Drug metabolism by flavin-containing monooxygenases of human and mouse

Ian R. Phillips [a,b] and Elizabeth A. Shephard [a,*]

[a]Institute of Structural and Molecular Biology, University College London, London WC1E 6BT, UK

[b] School of Biological and Chemical Sciences, Queen Mary University of London, London E1 4NS, UK

Qualifications:
IRP, PhD
EAS, MSc, PhD

Job Title:
IRP, Emeritus Professor QMUL and visiting professor UCL
EAS, Professor

* Corresponding author. Elizabeth A. Shephard, Institute of Structural and Molecular Biology, University College London, London WC1E 6BT, UK

Phone: +44 (0) 2076792321

Email: e.shephard@ucl.ac.uk

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1. Introduction

Flavin-containing monooxygenases (FMOs; EC 1.14.13.8) are present in a wide range of organisms, including bacteria, fungi, plants, invertebrates and vertebrates. In eukaryotes, the enzymes are located in the membranes of the endoplasmic reticulum and catalyze the oxidative metabolism of a broad range of structurally diverse chemicals, including therapeutic drugs, dietary-derived compounds and pesticides [1,2]. Substrates contain, as the site of oxygenation, a nucleophilic heteroatom, typically nitrogen, sulphur, phosphorus or selenium [3]. FMOs are one of the most important of the non-cytochrome P450 (CYP) enzymes involved in the phase-1 metabolism of drugs. Of 860 drugs surveyed, FMOs contribute to the metabolism of about 5%, catalyzing about 2% of the more than 4,000 oxidoreduction reactions undergone by these drugs [4]. However, as outlined below, this might be an underestimate.

In this review, we focus on the role of FMOs in the metabolism of drugs in human and mouse. We describe the FMO genes and proteins of human and mouse, the catalytic mechanism of FMOs and its significance for the metabolism of drugs. We outline differences between FMOs and CYPs; discuss factors contributing to potential underestimation of the contribution of FMOs to drug metabolism; summarise the developmental stage- and tissue-specific expression of *FMO* genes and how this differs between human and mouse; and describe factors that induce or inhibit FMOs. We describe the contribution of FMOs of human and mouse to the metabolism of drugs *in vitro* and *in vivo* and how genetic variation of FMOs affects drug metabolism *in vitro* and *in vivo*. Finally, we discuss the utility of animal models for FMO-mediated drug metabolism in humans.

2. FMOs of human and mouse

Humans possess five functional *FMO* genes, designated *FMO1* to *FMO5* [5,6]. *FMO1* to *FMO4* are clustered on chromosome 1, in the region q24.3 [6] (Figure 1). The cluster contains an additional *FMO* gene, *FMO6P*, classified as a pseudogene because of its inability to produce a correctly spliced mRNA [7]. *FMO5* is located ~26 Mb closer to the centromere, at 1q21.1 [6]. A second *FMO* gene cluster, composed entirely of pseudogenes, *FMO7P* to *FMO11P*, is present at 1q24.2 [6]. The human genome, therefore, contains 11 *FMO* genes, five of which are functional. In the mouse, five *Fmo* genes, designated *Fmo1* to *Fmo4* and *Fmo6*, which are orthologous to the corresponding genes of human, are clustered on Chromosome 1 (Figure 1) [6]. In mouse, as is the case in humans, the gene encoding FMO5 is located outside the main *Fmo* gene cluster, in this case on mouse Chromosome 3 [6]. A second *Fmo* gene cluster, which contains three genes, designated *Fmo9*, *Fmo12* and *Fmo13*, is located on mouse Chromosome 1, ~3.5 Mb from the main *Fmo* gene cluster [6]. *Fmo6, Fmo9, Fmo12* and *Fmo13* encode full-length open-reading frames and possess no obvious features that would categorize them as pseudogenes. The mouse genome, therefore, contains orthologues of the five functional *FMO* genes of human and four additional, potentially functional *Fmo* genes.

3. Catalytic mechanism

Much of our knowledge of the catalytic mechanism and substrate preferences of FMOs derives from the pioneering work of Dan Ziegler and colleagues (reviewed in [8-10]). For catalysis, FMOs require flavin adeninine dinucleotide (FAD) as a prosthetic group, NADPH as a cofactor and molecular oxygen as a cosubstrate. The catalytic mechanism (Figure 2) is unusual because FMOs activate oxygen, in the form

of the C4a hydroperoxide derivative of FAD, in the absence of a bound oxygenatable substrate. Steps 1 and 2 are fast and the enzyme is present as the active C4a-hydroperoxyflavin form, capable of oxygenating any soft nucleophile able to gain access to the active site.

The unusual mechanism of FMOs accounts for their broad substrate range [8-10]. The rate-limiting step is considered to be either the breakdown of FADH-OH to release water or the release of $NADP^+$, both of which occur after the oxygenation of substrate and the release of oxygenated product. Consequently, in almost all cases, the catalytic constant (k_{cat}) is independent of the structure of the substrate and the specificity constant (k_{cat}/K_M) is determined largely by the K_M for the substrate. Because FMOs do not form classical Michaelis-Menten enzyme-substrate complexes, K_M is a measure of the ease with which a substrate can gain access to the active site. Size and shape are important factors that restrict access to the active site [8-11]. Charge is also important [8-10]: the best substrates are uncharged or have a single positive charge. In contrast, zwitterions or compounds with more than one positive charge are excluded, as are compounds with a single negative charge, except in cases where the charge is located at an appropriate distance from the site of oxygenation.

4. Differences between FMOs and CYPs

There are distinct differences in the catalytic mechanisms of FMOs and CYPs. In contrast to FMOs, which accept electrons directly from NADPH, CYPs receive electrons from NADPH via an accessary protein, NADPH-cytochrome P450 reductase, and bind and activate oxygen only after binding oxygenatable substrate. Whereas FMOs stabilize the active hydroperoxy flavin intermediate, CYPs form a relatively unstable ferrous-O_2 complex. FMOs have a preference for nucleophilic

compounds, whereas CYPs accept less nucleophilic ones. FMOs and CYPs share some substrates in common, but often produce different metabolites: although CYPs, in common with FMOs, can effect heteroatom oxygenation of nitrogen or sulfur, they more often catalyze carbon hydroxylation, heteroatom release (dealkylation) or epoxidation [12].

CYPs oxidize chemicals via sequential one-electron processes and, thus, are more capable of producing reactive, potentially toxic products, some of which can inactivate or inhibit CYPs. In contrast, FMOs oxygenate substrates, at a nucleophilic heteroatom, via a two-electron mechanism that generally produces polar, readily excretable detoxification products. Products of FMO-catalyzed reactions, even those that are relatively reactive, do not inhibit the enzyme, but can sometimes inhibit or inactivate nearby proteins, including CYPs [13,14].

5. Factors leading to possible underestimation of the contribution of FMOs to drug metabolism

Methods by which microsomes are prepared and incubated may have lead to underestimation of the contribution of FMOs to drug metabolism. In the absence of NADPH, FMOs are thermally labile [15]. Consequently, liver perfusion *in situ*, to remove blood before preparation of microsomes, could reduce FMO activity. To avoid autooxidative reactions, to which CYPs are prone, microsomes are often incubated at 37 ^{o}C in the presence of substrate and the reaction initiated by the addition of NADPH. Preincubation in the absence of NADPH would diminish FMO activity. Optimal FMO activity can be maintained by preparing microsomes at 4 ^{o}C, adding an NADPH-generating system to the microsomes before preincubation and initiating reactions by addition of substrate [15].

To distinguish between the contributions of FMOs and CYPs to the metabolism of a drug, incubations can be carried out on microsomes that have been pre-heated at 55 ^{o}C in the absence of NADPH, which knocks out FMO activity [15]. To selectively knock out CYP activities microsomes can be incubated at pH 10, or in the presence of carbon monoxide, high concentrations of non-ionic detergents, an inhibitory antibody to NADPH CYP reductase, or chemical inhibitors of CYPs [15]. However, some CYP inhibitors, such as SKF525A, are substrates for FMO and, therefore, could act as an alternative substrate competitive inhibitor of FMO. The ability of an FMO to catalyze oxygenation of a particular drug can be confirmed by the use of recombinantly expressed FMO.

6. Differences in expression of *FMO* genes between human and mouse

There are distinct differences between human and mouse in the tissue-specific expression of FMOs and in mouse marked gender-specific differences. In humans, expression of the *FMO1* gene in liver is switched off at birth [16,17]. This is in contrast to mouse, in which FMO1 constitutes a major form of the enzyme present in adult liver [18,19]. Silencing of the *FMO1* gene in adult human liver may be due to the presence, upstream of the hepatic promoter P0, of a LINE-1 element that acts as a powerful transcriptional repressor [20]. In extra-hepatic tissues, alternative promoters, P1 and P2, are used [6,20]. In adult human, FMO1 is present in kidney, its main site of expression [21-23], and in small intestine [22]. In adult mouse, *Fmo1* is expressed in liver, kidney, lung [19], white adipose tissue [24] and, to a small extent, in whole brain, in which it is the most highly expressed FMO [19]. In mouse, expression of *Fmo1* in liver and lung is greater in females than in males, but in kidney is greater in females [19].

In both human and mouse, the main site of expression of *FMO2* is lung [1,19,25]. FMO2 is also present in kidney [1,19].

In humans, expression of *FMO3* in liver is switched on after birth [17,21] and continues to increase throughout childhood and adolescence, reaching a maximum in adulthood [17]. FMO3 declines during menstruation [26] but otherwise there is little difference in expression between males and females. In mouse, however, there is a profound gender difference in the expression of *Fmo3* [19,27]. In females, *Fmo3* expression is switched on after birth, increases to reach a peak at five weeks of age and is maintained, albeit at a slightly lower level, throughout adulthood; in males, it increases from birth to three weeks of age and is subsequently switched off, being undetectable by five weeks of age.

The *FMO4* gene is expressed in several tissues in human and mouse but, with the exception of human pancreas, in all cases the expression is very low [1,6,19].

The gene for FMO5 is expressed in the embryo and in many tissues of fetal and adult human and mouse [6]. In both organisms it is most highly expressed in adult liver and in this organ its abundance is similar to or greater than that of FMO3 [1,19]. In humans, *FMO5* is highly expressed also in stomach and pancreas [6] and in the small intestine, in which it is the most highly expressed *FMO* gene [1]. In mouse, it is expressed also in kidney [19] and in small and large intestine, where it is the most highly expressed isoform [28]. In mouse liver and kidney, *Fmo5* expression is higher in males than in females [19].

In mouse liver, mRNAs for FMO1, FMO2, FMO4 and FMO5 are present at birth. Those encoding FMO2 and FMO4 exhibit no significant age-related changes. In contrast, expression of *Fmo1* and *Fmo5* changes with age, increasing after birth, to reach a peak at five weeks post-partum [19]. As described above, *Fmo3* expression

exhibits gender-specific age-related changes. With the exception of FMO3 (see above), no equivalent information is available on the effect of age on *FMO* expression in humans.

7. Complement of FMOs in major organs involved in drug metabolism

The complement of FMOs present in major sites of drug metabolism, such as liver, kidney, lung and small intestine, differs qualitatively and quantitatively in both human and mouse and there are marked species differences [1,19] (Table 1). In adult human liver, the main FMOs are FMO3 and FMO5, with FMO1 being absent. FMO3 and FMO5 are abundant in female mouse liver, but FMO1 is also present. In adult male mouse liver, FMO1 and FMO5 are present, but FMO3 is absent. In human kidney, the most abundant FMO is FMO1, followed by FMO2. In female mouse kidney, FMO1 is the most abundant isoform, but in male mouse kidney, the abundance of FMO5 is similar to that of FMO1. In human lung, by far the most highly expressed FMO mRNA is that for FMO2. However, as discussed in Section 12, most humans do not produce functional FMO2 from the mRNA. In mouse lung, both FMO1 and FMO2 are expressed [19,29]. FMO5 is the main isoform present in the small intestine in both human and mouse.

Interindividual variations have been reported in the abundance in adult human kidney of FMO1 (up to 5-fold) [30], and in adult human liver of FMO3 (10- to 20-fold) [17,31,32] and FMO5 (10-fold) [31]. With the exception of FMO5, FMOs are not readily inducible by foreign chemicals (see Section 9). Consequently, individual variations in the abundance of FMO1 and FMO3 are likely due to genetic or physiological factors, but in the case of FMO5 differential exposure to foreign chemicals might contribute.

The presence of FMO1 and FMO5 in fetal human liver [6,23,33] suggests that drug substrates for these enzymes that can cross the placenta will be metabolized in fetal liver. The amount of FMO1 in adult human kidney [22] is more than that of CYPs [34]. Thus, in adult human, FMO1 is likely to contribute substantially to the renal metabolism of drugs. The amount of FMO3 in adult human liver [31] is ~ 60% of that of CYP3A4 [35], the most abundant hepatic CYP, which catalyzes almost 30% of all oxidoreduction reactions undergone by drugs [4]. Given the relative abundance of FMO3 in liver and FMO1 in kidney, it is perhaps surprising that only about 2% of oxidoreduction reactions undergone by drugs has been attributed to FMOs and suggests that their contribution to drug metabolism might be underestimated.

8. Regional-specific expression of FMOs

In adult female mouse liver, mRNAs encoding FMO2, FMO3 and FMO4 are expressed in the periportal region, the expression of FMO3 mRNA being far greater than that of FMO2 or FMO4. In contrast, mRNAs encoding FMO1 and FMO5 are expressed primarily in the perivenous region, with a gradient across the acini [19]. In the kidney, expression of all five FMO mRNAs is localized to the distal and proximal tubules and the collecting ducts [19]. FMO1 mRNA was detected also in the glomerulous [19]. In lung, FMO1, FMO2 and FMO5 mRNAs are localized to the endothelial lining of the alveoli and terminal bronchiole [19]. FMO3 mRNA is present only in the terminal bronchiole, and, unlike the situation in liver, in lung this isoform is present in both adult male and female mice [19].

Little information is available on the regional localization of FMOs in human tissues such as liver and kidney. However, in human skin, an organ that is exposed to numerous chemicals, FMO1, FMO3, FMO4 and FMO5 mRNAs are localized in the

epidermis, sebaceous glands and hair follicles [36].

9. Induction and inhibition of FMOs

In contrast to CYPs, FMOs are not readily inducible by foreign chemicals. An exception is FMO5, which is inducible in human hepatocytes by rifampicin [37], in HepG2 cells by hyperforin [38] and in a breast cancer cell line by the synthetic progestin R5020 [39]. In primary hepatocytes isolated from mice there are gender differences in *Fmo5* expression in response to some chemicals [40]. In response to progesterone, the abundance of FMO5 mRNA was increased in hepatocytes isolated from female mice, but reduced in cells isolated from males, and rifampicin induced *Fmo5* expression to a much greater extent in hepatocytes isolated from female than from male mice. In mouse, the abundance of FMO mRNAs can be influenced by diet [41].

The repression of *Fmo3* gene expression in adult male mouse liver can be reversed: FMO3 mRNA is induced ~ 6000-fold by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), but this is not accompanied by a corresponding increase in FMO catalytic activity [42]. The response of *Fmo3* expression in females to TCDD was modest. The increase in the abundance of FMO3 mRNA represents a reversal of the repression of *Fmo3* expression in the liver of adult male mice rather than an induction of gene expression. The results indicate that if mice are the selected species for investigation of FMO3 induction, studies should include both male and female animals.

In contrast to CYPs, FMOs are not readily irreversibly inhibited by foreign chemicals. Exceptions are the inhibition of the activity of porcine FMO1 *in vitro* by (*N, N*-dimethylamino) stilbene carboxylates [43] and of human FMO3 *in vitro* and *in*

vivo by dietary indoles, present in brassicas [44]. FMOs are, however, subject to competitive inhibition by alternative substrates. One such competitive inhibitor, methimazole, has been used to distinguish FMO- from CYP-mediated metabolism of drugs. However, this approach is problematic because the product of FMO-mediated *S*-oxygenation of methimazole, a sulfenic acid, results in a decrease in CYP content [13].

10. Drug substrates of FMOs

Of the FMOs, FMO1 has the broadest substrate range, followed by FMO3. FMO1 and FMO3 share some substrates in common, e.g., benzydamine, itopride, *N*-deacetyl ketoconazole, voriconazole and tamoxifen, but are relatively selective towards others, e.g., FMO1 for chlorpromazine, imipramine and quazepan, FMO3 for procainamide. FMO2 and FMO5 have a more restricted range of substrates. In comparison with other FMOs, FMO4 is extended at its C-terminus by 23 to 26 amino-acid residues [5,45]. Expression of full-length FMO4 has proved problematic. Consequently, almost nothing is known of its substrate range and, as FMO4 is expressed in very low amounts, it is thought not to play a significant role in drug metabolism.

Therapeutic drugs that are substrates for human FMO1, FMO2, FMO3 and FMO5 are listed in Tables 2, 3, 4 and 5, respectively. Most drug substrates of FMOs are tertiary amines, which are *N*-oxygenated to form the *N*-oxide, or sulfides, which are *S*-oxygenated to the sulfoxide. Others include primary amines, such as amphetamine [46], which are converted to an *N*-hydroxylamine, then, via a second oxygenation and loss of water, to an oxime; secondary amines, such as *N*-deacetyl ketoconazole [47], which are converted initially to an *N*-hydroxyamine, then, via a second oxygenation, to a nitrone; and thiocarbamides, such as ethionamide [29], which are converted to a sulfoxide. *N*-oxides and sulfoxides are polar and readily

excreted and, thus, FMO-mediated metabolism of tertiary amines and sulfides represents a detoxification. In contrast, some of the *N*-hydroxylated products of FMO-mediated metabolism of primary and secondary amines can inhibit CYPs and, in some cases, e.g., the hydroxylated product of *N*-deacetyl ketoconazole, have toxic effects [48].

FMOs can be stereoselective in the production of product. Examples include nicotine [49], pargyline [5], amphetamine [50], sulindac sulfide [30] and cimetidine [51]. In some cases, the stereoselectivity of FMOs differs. In the case of pargyline *N*-oxygenation, human FMO1 produces only the (+)-enantiomer, whereas human FMO3 produces predominantly the (-)-enantiomer [5]. For cimetidine *S*-oxygenation, human FMO1 produces more (-)- than (+)-enantiomer, but for human FMO3 the opposite is the case [51]. Rettie et al. showed that prochiral sulfoxidation of the non-drug substrate methyl *p*-tolyl sulfide was stereoselective for FMO1, which produces exclusively the (*R*)-sulfoxide, whereas FMO3 produces equal amounts of both the (*R*)- and (S)-sulfoxides [52]. Thus, this compound can be used to distinguish the activities of FMO1 and FMO3 in preparations of microsomes in which both of these enzymes are present [52].

FMO5 displays no or poor reactivity towards classical FMO substrates and, consequently, was thought not to play a significant role in drug metabolism. FMO5 is known to catalyze the *N*- or *S*-oxygenation of only a small number of drugs *in vitro* (Table 5). A comparison of mouse FMO1, FMO3 and FMO5 catalytic activity towards a range of substrates identified unique properties of FMO5 [53]. Recently, human FMO5 has been identified as a Baeyer-Villiger monooxygenase [54] able to catalyze oxidation of a wide range of carbonyl compounds, via insertion of an oxygen atom into a carbon-carbon bond adjacent to the carbonyl group (aldehyde or ketone),

to form an ester. Drugs that have been identified as being metabolized by FMO5-mediated Baeyer-Villiger oxidation include the anticancer compound E7016 [55] and the antibacterial MRX-1 [56]. Now that FMO5 has been shown to catalyze a Baeyer-Villiger reaction, more substrates for this enzyme are likely to be identified. Earlier work showed that porcine FMO1 is able to catalyze the Baeyer-Villiger oxidation of several carbonyl compounds [57]. However, it is not known whether human or mouse FMO1 has this ability.

In the majority of cases, drug substrates of FMOs are metabolized also by other enzymes, particularly CYPs, and the FMO-catalyzed reaction does not represent the major route of metabolism. However, for individuals who possess polymorphic variants that decrease catalytic activity of CYPs the contribution of FMOs to drug metabolism is likely to be greater. One such example is that of nicotine, which is metabolized predominantly by CYP2A6 [58], but also by FMO3 [49]. The oxidation of a substrate by CYPs and FMOs usually results in formation of distinct products. This is certainly the case for aliphatic tertiary amines, FMOs producing exclusively the *N*-oxide, whereas CYP-mediated oxidation results in *N*-dealkylation [59].

Drugs that are likely to be metabolized exclusively or predominantly by FMOs include benzydamine [60], itopride [61], olopatadine [62], pargyline [5], ranitidine [31,63] and xanomeline [64], via *N*-oxygenation, and albendazole [65], cimetidine [31,51,66], ethionamide [29] and sulindac sulfide [30], via *S*-oxygenation.

Another factor contributing to the potential underestimation of the contribution of FMOs to the *N*-oxygenation of drugs containing tertiary amines is that the *N*-oxides can be retro-reduced to the parent compound by CYPs or other reductases [67], such as quinone reductase [68] and aldehyde oxidase [69]. The

contribution of FMOs to the metabolism of drugs containing secondary amines may also be underestimated. In this case, the initial product of the FMO-catalyzed reaction, an *N*-hydroxyamine, is converted, via a second oxygenation, to a nitrone, which is hydrolyzed to produce an aldehyde and a hydroxylated primary amine [70], the latter being enzymatically reduced to a primary amine, the same product as would be produced from the initial secondary amine substrate by a CYP-mediated *N*-dealkylation [71].

In addition to drugs, FMOs catalyze the oxygenation of several other foreign chemicals, including the pro-neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (FMO1) [72], insecticides, such as phorate and disulfoton (FMO2) [73], and dietary-derived compounds, such as trimethylamine (FMO3) [74,75]. However, discussion of the involvement of FMOs in the metabolism of non-drug xenobiotics is beyond the scope of this review.

11. Role of FMOs in endogenous metabolism

Because of charge restrictions, discussed in Section 3, almost all small endogenous nucleophiles are excluded from the active site of FMOs. Exceptions include the biogenic amines tyramine [76] and phenethylamine [77], which are converted by human FMO3 to the *N*-oxide, then via a second, stereoselective, *N*-oxygenation to the *trans*-oxime. Human FMO3 can also catalyze *S*-oxygenation of methionine, but, as the K_M for the reaction is in the mM range, its physiological relevance is questionable. Porcine FMO1 can *S*-oxygenate cysteamine and cysteine *S*-conjugates, but again, in the latter case the K_M is high, casting doubt on its metabolic relevance.

The use of *Fmo*-knockout mouse lines has revealed that, in addition to contributing to the metabolism of drugs, FMO1 and FMO5 have roles in endogenous

metabolism, FMO1 as a regulator of energy balance [24] and FMO5 as a regulator of metabolic aging, acting via pleiotropic effects, including influencing weight gain and plasma concentrations of glucose and cholesterol [78].

12. Genetic variants of FMOs and their effect on drug metabolism

Most humans are homozygous for a nonsense mutation of *FMO2*, c.1414C>T (p.(Gln472*)) [79]. The resultant allele, *FMO2*2A*, encodes a truncated polypeptide, FMO2.2A, that lacks 64 amino-acid residues from its carboxy terminus and is catalytically inactive [79]. Thus, in contrast to mouse, most humans do not express functional FMO2. The ancestral allele, *FMO2*1*, which encodes a full-length polypeptide, FMO2.1, is present in populations of recent African descent, and in some regions of sub-Saharan Africa almost 50% of individuals possess at least one *FMO2*1* allele [80]. Other deleterious variants have been identified, but these occur on the *FMO2*2A* allele [81]. Individuals who possess an *FMO2*1* allele, thus, are likely to express a functional protein. The presence of full-length active FMO2 in lung microsomes isolated from an *FMO2*1* individual has been confirmed [82]. The distribution of *FMO2*1* and *FMO2*2A* alleles among world populations has implications for interethnic and, in African populations, interindividual variation in response to drugs, particularly those for which the lung is a target organ or route of entry, such as the antitubercular prodrugs ethionamide and thiacetazone.

Rare genetic variants that abolish or severely impair FMO3 activity cause primary trimethylaminuria [2,74,83-85]. Affected individuals are unable to metabolize dietary-derived trimethylamine, a substrate that is specific for FMO3 [75], to its non-odorous *N*-oxide and, consequently, excrete the smelly free amine in breath, sweat and urine [86]. It is expected that sufferers of trimethylaminuria will have

impaired ability to metabolize drugs that are substrates for FMO3, and this has been shown to be the case for benzydamine [87].

In addition to rare, loss-of-function variants, at least 15 nonsynonymous single-nucleotide polymorphic variants (SNPs) of *FMO3* have been identified [2,84,88]. A catalogue of genetic variants of *FMO3* and their effect on drug metabolism [89] can be accessed at the *FMO3* locus-specific database (http://databases.lovd.nl/shared/genes/FMO3). The effect of many of the SNPs on the metabolism of drugs has been tested *in vitro*. Individually, the most common SNPs, c.472G>A(p.(Glu158Lys)) and c.923A>G(p.(Glu308Gly)), have little or no effect on enzyme activity [1,2,90,91]. In Europeans and Asians, however, the two SNPs are often linked, occurring on the same chromosome [92-95]. The effect of the compound variant, (p.(Glu158Lys;Glu308Gly)), on enzyme activity is greater than that of either variant alone, and the extent to which it reduces activity depends on the substrate [1,2,90,91,94,96,97].

The influence of c.769G>A(p.(Val257Met)), a SNP that is common in Asian populations, also is substrate dependent. This is illustrated by its effect on the ability of FMO3 to catalyze the *N*-oxygenation of the anti-cancer aurora kinase inhibitors danusertib and tozasertib [98]. The variant significantly reduces the *N*-oxygenation of danusertib, but has no effect on production of tozasertib *N*-oxide. The consequences for an individual undergoing treatment with danusertib of possession of a c.769G>A(p.(Val257Met)) variant are not known.

Three other SNPs affect FMO3 activity: c.183C>A(p.(Asn61Lys)) causes a marked reduction in enzyme activity towards four different substrates [99]; c.613C>T(p.(Arg205Cys)) has a moderate effect on activity [97]; and c.1079T>C(p.(Leu360Pro)) increases activity by 2- to 5-fold [96]. However, each is

present at low frequency and/or occurs in a single population group [2] and, consequently, has limited significance for the general population.

Several SNPs have been identified in the promoter region of *FMO3* [100]. These segregate into 15 haplotypes. Of these, two markedly decrease transcription *in vitro*, whereas one increases transcription 8-fold [100]. The effect of the promoter-region SNPs on transcription of the *FMO3* gene *in vivo* is not known.

Drug-drug interactions are thought to be rare for substrates of FMO3 [59]. However, a study *in vitro* demonstrated that FMO3-catalyzed *N*-oxygenation of benzydamine was inhibited by several substrates, including itopride, tozatertib, methimazole and trimethylamine, and *S*-oxygenation of sulindac sulfide was inhibited by methimazole [101], but, as discussed in Section 9, the use of methimazole as an inhibitor of FMO activity can be problematic. It is suggested that individuals with reduced FMO3 activity, such as those with the (p.(Glu158Lys;Glu308Gly)) compound variant, might experience unexpected consequences in drug response if exposed to more than one FMO3 substrate.

FMO3 activity might be influenced by factors other than polymorphic variation. For instance, in the presence of excess nitric oxide, FMO3 undergoes *S*-nitrosylation, which reduces the ability of the enzyme to catalyze ranitidine *N*-oxygenation [102], and the availability of the transcription factors HNF-4 and NF-Y may influence *FMO3* expression [103].

Few nonsynonymous SNPs have been identified in *FMO1* and each occurs at low frequency [2,91,104]. Of those tested, only one had an effect on enzyme activity *in vitro*: c.1504C>T(p.(Arg502*)), which abolished activity towards methimazole, but had no effect on activity towards three other substrates [105]. A relatively common SNP, g.-9536C>A (the *FMO1*6* allele), located in a YY1 element of the fetal liver-

specific promoter of *FMO1*, prevented binding of the transcription factor and decreased promoter activity *in vitro*, but did not correlate with lower expression of *FMO1* in fetal liver *in vivo* [106].

Very few nonsynonymous SNPs have been identified in the genes encoding FMO4 and FMO5 [2,104]. Of these, most occur at very low frequency and none is known to affect enzyme activity [2,104].

Although *FMO* genetic variants, especially those affecting the activity of FMO3, are known to influence drug metabolism *in vitro*, an understanding of the impact of *FMO* variants *in vivo* is limited to a small number of studies in humans. In mouse, studies *in vivo* using *Fmo*-knockout lines have provided additional information (see Section 13).

Sulindac sulfoxide is a prodrug converted by gut bacteria to sulindac sulfide, the active form of the drug, which is metabolized by FMO3 back to the inactive sulfoxide and, then, to sulindac sulfone [30]. The FMO3 variants (p.(Glu158Lys)) and (p.(Glu308Gly)) decrease FMO3 activity towards sulindac *in vitro* [30]. In patients with familial adenomatous polyposis undergoing treatment with sulindac, the variants are associated with regression of existing polyps and protection against adenoma formation, particularly in individuals homozygous for the double variant (p.(Glu158Lys;Glu308Gly)) [107,108]. It is thought that reduction of FMO3 activity, as a consequence of these polymorphic variants, would result in higher circulating concentrations of the active sulindac sulfide.

Conversion of ranitidine to its *N*-oxide by FMO3 was demonstrated *in vitro* using human liver microsomes [31]. Subsequently, the double variant (p.(Glu158Lys;Glu308Gly)) was found to have a reduced capacity for ranitidine *N*-oxygenation [94]. *N*-oxygenation of ranitidine by FMO3 has been confirmed *in vivo*

[93]; individuals heterozygous or homozygous for the (p.(Glu158Lys;Glu308Gly)) double variant excreted lower amounts of ranitidine *N*-oxide in their urine than did individuals homozygous for the ancestral (p.(Glu158;Glu308)) allele [93,94].

Although benzydamine is effectively converted *in vitro* to benzydamine *N*-oxide by FMO1 and FMO3 [60], the lack of expression of FMO1 in adult human liver enables benzydamine to be used as a probe for FMO3 activity *in vivo*. The role of FMO3 in benzydamine metabolism *in vivo* was confirmed in a study of patients suffering from trimethylaminuria, caused by mutations that severely impair FMO3 activity, in whom plasma and urinary concentrations of benzydamine *N*-oxide were markedly reduced [87].

The antipsychotic olanzapine is converted *in vitro* to its *N*-oxide by FMO3 [109] and, through the action of CYPs, can be hydroxylated or *N*-demethylated [109,110]. The *N*-oxide can be formed also by CYP2D6 *in vitro*, but olanzapine metabolism *in vivo* is not influenced by *CYP2D6* genotype [111,112]. Olanzapine *N*-oxide formation *in vitro* by the FMO3 variant (p.(Glu158Lys;Glu308Gly)) was lower than by the ancestral form, (p.(Glu158;Glu308)) [113]. When the influence of this compound variant was examined *in vivo* the C/D (median dose-adjusted steady-state serum concentration) of olanzapine *N*-oxide in patients homozygous for (p.(Glu158Lys;Glu308Gly)) was ~50% lower than in individuals heterozygous or homozygous for the ancestral form, but the variant allele had no effect on the plasma concentration of olanzapine [114]. In a study of Japanese, several genotypes of *FMO3* and *CYP*s were found not to be associated with changes in olanzapine clearance, the conclusion being that the drug is subject to multi-pathway metabolism and if one pathway is less effective others compensate [112]. Variants of *FMO1* have been shown to alter olanzapine metabolism. In this case, the non-coding variant *FMO1*6*,

which decreases promoter activity *in vitro* (see above), was found to increase serum C/Ds in patients [114].

Itopride, a gastroprokinetic agent, undergoes *N*-oxygenation through the action of FMO3 *in vitro* [61] and *in vivo* [115] and this is considered the major route of metabolism. Chinese individuals homozygous for (p.(Glu158Lys;Glu308Gly)) had higher plasma concentrations of itopride and lower concentrations of the *N*-oxide than those homozygous for the ancestral form (p.(Glu158;Glu308)) [115].

The involvement of FMO3 in clozapine *N*-oxygenation was demonstrated *in vitro* [116]. A study *in vivo* found no correlation between clozapine *N*-oxide production and the (p.(Glu158Lys)), (p.(Val257Met)) or (p.(Glu308Gly)) variants [92].

FMO3 converts nicotine to its *N*-oxide [49] and, in humans, 4-7% of nicotine equivalents are excreted as the *N*-oxide. CYP2A6, however, is the most important contributor to nicotine metabolism and nicotine *N*-oxide excretion is increased to ~30% of the absorbed nicotine in individuals homozygous for a *CYP2A6* deletion [117]. Polymorphisms that influence FMO3 activity would, therefore, be expected to be of greater importance in individuals with compromised CYP2A6 activity.

13. Mouse models for FMO-mediated drug metabolism

A consequence of the marked differences between human and mouse in expression of FMO1 and FMO3 in adult liver and the lack of functional FMO2 in most humans is that there are distinct differences between the species in the metabolism of drugs *in vivo* by FMOs. For instance, drug substrates of FMO1 will be metabolized in mouse, but not human, liver; substrates of FMO2 will be metabolized in mouse, but not in the majority of humans; and substrates for FMO3 will be metabolized in liver of humans and female mice, but not of male mice. Therefore, wild-type mouse is not a good

animal model for FMO-mediated drug metabolism in humans. However, female mice, because they express FMO3 in adult liver, may be useful as a model for FMO3-mediated metabolism, and, if used in combination with adult male mice, which lack hepatic FMO3, for confirming the participation of FMO3 in metabolism of a drug.

Few studies have directly compared the contribution of FMOs to drug metabolism *in vivo* in humans and mice. A study of procainamide, using a urinary metabolite profiling approach, concluded that in humans *N*-oxidation represents a major metabolic route, producing procainamide *N*-oxide and *N*-acetyl procainamide *N*-oxide, whereas in mice this was a minor route [118]. Experiments *in vitro* confirmed that both FMO3 and FMO1 catalyze oxygenation of procainamide and *N*-acetyl procainamide, the rates of formation being higher for FMO3. However, the mouse study used adult males and the differences found between human and mouse likely result from the lack of FMO3 in adult male mouse liver.

Female mice in which the genes for FMO1, FMO2 and FMO4 have been knocked out (*Fmo1*$^{(-/-)}$, *Fmo2*$^{(-/-)}$, *Fmo4*$^{(-/-)}$) [119] express in adult liver FMO3 and FMO5, but no other FMOs, and, thus, for most humans, would be a good animal model for FMO-mediated drug metabolism. The use of *Fmo*-knockout mouse lines can also help assess the contribution of a FMO protein to the metabolism of a drug that undergoes multi-pathway metabolism *in vivo* [120]. For instance, the antidepressant imipramine is oxygenated only by FMO1, to produce the *N*-oxide [119]. Imipramine *N*-oxide undergoes retroreduction *in vivo*, thus, quantification of the oxygenated product in plasma or urine can miss or underestimate the contribution of FMO1 to overall metabolism and clearance of the drug. Using an *Fmo1*-null mouse model, imipramine was shown to cause sedation only in wild-type animals, i.e., those that produced imipramine *N*-oxide in brain [119]. Adverse behavioural changes, such

as tremor, were observed only in mice that lacked FMO1 (the knockout mice), which produced higher amounts in brain of desipramine, a product of CYP-mediated metabolism.

FMO2 catalyzes the *S*-oxygenation of the antitubercular prodrugs ethionamide and thiacetazone *in vitro* [29,121]. The triple knockout mouse (*Fmo1*$^{(-/-)}$, *Fmo2*$^{(-/-)}$, *Fmo4*$^{(-/-)}$) was used to better mimic the situation in human, with respect to expression of FMOs in lung, and to establish whether a lack of FMO2 influences the metabolism of ethionamide *in vivo* [122]. The plasma concentration of ethionamide was found to be higher in knockout than in wild-type mice and that of ethionamide *S*-oxide to be higher in wild-type than in knockout animals, confirming the involvement of FMO2 in the metabolism of ethionamide. To be effective ethionamide must be taken up by mycobacteria in the lungs and subsequently metabolized, by the bacterial monooxygenase EtaA, to the sulfenic acid, which acts as the therapeutic agent [123]. Metabolism of ethionamide in the lung by FMO2 to the sulfoxide and, subsequently, to a potentially toxic sulfenic acid, would be expected to reduce drug efficacy, by diminishing the amount of prodrug available for uptake by mycobacteria, and increase harmful side effects.

14. Conclusions

Five functional FMOs, FMOs 1-5, are present in humans. The catalytic mechanism of FMOs, in which oxygen is activated in the absence of bound substrate, is unusual. It enables FMOs to oxygenate any nucleophile able to gain access to the active site, thus accounting for their broad substrate range. Human, FMO5 has been identified as a Baeyer-Villiger enzyme that can oxygenate a range of electrophilic substrates. Baeyer-Villiger oxidations can also be catalyzed by porcine FMO1.

Whether this is the case for FMO1 of human or mouse remains to be established.

FMO-catalyzed reactions are generally detoxifications, producing polar, readily excretable metabolites. Most drug substrates of FMOs are metabolized also by other enzymes, particularly CYPs, but some are metabolized predominantly or exclusively by FMOs. The contribution of FMOs to drug metabolism might be underestimated, owing to the use of methods for preparation and incubation of microsomes that do not preserve FMO activity. In contrast to CYPs, FMOs are not readily induced or inhibited, but genetic variation, particularly of FMO2 and FMO3, affects the metabolism of drug substrates of these enzymes.

There are marked differences between human and mouse in developmental stage- and tissue-specific expression of FMOs. Consequently, human and mouse differ in the complement of FMOs present in liver, kidney and lung, the main sites of drug metabolism. The species differences are most striking in adult liver: in humans, the most abundant FMOs are FMO3 and FMO5, with FMO1 being absent; in mouse FMO1, along with FMO5, is present and, in females, but not in males, FMO3 also is present. Thus, mouse is not a good animal model for FMO-mediated drug metabolism in humans. However, female mice in which the *Fmo1*, *Fmo2* and *Fmo4* genes have been disrupted (*Fmo1*$^{(-/-)}$, *Fmo2*$^{(-/-)}$, *Fmo4*$^{(-/-)}$) express in adult liver FMO3 and FMO5, but no other FMOs, and, thus, for most humans are a good model for FMO-mediated drug metabolism.

15. Expert opinion

A number of drugs have been identified as substrates for FMOs, some being metabolized predominantly or exclusively by these enzymes. Research on Phase-1 drug metabolism has concentrated on CYPs, and methods commonly used for the

preparation and incubation of microsomes can result in selective loss of FMO activity, leading to potential underestimation of the contribution of FMOs to drug metabolism. Thus, despite the broad substrate range of FMOs and their relative abundance in liver, kidney and lung, the major sites of drug metabolism, the number of drugs identified as being metabolized by FMOs is relatively small. The adoption of methods for microsome preparation and incubation that favour retention of FMO activity, combined with selective ablation of CYP activity, would help achieve a more complete appreciation of the contribution of FMOs to drug metabolism. The identification of FMO5 as a Baeyer-Villiger monooxygenase indicates that FMO5 has a more important role in drug metabolism than hitherto suspected and that additional drug substrates of FMO5 are likely to be identified.

The substrate preferences and reactions catalyzed by FMO5 differ from those of other mammalian FMOs, suggesting that FMO5 may have a distinct mechanism of action. It will be important to establish whether this is the case. A detailed understanding of the mechanism of action of mammalian FMOs has been hindered by the lack of 3D structures of these proteins.

FMO-catalyzed reactions generally form polar, readily excretable detoxification products, whereas CYPs are more able to produce reactive, potentially toxic products. In contrast to CYPs, FMOs, with the exception of FMO5, are not readily induced or inhibited by foreign chemicals. These differences between FMOs and CYPs indicate that drugs that are metabolized predominantly by FMOs would be less likely to elicit drug-drug interactions and potentially harmful side effects, and that the design of such drugs would offer clinical advantages.

The human gut microbiome makes important contributions to the metabolism of orally administered drugs, for instance, in production of sulindac sulfide, the active

form of the prodrug sulindac, which is subsequently inactivated by metabolism by FMO3. There are considerable inter- and intra-individual differences in the composition of gut flora and the effect of these on drug metabolism is not well understood.

The general lack of induction or inhibition of FMOs indicates that the interindividual variation in abundance or activity of FMOs is largely the result of genetic or physiological factors. This offers the possibility that genotyping individuals for *FMO* variants would identify those who might experience problems when treated with drugs metabolized by FMOs.

Investigation of FMO-mediated drug metabolism would benefit greatly from the availability of an animal model that accurately reflects the metabolic capacity of human with respect to FMOs. The functional capabilities *in vitro* of FMOs of mouse are very similar to those of their human orthologues. However, owing to marked differences between human and mouse in the abundance of FMOs in the major organs of drug metabolism, particularly liver, mouse is not a good model for investigation of FMO-mediated metabolism *in vivo*. This problem can be overcome by the development and use of knockout-mouse lines in which selected *Fmo* genes have been disrupted. For instance, female *Fmo1*$^{(-/-)}$,*Fmo2*$^{(-/-)}$, *Fmo4*$^{(-/-)}$ mice express in adult liver FMO3 and FMO5, but not FMO1, a complement of FMOs similar to that in the liver of Europeans, Asians and most Africans, and, thus, are a good model for most humans. Wild-type adult male mice are natural liver-specific knockouts for *Fmo3* and, thus, in conjunction with wild-type females, which do express *Fmo3*, can be used to investigate the role of FMO3 in metabolism of a drug. It is predicted that the use of knockout-mouse lines will facilitate advances in understanding the contribution of FMOs to the metabolism of therapeutic drugs.

The discovery that FMO1 and FMO5 function in endogenous metabolism initiates an exciting new area of research and has important implications for drug therapy. Drugs that are substrates for these enzymes may compete with endogenous substrates and, thus, affect energy homeostasis, in the case of FMO1, or metabolic aging, in the case of FMO5, with potential effects on therapeutic response and endogenous metabolic functions. Induction of FMO5 by some therapeutics may also have adverse effects on the metabolic health of patients.

Article highlights box

- FMOs are involved in the metabolism of a number of therapeutic drugs; in most cases the drugs are metabolized also by other enzymes, particularly CYPs, but some are metabolized exclusively or predominantly by FMOs
- The contribution of FMOs to drug metabolism may be underestimated, in particular, the ability of FMO1 and FMO5 to catalyze Baeyer-Villiger reactions
- Human and mouse differ with respect to the complement of FMOs present in major organs of drug metabolism, consequently, wild-type mouse is not a good model for FMO-mediated metabolism in human
- Genetic variants of FMOs, particularly of FMO2 and FMO3, affect metabolism of substrates of these enzymes
- FMO1 and FMO5 function in endogenous metabolism, which has implications for drug therapy
- Female knockout mice in which the *Fmo1, Fmo2* and *Fmo4* genes have been disrupted are a good model for FMO-mediated drug metabolism in human

Figure Legends

Figure 1. Chromosomal localization and arrangement of *FMO* genes of human and mouse. Arrows represent direction of transcription. Diagram not to scale.

Figure 2. Catalytic cycle of mammalian FMOs. Evidence for the cycle is based on spectrophotometric and kinetic studies (reviewed in [8,10]). NADPH binds and reduces the prosthetic group FAD to $FADH_2$ (step 1). Molecular oxygen then binds and is reduced, forming C4a-hydroperoxyflavin, which is stabilized by $NADP^+$ (step 2). Substrate (S) is oxygenated via nucleophilic attack on the distal oxygen of the C4a-hydroperoxyflavin, leaving the prosthetic group in the form of C4a-hydroxyflavin (step 3). Water is then released, reforming FAD (step 4) and the final step is the release of $NADP^+$ (step 5). Adapted from [10].

References

1. Cashman JR, Zhang J. Human flavin-containing monooxygenases. Annu Rev Pharmacol Toxicol 2006;46:65–100

** An important review of FMOs of human.

2. Phillips I, Francois A, Shephard E. The Flavin-containing monoooxygenases (FMOs): genetic variation and its consequences for the metabolism of therapeutic drugs. Curr Pharmacogen 2007;5:292–313

** A comprehensive review of FMOs and their genetic variants.

3. Krueger SK, Williams DE. Mammalian flavin-containing monooxygenases: structure/function, genetic polymorphisms and role in drug metabolism. Pharmacol Ther 2005;106:357–87

** A key review of FMOs

4. Rendic S, Guengerich FP. Survey of human oxidoreductases and cytochrome P450 enzymes involved in the metabolism of xenobiotic and natural chemicals. Chem Res Toxicol 2015;28:38–42

5. Phillips IR, Dolphin CT, Clair P, et al. The molecular biology of the flavin-containing monooxygenases of man. Chem Biol Interact 1995;96:17–32

6. Hernandez D, Janmohamed A, Chandan P, et al. Organization and evolution of the flavin-containing monooxygenase genes of human and mouse: identification of novel gene and pseudogene clusters. Pharmacogenetics 2004;14:117–30

** A comprehensive description of human and mouse FMOs

7. Hines RN, Hopp KA, Franco J, et al. Alternative processing of the human FMO6 gene renders transcripts incapable of encoding a functional flavin-containing monooxygenase. Mol Pharmacol 2002;62:320–5

8. Ziegler DM. Recent studies on the structure and function of multisubstrate flavin-containing monooxygenases. Annu Rev Pharmacol Toxicol 1993;33:179–99

9. Poulsen LL, Ziegler DM. Multisubstrate flavin-containing monooxygenases: applications of mechanism to specificity. Chem Biol Interact 1995;96:57–73

10. Ziegler DM. An overview of the mechanism, substrate specificities, and structure of FMOs. Drug Metab Rev 2002;34:503–11

11. Kim YM, Ziegler DM. Size limits of thiocarbamides accepted as substrates by human flavin-containing monooxygenase 1. Drug Metab Dispos 2000;28:1003–6

12. Guengerich FP. Common and uncommon cytochrome P450 reactions related

to metabolism and chemical toxicity. Chem Res Toxicol 2001;14:611–50

13. Kedderis GL, Rickert DE. Loss of rat liver microsomal cytochrome P-450 during methimazole metabolism. Role of flavin-containing monooxygenase. Drug Metab Dispos 1985;13:58–61

14. Cerny MA, Hanzlik RP. Cyclopropylamine inactivation of cytochromes P450: role of metabolic intermediate complexes. Arch Biochem Biophys 2005;436:265–75

15. Cashman JR. Some distinctions between flavin-containing and cytochrome P450 monooxygenases. Biochem Biophys Res Commun 2005;338:599–604

** A review comparing FMOs and CYPs

16. Dolphin C, Shephard EA, Povey S, et al. Cloning, primary sequence, and chromosomal mapping of a human flavin-containing monooxygenase (FMO1). J Biol Chem 1991;266:12379–85

17. Koukouritaki SB, Simpson P, Yeung CK, et al. Human Hepatic Flavin-Containing Monooxygenases 1 (FMO1) and 3 (FMO3) Developmental Expression. Pediatric Res 2002;51:236–43

* Paper describing developmental regulation of FMO expression in human.

18. Cherrington NJ, Cao Y, Cherrington JW, et al. Physiological factors affecting protein expression of flavin-containing monooxygenases 1, 3 and 5. Xenobiotica 1998;28:673–82

19. Janmohamed A, Hernandez D, Phillips IR, Shephard EA. Cell-, tissue-, sex- and developmental stage-specific expression of mouse flavin-containing monooxygenases (Fmos). Biochem Pharmacol 2004;68:73–83

* Paper describing developmental and gender-specific expression and regional localization of FMOs in mouse.

20. Shephard EA, Chandan P, Stevanovic-Walker M, et al. Alternative promoters and repetitive DNA elements define the species-dependent tissue-specific expression of the FMO1 genes of human and mouse. Biochem J 2007;406:491–9

21. Dolphin CT, Cullingford TE, Shephard EA, et al. Differential developmental and tissue-specific regulation of expression of the genes encoding three members of the flavin-containing monooxygenase family of man, FMO1, FMO3 and FMO4. Eur J Biochem 1996;235:683–9

22. Yeung CK, Lang DH, Thummel KE, Rettie AE. Immunoquantitation of FMO1 in human liver, kidney, and intestine. Drug Metab Dispos 2000;28:1107–11

23. Zhang J, Cashman JR. Quantitative analysis of FMO gene mRNA levels in human tissues. Drug Metab Dispos 2006;34:19–26

24. Veeravalli S, Omar BA, Houseman L, et al. The phenotype of a flavin-containing monooyxgenase knockout mouse implicates the drug-metabolizing enzyme FMO1 as a novel regulator of energy balance. Biochem Pharmacol 2014;90:88–95

** First identification of an endogenous role for an FMO.

25. Siddens LK, Henderson MC, VanDyke JE, et al. Characterization of mouse flavin-containing monooxygenase transcript levels in lung and liver, and activity of expressed isoforms. Biochem Pharmacol 2008;75:570–9

26. Shimizu M, Cashman JR, Yamazaki H. Transient trimethylaminuria related to menstruation. BMC Med Genet 2007;8:2

27. Falls JG, Blake BL, Cao Y, et al. Gender differences in hepatic expression of flavin-containing monooxygenase isoforms (FMO1, FMO3, and FMO5) in mice. J Biochem Toxicol 1995;10:171–7

** Report of gender-specific differences in expression of FMOs of mouse.

28. Fu ZD, Selwyn FP, Cui JY, Klaassen CD. RNA sequencing quantification of xenobiotic-processing genes in various sections of the intestine in comparison to the liver of male mice. Drug Metab Dispos 2016;44:842–56

29. Henderson MC, Siddens LK, Morré JT, et al. Metabolism of the anti-tuberculosis drug ethionamide by mouse and human FMO1, FMO2 and FMO3 and mouse and human lung microsomes. Toxicol Appl Pharmacol 2008;233:420–7

30. Hamman MA, Haehner-Daniels BD, Wrighton SA, et al. Stereoselective sulfoxidation of sulindac sulfide by flavin-containing monooxygenases. Comparison of human liver and kidney microsomes and mammalian enzymes. Biochem Pharmacol 2000;60:7–17

31. Overby LH, Carver GC, Philpot RM. Quantitation and kinetic properties of hepatic microsomal and recombinant flavin-containing monooxygenases 3 and 5 from humans. Chem Biol Interact 1997;106:29–45

32. Cashman JR, Zhang J. Interindividual differences of human flavin-containing monooxygenase 3: genetic polymorphisms and functional variation. Drug Metab Dispos 2002;30:1043–52

33. Hines RN. Developmental expression of drug metabolizing enzymes: Impact on disposition in neonates and young children. Int J Pharm 2013;452:3–7

34. Jakobsson SV, Cintig DL. Studies on the cytochrome P-450-containing mono-oxygenase system in human kidney cortex microsomes. J Pharmacol Exper Ther 1973;185:226–34

35. Shimada T, Yamazaki H, Mimura M, et al. 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 Exper Ther 1994;270:414–23

36. Janmohamed A, Dolphin CT, Phillips IR, Shephard EA. Quantification and cellular localization of expression in human skin of genes encoding flavin-containing monooxygenases and cytochromes P450. Biochem Pharmacol 2001;62:777–86

37. Rae JM, Johnson MD, Lippman ME, Flockhart DA. Rifampin is a selective, pleiotropic inducer of drug metabolism genes in human hepatocytes: studies with cDNA and oligonucleotide expression arrays. J Pharmacol Exper Ther 2001;299:849–57

38. Krusekopf S, Roots I. St. John's wort and its constituent hyperforin concordantly regulate expression of genes encoding enzymes involved in basic cellular pathways. Pharmacogenet Genomics 2005;15:817–29

39. Miller MM, James RA, Richer JK, et al. Progesterone regulated expression of flavin-containing monooxygenase 5 by the B-isoform of progesterone receptors: implications for tamoxifen carcinogenicity. J Clin Endocrinol Metab 1997;82:2956–61

40. Houseman L, Edwards M, Phillips IR, Shephard EA. Isolation and culture of mouse hepatocytes: gender-specific gene expression responses to chemical treatments. Methods Mol Biol 2015;1250:3–12

41. Guo Y, Cui JY, Lu H, Klaassen CD. Effect of various diets on the expression of phase-I drug-metabolizing enzymes in livers of mice. Xenobiotica 2015;45:586–97

42. Celius T, Roblin S, Harper PA, et al. Aryl hydrocarbon receptor-dependent induction of flavin-containing monooxygenase mRNAs in mouse liver. Drug Metab Dispos 2008;36:2499–505

43. Clement B, Weide M, Ziegler DM. Inhibition of purified and membrane-bound flavin-containing monooxygenase 1 by (N,N-dimethylamino)stilbene carboxylates. Chem Res Toxicol 1996;9:599–604

44. Cashman JR, Xiong Y, Lin J, et al. In vitro and in vivo inhibition of human flavin-containing monooxygenase form 3 (FMO3) in the presence of dietary indoles. Biochem Pharmacol 1999;58:1047–55

45. Dolphin CT, Shephard EA, Povey P, et al. Cloning, primary sequence and chromosomal localization of human FMO2, a new member of the flavin-containing mono-oxygenase family. Biochem J 1992;287:261-7

* Following a name change in nomenclature the FMO referred to in this paper was renamed FMO4.

46. Cashman JR, Park SB, Berkman CE, Cashman LE. Role of hepatic flavin-containing monooxygenase 3 in drug and chemical metabolism in adult humans. Chem Biol Interact 1995;96:33–46

47. Rodriguez RJ, Miranda CL. Isoform specificity of N-deacetyl ketoconazole by human and rabbit flavin-containing monooxygenases. Drug Metab Dispos 2000;28:1083–6

48. Rodriguez RJ, Buckholz CJ. Hepatotoxicity of ketoconazole in Sprague-Dawley rats: glutathione depletion, flavin-containing monooxygenases-mediated bioactivation and hepatic covalent binding. Xenobiotica 2003;33:429–41

49. Park SB, Jacob P, Benowitz NL, Cashman JR. Stereoselective metabolism of (S)-(-)-nicotine in humans: formation of trans-(S)-(-)-nicotine N-1'-oxide. Chem Res Toxicol 1993;6:880–8

50. Cashman JR, Xiong YN, Xu L, Janowsky A. N-oxygenation of amphetamine and methamphetamine by the human flavin-containing monooxygenase (form 3): role in bioactivation and detoxication. J Pharmacol Exp Ther 1999;288:1251–60

51. Hai X, Adams E, Hoogmartens J, Van Schepdael A. Enantioselective in-line and off-line CE methods for the kinetic study on cimetidine and its chiral metabolites with reference to flavin-containing monooxygenase genetic isoforms. Electrophoresis 2009;30:1248–57

52. Rettie AE, Lawton MP, Sadeque AJ, et al. Prochiral sulfoxidation as a probe for multiple forms of the microsomal flavin-containing monooxygenase: studies with rabbit FMO1, FMO2, FMO3, and FMO5 expressed in Escherichia coli. Arch Biochem Biophys 1994;311:369–77

53. Zhang J, Cerny MA, Lawson M, et al. Functional activity of the mouse flavin-containing monooxygenase forms 1, 3, and 5. J Biochem Mol Toxicol 2007;21:206–15

54. Fiorentini F, Geier M, Binda C, et al. Biocatalytic characterization of human FMO5: unearthing Baeyer-Villiger reactions in humans. ACS Chem Biol 2016;11:1039–48

** Paper describing role of human FMO5 as a Baeyer-Villiger monooxygenase

55. Lai WG, Farah N, Moniz GA, Wong YN. A Baeyer-Villiger oxidation specifically catalyzed by human flavin-containing monooxygenase 5. Drug Metab Dispos 2011;39:61–70.

** First report of an FMO5-catalysed Baeyer-Villiger drug oxidation.

56. Meng J, Zhong D, Li L, et al. Metabolism of MRX-I, a novel antibacterial oxazolidinone, in humans: the oxidative ring opening of 2,3-Dihydropyridin-4-one catalyzed by non-P450 enzymes. Drug Metab Dispos 2015;43:646–59

57. Chen GP, Poulsen LL, Ziegler DM. Oxidation of aldehydes catalyzed by pig liver flavin-containing monooxygenase. Drug Metab Dispos 1995;23:1390–3

58. Nakajima M, Yamamoto T, Nunoya K, et al. Role of human cytochrome

P4502A6 in C-oxidation of nicotine. Drug Metab Dispos 1996;24:1212–7

59. Cashman JR. Role of flavin-containing monooxygenase in drug development. Expert Opin Drug Metab Toxicol 2008;4:1507–21

** Review of role of FMOs in drug development.

60. Lang DH, Rettie AE. In vitro evaluation of potential in vivo probes for human flavin-containing monooxygenase (FMO): metabolism of benzydamine and caffeine by FMO and P450 isoforms. Br J Clin Pharmacol 2000;50:311–4

61. Mushiroda T, Douya R, Takahara E, Nagata O. The involvement of flavin-containing monooxygenase but not CYP3A4 in metabolism of itopride hydrochloride, a gastroprokinetic agent: comparison with cisapride and mosapride citrate. Drug Metab Dispos 2000;28:1231–7

* Demonstration that itopride is metabolized by FMO3.

62. Kajita J, Inano K, Fuse E, et al. Effects of olopatadine, a new antiallergic agent, on human liver microsomal cytochrome P450 activities. Drug Metab Dispos 2002;30:1504–11

63. Chung W-G, Park CS, Roh HK, et al. Oxidation of ranitidine by isozymes of flavin-containing monooxygenase and cytochrome P450. Jpn J Pharmacol 2000;84:213–20

64. Ring BJ, Wrighton SA, Aldridge SL, et al. Flavin-containing monooxygenase-mediated N-oxidation of the M_1-muscarinic agonist xanomeline. Drug Metab Dispos 1999;27:1099–103

65. Rawden HC, Kokwaro GO, Ward SA, Edwards G. Relative contribution of cytochromes P-450 and flavin-containing monoxygenases to the metabolism of albendazole by human liver microsomes. Br J Clin Pharmacol 2000;49:313–22

66. Cashman JR, Park SB, Yang ZC, et al. Chemical, enzymatic, and human enantioselective S-oxygenation of cimetidine. Drug Metab Dispos 1993;21:587–97.

67. Parte P, Kupfer D. Oxidation of tamoxifen by human flavin-containing monooxygenase (FMO) 1 and FMO3 to tamoxifen-N-oxide and its novel reduction back to tamoxifen by human cytochromes P450 and hemoglobin. Drug Metab Dispos 2005;33:1446–52

68. Kitamura S, Sugihara K, Tatsumi K. A unique tertiary amine N-oxide reduction system composed of quinone reductase and heme in rat liver preparations. Drug Metab Dispos 1999;27:92–7

69. Kitamura S, Tatsumi K. Involvement of liver aldehyde oxidase in the reduction of nicotinamide N-oxide. Biochem Biophys Res Commun 1984;120:602–6

70. Ziegler DM. Flavin-containing monooxygenases: catalytic mechanism and substrate specificities. Drug Metab Rev 1988;19:1–32

71. Yamada H, Baba T, Hirata Y, et al. Studies on N-demethylation of methamphetamine by liver microsomes of guinea-pigs and rats: the role of flavin-containing mono-oxygenase and cytochrome P-450 systems. Xenobiotica 1984;14:861–6

72. Chiba K, Kobayashi K, Itoh K, et al. N-oxygenation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by the rat liver flavin-containing monooxygenase expressed in yeast cells. Eur J Pharmacol 1995;293:97–100

73. Henderson MC, Krueger SK, Siddens LK, et al. S-oxygenation of the thioether organophosphate insecticides phorate and disulfoton by human lung flavin-containing monooxygenase 2. Biochem Pharmacol 2004;68:959–67

74. Dolphin CT, Janmohamed A, Smith RL, et al. Missense mutation in flavin-containing mono-oxygenase 3 gene, FMO3, underlies fish-odour syndrome. Nat Genet 1997;17:491–4

** First report of a causative mutation for trimethylaminuria.

75. Lang DH, Yeung CK, Peter RM, et al. Isoform specificity of trimethylamine N-oxygenation by human flavin-containing monooxygenase (FMO) and P450 enzymes: selective catalysis by FMO3. Biochem Pharmacol 1998;56:1005–12

76. Lin J, Cashman JR. Detoxication of tyramine by the flavin-containing monooxygenase: stereoselective formation of the trans oxime. Chem Res Toxicol 1997;10:842–52

77. Lin J, Cashman JR. N-oxygenation of phenethylamine to the trans-oxime by adult human liver flavin-containing monooxygenase and retroreduction of phenethylamine hydroxylamine by human liver microsomes. J Pharmacol Exp Ther 1997;282:1269–79

78. Gonzalez Malagon SG, Melidoni AN, et al. The phenotype of a knockout mouse identifies flavin-containing monooxygenase 5 (FMO5) as a regulator of metabolic ageing. Biochem Pharmacol 2015;96:267–77

** First report identifying an endogenous role for FMO5.

79. Dolphin CT, Beckett DJ, Janmohamed A, et al. The Flavin-containing monooxygenase 2 gene (FMO2) of humans, but not of other primates, encodes a truncated, nonfunctional protein. J Biol Chem 1998;273:30599–607.

** Identification of a nonsense mutation of FMO2 present in most humans.

80. Veeramah KR, Thomas MG, Weale ME, et al. The potentially deleterious functional variant flavin-containing monooxygenase 2*1 is at high frequency throughout sub-Saharan Africa. Pharmacogenet Genomics 2008;18:877–86

81. Krueger SK, Siddens LK, Henderson MC, et al. Haplotype and functional analysis of four flavin-containing monooxygenase isoform 2 (FMO2) polymorphisms in Hispanics. Pharmacogenet Genomics 2005;15:245–56

82. Krueger SK, Martin SR, Yueh M-F, et al. Identification of active flavin-containing monooxygenase isoform 2 in human lung and characterization of expressed protein. Drug Metab Dispos 2002;30:34–41

83. Treacy EP, Akerman BR, Chow LM, et al. Mutations of the flavin-containing monooxygenase gene (FMO3) cause trimethylaminuria, a defect in detoxication. Hum Mol Genet 1998;7:839–45

84. Yamazaki H, Shimizu M. Survey of variants of human flavin-containing monooxygenase 3 (FMO3) and their drug oxidation activities. Biochem Pharmacol 2013;85:1588–93

85. Shephard EA, Treacy EP, Phillips IR. Clinical utility gene card for: Trimethylaminuria - update 2014. Eur J Hum Genet 2015; doi:10.1038/ejhg.2014.226

86. Phillips IR, Shephard EA. Primary Trimethylaminuria. In: GeneReviews at GeneTests:Medical Genetics Information Resource (database online), 2015. Copyright University of Washington, Seattle 1993-2016. Available at www.ncbi.nlm.gov/NBK1103/

87. Mayatepek E, Flock B, Zschocke J. Benzydamine metabolism in vivo is impaired in patients with deficiency of flavin-containing monooxygenase 3. Pharmacogenetics 2004;14:775–7

* In vivo study confirming role of FMO3 in benzydamine metabolism.

88. Phillips IR, Shephard EA. Flavin-containing monooxygenases: mutations, disease and drug response. Trends Pharmacol Sci 2008;29:294–301

89. Hernandez D, Addou S, Lee D, et al. Trimethylaminuria and a human FMO3 mutation database. Hum Mutat 2003;22:209–13

90. Cashman JR. The implications of polymorphisms in mammalian flavin-containing monooxygenases in drug discovery and development. Drug Discov Today 2004;9:574–81

91. Koukouritaki SB, Hines RN. Flavin-containing monooxygenase genetic polymorphism: impact on chemical metabolism and drug development. Pharmacogenomics 2005;6:807–22

92. Sachse C, Ruschen S, Dettling M, et al. Flavin monooxygenase 3 (FMO3) polymorphism in a white population: allele frequencies, mutation linkage, and functional effects on clozapine and caffeine metabolism. Clin Pharmacol Ther 1999;66:431–8

93. Kang J-H, Chung W-G, Lee K-H, et al. Phenotypes of Flavin-containing monooxygenase activity determined by ranitidine N-oxidation are positively

correlated with genotypes of linked FMO3 gene mutations in a Korean population. Pharmacogenetics 2000;10;67-78

94. Park C-S, Kang J-H, Chung W-G, et al. Ethnic differences in allelic frequency of two Flavin- containing monooxygenase 3 (FMO3) polymorphisms: linkage and effects on in vivo and in vitro FMO activities. Pharmacogenet Genomics 2002;12:1–4

95. Allerston CK, Shimizu M, Fujieda M, et al. Molecular evolution and balancing selection in the flavin-containing monooxygenase 3 gene (FMO3). Pharmacogenet Genomics 2007;17:827–39.

96. Lattard V, Zhang J, Tran Q, et al. Two new polymorphisms of the FMO3 gene in Caucasian and African-American populations: comparative genetic and functional studies. Drug Metab Dispos 2003;31:854–60

97. Shimizu M, Yano H, Nagashima S, et al. Effect of genetic variants of the human Flavin-containing monooxygenase 3 on N- and S-oxygenation activities. Drug Metab Dispos 2007;35:328–30

98. Catucci GG, Occhipinti AA, Maffei MM, et al. Effect of human flavin-containing monooxygenase 3 polymorphism on the metabolism of aurora kinase inhibitors. Int J Mol Sci 2013;14:2707–16

99. Koukouritaki SB, Poch MT, Henderson MC, et al. Identification and functional analysis of common human Flavin-containing monooxygenase 3 genetic variants. J Pharmacol Exper Ther 2007;320:266–73

100. Koukouritaki SB, Poch MT, Cabacungan ET, et al. Discovery of novel flavin-containing monooxygenase 3 (FMO3) single nucleotide polymorphisms and functional analysis of upstream haplotype variants. Mol Pharmacol 2005;68:383–92

101. Shimizu M, Shiraishi A, Sato A, et al. Potential for drug interactions mediated by polymorphic flavin-containing monooxygenase 3 in human livers. Drug Metab Pharmacokinet 2015;30:70–4

102. Ryu S-D, Yi H-G, Cha Y-N, et al. Flavin-containing monooxygenase activity can be inhibited by nitric oxide-mediated S-nitrosylation. Life Sci 2004;75:2559–72

103. Nagashima S, Shimizu M, Yano H, et al. Inter-individual variation in flavin-containing monooxygenase 3 in livers from Japanese: correlation with hepatic transcription factors. Drug Metab Pharmacokinet 2009;24:218–25

104. Furnes B, Feng J, Sommer SS, Schlenk D. Identification of novel variants of the flavin-containing monooxygenase gene family in African Americans. Drug Metab Dispos 2003;31:187–93

105. Furnes B, Schlenk D. Evaluation of xenobiotic N- and S-oxidation by variant flavin-containing monooxygenase 1 (FMO1) enzymes. Toxicol Sci 2004;78:196–203

106. Hines RN, Luo Z, Hopp KA, et al. Genetic variability at the human FMO1 locus: significance of a basal promoter yin yang 1 element polymorphism (FMO1*6). J Pharmacol Exper Ther 2003;306:1210–8

107. Hisamuddin IM, Wehbi MA, Chao A, et al. Genetic polymorphisms of human flavin monooxygenase 3 in sulindac-mediated primary chemoprevention of familial adenomatous polyposis. Clin Cancer Res 2004;10:8357–62

* Identification that genetic variants of FMO3 enhance response to sulindac

108. Hisamuddin IM, Wehbi MA, Schmotzer B, et al. Genetic polymorphisms of flavin monooxygenase 3 in sulindac-induced regression of colorectal adenomas in familial adenomatous polyposis. Cancer Epidemiol Biomarkers Prev 2005;14:2366–9

* Identification that genetic variants of FMO3 enhance response to sulindac

109. Ring BJ, Catlow J, Lindsay TJ, et al. Identification of the human cytochromes P450 responsible for the in vitro formation of the major oxidative metabolites of the antipsychotic agent olanzapine. J Pharmacol Exper Ther 1996;276:658–66

110. Korprasertthaworn P, Polasek TM, Sorich MJ, et al. In vitro characterization of the human liver microsomal kinetics and reaction phenotyping of olanzapine metabolism. Drug Metab Dispos 2015;43:1806–14

111. Söderberg MM, Dahl M-L. Pharmacogenetics of olanzapine metabolism. Pharmacogenomics 2013;14:1319–36

112. Okubo M, Narita M, Murayama N, et al. Individual differences in in vitro and in vivo metabolic clearances of the antipsychotic drug olanzapine from non-smoking and smoking Japanese subjects genotyped for cytochrome P4502D6 and flavincontaining monooxygenase 3. Hum Psychopharmacol 2016;31:83–92

113. Cashman JR, Zhang J, Nelson MR, Braun A. Analysis of flavin-containing monooxygenase 3 genotype data in populations administered the anti-schizophrenia agent olanzapine. Drug Metab Lett 2008;2:100–14

114. Söderberg MM, Haslemo T, Molden E, Dahl M-L. Influence of FMO1 and 3 polymorphisms on serum olanzapine and its N-oxide metabolite in psychiatric patients. Pharmacogenomics J 2013;13:544–50

115. Zhou L-P, Tan Z-R, Chen H, et al. Effect of two-linked mutations of the FMO3 gene on itopride metabolism in Chinese healthy volunteers. Eur J Clin Pharmacol 2014;70:1333–8

116. Tugnait M, Hawes EM, McKay G, et al. N-oxygenation of clozapine by flavin-containing monooxygenase. Drug Metab Dispos 1997;25:524–7

117. Yamanaka H, Nakajima M, Nishimura K, et al. Metabolic profile of nicotine

in subjects whose CYP2A6 gene is deleted. Eur J Pharm Sci 2004;22:419–25

118. Li F, Patterson AD, Krausz KW, et al. Metabolomics reveals the metabolic map of procainamide in humans and mice. Biochem Pharmacol 2012;83:1435–44

119. Hernandez D, Janmohamed A, Chandan P, et al. Deletion of the mouse Fmo1 gene results in enhanced pharmacological behavioural responses to imipramine. Pharmacogenet Genomics 2009;19:289–99

** Demonstrates the importance in vivo of FMO1 in imipramine metabolism and response.

120. Shephard EA, Phillips IR. The potential of knockout mouse lines in defining the role of flavin-containing monooxygenases in drug metabolism. Expert Opin Drug Metab Toxicol 2010;6:1083–94

121. Francois AA, Nishida CR, Ortiz de Montellano PR, et al. Human flavin-containing monooxygenase 2.1 catalyzes oxygenation of the antitubercular drugs thiacetazone and ethionamide. Drug Metab Dispos 2009;37:178–86

122. Palmer A, Leykam V, Larkin A, et al. Metabolism and pharmacokinetics of the anti-tuberculosis drug ethionamide in a Flavin-containing monooxygenase null mouse. Pharmaceuticals 2012;5:1147–59

123. Vannelli TA, Dykman A, Ortiz de Montellano PR. The antituberculosis drug ethionamide is activated by a flavoprotein monooxygenase. J Biol Chem 2002;277:12824–9

124. Shaffer CL, Gunduz M, Scialis RJ, Fang AF. Metabolism and disposition of a selective α_7 nicotinic acetylcholine receptor agonist in humans. Drug Metab Dispos 2007;35:1188–95

125. Potega A, Dabrowska E, Niemira M, et al. The imidazoacridinone antitumor drug, C-1311, is metabolized by flavin monooxygenases but not by cytochrome P450s. Drug Metab Dispos 2011;39:1423–32

126. Szökő É, Tábi T, Borbás T, et al. Assessment of the N-oxidation of deprenyl, methamphetamine, and amphetamine enantiomers by chiral capillary electrophoresis: an in vitro metabolism study. Electrophoresis 2004;25:2866–75

127. Pike MG, Mays DC, Macomber DW, Lipsky JJ. Metabolism of a disulfiram metabolite, S-methyl N,N-diethyldithiocarbamate, by flavin monooxygenase in human renal microsomes. Drug Metab Dispos 2001;29:127–32

128. Joo J, Wu Z, Lee B, et al. In vitro metabolism of an estrogen-related receptor γ modulator, GSK5182, by human liver microsomes and recombinant cytochrome P450s. Biopharm Drug Dispos 2015;36:163–73

129. Usmani KA, Chen WG, Sadeque AJM. Identification of human cytochrome P450 and flavin-containing monooxygenase enzymes involved in the

metabolism of lorcaserin, a novel selective human 5-hydroxytryptamine 2C agonist. Drug Metab Dispos 2012;40:761–71

130. Ohmi N, Yoshida H, Endo H, et al. S-oxidation of S-methyl-esonarimod by flavin-containing monooxygenases in human liver microsomes. Xenobiotica 2003;33:1221–31

131. Ballard JE, Prueksaritanont T, Tang C. Hepatic metabolism of MK-0457, a potent aurora kinase inhibitor: interspecies comparison and role of human cytochrome P450 and flavin-containing monooxygenase. Drug Metab Dispos 2007;35:1447–51.

132. Karanam BV, Hop CECA, Liu DQ, et al. In vitro metabolism of MK-0767 [(+/-)-5-[(2,4-dioxothiazolidin-5-yl)methyl]-2-methoxy-N-[[(4-trifluoromethyl) phenyl]methyl]benzamide], a peroxisome proliferator-activated receptor alpha/gamma agonist. I. Role of cytochrome P450, methyltransferases, flavin monooxygenases, and esterases. Drug Metab Dispos 2004;32:1015–22

133. Yu J, Brown DG, Burdette D. In vitro metabolism studies of nomifensine monooxygenation pathways: metabolite identification, reaction phenotyping, and bioactivation mechanism. Drug Metab Dispos 2010;38:1767–78

134. Xie G, Wong CC, Cheng K-W, et al. Regioselective oxidation of phospho-NSAIDs by human cytochrome P450 and flavin monooxygenase isoforms: implications for their pharmacokinetic properties and safety. Br J Pharmacol 2012;167:222–32

135. Miura M, Ohkubo T. In vitro metabolism of quazepam in human liver and intestine and assessment of drug interactions. Xenobiotica 2004;34:1001–11

136. Hai X, Nauwelaers T, Busson R, et al. A rapid and sensitive CE method with field-enhanced sample injection and in-capillary derivatization for selenomethionine metabolism catalyzed by flavin-containing monooxygenases. Electrophoresis 2010;31:3352–61

137. Washio T, Arisawa H, Kohsaka K, Yasuda H. Identification of human drug-metabolizing enzymes involved in the metabolism of SNI-2011. Biol Pharm Bull 2001;24:1263–6

138. Attar M, Dong D, Ling K-HJ, Tang-Liu DD-S. Cytochrome P450 2C8 and flavin-containing monooxygenases are involved in the metabolism of tazarotenic acid in humans. Drug Metab Dispos 2003;31:476–81

139. Kousba A, Soll R, Yee S, Martin M. Cyclic conversion of the novel Src kinase inhibitor [7-(2,6-dichloro-phenyl)-5-methyl-benzo[1,2,4]triazin-3-yl]-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-amine (TG100435) and Its N-oxide metabolite by flavin-containing monoxygenases and cytochrome P450 reductase. Drug Metab Dispos 2007;35:2242–51

140. Qian L, Ortiz de Montellano PR. Oxidative activation of thiacetazone by the Mycobacterium tuberculosis flavin monooxygenase EtaA and human FMO1

and FMO3. Chem Res Toxicol 2006;19:443–9

141. Yanni SB, Annaert PP, Augustijns P, et al. Role of flavin-containing monooxygenase in oxidative metabolism of voriconazole by human liver microsomes. Drug Metab Dispos 2008;36:1119–25

142. Wagmann L, Meyer MR, Maurer HH. What is the contribution of human FMO3 in the N-oxygenation of selected therapeutic drugs and drugs of abuse? Toxicol Lett 2016;258:55–70

143. Salva M, Jansat JM, Martinez-Tobed A, Palacios JM. Identification of the human liver enzymes involved in the metabolism of the antimigraine agent almotriptan. Drug Metab Dispos 2003;31:404–11

144. Störmer E, Roots I, Brockmöller J. Benzydamine N-oxidation as an index reaction reflecting FMO activity in human liver microsomes and impact of FMO3 polymorphisms on enzyme activity. Br J Clin Pharmacol 2000;50:553–61

145. Wang L, Christopher LJ, Cui D, et al. Identification of the human enzymes involved in the oxidative metabolism of dasatinib: an effective approach for determining metabolite formation kinetics. Drug Metab Dispos 2008;36:1828–39

146. Zhou S, Kestell P, Paxton JW. 6-methylhydroxylation of the anti-cancer agent 5,6-dimethylxanthenone-4-acetic acid (DMXAA) by flavin-containing monooxygenase 3. Eur J Drug Metab Pharmacokinet 2002;27:179–83

147. Pike MG, Martin YN, Mays DC, et al. Roles of FMO and CYP450 in the metabolism in human liver microsomes of S-methyl-N,N-diethyldithiocarbamate, a disulfiram metabolite. Alcohol Clin Exp Res 1999;23:1173–9

148. Jacobsen W, Christians U, Benet LZ. In vitro evaluation of the disposition of A novel cysteine protease inhibitor. Drug Metab Dispos 2000;28:1343–51

149. Prueksaritanont T, Lu P, Gorham L, et al. Interspecies comparison and role of human cytochrome P450 and flavin-containing monooxygenase in hepatic metabolism of L-775,606, a potent 5-HT(1D) receptor agonist. Xenobiotica 2000;30:47–59

150. Luo JP, Vashishtha SC, Hawes EM, et al. In vitro identification of the human cytochrome p450 enzymes involved in the oxidative metabolism of loxapine. Biopharm Drug Dispos 2011;32:398–407

151. Hanlon SP, Camattari A, Abad S, et al. Expression of recombinant human flavin monooxygenase and moclobemide-N-oxide synthesis on multi-mg scale. Chem Commun 2012;48:6001–3

152. Bloom AJ, Murphy SE, Martinez M, et al. Effects upon in-vivo nicotine metabolism reveal functional variation in FMO3 associated with cigarette consumption. Pharmacogenet Genomics 2013;23:62–8

153. Jin X, Pybus BS, Marcsisin R, et al. An LC-MS based study of the metabolic profile of primaquine, an 8-aminoquinoline antiparasitic drug, with an in vitro primary human hepatocyte culture model. Eur J Drug Metab Pharmacokinet 2014;39:139–46

154. Reid JM, Walker DL, Miller JK, et al. The metabolism of pyrazoloacridine (NSC 366140) by cytochromes P450 and flavin monooxygenase in human liver microsomes. Clin Cancer Res 2004;10:1471–80

155. Pichard-Garcia L, Weaver RJ, Eckett N, et al. The olivacine derivative S 16020 (9-hydroxy-5,6-dimethyl-N-[2-(dimethylamino)ethyl)-6H-pyrido(4,3-B)-carbazole-1-carboxamide) induces CYP1A and its own metabolism in human hepatocytes in primary culture. Drug Metab Dispos 2004;32:80–8

156. Lomri N, Yang Z, Cashman JR. Expression in Escherichia coli of the flavin-containing monooxygenase D (form II) from adult human liver: determination of a distinct tertiary amine substrate specificity. Chem Res Toxicol1993;6:425–9

Table 1. **Major FMO forms expressed in adult human and mouse tissues**

	Human	Mouse male	Mouse female
Liver	FMO3, FMO5	FMO1, FMO5	FMO1, FMO3, FMO5
Lung	FMO2	FMO1, FMO2,	FMO1, FMO2
Kidney	FMO1	FMO1, FMO5	FMO1

Table 2. Drug substrates of human FMO1

Substrate	Type of drug	Reaction	Analytical system	Reference
N-(3*R*)-1-azabicyclo[2.2.2]oct-3-ylfuro[2,3-*c*]pyridine-5-carboxamide	α_7 nicotinic acetylcholine receptor agonist	*N*-oxygenation	in vivo; kidney microsomes; recombinant protein	[124]
Benzydamine	nonsteroidal antiinflammatory	*N*-oxygenation	recombinant protein	[60]
C-1311	antitumour agent	*N*-oxygenation	recombinant protein	[125]
Cimetidine	antiulcerative, histamine H2-receptor antagonist	*S*-oxygenation	recombinant protein	[51]
Deprenyl	monoamine oxidase type B inhibitor	*N*-oxygenation	recombinant protein	[126]
Disulfiram metabolite, *s*-methyl *n,n*-diethyldithiocarbamate[a]	antialcoholic	*S*-oxygenation	kidney microsomes; recombinant protein	[127]
Ethionamide	antitubercular	*S*-oxygenation	recombinant protein	[29]
GSK5182	antidiabetic, estrogen-related receptor γ modulator	*N*-oxygenation	recombinant protein	[128]
Imipramine	antidepressant, 5HT/noradrenalin re-uptake inhibitor	*N*-oxygenation	recombinant protein	[105]
Itopride	dopamine D2 blocker and acetylcholinesterase inhibitor	*N*-oxygenation	recombinant protein	[61]
N-deacetyl ketoconazole[b]	antifungal	*N*-hydroxylation	recombinant protein	[47]
Lorcaserin	selective human 5-hydroxytryptamine 2C agonist	*N*-hydroxylation	kidney microsomes; recombinant protein	[129]
S- and *R*-Metamphetamine	psychostimulant	*N*-hydroxylation [c]	recombinant protein	[126]
Methimazole	thyroperoxidase inhibitor	*S*-oxygenation	recombinant protein	[11]
S-methyl esonarimod[d]	cytokine production inhibitor	*S*-oxygenation	recombinant protein	[130]
MK-0457 (Tozasertib)	aurora kinase inhibitor	*N*-oxygenation	recombinant protein	[131]
MK-0767 methyl sulphide	peroxisome proliferator receptor activator	*S*-oxygenation	recombinant protein	[132]
Nomifensine	antidepressant	*N*-oxygenation	recombinant protein	[133]
Olopatadine	antihistamine,	*N*-oxygenation	recombinant	[62]

	histamine H1 receptor-selective antagonist		protein	
Pargyline	monoamine oxidase type B inhibitor	*N*-oxygenation	recombinant protein	[5]
Phospho-sulindac	nonsteroidal anti-inflammatory	*S*-oxygenation	recombinant protein	[134]
Procainamide	type I antiarrhythmic agent	*N*-oxygenation	recombinant protein	[118]
Quazepam	benzodiazepine	Desulfuration (2-oxo form)	small intestine microsomes; recombinant protein	[135]
Selenomethionine	anticancer agent	*Se*-oxygenation	recombinant protein	[136]
SNI-2011	muscarinic agagonist	*N*-oxygenation	kidney microsomes; recombinant protein	[137]
Sulindac sulfide[e]	nonsteroidal anti-inflammatory (colorectal cancer)	*S*-oxygenation	kidney microsomes	[30]
Tamoxifen	antiestrogen, estrogen receptor modulator	*N*-oxygenation	recombinant protein	[67]
Tazarotenic acid[f]	retinoic acid receptor modulator	*S*-oxygenation	recombinant protein	[138]
TG100435	src kinase Inhibitor	*N*-oxygenation	recombinant protein	[139]
Thiacetazone	antitubercular	*S*-oxygenation	recombinant protein	[121,140]
Voriconazole	antifungal	*N*-oxygenation	recombinant protein	[141]
Xanomeline	M1 muscarinic agonist	*N*-oxygenation	kidney microsomes; recombinant protein	[64]

[a] metabolite of disulfiram, product is a sulfine; [b]major metabolite of ketoconazole; [c]metamphetamine hydroxylamine is further converted by FMO action to a mixture of nitrones, which are subsequently hydrolysed to yield an aldehyde and a hydroxylated primary amine, the latter then being reduced to a primary amine; [d]active metabolite of esonarimod; [e]the active metabolite of the prodrug sulindac; [f]major metabolite of tazarotene.

Table 3. Drug substrates of human FMO2.1

Substrate	Type of drug	Reaction	Analytical system	Reference
Ethionamide	antitubercular	*S*-oxygenation	Recombinant protein	[29]
Methimazole	thyroperoxidase inhibitor	*S*-oxygenation	Recombinant protein	[82]
Thiacetazone	antitubercular	*S*-oxygenation	Recombinant protein	[121]

Table 4. Drug substrates of human FMO3

Drug	Substrate	Reaction	Analytical system	Reference
Albendazole	anthelmitic	*S*-oxygenation	liver microsomes; recombinant protein	[65]
N,*N*-diallyltryptamine	pschychostimulant	*N*-oxygenation	liver microsomes; recombinant protein	[142]
Almotriptan	antimigraine 5-HT1B, 1D receptor agonist	*N*-oxygenation	liver microsomes; recombinant protein	[143]
Amphetamine[a]	dopamine transporter ligand (antipsychotic)	*N*-oxygenation	recombinant protein	[50]
N-(3*R*)-1-azabicyclo[2.2.2]oct- 3-ylfuro[2,3-*c*]pyridine-5-carboxamide	α_7 nicotinic acetylcholine receptor agonist	*N*-oxygenation	in vivo; liver microsomes; recombinant protein	[124]
Benzydamine	nonsteroidal antiinflammatory	*N*- oxygenation	in vivo; liver microsomes; recombinant protein	[60,87,144]
Cimetidine	histamine H2-receptor antagonist	*S*-oxygenation	in vivo; liver microsomes; recombinant protein	[31,66]
Clozapine	antipsychotic dopamine D2, 5'HT2 and 5'HT1C receptor antagonist	*N*-oxygenation	liver microsomes; purified protein	[116]
Danusertib	aurora kinase inhibitor	*N*-oxygenation	recombinant protein	[98]
Dasatinib	BCR-ABL and SRC family kinase inhibitor	*N*-oxygenation	liver microsomes; recombinant protein	[145]
Deprenyl	monoamine oxidase type B inhibitor	*N*-oxygenation	liver microsomes; recombinant protein	[126]
C-1311	antitumour agent	*N*-oxygenation	liver microsomes; recombinant protein	[125]
5,6-dimethylxanthenone-4-acetic acid (vadimezan)	anticancer agent, cytokine inducer	methyl hydroxylation	liver microsomes; recombinant protein	[146]
Disulfiram metabolite, *s*-methyl *n*,*n*-	antialcoholic	*S*-oxygenation	liver microsomes;	[147]

diethyldithiocarbamate[b]			recombinant protein	
Ethionamide	antitubercular	*S*-oxygenation	recombinant protein	[29]
GSK5182	antidiabetic, estrogen-related receptor γ modulator	*N*-oxygenation	liver microsomes; recombinant protein	[128]
Itopride	dopamine D2 blocker and acetylcholinesterase inhibitor	*N*-oxygenation	in vivo; microsomes; recombinant protein	[61,115]
K11777	peptidomimetic, cysteine protease inhibitor	*N*-oxygenation	liver microsomes; recombinant protein	[148]
N-deacetyl ketoconazole[c]	antifungal	*N*-hydroxylation	recombinant protein	[47]
L-775,606	5-HT(1D) receptor agonist	*N*-oxygenation	liver microsomes	[149]
Loxapine	tricyclic antipsychotic	*N*-oxygenation	liver microsomes	[150]
S-Methamphetamine[d]	psychostimulant	*N*-hydroxylation	recombinant protein	[50]
Methimazole	thyroperoxidase inhibitor	*S*-oxygenation	liver microsomes; recombinant protein	[31]
S-methyl esonarimod[e]	cytokine production inhibitor	*S*-oxygenation	liver microsomes; recombinant protein	[130]
MK-0457 (Tozasertib)	aurora kinase inhibitor	*N*-oxygenation	liver microsomes; recombinant protein	[98,131]
MK-0767 methyl sulphide	peroxisome proliferator receptor activator	*S*-oxygenation	liver microsomes; recombinant protein	[132]
Moclobemide	monoamine oxidase type A inhibitor	*N*-oxygenation	recombinant protein	[151]
Nicotine	adenosine receptor ligand, stimulant	*N*-oxygenation	in vivo; recombinant protein	[49,152]
Nomifensine	antidepressant	*N*-oxygenation	recombinant protein	[133]
Olanzapine	multi receptor antagonist, antipsychotic	*N*-oxygenation	liver microsomes; in vivo	[109,114]
Olopatadine	antihistamine, histamine H1 receptor-selective antagonist	*N*-oxygenation	recombinant protein	[62]
Pargyline	monoamine oxidase type B inhibitor	*N*-oxygenation	recombinant protein	[5]
Phospho-sulindac	nonsteroidal anti-	*S*-oxygenation	liver	[134]

	inflammatory		microsomes; recombinant protein	
Primaquine	antimalarial	not known	recombinant protein; primary hepatocyes	[153]
Procainamide	type I antiarrhythmic agent	*N*-oxygenation	in vivo; liver microsomes; recombinant protein	[118]
Pyrazolacridine	antitumour	*N*-oxygenation	liver microsomes; recombinant protein	[154]
Ranitidine	histamine H2-receptor antagonist, antiulcerative	*N*- and *S*-oxygenation	liver microsomes; recombinant protein	[31,63]
S 16020	topoisomerase II inhibitor, antitumour	*N*-oxygenation	primary hepatocytes; recombinant protein	[155]
Selenomethionine	anticancer agent	*Se*-oxygenation	recombinant protein	[136]
Sulindac sulfide[f]	nonsteroidal anti-inflammatory	*S*-oxygenation	liver microsomes; recombinant protein	[30]
Tamoxifen	antiestrogen, estrogen receptor modulator	*N*-oxygenation	liver microsomes; recombinant protein	[67]
Tazarotenic Acid[g]	retinoic acid receptor modulator	*S*-oxygenation	liver microsomes; recombinant protein	[138]
TG100435	Src kinase inhibitor	*N*- oxygenation	liver microsomes; recombinant protein	[139]
Thiacetazone	antitubercular	*S*-oxygenation	recombinant protein	[121,140]
Trifluoperazine	calmodulin antagonist	*N*-oxygenation	liver microsomes; recombinant protein	[156]
Voriconazole	antifungal	*N*- oxygenation	liver microsomes; recombinant protein	[141]
Xanomeline	M1 muscarinic agonist	*N*- oxygenation	liver microsomes; recombinant protein	[64]

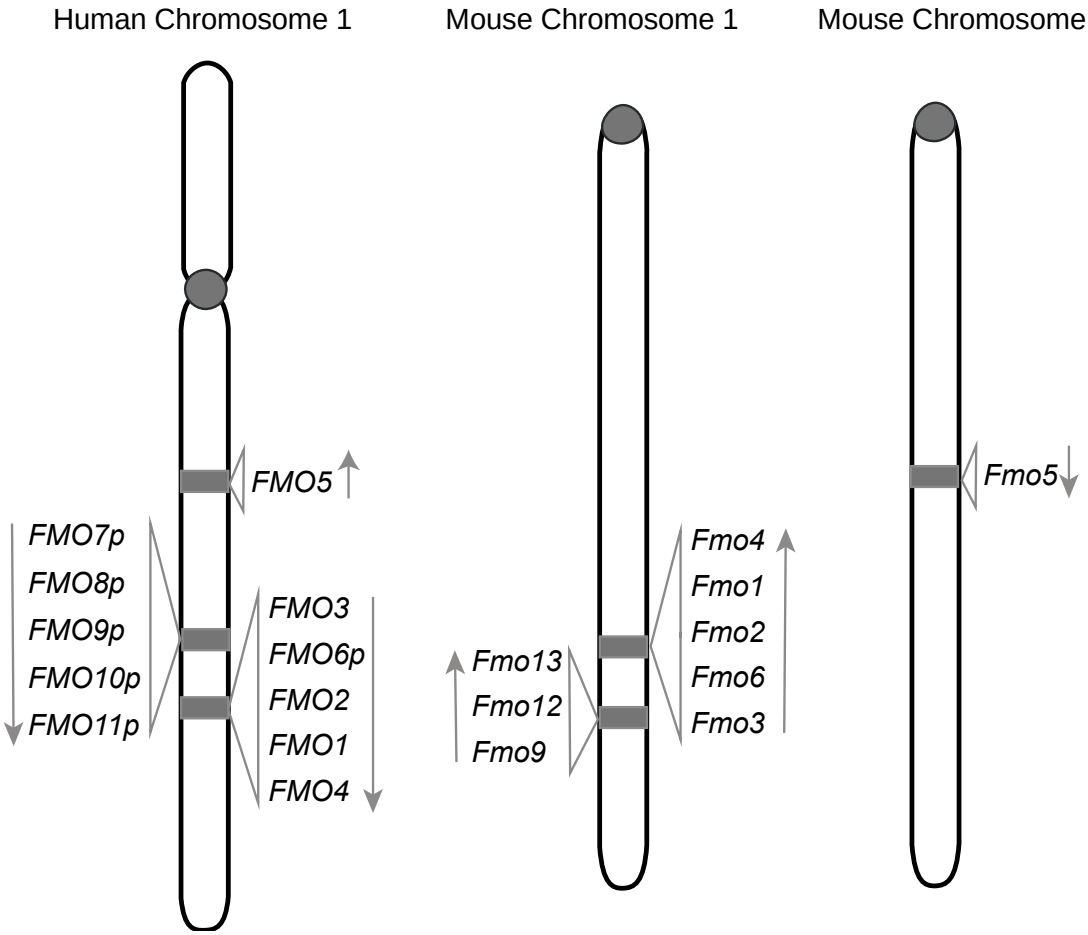
[a]the initial product, an *N*-hydroxylamine, is converted, via a second oxygenation and loss of water, to an oxime; [b]metabolite of disulfiram, product is a sulfine; [c]major metabolite of ketoconazole; [d]metamphetamine hydroxylamine is further converted by FMO action to a mixture of nitrones, which are subsequently hydrolysed to yield an aldehyde and a hydroxylated primary amine, the latter then

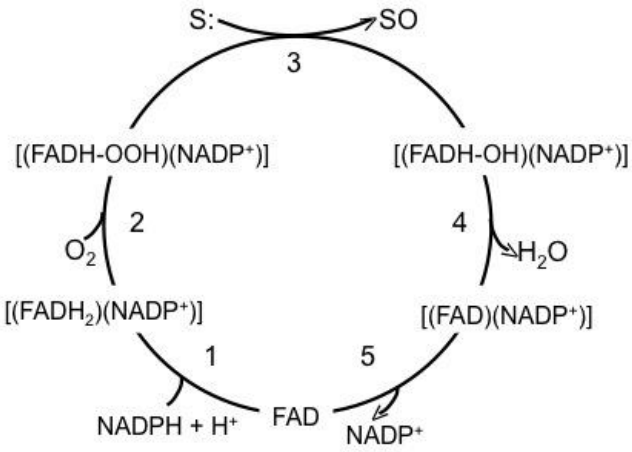
being reduced to a primary amine; [e]active metabolite of esonarimod; [f]the active metabolite of the prodrug sulindac; [g]major metabolite of tazarotene.

Table 5. Drug substrates of human FMO5

Substrate	Type	Reaction	Analytical system	Reference
S-methyl esonarimod[a]	cytokine production inhibitor	*S*-oxygenation	liver microsomes; recombinant protein	[130]
E7016	anticancer agent, inhibitor of poly(ADP-ribose) polymerase	BV oxidation	recombinant protein, fortified with liver cytosol	[55]
MRX-I	antibacterial	BV oxidation	recombinant protein, fortified with liver cytosol	[56]
Nomifensine	antidepressant	*N*-oxygenation	recombinant protein	[133]
Phospho-sulindac	nonsteroidal anti-inflammatory	*S*-oxygenation	liver microsomes; recombinant protein	[134]
Ranitidine	histamine H2-receptor antagonist, antiulcerative	*S*-oxygenation	purified protein	[63]

[a]active metabolite of esonarimod; BV, Baeyer-Villiger.


Human Chromosome 1
Mouse Chromosome 1
Mouse Chromosome 3
FMO5
FMO7p
FMO8p
FMO9p
FMO10p
FMO11p
FMO3
FMO6p
FMO2
FMO1
FMO4
Fmo13
Fmo12
Fmo9
Fmo4
Fmo1
Fmo2
Fmo6
Fmo3
Fmo5


S:
SO
3
[(FADH-OOH)(NADP+)]
[(FADH-OH)(NADP+)]
2
4
O2
H2O
[(FADH2)(NADP+)]
[(FAD)(NADP+)]
1
5
NADPH + H+
FAD
NADP+