Flavin-containing monooxygenase 3 (FMO3): genetic variants and their consequences for drug metabolism and disease

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Abstract

1. The review focuses on genetic variants of human flavin-containing monooxygenase 3 (FMO3) and their impact on enzyme activity, drug metabolism and disease.

2. The majority of FMO-mediated metabolism in adult human liver is catalyzed by FMO3. Some drugs are metabolized in human liver predominantly by FMO3, but most drug substrates of FMO3 are metabolized also by other enzymes, particularly cytochromes P-450, and the FMO3-catalyzed reaction is not the major route of metabolism.

3. Rare variants that severely affect production or activity of FMO3 cause the disorder trimethylaminuria and impair metabolism of drug substrates of FMO3. More common variants, particularly p.[(Glu158Lys);(Glu308Gly)], can moderately affect activity of FMO3 *in vitro* and reduce metabolism of drug substrates *in vivo*, in some cases increasing drug efficacy or toxicity.

4. Common variants of *FMO3* have been associated with a number of disorders, but additional studies are needed to confirm or refute such associations.

5. Elevated plasma concentrations of trimethylamine *N*-oxide, a product of an FMO3-catalyzed reaction, have been implicated in certain diseases, particularly cardiovascular disease. However, the evidence is often contradictory and additional work is required to establish whether trimethylamine *N*-oxide is a cause, effect or biomarker of the disease.

6. Genetic variants of other *FMO*s are also briefly discussed.

Keywords: allele frequency; cardiovascular disease; clinical effects; FMO1; FMO2; human; mutation; polymorphism; trimethylamine *N*-oxide; trimethylaminuria

Introduction

Flavin-containing monooxygenases (FMOs; EC 1.14.13.8) catalyze the oxidative metabolism of a broad range of foreign chemicals, including therapeutic drugs and dietary-derived

compounds (Krueger & Williams, 2005; Phillips et al., 2007; Phillips & Shephard, 2017). Much of our knowledge of the catalytic mechanism and substrate preferences of FMOs derives from the pioneering work of Dan Ziegler and colleagues (Ziegler, 1993; Poulsen & Ziegler, 1995; Ziegler, 2002). For catalysis, FMOs require FAD as a prosthetic group, NADPH as a cofactor and molecular oxygen as a cosubstrate. Preferred substrates contain, as the site of oxygenation, a soft nucleophilic heteroatom, typically nitrogen or sulfur (Krueger & Williams, 2005). The mechanism of action of FMOs is unusual, as it enables the enzymes to activate oxygen, in the form of a stable C4a hydroperoxide derivative of FAD, in the absence of a bound oxygenatable substrate (Fig. 1). Consequently, the enzymes are present in in an activated 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. The slowest (rate-limiting) steps in the catalytic cycle are the breakdown of FADH-OH to release H_2O and the release of $NADP^+$ (Fig. 1, steps 4 and 5). As both occur after the oxygenation of substrate and release of oxygenated product (Fig. 1, step 3), the catalytic constant (k_{cat}) is usually 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. Factors that restrict access to the active site are size, shape and charge; the best substrates are uncharged or have a single positive charge (Ziegler, 1993; Poulsen & Ziegler, 1995; Ziegler, 2002). The catalytic cycle can also undergo uncoupling, producing H_2O_2 with the release of $NADP^+$ (Siddens et al., 2014) (Fig. 1), enabling FMOs to moonlight as NADPH oxidases. The rate of uncoupling is higher in the

presence of substrate, with as much as 30-50% of bound oxygen being released as H_2O_2 (Siddens et al., 2014).

The catalytic mechanism of FMOs differs from that of cytochromes P450 (CYPs). In contrast to FMOs, which accept electrons directly from NADPH, CYPs receive electrons from NADPH via an accessory protein, CYP reductase, and bind and activate oxygen only after binding oxygenatable substrate (reviewed in Guengerich, 2007).

Humans possess five functional *FMO* genes, designated *FMO1, 2, 3, 4* and *5* (Lawton et al., 1994; Phillips et al., 1995; Hernandez et al., 2004). *FMO*s *1, 2, 3* and *4* are clustered on chromosome 1, in the region q24.3 (Hernandez et al., 2004). The cluster contains an additional *FMO* gene, *FMO6P*, classified as a pseudogene because it is unable to produce a correctly spiced mRNA (Hines et al., 2002). *FMO5* is located ~26 Mb closer to the centromere, at 1q 21.1 (Hernandez et al., 2004). This review focuses on human *FMO3* and the protein it encodes.

The *FMO3* gene and its expression

The *FMO3* gene comprises nine exons, the first of which is non-coding (Dolphin et al., 1997a), and encodes a polypeptide of 532 amino-acid residues and molecular mass 60,047 (Phillips et al., 1995; Dolphin et al., 1996).

In adult humans, the main site of expression of *FMO3* is the liver, in which, together with FMO5, it is the most abundant FMO isoform (Phillips et al., 1995; Dolphin et al., 1996) (Hernandez et al., 2004). *FMO3* is also expressed in skin (Janmohamed et al., 2001), pancreas and in adrenal medulla and cortex (Hernandez et al., 2004). Humans do not express *FMO1* in

adult liver (Dolphin et al., 1991, 1996; Phillips et al., 1995; Yeung et al., 2000; Koukouritaki et al., 2002) T. This is also the case in *Cynomologous macaque*, an old-world monkey (Uno et al., 2013). In contrast, in all other mammals investigated, including marmoset (Uehara et al., 2017), a new-world monkey, FMO1 represents a major hepatic form of the enzyme (reviewed in Phillips et al., 2007). Silencing of the *FMO1* gene in adult human liver might be a consequence of the presence, upstream of the core liver promoter of *FMO1*, of LINE-1 elements that act as powerful transcriptional repressors (Shephard et al., 2007). These elements are absent from species in which the gene is expressed in adult liver. Expression of *FMO1* in human extra-hepatic tissues, such as kidney and intestine, is through the use of alternative promoters (Hernandez et al., 2004; Shephard et al., 2007).

In humans, expression of *FMO3* is switched on within the first two years after birth (Dolphin et al., 1996; Koukouritaki et al., 2002) and increases throughout childhood and adolescence to reach a maximum in adulthood (Koukouritaki et al., 2002). Interindividual variations of 10- to 20-fold in the abundance of FMO3 in adult human liver have been reported (Overby et al., 1997; Cashman & Zhang 2002; Koukouritaki et al., 2002), with the amount of FMO3 reaching that of CYP2C9 and ~ 60% of that of CYP3A4, the most abundant hepatic CYP (Shimada et al., 1994). In human skin, the abundance of FMO3 mRNA is as great as that of mRNAs encoding CYP2A6 and CYP3A4 (Janmohamed et al., 2001).

Factors that affect FMO3 expression or activity

In contrast to CYPs, FMO3 is not readily inducible by foreign chemicals. Consequently, individual variation in the expression of FMO3 is likely due to genetic or physiological factors. The abundance of FMO3 declines during menstruation (Zhang et al., 1996; Shimizu, et al., 2007a), probably because of a decrease in expression of the *FMO3* gene in response to

female sex hormones (Coecke et al., 1998). *FMO3* expression may also be influenced *in vivo* by the availability of the transcription factors HNF-1 and NF-Y (Nagashima et al., 2009).

FMO3, in contrast to CYPs, is not readily irreversibly inhibited by foreign chemicals. However, the activity of FMO3 is inhibited *in vitro* and *in vivo* by dietary indoles present in brassicas (Cashman et al., 1999a) and by nitric oxide-mediated *S*-nitrosylation (Ryu et al., 2004). The enzyme is also subject to competitive inhibition by alternative substrates (Shimizu et al., 2015).

Genetic variants of *FMO3*

Variants that cause trimethylaminuria

A major impetus for investigating FMOs of human was to identify the molecular basis of the recessively inherited disorder trimethylaminuria (OMIM 602079) and build on the earlier work of Bob Smith and colleagues in characterizing the disorder and establishing its mode of inheritance (Al-Waiz et al., 1987; reviewed in Mitchell & Smith, 2001). The disorder is characterized by the excretion of excessive amounts of the smelly tertiary amine trimethylamine in urine, sweat, breath and reproductive fluids, which imparts an odour characteristic of rotten fish (Mitchell & Smith, 2001, 2003). The trimethylamine is derived from dietary precursors, via the action of gut bacteria (reviewed in Fennema et al., 2016). Affected individuals are defective in the hepatic metabolism of trimethylamine to its non-odorous *N*-oxide (reviewed in Mitchell & Smith, 2001; Phillips & Shephard, 2015; Shephard et al., 2015), a reaction that is selectively catalyzed by FMO3 (Lang et al., 1998). Although no overt physical symptoms are associated with trimethylaminuria, the unpleasant odour characteristic of the disorder often results in psychological and social problems, including

anxiety, chronic depression and, in extreme cases, suicidal tendencies (Mitchell & Smith, 2001).

Primary trimethylaminuria is caused by homozygous or compound heterozygous variants of the *FMO3* gene (OMIM 136132) that abolish or severely affect the production or activity of FMO3. The first causative variant identified was c.458C>T[p.(Pro153Leu)] (Dolphin et al., 1997b; Treacy et al., 1998). In this review, numbering of coding-region variants is based on the transcript reference sequence NM 001002294.2. Subsequently, more than 40 genetic variants that cause trimethylaminuria have been identified (reviewed in Phillips et al., 2007; Phillips & Shephard, 2008; Yamazaki & Shimizu, 2013; Gao et al., 2016). These include missense and nonsense variants, small (1- or 2-bp) deletions and a large (12.2-kb) deletion. In one case, the haplotype in which a variant occurs is important: c.560T>C[p.(Val187Ala)] has no effect on FMO3 activity, but when it occurs in *cis* with the common polymorphic variant c.472G>A[p.(Glu158Lys)] (see below), that is, p.[(Glu158Lys);(Val187Ala)], it markedly decreases enzyme activity and contributes to severe trimethylaminuria (Motika et al., 2009). A human *FMO3* locus-specific database has been established (Hernandez et al., 2003) (https://databases.lovd.nl/shared/genes/FMO3) that catalogues genetic variants of *FMO3*, including those causative of trimethylaminuria. A comprehensive description of genetic variants of *FMO3* that cause trimethylaminuria is beyond the scope of this review.

Common coding-region variants and their effect on FMO3 *activity* in vitro

In addition to rare variants causative of trimethylaminuria, almost 200 other variants have been identified in the coding region of *FMO3* (dbSNP, build 152) (https://www.ncbi.nih.gov/snp/), some of which are present at relatively high frequency (reviewed in Phillips et al., 2007; Phillips & Shephard, 2008;Yamazaki & Shimizu, 2013). A

catalogue of genetic variants of *FMO3* and their effect on drug metabolism can be accessed at https://databases.lovd.nl/shared/genes/FMO3. In this review, we focus on variants that are common, that is, are present in at least one population group at an allelic frequency of >1%, and, thus, would be of significance for the general population (Table 1). Frequencies of variants are taken from the relevant Reference SNP (rs) Report of dbSNP (build 152) (https://www.ncbi.nih.gov/snp/).

Seven nonsynonomous and two synonomous variants are present at an allelic frequency of >1% in at least one population group. The most common nonsynonomous variant, c.472G>A[p.(Glu158Lys)] (Brunelle et al., 1997; Dolphin et al., 1997b), is present at high allelic frequency in all population groups: ~45% in Africans, 40-45% in Europeans and ~30% in Asians. Another variant, c.923A>G[p.(Glu308Gly)] (Treacy et al., 1998), is relatively common in Europeans and Asians, at frequencies of ~20% and ~10%, respectively, but is less common in Africans (~4%). Studies *in vitro* reveal that, individually, each of these variants has little or no effect on the activity of the enzyme against a range of substrates (reviewed in Cashman, 2004; Krueger & Williams 2005; Koukouritaki & Hines 2005; Cashman & Zhang 2006; Phillips et al., 2007). In Europeans and Asians, however, in almost all cases the two variants are linked, occurring in *cis* on the same chromosome (Sachse et al., 1999; Kang et al., 2000; Cashman et al., 2001; Park et al., 2002; Allerston et al., 2007). In Europeans and Japanese the allele frequency of the compound variant, p.[(Glu158Lys);(Glu308Gly)], can be as high as 20% (Mao et al., 2009). Consequently, the proportion of individuals who possess at least one copy of the compound variant will be considerably higher, reaching more than 35% in some populations (Mao et al., 2009). The effect on enzyme activity of the compound variant p.[(Glu158Lys);(Glu308Gly)] is greater than that of either variant alone, resulting in a moderate reduction in activity, the extent of which is dependent on the substrate (Park et al.,

2002; Lattard et al, 2003; Cashman, 2004; Koukouritaki & Hines, 2005; Krueger & Williams, 2005; Cashman & Zhang 2006; Phillips et al., 2007).

A third variant, c.769G>A[p.(Val257Met)] (Treacy et al., 1998), is relatively common in Asians (~13% allele frequency), but less so in Europeans (6-7%) and Africans (<4%). The variant significantly reduces the ability of the enzyme to catalyze *N*-oxygenation of the anticancer aurora kinase inhibitor danusertib, but has no effect on the *N*-oxygenation of another aurora kinase inhibitor, tozasertib (Catucci et al., 2013), or on the oxygenation of a range of other substrates (Dolphin et al., 2000; Cashman 2004; Krueger & Williams, 2005; Koukouritaki & Hines, 2005; Cashman & Zhang, 2006; Shimizu, et al., 2007b). The variant c.394G>C[p.(Asp132His)] (Furnes et al., 2003), which is present at a frequency of ~4% in Africans, but is extremely rare in Europeans and Asians, moderately reduces activity towards trimethylamine and methimazole, but not 10-(N,N-dimethylaminopentyl)-2-(trifluoromethyl) phenothiazine (Lattard et al., 2003).

Of the other three relatively common nonsynonomous variants, c.539G>T[p.(Gly180Val)], which is present in Europeans at a frequency of 1-2%, has no effect on activity (Dolphin et al., 2000). The effect of the remaining two, c.830T>C[p.(Val277Ala)] (Cashman, 2002) and c.1084G>C[p.(Glu362Gln)] (Cashman 2002; Furnes et al., 2003), which are present only in Africans, at frequencies of ~5% and ~1.5%, respectively, has not been determined.

Another variant, c.183C>A[p.(Asn61Lys)] (Koukouritaki et al., 2005), abolishes or markedly reduces the activity of FMO3 towards four different substrates: trimethylamine, ethylenethiourea, methimazole and sulindac (Koukouritaki et al., 2007). Although the variant has been detected in African-American and non-Latino white populations at frequencies of

3.5 and 5% respectively (Koukouritaki et al., 2005), it is not present in dbSNP (build 152). The effect of the variant on oxygenation of trimethylamine indicates that it would be causative of trimethylaminuria, which, together with its absence from dbSNP, suggests that its frequency in the wider population would be much lower.

All of the other nonsynonomous *FMO3* variants are present at low frequency, <1%, and are often confined to a single population group. For most, the effect of the variant on enzyme activity is unknown. For those that have been investigated, the variant either has no or little effect on activity, except for c.613C>T[p.(Arg205Cys)] (Fujieda et al., 2003), which moderately reduces activity (Yamazaki & Shimizu, 2007), and c.1079T>C[p.(Leu360Pro)] (Furnes et al., 2003), which increases activity by 2- to 5-fold (Lattard et al., 2003). The variant c.613C>T[p.(Arg205Cys)] is present in Europeans and Asians at a frequency of <0.1% and is absent from Africans, whereas c.1079T>C[p.(Leu360Pro)] is present in Africans at a frequency of ~0.5%, but does not occur in Europeans and Asians. Consequently, neither variant would have a significant impact on the general population.

There are two common synonomous variants of the *FMO3* gene (Cashman, 2002): c.855C>T[p.(Asn285=)] is present at allele frequencies of ~45% in Africans, ~ 40% in Japanese and ~25% in Europeans, whereas c.441C>T[p.(Ser147=)] is present at frequencies of 8% in Africans, 5% in Europeans and ~25% in Japanese (Table 1). Although synonomous variants do not change the sequence of the encoded protein, in some cases they can affect processing of the mRNA, resulting in production of reduced amounts of protein or of an abnormal protein (Sauna & Kimchi-Sarfaty, 2011). Although not investigated, based on the relatively high frequency of the variants, it is thought that neither has any effect.

Variants in the upstream regulatory region

Seven variants have been identified in the 5'-flanking region of the *FMO3* gene: g.-2650C>A, g.-2589C>T, g.-2543T>A, g.-2177G>C, g.-2106G>A, g.-2099A>G, g.-1961T>C (Koukouritaki et al., 2005). The variants were inferred to segregate into 14 variant haplotypes, six of which were considered common (frequency >1%) (Koukouritaki et al., 2005). Of the six common variant haplotypes, the transcriptional activity *in vitro* of three was similar to that of the ancestral *FMO3* upstream region, whereas one increased transcription 8-fold and the remaining two markedly reduced transcription (Koukouritaki et al., 2005). The haplotype that increased transcription was relatively common, being present at frequencies of 29, 11 and 6%, respectively, in Hispanic Americans of Mexican origin, African Americans and whites of northern-European descent, but the two that reduced transcription were each present at low frequency in a single population group, one in the white group, at 4%, and the other in the African-American group, at 1.5% (Koukouritaki et al., 2005). It is not known whether the promoter-region variants affect transcription of the *FMO3* gene *in vivo*.

An extended haplotype analysis found that the upstream variant haplotype that increased transcription *in vitro* was associated with c.472G>A[p.(Glu158Lys)], c.923A>G[p.(Glu308Gly)] or the double variant p.[(Glu158Lys);(Glu308Gly)] (Koukouritaki et al., 2007). In the latter case, an increase in the amount of protein produced, as a consequence of promoter variants, might compensate for an enzyme that has lower activity because of coding-region variants.

Natural selection of* FMO3 *haplotypes

Analysis of genetic variation in *FMO3* indicates that the gene has been the subject of balancing natural selection (Allerston et al., 2007), which results in two or more haplotypes

being maintained at frequencies higher than expected under a neutral model of evolution. Three *FMO3* haplotypes appear to be targets for selection: the ancestral haplotype and two variant haplotypes, one containing both the c.472G>A[p.(Glu158Lys)] and c.923A>G[p.(Glu308Gly)] variants and, thus, would encode an enzyme with reduced catalytic activity, the other containing the 5'-flanking-region variants associated with increased promoter activity (see above). The natural selection of haplotypes that increase the amount or decrease the activity of FMO3 suggests that the resulting metabolic versatility conferred an evolutionary advantage.

Drug substrates of FMO3

Drugs that are substrates for human FMO3 are listed in Table 2. Many are substrates also for FMO1. However, owing to the absence of FMO1 in adult human liver (see above), the majority of FMO-mediated metabolism in this organ is carried out by FMO3.

Most drug substrates of FMO3 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 (Cashman et al., 1999b), 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 (Rodriguez & Miranda, 2000), which are converted initially to an *N*-hydroxyamine, then, via a second oxygenation, to a nitrone; and thiocarbamides, such as ethionamide (Henderson et al., 2008), which are converted to a sulfoxide. *N*-oxides and sulfoxides are polar and readily excretable and, thus, FMO3-mediated metabolism of tertiary amines and sulfides represents a detoxification. In contrast, some of the *N*-hydroxylation products of primary and secondary amines can inhibit CYPs, with potential toxic effects.

In the case of some substrates, for instance, amphetamine (Cashman et al., 1999b), cimetidine (Cashman et al., 1993; Hai et al., 2009), nicotine (Park et al., 1993), pargyline (Phillips et al., 1995) and sulindac sulfide (Hamman et al., 2000), FMO3 is stereoselective in the production of product.

Most drug substrates of FMO3 are also substrates of other enzymes, particularly CYPs, and the FMO3-catalyzed reaction does not represent the major route of metabolism. However, in individuals who possess genetic variants that decrease the catalytic activity of CYPs, the contribution of FMO3 to drug metabolism is likely to be greater. An example is that of nicotine, which is metabolized predominantly by CYP2A6 (Cashman et al., 1992; Nakajima et al., 1996), but also by FMO3 (Park et al., 1993) (see below). The metabolism of a drug by CYPs and FMOs usually results in the production of distinct products; in the case of aliphatic tertiary amines, CYP-mediated oxidation usually results in *N*-dealkylation, whereas FMOs produce exclusively the *N*-oxide (Cashman, 2008).

Drugs that are likely to be metabolized exclusively or predominantly by FMOs include the nonsteroidal anti-inflammatory benzydamine (Lang & Rettie, 2001), the gastroprokinetic dopamine D2 blocker itopride (Mushiroda et al., 2000), the antihistamine olopatidine (Kajita et al., 2002), the monoamine oxidase type B inhibitor pargyline (Phillips et al., 1995), the antiulcerative histamine H2-receptor antagonist ranitidine (Overby et al., 1997; Chung et al., 2000) and the M1 muscarinic agonist xanomeline (Ring et al., 1999), via *N*-oxygenation, and the antihelmitic albendazole (Rawden et al., 2000), the histamine H2-receptor antagonist cimetidine (Cashman et al., 1993; Overby et al., 1997; Hai et al., 2009), the antitubercular ethionamide (Henderson et al., 2008) and sulindac sulfide, the active metabolite of the nonsteroidal anti-inflammatory prodrug sulindac (Hamman et al., 2000), via *S*-oxygenation.

In the case of adult human liver the FMO that catalyzes the oxygenation of these drugs is FMO3.

Clinical effects of genetic variants of *FMO3*

Trimethylaminuria

Individuals who are homozygous or compound heterozygous for genetic variants that abolish or severely impair the production or activity of FMO3 have a markedly reduced capacity for metabolizing dietary-derived trimethylamine to its *N*-oxide and, consequently, suffer from the inherited disorder primary trimethylaminuria (see above) (reviewed in Mitchell & Smith, 2000; Phillips et al., 2007; Phillips & Shephard 2008, 2015; Shephard et al., 2015).

The incidence of heterozygous carriers in the white British population is 0.5-1.0% (Al-Waiz et al., 1987), giving an estimated frequency of the disorder in this population of about 1 in 40,000 (Shephard et al., 2015), but carrier incidence may be higher in other populations (Mitchell et al., 1997).

When present in the homozygous state, the relatively common compound variant p.[(Glu158Lys); (Glu308Gly)] may cause mild or transient trimethylaminuria, particularly in infants and young children (Zschocke et al., 1999; Zschocke & Mayatepek, 2000), who have low amounts of FMO3 as a consequence of immature expression of the *FMO3* gene (Koukouritaki et al., 2002).

Effects on drug metabolism

Individuals who possess genetic variants causative of trimethylaminuria are expected to exhibit an impaired ability to metabolize any drug substrate of FMO3 and, in the case of

benzydamine, this has been confirmed (Mayatepek et al., 2004). However, one such variant, c.182A>G[p.(Asn61Ser)], although abolishing the ability of FMO3 to catalyze *N*-oxygenation of trimethylamine, has no effect on the *S*-oxygenation of methimazole (Dolphin et al., 2000). In addition, common genetic variants of *FMO3* are known to influence the metabolism of drug substrates of FMO3 *in vitro* (see above), and a number of studies have contributed to our understanding of the impact of these variants on drug metabolism *in vivo*.

Sulindac, a nonsteroidal anti-inflammatory used for the treatment of familial adenomatous polyposis, is a prodrug. It is converted by gut bacteria from a sulfoxide to sulindac sulfide, the active form of the drug, which is then absorbed (Duggan et al., 1977; Etienne et al., 2003) and metabolized by FMO3 back to the inactive sulfoxide and, then, to sulindac sulfone (Hamman et al., 2000). In patients with familial adenomatous polyposis undergoing treatment with sulindac, the FMO3 variants c.472G>A[p.(Glu158Lys)] and c.923A>G[p.(Glu308Gly)] are associated with regression of existing polyps and protection against adenoma formation, particularly in those homozygous for the compound variant p.[(Glu158Lys);(Glu308Gly)] (Hisamuddin et al., 2004, 2005). Both variants decrease the ability of FMO3 to catalyze the oxygenation of sulindac *in vitro* (Hamman et al., 2000) and healthy individuals homozygous for p.[(Glu158Lys);(Glu308Gly)] metabolize the active drug more slowly than those homozygous for the ancestral allele (Tang et al., 2017). It is thought that reduction of FMO3 activity *in vivo*, as a consequence of these variants, would result in higher, more persistent concentrations of active drug, leading to increased drug efficacy and a positive effect on clinical outcome.

A genetic variant of *FMO3* can result in an increase in the toxic side effects of a drug. Although c.769G>A[p.(Val257Met)] has little effect on FMO3-catalyzed *S*-oxygenation of

methimazole *in vitro* (Dolphin et al., 2000), the variant is associated with antithyroid (methimazole) drug-induced agranulocytosis in Han Chinese being treated for Graves' disease (He et al., 2019). The results suggest that the variant, which is common in this population, would be a useful predictor of antithyroid drug-induced agranulocytosis in Chinese patients undergoing methimazole treatment of hyperthyroidism.

The *N*-oxygenation of the antihistamine ranitidine is catalyzed by FMO3 both *in vitro* (Overby et al., 1997) and *in vivo* (Kang et al., 2000). The compound variant p.[(Glu158Lys);(Glu308Gly)] decreases ranitidine *N*-oxygenation *in vitro* (Park et al., 2002) and individuals heterozygous or homozygous for p.[(Glu158Lys);(Glu308Gly)] excrete lower amounts of ranitidine *N*-oxide in their urine than those homozygous for the ancestral, p.[(Glu158);(Glu308)], allele (Kang et al., 2000; Park et al., 2002).

N-oxygenation of the gastroprokinetic agent itopride also is catalyzed by FMO3 *in vitro* (Mushiroda et al., 2000) and *in vivo* (Zhou et al., 2014) and is considered the major route of metabolism of the drug. In a study of Chinese, individuals homozygous for p.[(Glu158Lys);(Glu308Gly)] had higher plasma concentrations of itopride and lower concentrations of itopride *N*-oxide than those homozygous for the ancestral allele, p.[(Glu158);(Glu308)] (Zhou et al., 2014). In the case of ranitidine and itopride, it is not known whether reduction in the metabolism of the drug, as a consequence of the compound variant allele p.[(Glu158Lys);(Glu308Gly)], translates into improved efficacy and clinical outcome or to an increase in drug toxicity.

Although FMO3 catalyzes the *N*-oxygenation of the antipsychotic clozapine *in vitro* (Tugnait et al., 1997), none of the variant alleles c.472G>A[p.(Glu158Lys)],

c.923A>G[p.(Glu308Gly)] or c.769G>A[p.(Val257Met)] had an effect on clozapine *N*-oxide production *in vivo* (Sachse et al., 1999).

The antipsychotic olanzapine can be hydroxylated or *N*-demethylated *in vitro* by CYPs, in particular CYP1A2, CYP2D6 and CYP2C8 (Ring et al., 1996; Korprasertthaworn et al., 2015), and *N*-oxygenated by FMO3 (Ring et al., 1996). The formation of the *N*-oxide *in vitro* by the FMO3 compound variant p.[(Glu158Lys);(Glu308Gly)] was less than that by the ancestral form, p.[(Glu158);(Glu308)] (Cashman et al., 2008). Studies *in vivo* found that in patients homozygous for p.[(Glu158Lys);(Glu308Gly)], the C/D (mean dose-adjusted steady-state serum concentration) of olanzapine *N*-oxide was ~50% lower than in those heterozygous or homozygous for the ancestral form, p.[(Glu158);(Glu308)], but that the variant allele had no effect on the plasma concentration of olanzapine (Söderberg et al., 2013). In a study of Japanese, genotypes of *CYP2D6* and *FMO3* were found not to be associated with the rate of clearance of olanzapine, the conclusion being that the drug is subject to multi-pathway metabolism and if one pathway is less effective then others compensate (Okubo et al., 2016). The predominant pathway of olanzapine metabolism *in vivo* is direct glucuronidation, mediated by uridine diphosphate-glucuronosyltransferase 1A4 (UGT1A4) (Linnet, 2002). Thus, although variants of *FMO3* can have a considerable effect on the amount of the metabolite produced by the enzyme, olanzapine *N*-oxide, they would have little impact on the overall clearance of the drug.

Another example of a drug substrate of FMO3 that is subject to multi-pathway metabolism is the stimulant nicotine. The major pathway of nicotine metabolism is mediated by CYP2A6 (Cashman et al., 1992; Nakajima et al., 1996), with only 4-7% of nicotine equivalents being excreted as its *N*-oxide (Benowitz et al., 1994), a product formed by FMO3 (Cashman et al.,

1992). However, in individuals homozygous for a deletion of *CYP2A6* as much as 30% of absorbed nicotine is excreted as the *N*-oxide (Yamanaka et al., 2004). Genetic variants of *FMO3* that influence nicotine metabolism (Teitelbaum et al., 2018), would, therefore, be expected to be of greater importance in individuals with compromised CYP2A6 activity, and there is evidence that this is the case (Perez-Paramo et al., 2019).

Although drug-drug interactions are thought to be rare for substrates of FMO3 (Cashman, 2008), the enzyme is subject to competitive inhibition *in vitro* by alternative substrates (Shimizu et al., 2015). FMO3-catalyzed *N*-oxygenation of benzydamine was inhibited by trimethylamine, methimazole, itopride and tozasertib, and *S*-oxygenation of sulindac sulfide by methimazole. In some cases, suppression of activity was greater for variants of FMO3, such as p.[(Glu158Lys);(Glu308Gly)], than for the ancestral form of the enzyme. The results suggest that individuals exposed to more than one FMO3 substrate might experience unexpected consequences in drug response, particularly those homozygous for genetic variants that reduce enzyme activity.

Disease association

Genetic variants of *FMO3* have been implicated in a number of diseases other than trimethylaminuria. FMO3 is involved in the metabolism of nicotine (Benowitz et al., 1994) (see above). In cases of sudden infant death syndrome homozygotes for the variant c.472G>A[p.(Glu158Lys)] were more common than in controls, particularly in cases where the mother was a heavy smoker, suggesting that c.472G>A[p.(Glu158Lys)] is a genetic risk factor in children whose mothers smoke (Poetsch et al., 2010).

Although FMO3-catalyzed *N*-oxygenation is a relatively minor pathway of nicotine metabolism (Benowitz et al., 1994), the variant c.923A>G[p.(Glu308Gly)] is associated with reduced nicotine dependence (Teitelbaum et al., 2018). This is thought to be due to decreased *N*-oxygenation of nicotine in the brain, an organ in which FMO3, but not CYP2A6 (the major nicotine-metabolizing enzyme in the liver), is expressed.

The c.472G>A[p.(Glu158Lys)] variant has been reported to be associated in a Russian population with increased risk of hypertension in smokers but not in non-smokers (Bushueva et al., 2014). In a Turkish population, both the c.472G>A[p.(Glu158Lys)] and c.923A>G[p.(Glu308Gly)] variants were found to be associated with increased risk of ischemic stroke in hypertensive individuals, whereas in obese individuals the risk was higher for the ancestral form of the alleles (Türkanoğlu Özçelik et al., 2013). Other studies, however, in Irish and European- and African-Americans, found no association between these variants and hypertension (Dolan et al., 2005; Bryant et al., 2019).

A genome-wide association study identified a single-nucleotide polymorphism (SNP) (rs1795240) located just downstream of the *FMO3* gene as being the most highly associated with differences in the volume of the lentiform nucleus (Hibar et al., 2013), which has been implicated in several heritable degenerative and psychiatric disorders, including Parkinsonian syndromes, Huntingdon's disease, Wilson's disease, Tourette's syndrome and attention deficit hyperactivity disorder. However, the mechanism by which the SNP might affect lentiform nucleus volume is unknown.

In comparison with children homozygous for the ancestral allele, p.[(Glu158);(Glu308)], those homozygous for the p.[(Glu158Lys);(Glu308Gly)] variant had lower plasma insulin

concentrations, better insulin sensitivity and were protected against obesity (Morandi et al., 2018).

Studies on mice have suggested a number of roles for FMO3 itself in health and disease, including modulation of glucose and lipid homeostasis (Shih et al., 2015), involvement in cholesterol metabolism and reverse cholesterol transport (Bennett et al., 2013; Warrier et al., 2015), and as a target for down-regulation by insulin (Miao et al., 2015).

Trimethylamine* N-*oxide and disease

Trimethylamine *N*-oxide, a product of FMO3-catalyzed oxygenation of the dietary-derived compound trimethylamine (see above), has been implicated in a number of diseases as a cause, consequence or biomarker (reviewed in Fennema et al., 2016).

Cardiovascular disease

It has been suggested that elevated plasma concentrations of trimethylamine *N*-oxide, as a consequence of production of trimethylamine from dietary supplements of choline, carnitine or trimethylamine *N*-oxide by gut flora and its subsequent oxygenation by FMO3 in the liver, increase the risk of cardiovascular disease (Wang et al., 2011; Bennett et al., 2013; Koeth et al., 2013; Tang et al., 2013; Obeid et al., 2016) and are a predictive marker for near- and long-term cardiovascular events (Li et al., 2017).

Identification of trimethylamine *N*-oxide as a causative factor for cardiovascular diseases is controversial. A causative link between trimethylamine *N*-oxide and cardiovascular disease is counterintuitive because consumption of marine fish, the richest dietary source of trimethylamine *N*-oxide (Zhang et al., 1999), is strongly associated with a lower incidence of

the disease (Takata et al., 2013). Other studies found either that trimethylamine *N*-oxide was not correlated with biomarkers of cardiovascular disease (Miller et al., 2014) or that its effect was protective (Fukami et al., 2015) and carnitine, a dietary precursor of trimethylamine, has proven efficacious for treatment of cardiovascular disease (Flanagan et al., 2010; DiNicolantonio et al., 2013). Furthermore, no health benefits have been reported as a consequence of the lack or low production of trimethylamine *N*-oxide in those affected by primary trimethylaminuria.

Much of the work that implicates trimethylamine *N*-oxide as a causative factor in cardiovascular disease is based on studies of genetically modified (*ApoE*$^{-/-}$) mice predisposed to development of atherosclerosis and involves chronic administration of precursors of trimethylamine in amounts far in excess of those present in normal diets or therapeutic supplements. In one such study mice ingested, over a 15-week period, ~2g/kg/day of L-carnitine (Koeth et al., 2013), a dose ~1,000-fold greater than that obtained by a 70-kg human by daily consumption of a 250-g steak. In contrast, a study of wild-type mice and two different *Fmo*-knockout mouse lines fed a standard chow diet found that trimethylamine *N*-oxide is negatively correlated with plasma cholesterol concentration and not correlated with an index of atherosclerosis, (total plasma cholesterol - HDL cholesterol/HDL cholesterol) (Veeravalli et al., 2018), indicating that under normal dietary conditions trimethylamine *N*-oxide does not act as a proatherogenic molecule. Although atherosclerosis indices increased in mice in response to a high-fat diet, supplementation of the high-fat diet with trimethylamine *N*-oxide resulted in a significant decrease in atherosclerosis indices to below that of mice fed a standard chow diet (Gao et al., 2014).

ApoE$^{-/-}$ mice have been extensively used as a model of atherosclerosis; however, they lack cholesteryl-ester transfer protein (CETP), a key enzyme in reverse cholesterol transport (Barter et al., 2003). *ApoE*$^{-/-}$ mice transfected with CETP provide a more appropriate model for progression of human cardiovascular disease. Studies of such mice found that trimethylamine *N*-oxide was protective against, not causative of, cardiovascular disease (Collins et al., 2016).

An additional problem associated with extrapolation of experimental results from mouse to human is the marked species-dependent gender difference in expression of FMO3 between mouse and human. In mice, at about six weeks of age expression of the *Fmo3* gene is switched off in the liver of males, but not of females (Falls et al., 1995; Janmohamed et al., 2004). After this age male mice produce no detectable hepatic FMO3 and, thus, in comparison with females, produce far less trimethylamine *N*-oxide.

Other diseases

Trimethylamine *N*-oxide has also been implicated in prostate (Mondul et al., 2015) and colorectal cancer (Xu et al., 2015). However, as the authors of the latter study point out, the *N*-oxide is a gut microbial metabolite of dietary meat and fat and, thus, may be a marker, rather than a direct cause, of the disease. Another study found that high plasma concentrations of trimethylamine *N*-oxide were not associated with an increased risk of colorectal cancer (Guertin et al., 2017).

Trimethylamine *N*-oxide has been implicated in chronic kidney disease (Tang et al., 2015), although, because the *N*-oxide is released from the renal medulla into plasma following kidney damage (Serkova et al., 2005), its plasma concentration may be a proxy for the extent

of renal damage. Another study of chronic kidney disease found that patients heterozygous or homozygous for the c.472G>A[p.(Glu158Lys)] variant had higher plasma concentrations of trimethylamine *N*-oxide and an ~2-fold increased mortality risk, but that trimethylamine *N*-oxide itself was not associated with clinical outcome (Robinson-Cohen et al., 2016). In this case, elevated trimethylamine *N*-oxide may be a marker for variability of FMO3 activity.

Higher plasma concentrations of trimethylamine *N*-oxide are associated with type-2 diabetes in humans (Dambrova et al., 2016), and, in mice fed a high-fat diet, dietary trimethylamine *N*-oxide was found to exacerbate impaired glucose tolerance and perturb the hepatic insulin signaling pathway (Gao et al., 2014). In contrast, lower plasma concentrations of trimethylamine *N*-oxide have been associated with inflammatory bowel disease and with active versus inactive ulcerative colitis (Wilson et al., 2015).

Knockdown of *Fmo3* expression protects mice from high-fat-diet-induced obesity by stimulation of 'beiging' of white adipose tissue, that is, the conversion of the tissue to brown adipose tissue (Schugar et al., 2017). However, dietary provision of trimethylamine *N*-oxide was unable to reverse *Fmo3*-knockdown-driven reduction of body weight or of enhanced beiging of white adipose tissue, suggesting that the mechanism by which FMO3 modulates these factors is independent of trimethylamine *N*-oxide. It should be noted that, as a consequence of the gender-specific silencing of expression of *Fmo3* (Falls et al., 1995; Janmohamed et al., 2004) (see above), adult male mice are natural liver-specific knockouts of *Fmo3*.

Variation in the gut microbiome has been linked to several disease states. As trimethylamine *N*-oxide is a metabolite of a gut-microbiome-derived molecule (trimethylamine), its plasma

concentration may reflect changes in gut microbiome composition. Thus, elevated plasma concentrations of trimethylamine *N*-oxide may be a consequence of changes in the gut microbiome, which in turn could be an underlying cause or effect of the disease. In either case, trimethylamine *N*-oxide would be a biomarker, rather than a causative factor of the disease.

The physiological roles of trimethylamine *N*-oxide and the evidence for and against trimethylamine *N*-oxide being detrimental for health have been reviewed (Ussher et al., 2013; Ufnal et al., 2015; Cho & Caudill, 2017; Nowiński & Ufnal, 2018).

Genetic variants of other FMOs

FMO2

The variant c.1414C>T [p.(Gln472*)] (Dolphin et al., 1998), the *FMO2*2A* allele, is present at a frequency of essentially 100% in all populations, with the exception of individuals of recent African descent (Dolphin et al., 1998; Whetstine et al., 2000). *FMO2*2A* encodes a truncated polypeptide that lacks 64 amino-acid residues from its carboxy-terminus and is catalytically inactive (Dolphin et al., 1998). The ancestral allele, *FMO2*1*, which encodes a full-length catalytically active protein (FMO2.1) (Dolphin et al., 1998), is present throughout sub-Saharan Africa, in some regions attaining frequencies of up to 26%, with almost 50% of individuals possessing at least one *FMO2*1* allele (Veeramah et al., 2008). Several other variants have been identified (Whetstine et al., 2000; Furnes et al., 2003), some of which abolish catalytic activity (Krueger et al., 2005). However, the deleterious variants occur on the *FMO2*2A* allele (Krueger et al., 2005). Individuals who possess a *FMO2*1* allele, thus, are likely to express an active protein. The main site of expression of *FMO2* is the lung

(Hernandez et al., 2004) and the presence of full-length active FMO2 in lung microsomes isolated from an *FMO2*1* individual has been confirmed (Krueger et al., 2002).

FMO2 catalyzes the *S*-oxygenation of the organophosphate insecticides phorate and disulfoton (Henderson et al., 2004a), which represents a detoxification pathway, and of thioureas (Henderson et al., 2004b), in this case producing sulfenic or sulfinic acid derivatives, which are more toxic than the parent compound. Thus, individuals who express functional FMO2 may have reduced risk of toxicity if exposed to organophosphates, but a potentially greater risk of pulmonary toxicity following exposure to thioureas.

Drug substrates of FMO2 include the thyroperoxidase inhibitor methimazole (Krueger et al., 2002) and the antituberculars ethionamide (Henderson et al., 2008; Francois et al., 2009) and thiacetazone (Francois et al., 2009). In the case of ethionamide, the importance of FMO2 in the metabolism of the drug has been confirmed *in vivo* through the use of a knockout-mouse line (Palmer et al., 2012). To be effective, ethionamide must be taken up by mycobacteria in the lungs and metabolized, by the bacterial monooxygenase EtaA, to the *S*-oxide and subsequently to a sulfinic acid metabolite. The latter or a product of its decomposition is thought to be the therapeutic agent (Vannelli et al., 2002). Metabolism of ethionamide in the lungs by FMO2 to the sulfoxide and, subsequently, to potentially toxic sulfenic and sulfinic acids would be expected to reduce drug efficacy, by decreasing the amount of prodrug available for uptake by mycobacteria, and increase harmful side effects. Therefore, the distribution of *FMO2*1* and *FMO2*2A* alleles among world populations has implications for interethnic and, in Africans, interindividual variation in susceptibility to toxic chemicals and in response to drugs that are substrates of FMO2.

FMOs 1, 4 and 5

Few nonsynonomous variants have been identified in the genes encoding FMO1, FMO4 and FMO5 and each occurs at low or very low frequency and is often restricted to a single population group (Furnes et al., 2003; Koukouritaki & Hines, 2005; Phillips et al., 2007). Of these, only one is known to affect enzyme activity *in vitro*: c.1504C>T[p.(Arg502*)] of FMO1, which abolishes activity towards methimazole, but has little or no effect on activity towards three other substrates (Furnes & Schlenk, 2004). A relatively common variant, 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 transcription *in vitro*, but was not correlated with lower *FMO1* expression in fetal liver *in vivo* (Hines et al., 2003).

In the case of FMO1 and FMO5, the paucity of genetic variants is consistent with the roles of these enzymes in the regulation of endogenous metabolic processes, including energy metabolism (FMO1) (Veeravalli et al., 2014) and metabolic ageing and glucose homeostasis (FMO5) (Gonzalez Malagon et al., 2015; Scott et al., 2017).

Conclusions

In humans, in contrast to almost all other mammals investigated, FMO1 is not expressed in adult liver and the major hepatic drug-metabolizing FMO is FMO3. Although FMO3 can catalyze the oxygenation of a broad range of drugs *in vitro*, most are metabolized *in vivo* by other enzymes, particularly CYPs, and the FMO3-catalyzed reaction is not the major route of metabolism. Some drugs, however, are metabolized exclusively or predominantly in human liver by FMO3.

Rare genetic variants that severely affect the production or activity of FMO3 give rise to the inherited disorder primary trimethylaminuria. In addition to defective *N*-oxygenation of trimethylamine, affected individuals will have an impaired ability to metabolize drug substrates of FMO3. More common, coding-region variants of *FMO3* have been identified that affect activity of the enzyme, particularly the double variant p.[(Glu158Lys);(Glu308Gly)], which results in a moderate reduction in activity that is substrate-dependent. Individuals that are heterozygous or homozygous for this variant exhibit moderately impaired metabolism of some drug substrates of FMO3 *in vivo*, which can result in an increase in drug efficacy or toxicity. Although FMO3 may make a minor contribution to the metabolism of a drug, in cases where an individual is homozygous for a genetic variant that results in a substantial deficiency in production or activity of the major metabolic enzyme the contribution of FMO3 will become more significant and genetic variants of *FMO3* more relevant to metabolism of the drug.

Common genetic variants of *FMO3* have been associated with an increased or decreased risk of certain disorders, including sudden infant death syndrome, hypertension, some neurological conditions and obesity. In some cases the evidence is contradictory and for all cases additional studies are needed to confirm or refute such associations.

High plasma concentrations of trimethylamine *N*-oxide have been associated with a number of diseases, particularly cardiovascular disease. Trimethylamine *N*-oxide is the product of an FMO3-mediated host-microbiome metabolic axis (Fennema et al., 2016); its production is influenced by diet, gut microbiome composition and the amount and activity of hepatic FMO3. The causative effect of trimethylamine *N*-oxide in disease states is controversial, with some studies indicating that it has no pathological effect or that it is protective. Evidence

suggests that for some diseases an elevated plasma concentration of trimethylamine *N*-oxide is a biomarker of the condition, rather than a direct causative factor. As such, trimethylamine *N*-oxide would not represent a therapeutic target, but would be an indicator of disease risk or progress.

In contrast to *FMO3*, the genes encoding FMO1, FMO4 and FMO5 have few polymorphic variants. In the case of *FMO2*, most of the world's population is homozygous for a nonsense mutation, and, thus, do not produce functional FMO2. An exception being individuals of recent African descent, many of whom express a full-length functional protein. Consequently, in African populations, there is interindividual variation in the metabolism of environmental pollutants and therapeutic drugs that are substrates of FMO2.

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Figure Caption

Figure 1. Catalytic cycle of mammalian FMOs. 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-hydroflavin (step 3). Water is then released, reforming FAD (step 4), followed by the release of $NADP^+$ (step 5). The cycle can also undergo uncoupling, producing H_2O_2 from the C4a-hydroperoxyflavin, with the release of $NADP^+$, to reform FAD (dashed lines). Based on Ziegler (2002) and Siddens et al. (2014).

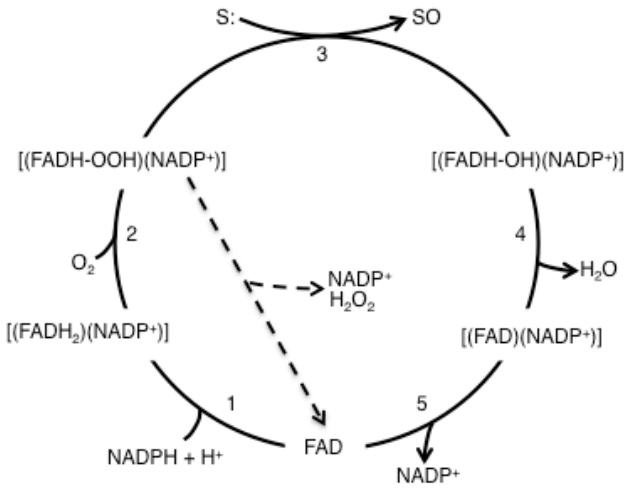

Table 1. Common genetic variants in the coding region of *FMO3*

Variant	rs number	Minor allele frequency[a]			Effect on activity *in vitro*	Reference
		Afr	Asn	Eur		
c.394G>C[p.(Asp132His)]	12072582	0.042	0.0001	0.00007	substrate-dependent decrease	Furnes et al., 2003; Lattard et al., 2003
c.441C>T[p.(Ser147=)]	1800822	0.080	0.190	0.053	no effect	Cashman, 2002
c.539G>T[p.(Gly180Val)]	75904274	0.003	0.004	0.016	no effect	Dolphin et al., 2000
c.472G>A[p.(Glu158Lys)]	2266782	0.464	0.283	0.420	moderate substrate-dependent decrease	Brunelle et al., 1997; Dolphin et al., 1997b
c.769G>A[p.(Val257Met)]	1736557	0.035	0.132	0.065	substrate-dependent decrease	Treacy et al., 1998; Dolphin et al., 2000
c.830T>C[p.(Val277Ala)]	2066530	0.049	0.0001	0.00006	n.d.	Cashman, 2002
c.855C>T[p.(Asn285=)]	909530	0.440	0.284	0.252	no effect	Cashman, 2002
c.923A>G[p.(Glu308Gly)]	2266780	0.035	0.096	0.202	moderate substrate-dependent decrease	Treacy et al., 1998
c.1084G>C[p.(Glu362Gln)]	2066532	0.014	0	0.00003	n.d.	Cashman, 2002; Furnes et al., 2003

[a] Frequencies are from the genome Aggregation Exomes Database (gnomAD-Exomes) and are based on the number of minor alleles in more than 15,000 chromosomes from African (Afr), 47,000 from Asian (Asn) and 130,000 from European (Eur) individuals.

n.d., not determined.

Table 2. Drug substrates of human FMO3

Drug	Class	Reaction	Analytical system	References
Albendazole	anthelmitic	*S*-oxygenation	liver microsomes; recombinant protein	Rawden et al., 2000
Almotriptan	antimigraine 5-HT1B, 1D receptor agonist	*N*-oxygenation	liver microsomes; recombinant protein	Salva et al., 2003
Amphetamine[a]	dopamine transporter ligand (antipsychotic)	*N*-oxygenation	recombinant protein	Cashman, et al., 1999b
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	liver microsomes; recombinant protein; in vivo	Shaffer et al., 2007
Benzydamine	nonsteroidal antiinflammatory	*N*- oxygenation	liver microsomes; recombinant protein; in vivo	Lang & Rettie, 2001; Störmer et al., 2001; Mayatepek et al., 2004
C-1311 (5-diethylaminoethylamino-8-hydroxyimidazoacridinone)	antitumor agent	*N*-oxygenation	liver microsomes; recombinant protein	Potega et al., 2011
Cimetidine	histamine H2-receptor antagonist	*S*-oxygenation	liver microsomes; recombinant protein; in vivo	Cashman et al., 1993; Overby et al., 1997
Clozapine	antipsychotic dopamine D2, 5'HT2 and 5'HT1C receptor antagonist	*N*-oxygenation	liver microsomes; purified protein	Tugnait et al., 1997
Danusertib	aurora kinase inhibitor	*N*-oxygenation	recombinant protein	Catucci et al., 2013
Dapsone	sulfone antibiotic	arylamine oxidation	keratinocytes; recombinant protein	Vyas et al., 2006

Dasatinib	BCR-ABL and SRC family kinase inhibitor	*N*-oxygenation	liver microsomes; recombinant protein	Wang et al., 2008
Deprenyl	monoamine oxidase type B inhibitor	*N*-oxygenation	liver microsomes; recombinant protein	Szökő et al., 2004
N,N-diallyltryptamine	pschychostimulant	*N*-oxygenation	liver microsomes; recombinant protein	Wagmann et al., 2016
Disulfiram metabolite, *s*-methyl *n*,*n*- diethyldithiocarbamate[b]	antialcoholic	*S*-oxygenation	liver microsomes; recombinant protein	Pike et al., 1999
Ethionamide	antitubercular	*S*-oxygenation	recombinant protein	Henderson et al., 2008
GSK5182 (4-[(Z)-1-[4-(2-dimethylaminoethyloxy)phenyl]-hydroxy-2-phenylpent-1-enyl]phenol	antidiabetic, estrogen-related receptor γ modulator	*N*-oxygenation	liver microsomes; recombinant protein	Joo et al., 2015
Itopride	dopamine D2 blocker and acetylcholinesterase inhibitor	*N*-oxygenation	liver microsomes; recombinant protein; in vivo	Mushiroda et al., 2000; Zhou et al., 2014
K11777	peptidomimetic, cysteine protease inhibitor	*N*-oxygenation	liver microsomes; recombinant protein	Jacobsen et al., 2000
N-deacetyl ketoconazole[c]	antifungal	*N*-hydroxylation	recombinant protein	Rodriguez & Miranda, 2000
L-775,606	5-HT(1D) receptor agonist	*N*-oxygenation	liver microsomes	Prueksaritanont et al., 2000
Loxapine	Tricyclic antipsychotic	*N*-oxygenation	liver microsomes	Luo et al., 2011
S-Methamphetamine[d]	psychostimulant	*N*-hydroxylation	recombinant protein	Cashman et al., 1999b
Methimazole	thyroperoxidase inhibitor	*S*-oxygenation	liver microsomes; recombinant protein	Overby et al., 1997

S-methyl esonarimod[e]	cytokine production inhibitor	*S*-oxygenation	liver microsomes; recombinant protein	Ohmi et al., 2003
MK-0457 (Tozasertib)	aurora kinase inhibitor	*N*-oxygenation	liver microsomes; recombinant protein	Ballard et al., 2007; Catucci et al., 2013
MK-0767 methyl sulphide	peroxisome proliferator receptor activator	*S*-oxygenation	liver microsomes; recombinant protein	Karanam et al., 2004
Moclobemide	monoamine oxidase type A inhibitor	*N*-oxygenation	recombinant protein	Hanlon et al., 2012
Nicotine	adenosine receptor ligand, stimulant	*N*-oxygenation	recombinant protein; in vivo	Park et al., 1993; Bloom et al., 2013
Nomifensine	antidepressant	*N*-oxygenation	recombinant protein	Yu et al., 2010
Olanzapine	multi receptor antagonist, antipsychotic	*N*-oxygenation	liver microsomes; in vivo	Ring et al., 1996; Söderberg et al., 2013
Olopatadine	antihistamine, histamine H1 receptor-selective antagonist	*N*-oxygenation	recombinant protein	Kajita et al., 2002
Pargyline	monoamine oxidase type B inhibitor	*N*-oxygenation	recombinant protein	Phillips et al., 1995
Perazine	antipsychotic	*N*-oxygenation	liver microsomes; recombinant protein	Störmer et al., 2000
Phospho-sulindac	nonsteroidal anti-inflammatory	*S*-oxygenation	liver microsomes; recombinant protein	Xie et al., 2012
Primaquine	antimalarial	not known	recombinant protein; primary hepatocyes	Jin et al., 2014
Procainamide	type I antiarrhythmic agent	*N*-oxygenation	liver microsomes; recombinant protein; in vivo	Li et al., 2012
Pyrazolacridine	antitumour	*N*-oxygenation	liver microsomes; recombinant protein	Reid et al., 2004

Ranitidine	Histamine H2-receptor antagonist, antiulcerative	*N*- and *S*-oxygenation	liver microsomes; recombinant protein; in vivo	Overby et al., 1997; Chung et al., 2000; Kang et al., 2000
S16020	topoisomerase II inhibitor, antitumour	*N*-oxygenation	primary hepatocytes; recombinant protein	Pichard-Garcia et al., 2004
Selenomethionine	anticancer agent	*Se*-oxygenation	recombinant protein	Hai et al., 2010
Sulfamethoxazole	Sulphonamide bacteriostatic antibiotic	arylamine oxidation	keratinocytes; recombinant protein	Vyas et al., 2006
Sulindac sulfide[f]	nonsteroidal anti-inflammatory	*S*-oxygenation	liver microsomes; recombinant protein; in vivo	Hamman et al., 2000; Tang et al., 2017
Tamoxifen	antiestrogen, estrogen receptor modulator	*N*-oxygenation	liver microsomes; recombinant protein	Parte & Kupfer, 2005
Tazarotenic Acid[g]	retinoic acid receptor modulator	*S*-oxygenation	liver microsomes; recombinant protein	Attar et al., 2003
TG100435 [7-(2,6- Dichloro-phenyl)-5-methyl-benzo[1,2,4]triazin-3-yl]-[4-(2-pyrrolidin-1-yl-ethoxy)-phenyl]-amine	Src kinase inhibitor	*N*- oxygenation	liver microsomes; recombinant protein	Kousba et al., 2007
Thiacetazone	antitubercular	*S*-oxygenation	recombinant protein	Qian & Ortiz de Montellano, 2006; Francois et al., 2009
Trifluoperazine	calmodulin antagonist	*N*-oxygenation	liver microsomes; recombinant protein	Lomri et al., 1993
Vadimezan (5,6-dimethylxanthenone-4-acetic acid (DMXXA)	anticancer agent, cytokine inducer	methyl hydroxylation	liver microsomes; recombinant protein	Zhou et al., 2002

Voriconazole	antifungal	*N*- oxygenation	liver microsomes; recombinant protein	Yanni et al., 2008
Xanomeline	M1 muscarinic agonist	*N*- oxygenation	liver microsomes; recombinant protein	Ring et al., 1999

[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]methamphetamine hydroxylamine is further converted by FMO action to a mixture of nitrones, which are subsequently hydrolyzed 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.