing *POR* variant most frequently found in Caucasians (1, 2). However, it is remarkable that even heterozygosity for this allele was sufficient to clearly

Table 2 Results of cytochrome P450 (*CYP*) genotyping in the patient with P450 oxidoreductase (*POR*) deficiency (ORD) due to homozygous *POR* A287P (subject 1) and her clinically unaffected, heterozygous mother (subject 2), listing the identified variant alleles (*) of *CYP2C9*, *CYP2C19* and *CYP2D6* previously described to affect enzymatic activity.

Drug-metabolizing CYP enzymes	Predicted drug metabolizer phenotype (according to variant CYP allele genotype)	
	Subject 1 (A287P/A287P)	Subject 2 (A287P/WT)
CYP2C9	Intermediate (*1/*2)	Rapid (*1/*1)
CYP2C19	Rapid (*1/*1)	Rapid (*1/*1)
CYP2D6	Ultrarapid (2×*2/*1)	Rapid (*1/*1)

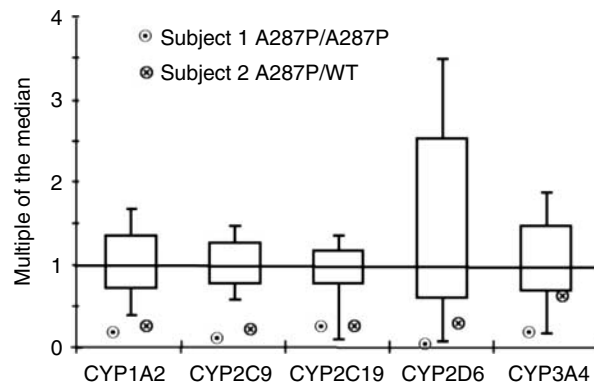


Figure 2 *In vivo* low dose cocktail phenotyping for assessment of drug-metabolizing CYP enzyme activities in P450 oxidoreductase deficiency. The results of the cocktail phenotyping in subject 1 (patient, A287P/A287P) and subject 2 (mother, A287P/WT) projected on to the reference range obtained in normal controls, with the boxes representing the 25th to 75th percentile and the whiskers indicating 5th and 95th percentiles respectively. Results are represented as multiples of the median enzyme activity observed in the respective normal reference cohorts (for details, see the Methods section).

decrease metabolic capacities of all five major drug-metabolizing CYP enzymes, as demonstrated in the patient's mother. Of note, the clinically observed drug-metabolizing CYP enzyme activities in the two subjects were severely decreased despite CYP genotyping results predictive of normal to high activities. This suggests that the effect of genetic variation in *POR* on interindividual variability in drug metabolism may be at least as important as that of variant CYP alleles.

We have previously shown that *POR* A287P differentially affects the activity of electron-accepting steroidogenic CYP enzymes, namely a preferential inhibition of 17-hydroxylase over 21-hydroxylase, as demonstrated *in vitro* and also *in vivo* by increased urinary excretion of corticosterone over cortisol metabolites, causing a relative accumulation of steroids with mineralocorticoid-like activity (24). The clinical significance of this finding is clearly reflected by the clinical phenotype of the current patient who presented with manifest arterial hypertension at the age of 24 years, most likely a consequence of mild mineralocorticoid excess. In addition, it has been shown that *POR* A287P does not result in any inhibition of CYP19A1 activity (25), further highlighting the differential effects of this mutant. Thus, it is entirely possible that distinct *POR* variants may have only modest effects on steroidogenic CYP enzymes but could severely disrupt drug-metabolizing CYP enzymes.

POR is a highly polymorphic gene with more than 140 documented sequence variants in the *POR*-coding region, including 40 present on more than 1% of alleles (26). A number of *in vitro* studies and a recent *in vivo* study have attempted to address the correlation between genetic variation in *POR* and drug-metabolizing CYP

enzyme activities (10–14, 27). A recent study demonstrated reduced *in vitro* CYP3A4 activity due to *POR* A287P (13). However, another study demonstrated complete abolishment of CYP1A2 and CYP2C19 activities by *POR* A287P *in vitro* (10), which contrasts with our *in vivo* finding of clearly diminished albeit still detectable CYP1A2 and CYP2C19 activities in our patient carrying homozygous A287P. This emphasizes that *in vitro* assays are valuable, but will not always correctly reflect complex systems such as the CYP–*POR* drug-metabolizing complex, where genetic variants within more than one gene may impact on the enzymatic activities observed *in vivo*. Obviously, we cannot exclude that the observed reduction in drug-metabolizing enzyme activities in our two subjects of similar familial genetic background could be due to another, hitherto not yet identified genetic variation. However, the significance of *POR* for drug metabolism was also indicated by previous rodent-based studies analysing liver-specific *Por* deletion, demonstrating disruption of cholesterol and bile acid metabolism (28) and reduced activation of the chemotherapeutic pro-drug cyclophosphamide (29). Another possible caveat is that the heterozygous mother was 78 years of age, and the drug metabolism profile was compared with CYP genotype-matched controls but could not be directly compared with age-matched controls as such data are not available. However, there is no general loss of hepatic CYP activity with age, as previously shown *in vitro* (30) and *in vivo* (31), which would explain the uniform low activity in the mother.

Of note, our patient developed breast cancer at the very early age of 37 years, despite a negative family history. It is tempting to speculate about the possible contribution of mutant *POR* to the development of this early onset breast cancer. CYP3A4 is the major enzyme involved in the inactivation of steroid hormones including glucocorticoids and sex steroids (9), and we found that *in vivo* CYP3A4 activity was grossly reduced in our patient, who had received standard dose oestrogen replacement for 20 years prior to being diagnosed with breast cancer. Impairment of CYP3A4 activity, as documented in our patient, will have undoubtedly resulted in delayed breakdown of and increased exposure to oestrogen, a well-known risk factor for breast cancer. CYP3A4 activity is known to display broad interindividual variability and, in contrast to other drug-metabolizing CYP enzymes, convincing associations between variant CYP3A4 alleles and observed *in vivo* enzymatic activity are lacking (32). Thus, variant *POR* might be a more important modulator of CYP3A4 function than genetic variation in CYP3A4 itself.

Our finding of grossly impaired drug-metabolizing activities in our patient despite carrying variant CYP alleles predictive of high enzymatic activities inevitably poses the question whether this should alter clinical management. We feel that based on our results, low

dose cocktail phenotyping, a low risk diagnostic procedure, should be considered in all patients with CAH caused by ORD to determine whether future drug therapy and, importantly, also steroid replacement therapy including glucocorticoid and sex steroids require individually tailored dosing.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported by the Medical Research Council United Kingdom (Programme Grant 0900567 to W Arlt).

References

- Arlt W, Walker EA, Draper N, Ivison HE, Ride JP, Hammer F, Chalder SM, Borucka-Mankiewicz M, Hauffa BP, Malunowicz EM, Stewart PM & Shackleton CH. Congenital adrenal hyperplasia caused by mutant P450 oxidoreductase and human androgen synthesis: analytical study. *Lancet* 2004 **363** 2128–2135. (doi:10.1016/S0140-6736(04)16503-3)
- Fluck CE, Tajima T, Pandey AV, Arlt W, Okuhara K, Verge CE, Jabs EW, Mendonça BB, Fujieda K & Miller WL. Mutant P450 oxidoreductase causes disordered steroidogenesis with and without Antley–Bixler syndrome. *Nature Genetics* 2004 **36** 228–230. (doi:10.1038/ng1300)
- Fukami M, Horikawa T, Nagai T, Tanaka T, Naiki Y, Sato N, Okuyama T, Nakai H, Soneda S, Tachibana K, Matsuo N, Sato S, Homma K, Nishimura G, Hasegawa T & Ogata T. Cytochrome P450 oxidoreductase gene mutations and Antley–Bixler syndrome with abnormal genitalia and/or impaired steroidogenesis: molecular and clinical studies in 10 patients. *Journal of Clinical Endocrinology and Metabolism* 2005 **90** 414–426. (doi:10.1210/jc.2004-0810)
- Kelley RI, Kratz LE, Glaser RL, Netzloff ML, Wolf LM & Jabs EW. Abnormal sterol metabolism in a patient with Antley–Bixler syndrome and ambiguous genitalia. *American Journal of Medical Genetics* 2002 **110** 95–102. (doi:10.1002/ajmg.10510)
- Schmidt K, Hughes C, Chudek JA, Goodyear SR, Aspdren RM, Talbot R, Gundersen TE, Blomhoff R, Henderson C, Wolf CR & Tickle C. Cholesterol metabolism: the main pathway acting downstream of cytochrome P450 oxidoreductase in skeletal development of the limb. *Molecular and Cellular Biology* 2009 **29** 2716–2729. (doi:10.1128/MCB.01638-08)
- Ribes V, Otto DM, Dickmann L, Schmidt K, Schuhbauer B, Henderson C, Blomhoff R, Wolf CR, Tickle C & Dollé P. Rescue of cytochrome P450 oxidoreductase (Por) mouse mutants reveals functions in vasculogenesis, brain and limb patterning linked to retinoic acid homeostasis. *Developmental Biology* 2007 **303** 66–81. (doi:10.1016/j.ydbio.2006.10.032)
- Porter JA, Ekker SC, Park WJ, von Kessler DP, Young KE, Chen CH, Ma Y, Woods AS, Cotter RJ, Koonin EV & Beachy PA. Hedgehog patterning activity: role of a lipophilic modification mediated by the carboxy-terminal autoprocessing domain. *Cell* 1996 **86** 21–34. (doi:10.1016/S0092-8674(00)80074-4)
- Nebert DW & Russell DW. Clinical importance of the cytochromes P450. *Lancet* 2002 **360** 1155–1162. (doi:10.1016/S0140-6736(02)11203-7)
- Guengerich FP. A malleable catalyst dominates the metabolism of drugs. *PNAS* 2006 **103** 13565–13566. (doi:10.1073/pnas.0606333103)
- Agrawal V, Huang N & Miller WL. Pharmacogenetics of P450 oxidoreductase: effect of sequence variants on activities of CYP1A2 and CYP2C19. *Pharmacogenetics and Genomics* 2008 **18** 569–576. (doi:10.1097/FPC.0b013e32830054ac)
- Hart SN, Wang S, Nakamoto K, Wesselman C, Li Y & Zhong XB. Genetic polymorphisms in cytochrome P450 oxidoreductase influence microsomal P450-catalyzed drug metabolism. *Pharmacogenetics and Genomics* 2008 **18** 11–24. (doi:10.1097/FPC.0b013e3282f2f121)
- Kranendonk M, Marohnic CC, Panda SP, Duarte MP, Oliveira JS, Masters BS & Rueff J. Impairment of human CYP1A2-mediated xenobiotic metabolism by Antley–Bixler syndrome variants of cytochrome P450 oxidoreductase. *Archives of Biochemistry and Biophysics* 2008 **475** 93–99. (doi:10.1016/j.abb.2008.04.014)
- Nicolo C, Fluck CE, Mullis PE & Pandey AV. Restoration of mutant cytochrome P450 reductase activity by external flavin. *Molecular and Cellular Endocrinology* 2010 **321** 245–252. (doi:10.1016/j.mce.2010.02.024)
- Oneda B, Crettol S, Sirot EJ, Bochud M, Ansermot N & Eap CB. The P450 oxidoreductase genotype is associated with CYP3A activity *in vivo* as measured by the midazolam phenotyping test. *Pharmacogenetics and Genomics* 2009 **19** 877–883. (doi:10.1097/FPC.0b013e32833225e7)
- Kirchheiner J, Bauer S, Meineke I, Rohde W, Prang V, Meisel C, Roots I & Brockmüller J. Impact of CYP2C9 and CYP2C19 polymorphisms on tolbutamide kinetics and the insulin and glucose response in healthy volunteers. *Pharmacogenetics* 2002 **12** 101–109. (doi:10.1097/00008571-200203000-00004)
- de Morais SM, Wilkinson GR, Blaisdell J, Nakamura K, Meyer UA & Goldstein JA. The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. *Journal of Biological Chemistry* 1994 **269** 15419–15422.
- Sachse C, Brockmüller J, Bauer S & Roots I. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. *American Journal of Human Genetics* 1997 **60** 284–295.
- Fuhr U, Jetter A & Kirchheiner J. Appropriate phenotyping procedures for drug metabolizing enzymes and transporters in humans and their simultaneous use in the "cocktail" approach. *Clinical Pharmacology and Therapeutics* 2007 **81** 270–283. (doi:10.1038/sj.clpt.6100050)
- Tomalik-Scharte D, Jetter A, Kinzig-Schippers M, Skott A, Sörgel F, Klaassen T, Kasel D, Harlfinger S, Doroshenko O, Frank D, Kirchheiner J, Bräter M, Richter K, Gramatté T & Fuhr U. Effect of propiverine on cytochrome P450 enzymes: a cocktail interaction study in healthy volunteers. *Drug Metabolism and Disposition* 2005 **33** 1859–1866. (doi:10.1124/dmd.105.005272)
- Tantcheva-Poor I, Zaigler M, Rietbrock S & Fuhr U. Estimation of cytochrome P-450 CYP1A2 activity in 863 healthy Caucasians using a saliva-based caffeine test. *Pharmacogenetics* 1999 **9** 131–144.
- Jetter A, Kinzig-Schippers M, Skott A, Lazar A, Tomalik-Scharte D, Kirchheiner J, Walchner-Bonjean M, Hering U, Jakob V, Rodamer M, Jabrane W, Kasel D, Brockmüller J, Fuhr U & Sörgel F. Cytochrome P450 2C9 phenotyping using low-dose tolbutamide. *European Journal of Clinical Pharmacology* 2004 **60** 165–171. (doi:10.1007/s00228-004-0754-z)
- Chang M, Dahl ML, Tybring G, Gotharson E & Bertilsson L. Use of omeprazole as a probe drug for CYP2C19 phenotype in Swedish Caucasians: comparison with S-mephenytoin hydroxylation phenotype and CYP2C19 genotype. *Pharmacogenetics* 1995 **5** 358–363. (doi:10.1097/00008571-199512000-00004)
- Tomalik-Scharte D, Doroshenko O, Kirchheiner J, Jetter A, Lazar A, Klaassen T, Frank D, Wyen C, Fätkenheuer G & Fuhr U. No role for the CYP3A5*3 polymorphism in intestinal and hepatic metabolism of midazolam. *European Journal of Clinical Pharmacology* 2008 **64** 1033–1035. (doi:10.1007/s00228-008-0503-9)
- Dhir V, Ivison HE, Krone N, Shackleton CH, Doherty AJ, Stewart PM & Arlt W. Differential inhibition of CYP17A1 and

- CYP21A2 activities by the P450 oxidoreductase mutant A287P. *Molecular Endocrinology* 2007 **21** 1958–1968. (doi:10.1210/me.2007-0066)
- 25 Pandey AV, Kempna P, Hofer G, Mullis PE & Fluck CE. Modulation of human CYP19A1 activity by mutant NADPH P450 oxidoreductase. *Molecular Endocrinology* 2007 **21** 2579–2595. (doi:10.1210/me.2007-0245)
- 26 Huang N, Agrawal V, Giacomini KM & Miller WL. Genetics of P450 oxidoreductase: sequence variation in 842 individuals of four ethnicities and activities of 15 missense mutations. *PNAS* 2008 **105** 1733–1738. (doi:10.1073/pnas.0711621105)
- 27 Gomes AM, Winter S, Klein K, Turpeinen M, Schaeffeler E, Schwab M & Zanger UM. Pharmacogenomics of human liver cytochrome P450 oxidoreductase: multifactorial analysis and impact on microsomal drug oxidation. *Pharmacogenomics* 2009 **10** 579–599. (doi:10.2217/pgs.09.7)
- 28 Henderson CJ, Otto DM, Carrie D, Magnuson MA, McLaren AW, Rosewell I & Wolf CR. Inactivation of the hepatic cytochrome P450 system by conditional deletion of hepatic cytochrome P450 reductase. *Journal of Biological Chemistry* 2003 **278** 13480–13486. (doi:10.1074/jbc.M212087200)
- 29 Henderson CJ, Pass GJ & Wolf CR. The hepatic cytochrome P450 reductase null mouse as a tool to identify a successful candidate entity. *Toxicology Letters* 2006 **162** 111–117. (doi:10.1016/j.toxlet.2005.10.016)
- 30 Parkinson A, Mudra DR, Johnson C, Dwyer A & Carroll KM. The effects of gender, age, ethnicity, and liver cirrhosis on cytochrome P450 enzyme activity in human liver microsomes and inducibility in cultured human hepatocytes. *Toxicology and Applied Pharmacology* 2004 **199** 193–209. (doi:10.1016/j.taap.2004.01.010)
- 31 Bebia Z, Buch SC, Wilson JW, Frye RF, Romkes M, Cecchetti A, Chaves-Gnecco D & Branch RA. Bioequivalence revisited: influence of age and sex on CYP enzymes. *Clinical Pharmacology and Therapeutics* 2004 **76** 618–627. (doi:10.1016/j.clpt.2004.08.021)
- 32 Perera MA. The missing linkage: what pharmacogenetic associations are left to find in CYP3A? *Expert Opinion on Drug Metabolism & Toxicology* 2010 **6** 17–28. (doi:10.1517/17425250903379546)

Received 6 September 2010

Accepted 15 September 2010