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The implications of polymorphisms in mammalian flavin-containing monooxygenases in drug discovery and development

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Use of the human flavin-containing monooxygenases (FMOs) in drug design and discovery could represent a paradigm shift in drug development and basic research. Although FMOs have been previously viewed as minor contributors to drug metabolism, the advantages associated with using FMOs to diversify the metabolism of a drug are now being recognized. Because FMOs typically oxygenate a wide variety of nucleophilic compounds to polar, benign metabolites, and because drugs do not induce expression of FMOs or inhibit their activity, potential drug–drug interactions are minimized. Interindividual variation for this class of enzyme is largely dependent on genetic variation. Examples of FMO allelic variation and splicing variants suggest that these genetic mutations could contribute to the interindividual and interethnic variability of FMO-mediated metabolism.

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▼ The flavin-containing monooxygenases (FMOs) comprise a family of FAD-, NADPH- and molecular oxygen-dependent microsomal enzymes [1,2]. FMOs constitute what is arguably the second most important human monooxygenase system, after cytochrome P450s (CYPs), and catalyze the oxygenation of many nitrogen-, sulfur-, phosphorous-, selenium- and other nucleophilic heteroatom-containing chemicals and drugs [3,4]. To date, research has identified five different genes that encode for functional forms of FMO proteins that show 50–58% amino acid identity across species lines. With the characterization of human genes encoding for FMOs, a nomenclature based on nucleotide sequence identity was adopted [5]; *FMO* genes with ≥82% identity are grouped within a family. Recently, evidence for several splicing variants has considerably altered the picture of the FMO family of proteins (Figure 1). In principle, this variation in FMO splicing could have a considerable affect on the interindividual variation of functional activity [6]. In addition to splicing variants, a significant number of allelic variants have been observed for FMOs. Single nucleotide polymorphisms (SNPs), other missense, nonsense, deletion and truncation mutants of human FMO have also been reported [7–9].

Of the five functional human FMOs known, FMO3 appears to be the most important FMO present in adult human liver. Based on immunoreactivity investigations, FMO3 is expressed at levels approaching 60% of the expression levels of the major CYP present in adult human liver (CYP3A4) [10]. Interestingly, FMO3 is not detectable in fetal liver, where FMO1 predominates attaining expression levels of ~32% of expressed CYP3A7 (based on relative-activity measurements) [11]. Thus, the *FMO* gene family appears to show a pronounced tissue- and temporal-selective expression pattern. Considering FMO1, the functional activity of this protein is temporally linked to the birth process but not gestational age. By contrast, birth is a requirement for the expression of FMO3, but the birth process alone is not sufficient to induce FMO3 expression; therefore, other, unknown factors must also induce FMO3 expression. Furthermore, FMO3 expression is low or absent in neonates [12]. At present, the molecular basis for the regulation of the postnatal expression of FMO3 is not fully understood. With the exception of FMO5, which reaches protein expression levels that

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approximate those of FMO3, other FMOs are not expressed to a discernible degree in adult human liver.

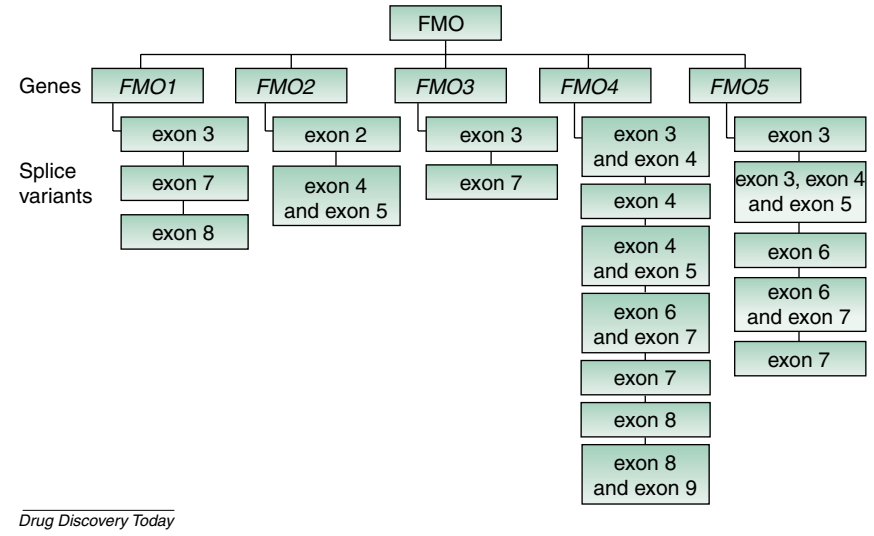

Figure 1. FMO splice variants. The genes encoding for FMOs (i.e. *FMO1–FMO5*) each have a variable number of prominent splice variants at the exons indicated in the boxes (i.e. *FMO1* has prominent splice variants at exons 3, 7 and 8). Abbreviation: FMO, flavin-containing monooxygenase.

Interindividual differences in FMO

Although the role of FMOs in human drug metabolism is not as widely documented as that of CYPs, recent examples indicate that FMOs play a prominent role in the metabolism of clinically important drugs. FMOs typically catalyze the oxygenation of nucleophilic compounds to benign, polar metabolites that are readily excreted, which could be considered advantageous from a pharmacokinetic standpoint [13]. Furthermore, drugs do not readily induce expression of FMOs or inhibit their functional activity. Consequently, the lack of induction of FMO expression by small molecules suggests that drug–drug interactions might be decreased for those small molecules that are predominantly metabolized by FMO, thus the contribution of FMO to the metabolism and detoxication of a chemical or drug potentially presents an attractive reason for their incorporation into the drug development process [4,13]. Although alternate substrates of FMO could be competitively inhibited by strong nucleophiles (e.g. strongly nucleophilic S-containing compounds), the majority of amine drugs are unlikely to act as competitive inhibitors. Furthermore, a lack of inhibition of FMO expression could also reduce drug–drug interactions.

For drugs that are cleared primarily by oxidative processes, it is thought that successful drug candidates are metabolized by multiple enzyme systems, including FMO, thus decreasing the reliance on one dominant enzyme. For example, if a drug is principally cleared by CYP2D6 or CYP2C19, which show genetic polymorphisms, considerable individual variability to clinical response might be observed. If one enzyme is largely responsible for the metabolism of a drug, the inhibition or induction of this enzyme could lead to abnormal levels of the drug, which could increase drug–drug interactions or decrease efficacy. The advantages associated with using the FMO system in drug detoxication might spur an increased use of functional groups in drug candidates that favor oxygenation by FMO. The observation that environmental chemical or drug exposure does not induce FMO suggests that it is the genetic variation observed for this family of enzymes that is responsible for interindividual variation. Understanding FMO pharmacogenetics in drug development might help to identify individuals that will respond more favorably to a drug, lead to a reduction in the time taken to get the drug to market and decrease the failure rate of new drug candidates.

Interindividual differences of FMO from *in vitro* studies

Traditionally, human liver microsomes have provided an effective *in vitro* measure of human drug metabolism. Studies of FMO3 functional activity in adult human liver microsomes have shown considerable intersample variability. For example, in the presence of highly characterized human liver microsomes, a 6.6-fold variation in (*S*)-nicotine *N*-1′-oxygenation activity [14] and a 2.4-fold variation in cimetidine S-oxygenation activity [15] were observed. For such studies, it is important to use microsomes in which selective functional FMO substrate oxygenation is highly correlated with immunoreactivity [16]. Furthermore, it is important to identify and to differentiate FMO functional activity variability that is the result of the thermal inactivation of microsomes [17] (i.e. resulting from mishandling of the tissues, improper preparation of the microsomes or storage of the microsomes) [12]. Thermal inactivation of FMO leading to a loss of activity can also occur during prewarming of the microsomes in the absence of NADPH. Thus, it is prudent to initiate FMO-dependent metabolism by addition of the substrate and not NADPH. In view of the thermal instability and the variability of FMO3, it is important to use well-characterized microsomes (i.e.

Table 1. Amino acid substitutions and functional activity of human FMO1, FMO2, FMO4 and FMO5 genetic variants

Protein	Amino acid substitution	Predicted or apparent functional activity of variant[a]	Refs
FMO1	H97Q	~WT	[40]
	T249T	WT	[7]
	I303V	~WT	[40]
	I303T	~WT	[40]
	V396V	WT	[7]
	R502X	Decreased	[40]
FMO2	D36G	Decreased	[7]
	D71insertion	Decreased	[7]
	F81S	~WT	[7]
	F182S	~WT	[7]
	S195L	~WT	[7]
	R238Q	Decreased	[40]
	R249X	Decreased	[7]
	E314G	Decreased	[7]
	R391T	Decreased	[40]
	N413K	Decreased	[7]
	X472Q	Decreased	[40]
FMO4	I37T	~WT	[40]
	V323A	~WT	[7]
	E339Q	Decreased	[40]
FMO5	P337P	WT	[7]
	S351P	Decreased	[7]
	P457L	Decreased	[40]

[a]Functional activity based on *in vitro* or *in vivo* measurements or 'best guess estimate'.
Abbreviations: FMO, flavin-containing monooxygenase; WT, wild-type.

microsomes with high correlation coefficients for functional activity and immunoreactivity) and, perhaps, to genotype the *FMO* of the microsomes used.

Common FMO polymorphisms

Significant variation in the frequencies of single- and multiple-site alleles, haplotypes and genotypes of FMO has been observed in DNA from healthy individuals. For example, based on the *Homo sapiens* chromosome 1 working draft sequence from the locus link of GenBank® (http://www.ncbi.nlm.nih.gov/Genbank/index.html), FMO1, FMO2, FMO3, FMO4 and FMO5 have 34, 57, 40, 30, and 40 genomic DNA variants present, respectively [7]. The FMO genomic variants translated into 2, 9, 19, 1 and 2 coding region variants for FMO1, FMO2, FMO3, FMO4 and FMO5, respectively (examples for FMO1, FMO2, FMO4 and FMO5 are presented in Table 1). The large number of *FMO3* variants reported in GenBank® could be because more data has been deposited for that gene. A similar picture has emerged from the data generated by the Environmental Genome Project (http://www.niehs.nih.gov/envgenom/home.htm).

FMO3 functional activity and polymorphisms

Full-length mRNA encoding for FMO3 is abundant in adult human liver but is rare in adult human kidney and fetal liver [18,19]. In tissue where the expression level of FMO3 is lower than typical levels or where other FMOs are present, the dominant FMO functional activity is less clear. In general, human FMO3 appears to oxygenate nucleophilic heteroatom-containing substrates that are slightly smaller than those accepted by human FMO1. Analogous to other FMOs, splicing variants are apparent for human FMO3. In total adult human and fetal brain extracts, FMO3 transcripts were not detectable. Two-step PCR amplification showed that two splice variants (skipping of exon 3 and exon 7) were observed in all tissues examined (adult liver, adult kidney and fetal liver), but only low levels were detected in adult human or fetal brain tissues. However, two-step PCR amplification showed that full-length FMO3 expression was the prominent FMO3 species in all the human brain, kidney and liver samples examined [6]. Expression of FMO3 from exon-deleted cDNA resulted in the production of an enzyme that was unable to catalyze the *N*- or S-oxygenation of standard substrates [6]. Thus, loss of 63 amino acids probably leads to the modification of protein structure and loss of FMO3 functional activity.

Investigation of the allelic frequencies of prominent *FMO3* polymorphisms in the DNA of healthy male and female Caucasians, Hispanics, Asians and African Americans [8,20,21] revealed significant differences for *FMO3* allelic frequencies between these ethnic groups. Emerging data suggests that particular populations could be predisposed to the abnormal metabolism of drugs or chemicals by FMO3. Thus far, several apparent pharmacogenetic effects of FMO3 on drug metabolism have been observed. For example, *N*-oxygenation of ranitidine has been correlated to FMO3 expression in investigations using Korean volunteers [22]. Abnormal FMO3-mediated *N*-oxygenation of trimethylamine (TMA) also appears to be linked to *FMO3* polymorphisms [23]. However, *N*-oxygenation of clozepine is not an effective probe *in vivo* for FMO3 activity, possibly because the K_m value for the catalysis of the oxygenation reaction is high [24]. It has been suggested that *N*-oxygenation of caffeine could be used as an *in vivo* marker for FMO3 functional activity, but the viability of this has yet to be confirmed [25]. Perhaps the best studied relationship

between *FMO3* polymorphisms and function is the association between *FMO3* genetic variants and TMA metabolism [9,26,27]. The prominent polymorphisms, amino acid substitutions and predicted functional activity of FMO3 variants are listed in Table 2.

Functional substrates for phenotyping FMO3

An important aspect of phenotyping FMO3 activity is to use substrates that are highly selective for FMOs such as TMA [28], benzydamine [23], (*S*)-nicotine [14], cimetidine [15], itopride [29], clozapine [24,30] and ranitidine [22].

In some cases [e.g. (*S*)-nicotine and cimetidine], stereoselective oxygenation has been used to advantage in the phenotyping of human FMO3 *in vivo* [31,32]. Adult human FMO3 catalyzes the *N*-oxygenation of (*S*)-nicotine to form the *trans*-isomer of (*S*)-nicotine *N*-1′-oxide exclusively, whereas oxygenation with FMO1 produces a mixture of *cis*- and *trans*-nicotine *N*-1′-oxide [14]. Studies have shown that following administration of (*S*)-nicotine to humans, the only product detected is *trans*-(*S*)-nicotine *N*-1′-oxide. Further investigations revealed that the interindividual variation in the concentration of *trans*-(*S*)-nicotine *N*-1′-oxide in the urine of human smokers, smokers infused with (*S*)-nicotine-d_2 and smokers infused with (*S*)-nicotine administered via the dermal route varied by 3.3-fold, 11.3-fold and 7.1-fold, respectively [32]. These results are in agreement with previous studies that showed significant interindividual variability in (*S*)-nicotine metabolism in humans [33]. However, the high K_m of (*S*)-nicotine oxygenation by human FMO3 and the low concentration of *trans*-(*S*)-nicotine *N*-1′-oxide formed *in vivo* illustrate the disadvantages of using (*S*)-nicotine as a probe substrate of FMO3 in human smokers [33].

The interindividual variation of the S-oxygenation of cimetidine is significant but is not as marked as that observed for the *N*-1′-oxygenation of (*S*)-nicotine [15]. The interindividual variability pharmacokinetic parameters (i.e. area under the curve, $t_{1/2}$ and clearance) for S-oxygenation of cimetidine were determined to be in the range 1.7–2.6, which could be the result of FMO genetic variability, presystemic intestinal metabolism or small population sampling issues. Despite the low variability calculated for the pharmacokinetic parameters, the similarity in the ratios of the stereoisomers of cimetidine S-oxide formed in human liver microsomes and that observed *in vivo* suggests that cimetidine S-oxide formation is a useful stereoselective probe of human FMO3 functional activity.

Other FMOs: functional activity and polymorphisms

Pig liver FMO1 is the most extensively studied member of the FMO family and much of our knowledge about FMO enzyme mechanism and substrate specificity has been derived from studies of this enzyme [34,35]. Whereas the livers of most small animals are rich in FMO1, it is not abundant in adult human liver; however, functional FMO1 is the predominant FMO found in fetal human liver. Although FMO1 is the most abundant FMO in adult human kidney and intestine, and is also present in the esophagus and nasal mucosa [36], it is possible that FMO1 does not contribute to the oxygenation of chemicals or the *N*-oxygenation of amine functionalities, unless the chemical or amine is present at elevated substrate concentrations or has a low K_m value. It appears that human FMO1 contains a more restricted substrate-binding domain than pig FMO1 [37] and, therefore, the substrates accepted by human

Table 2. Amino acid substitutions and functional activity of human FMO3 genetic variants

Amino acid substitution	Predicted or apparent functional activity of variant[a]	Refs
E32K	Decreased	[49]
A52T	Decreased	[56]
N61S	Decreased	[60]
M66I	Inactive	[51]
M82T	Decreased	[60]
D132H	Decreased	[8]
S147S	WT	[7]
G148X	Inactive	[59]
P153L	Inactive	[51,55]
E158K	Decreased	[51]
G180V	~WT	[60]
I199N	Decreased	[7]
F239F	WT	[7]
V257M	~WT	[51,60]
V277A	~WT	[7]
E305X	Inactive	[51,55]
E308G	Decreased	[51,58]
E314X	Inactive	[57]
L360P	Increased	[21]
E362Q	Decreased	[7]
R387L	Inactive	[57]
M434I	~WT	[60]
I486M	~WT	[7]
R492W	Inactive	[57]
G503R	Decreased	[40]
M661I	~WT	[26]

[a]Functional activity based on *in vitro* or *in vivo* measurements or 'best guess estimate'.
Abbreviations: FMO, flavin-containing monooxygenase; WT, wild-type.

FMO1 could be somewhat different from substrates that have been proposed based on data generated from small-animal FMO studies. Human FMO1 appears to accept larger *N*- and S-containing nucleophiles than human FMO3 [37]. For example, the side chains of the tertiary amine nitrogen of imipramine, orphenadrine and chlorpromazine are *N*-oxygenated by human FMO1 [37]. Disulfiram, which is an alcohol-deterrent agent, could potentially be a tissue-selective FMO1 substrate. Reduction of disulfiram to *N*,*N*-diethyldithiocarbamate followed by metabolic S-methylation produces an S-methyl metabolite that is efficiently S-oxygenated by FMO1 [38]. Although the contribution of human kidney microsomal FMO1-mediated S-oxygenation of the S-methyl metabolite is twofold to threefold greater than CYPs, the clinical significance of these results is unclear because the human kidney has at least 14-fold less metabolic capacity than the human liver.

Full-length FMO1 is the predominant species in adult kidney, fetal liver and fetal brain, but not in adult brain and adult liver [10,11,39]. An alternative splice variant (which could be the result of SNPs) that lacked exon 3 (i.e. a 189 bp fragment) was detected in all tissues. Skipping of exon 7 or exon 8 results in a frameshift, but skipping of exon 3 does not modify the reading frame [6]. Significant interindividual differences in expression have been noted for FMO1 [12]. Initially, two SNPs were detected in the human *FMO1* gene. However, the variation did not result in changes in the amino acid sequence of the protein [7]. More recently, seven SNPs were identified in DNA from African American [40] individuals (Table 1) that were not detected in DNA from Caucasian individuals [41].

To date, no information about the cDNA-expression of FMO1 variants has been reported. However, previous studies have shown that truncated FMO1 (23 amino acids removed) possessed 7% of the activity of wild-type FMO1 [42]. Based on this observation, it is possible that the R502X truncation variant will have some, but not full, FMO1 functional activity. Many of the other FMO1 variants are synonymous or encode amino acid changes for a structurally similar amino acid. It is thought that the activity of FMO1 would be largely unaffected by the amino acid substitutions that have been observed. The lack of functional substrates that are highly selective for FMO1 and the smaller contribution of the kidney, intestine and other non-hepatic tissues to drug metabolism could make the *in vivo* characterization of the role of FMO1 variants in abnormal chemical or drug metabolism difficult. However, because FMO1 is the major FMO detected in fetal human liver, there could be instances of altered metabolism in the fetus that might produce drug–drug interactions that are formed by FMO1 variants. The role of FMO1 as a metabolic sentry enzyme for other tissues could also be important.

FMO2 functional activity and polymorphisms

FMO2 is expressed at high levels in the lungs of small animals, with the exception of the domestic rat [43]. Although FMO2 is also expressed at high levels in non-human primates, it is not a prominent enzyme in human lung [44]. To date, all Caucasians and Asians examined are homozygous for a Q472X truncation mutation that results in the expression of non-functional protein [45]. Approximately 26.0% of African Americans have one normal allele and express full-length functional protein, whereas 4.5% of the Hispanic population expresses the functional protein [27]. Other polymorphisms affecting expression of FMO2 have been reported [46].

Recombinant full-length rabbit, monkey and human FMO2 have been expressed and evaluated for functional activity [45,47]. The Q472X truncation mutant was also expressed. No functional activity was observed for the Q472X truncation mutant but the wild-type enzyme possessed good activity for S-oxygenation of S-containing chemicals such as thioureas and thioamides [27,47]. Wild-type FMO2 appears to oxygenate S-containing chemicals more efficiently than *N*-containing chemicals. Because the sulfenic acids, which are formed from thioureas, are chemically reactive, humans with full-length FMO2 might be more susceptible to the toxicity of particular S-containing chemicals [48].

FMO4, FMO5 and FMO6 functional activity and polymorphisms

The abundance of adult hepatic mRNA encoding for FMOs is in the order FMO3≫FMO5≫FMO4 [49]. Because FMO4 is present at low concentrations and FMO5 appears to be an atypical FMO that is devoid of the functional activity associated with FMOs, FMO3 is the protein that is linked to the majority of FMO-mediated hepatic metabolism. One difficulty encountered with the characterization of FMO4 and FMO5 functional activity is the lack of a highly selective substrate, which significantly detracts from establishing a role of SNPs in the metabolism of drugs or chemicals.

Human FMO4 splice variants are the most complex scenario of all the FMO family members examined [6]. Full-length and splice variant forms (i.e. exon 4, exon 7 or exon 6 and exon 7 skipping variants) of FMO4 have been identified. Full-length FMO4 was detected in kidney and liver and was detected in only low levels in brain. Two-step PCR amplification showed that a complex pattern of FMO4 transcripts were present in the tissues examined [6] (Figure 1).

A few SNPs for human FMO4 and FMO5 have been reported [7] (amino acid changes predicted from the coding region of variants of FMO4 and FMO5 are listed in Table 1). The V323A variant is unlikely to possess significantly different functional activity compared to wild-type enzyme. For FMO5, the P337P SNP did not result in an amino acid change, but the S351P variant is likely to alter the functional activity of FMO5 significantly. Predicting changes in functional activity of FMO4 and FMO5 is hindered by a deficiency of selective substrates and a lack of robust functional activity. To date, neither FMO4 nor FMO5 have been observed to have high levels of functional activity. It is not clear whether this arises from proteolytic instability or some other mechanism that leads to enzyme inhibition. A sixth form of human FMO (i.e. FMO6) has been observed in humans [50], but this is a pseudogene and is unlikely to contribute to FMO-mediated metabolism.

FMO variants and human disease: clinical considerations

Among the human FMOs, FMO3 exclusively *N*-oxygenates TMA (TMA is not readily *N*-oxygenated by FMO1 or other FMOs) and, thus, provides a convenient means to phenotype human FMO3 [28,51]. Furthermore, the catalytic oxygenation of TMA by FMO3 facilitates the study of trimethylaminuria, which is a rare condition that causes the accumulation of unmetabolized TMA in the urine, sweat and breath that consequently results in an abnormal odor-syndrome [27]. There could be complex reasons for the manifestation of trimethylaminuria, but the best understood form that accounts for the majority of reported cases is the primary genetic form [27]. Other forms of trimethylaminuria exist, but it is the FMO3 common allelic variation and more rare mutations [7–9] that are responsible for the mild and more severe forms of this condition, respectively. Typically, human FMO3 converts greater than 95% of TMA to TMA *N*-oxide and an unaffected individual has 0–9% unmetabolized TMA in their urine, sweat or breath. Individuals with 10–39% unmetabolized TMA suffer from mild trimethylaminuria, whereas individuals having unmetabolized urinary TMA of greater than 40% are classified as suffering from severe trimethylaminuria. The incidence of trimethylaminuria has been reported to be between 0.1% and 1.0% in Caucasian populations [26], but it is probable that this condition is more prevalent in populations in the tropics; however, the molecular basis for this prevalence is unknown [52]. A study of North American individuals suffering from self-reported trimethylaminuria showed that 35% of the individuals had the syndrome [53]. For example, the allele frequency of E158K and E308G is as high as 20% in some European populations, and 2–5% of the healthy, otherwise normal general population probably carries the homozygous variant allele. It is thought that individuals with the dual mutant (i.e. E158K and E308G) might have mild trimethylaminuria and possibly other impaired sulfide and/or amine metabolism [54].

Studies of the British and Australian populations identified a phenotype–genotype relationship that showed some rare mutations (including P153L, M66I and E305X) caused severe trimethylaminuria [7,9,27]. cDNA-expression of the rare variants associated with severe trimethylaminuria indicated that defective FMO3 exhibited minimal or no TMA *N*-oxygenation functional activity. Additional trimethylaminuria-causing mutations have been identified in individuals from North America (e.g. E32K, A52T, E314X, R387L, G475D and M66I) [55–61] and additional rare mutations continue to be reported [9] (Table 2). Recently, a deletion mutation was observed that resulted in a frame shift and caused premature termination of the *FMO3* gene immediately after codon 65 [61]; the individual carrying this mutated DNA developed trimethylaminuria.

FMO in drug metabolism and development

Recently, several examples have been reported that highlight the potential advantages of using FMO-mediated transformations in drug metabolism and, in particular, in drug development. The tertiary amine itopride is primarily *N*-oxygenated to the tertiary amine *N*-oxide by FMO [29]. By contrast, the related gastroprokinetic agents cisapride and mosapride are largely metabolized by CYP3A4-mediated *N*-dealkylation. The change in metabolism pathway from CYP to FMO probably results in a decrease in the potential drug–drug interactions for itopride. However, the life-threatening ventricular arrhythmias and QT-interval prolongation that are associated with the concomitant administration of CYP3A4 inhibitors (which cause elevated plasma concentrations of cisapride) are unlikely to occur with the use of itopride. Because FMO3 activity is not readily inhibited by drugs, the probability of observing the accumulation of unmetabolized itopride is low. Thus, for itopride, a metabolism shift from CYP3A4-mediated metabolism to FMO-mediated metabolism could potentially result in fewer drug–drug interactions.

Another example that illustrates the characteristics of FMO metabolites that could be exploited in drug development stems from the observation that some *N*-oxygenated amines possess superior pharmaceutical properties when compared with the parent amines [13]. For example, the *N*-oxygenation of amidines produces amidoximes. Amidines have poor absorption and bioavailability properties but amidoximes are five pK_a units less basic than the parent amidine and have much more favorable pharmaceutical

properties [62]. Although not all amidines are substrates for FMO, the recognition that amidoximes could be prodrugs for drugs such as pentamidine, sibrafiban and melagatran, and that FMO-mediated metabolism could considerably improve the bioavailability of these drugs, has led to the use of this approach in drug development [62]. Furthermore, tertiary amine *N*-oxides are used as prodrugs for anticancer agents that are selectively reduced to the active compound in tumor cells [63].

Conclusions

Because environmental chemical exposure does not induce expression of FMO, any functional variation observed for this class of enzyme is largely dependent on genetic variation. Studies of human FMO have shown that FMO3 contributes to the oxygenation of nucleophilic heteroatom-containing chemicals and drugs and this affords more polar metabolites that are, therefore, more water-soluble and more readily excreted. Although exceptions have been observed, FMO is generally considered a detoxication catalyst. It is possible that FMOs evolved to protect humans from exposure to nucleophilic plant toxins and to convert plant materials to non-toxic metabolites. In this regard, it is speculated that different populations in specific geographical regions maintained variant FMO in the gene pool to detoxify particular toxins and foodstuffs. Diversification of the *FMO3* gene might have conferred a selective advantage on an individual carrying the mutated gene, because this mutation could lead to the expression of a protein that has a novel functional activity.

Recent studies of human *FMO3* have shown that several rare alleles are responsible for the underlying molecular mechanism that is responsible for trimethylaminuria. The observation that mutations in the *FMO3* gene lead to abrogated or significantly decreased TMA metabolism could signify differences in the FMO-mediated metabolism of chemicals or drugs by diverse populations. High-throughput-genotyping methods have identified several common genetic polymorphisms. Future research could reveal that the common *FMO3* variants contribute to the intermediate phenotypes that make up some common diseases or syndromes. Furthermore, examples of *FMO3* allelic variation show that these mutations could contribute to the interindividual and interethnic variability of FMO-mediated metabolism.

A growing body of information suggests that there are advantages associated with FMO-mediated metabolism of a drug or chemical. The structures of several drugs have been modified to incorporate FMOs as part of a multiple metabolic and detoxication pathway, which consequently diversifies the dependence on CYP and other metabolic enzymes. As drug development strategies incorporate the use of FMO3 in the metabolism and disposition of new chemical entities, a focus on individuals with different *FMO3* genotypes will undoubtedly be a consideration in the successful development of a drug.

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