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HUMAN FLAVIN-CONTAINING MONOOXYGENASES

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■ **Abstract** This review summarizes recent information concerning the pharmacological and toxicological significance of the human flavin-containing monooxygenase (FMO, EC 1.14.13.8). The human FMO oxygenates nucleophilic heteroatom-containing chemicals and drugs and generally converts them into harmless, polar, readily excreted metabolites. Sometimes, however, FMO bioactivates chemicals into reactive materials that can cause toxicity. Most of the interindividual differences of FMO are due to genetic variability and allelic variation, and splicing variants may contribute to interindividual and interethnic variability observed for FMO-mediated metabolism. In contrast to cytochrome P450 (CYP), FMO is not easily induced nor readily inhibited, and potential adverse drug-drug interactions are minimized for drugs prominently metabolized by FMO. These properties may provide advantages in drug design and discovery, and by incorporating FMO detoxication pathways into drug candidates, more drug-like materials may be forthcoming. Although exhaustive examples are not available, physiological factors can influence FMO function, and this may have implications for the clinical significance of FMO and a role in human disease.

INTRODUCTION

N- or *S*-oxygenation of nucleophilic heteroatom-containing small molecules has been known for many years, but it was assumed these reactions were catalyzed by cytochrome P450 (CYP)* (1). It was not until another hepatic microsomal enzyme was purified to homogeneity and shown to oxygenate dimethylaniline in the presence of NADPH that it was concluded that the flavin-containing monooxygenase (FMO) was distinct from CYP-mediated activity (2). The stoichiometry, substrate

*Abbreviations: Cytochrome P450, CYP; flavin-containing monooxygenase, FMO; trimethylamine, TMA; quantitative polymer chain reaction, Q PCR; hypoxanthine phosphoribosyl transferase, HPRT; single nucleotide polymorphism, SNP; nitric oxide, NO; area under the curve, AUC; clearance, CL; total parental nutrition, TPN; familial adenomatous polyposis, FAP.

specificity, and biochemical properties of FMO from pig liver microsomes were described in the 1970s largely by Ziegler & Poulson (3). Today, the significance of the human enzyme has increased as more compounds have been recognized to be substrates for FMO (4, 5). In the early 1980s, two groups observed that the pulmonary form of FMO was distinct from the hepatic form with regard to enzyme properties and substrate specificity (6, 7). Despite these important studies, information about FMO was meager until FMOs from multiple species and tissues were either cloned or purified (8, 9). Important contributions from Philpot and colleagues (9), Phillips and colleagues (10), Hines and colleagues (8, 11), Ballou and colleagues (12), Hodgson and colleagues (13), Williams and colleagues (14), and Rettie and colleagues (15) all contributed to advancing knowledge of FMO mechanism, regulation, and structure-function relations. In the 1990s, considerable knowledge of general catalytic and regulatory aspects was developed (16). The number of reported *FMO* genes in humans is six, although *FMO6* is a pseudogene (11). Recently, an additional cluster of five human FMO genes was discovered that apparently are also pseudogenes (17).

The general function of FMO is considered to be a xenobiotic detoxication catalyst. Similar to CYP, it is believed that FMO evolved to protect mammals from an onslaught of lipophilic nucleophilic chemicals in the early environment (18). In some cases, FMO function can be quite selective. This is exemplified by the detoxication and deoderation of trimethylamine (TMA) (19). The inability to *N*-oxygenate TMA because of defective FMO3 causes a condition called trimethylaminuria (20). On the other hand, FMO is involved in the oxygenation of a wide range of heteroatom-containing compounds, presumably to remove unwanted natural products in the diet (e.g., amine-, sulfide-, phosphorus-, and other nucleophilic heteroatom–containing compounds). Generally, FMO converts lipophilic xenobiotics to more polar, oxygenated, readily excreted metabolites. The catalytic rate of oxygenation is nominally about twice that of the catalytic rate for a typical CYP substrate (14). Like CYP, FMO appears to sacrifice considerable enzyme velocity to bind a wide range of substrates in its substrate binding domain (21). Sometimes, FMO oxygenates endogenous or physiological compounds to the corresponding oxide, and generally this decreases the pharmacological activity of the compound. It is notable that FMO strongly prefers not to accept physiologic nucleophiles (i.e., cysteine, glutathione) that would otherwise place a nonproductive futile cycle of NADPH consumption and thiol oxidation in place (8). On the other hand, examples exist where nucleophiles are converted to more oxygenated compounds with greater electrophilicity and participate in toxicological outcome (22–24). With the advent of more advanced technology based on PCR, additional roles of FMO in physiological processes of different organisms likely will be established.

NOMENCLATURE

FMO (EC 1.14.13.8) is an FAD- and NADPH-dependent microsomal enzyme that catalyzes the oxygenation of nucleophilic nitrogen-, sulfur-, phosphorus-, and other nucleophilic heteroatom–containing chemicals (5, 25). Initially defined as

dimethylaniline monooxygenase (26), the enzyme system is now recognized as a broad spectrum monooxygenase that accepts substrates as diverse as hydrazines, phosphines, boron-containing compounds, sulfides, selenides, iodide, as well as primary, secondary, and tertiary amines (5). However, not all enzymes that meet the above description are included in this family because of low sequence similarity (e.g., bacterial cyclohexanone monooxygenases). Over the years, various names have been given to the FMOs, but a systematic nomenclature was developed based on primary sequence identity (8, 9). Other terms for FMO have included flavin-mixed function monooxygenase and Ziegler's enzyme. Today, the designation FMO is for protein, and the prefix *FMO* is usually used to designate the genes.

The *FMO* gene family probably arose as a duplication of an ancestral gene or via a series of independent gene duplication events (17). FMOs with sequence identity >40% have been grouped in the same family, indicated by the first numeral (i.e., 1, 2, etc.) (8, 9). For the five forms of functional human FMO (i.e., FMO1 to FMO5), sequence identity ranges between 52% and 60%, with the exception of FMO3 and FMO6, which share 71% identity. *FMO* genes are located on the long arm of chromosome 1 (27, 28). A second cluster of human *FMO* genes are also located in another region of chromosome 1, but these are pseudogenes (17). The translation products are 532–558 amino acids in length and contain highly conserved FAD- and NADPH-binding domains (29, 30). Small base changes that may affect enzyme function are alleles, and as described below, they introduce considerable variability into the FMO enzymes (16, 31).

REGULATION AND SPECIES DIFFERENCES

FMO can be regulated at a number of levels, including expression of enzyme and modulation by cofactor supply, physiological factors, and diet. For active protein expression, FMO expression is both tissue and species dependent (25, 32). The developmental- and tissue-specific expression of FMOs has been characterized in a number of animal species, such as humans, mice, rats, and rabbits (8), using a number of methods, such as RNase protection assays to quantify mRNA (33, 34), Western blot analysis (35), Q PCR, and microsomal activity assays (36, 37), and has mainly focused on liver and kidney, where the majority of the metabolism of exogenous chemicals occur. Only limited animal studies, mostly in rat (38), indicated that FMO activity exists in brain, where substantial amounts of biogenic amines are present and carry out neurotransmission and other essential biological functions (16). Developmental- and tissue-specific expression profiles of FMOs are fairly distinct among different animal species. Therefore, studies in rat brain are not easily translatable for the understanding of FMO expression regulation in human brain. Utilizing real-time RT-PCR, we have systematically quantified mRNA levels of FMO1 to FMO5 in human brain tissues and examined developmental regulation and gender-dependence aspects (39). This study provided a systematic comparison of the mRNA level of FMO1 to FMO5 in the major organs of humans. As described below, FMO expression in human brain is very low.

Expression of Human FMOs 1–5

FMO1 is the most prevalent FMO in the adult kidney, and FMO1 expression in fetal liver and small intestine is 10- to 14-fold less than that in adult kidney. Expression of FMO1 in the lung constitutes only approximately 2.8% of FMO1 in the kidney. FMO1 in the brain is less than 1% of that of the kidney and is downregulated after birth. FMO2 is the dominant FMO in the adult lung, and FMO2 expression in the kidney is approximately sevenfold less than in the lung. Fetal liver and adult liver or small intestine FMO2 is expressed at only approximately 1% and 2% of that of FMO2 in lung, respectively. Brain FMO2 is expressed at less than 1% that of the lung and the amount is not different among any age group. FMO3 is a major FMO form in adult human liver, whereas for lung, kidney, fetal liver, and small intestine, FMO3 is present at 4.5%, 3.7%, 2.1%, and 1%, respectively, of the amount of adult liver. Brain FMO3 constitutes less than 1% of adult liver FMO3, and there is no difference in the amount of adult FMO3 as a function of age. FMO4 is most prevalent in adult liver and kidney, whereas fetal liver, small intestine, and lung contain approximately 10.9%, 10.8%, and 7.0%, respectively, of FMO4 of adult liver. Brain FMO4 represents less than 1% of the amount of FMO4 present in the liver and is not changed as a function of age. FMO5 was the most plentiful FMO present in adult liver, although fetal liver, small intestine, kidney, and lung contained considerable FMO5 at 18.8%, 12.8%, 9.8%, and 4%, respectively, of the amount present in adult liver. FMO5 is present in brain less than 1% that of adult liver and the concentration does not change as a consequence of age.

In contrast to previous human FMO reports (1, 4, 5), hepatic expression of FMO5 is equal to or greater than FMO3, the commonly reported dominant FMO form expressed in adult liver. This is in agreement with the report of elevated amounts of FMO5 expression in mouse liver (34). Although FMO5 represents $\geq$50% of the total FMO transcripts in adult human liver, the contribution of FMO5 enzyme functional activity has not been clearly established. FMO5 apparently does not catalyze the oxygenation of common FMO substrates (i.e., methimazole, ranitidine, cimetidine) (36), and a role of FMO5 in drug or chemical metabolism remains to be established. Another notable finding is the observation that the expression of fetal FMO1 is 10-fold downregulated compared with FMO1 in adult liver, and adult FMO3 is 50-fold upregulated compared with fetal FMO3, confirming a previous finding (35). Another finding is that, with the exception of FMO1, all other FMOs are expressed at greater levels in adult liver and adult brain compared with fetal liver and fetal brain. The results show that FMOs exhibit tissue- and developmental stage–specific patterns of expression (Table 1).

GENE ORGANIZATION

As described above, *FMO* genes are clustered together in chromosome 1. In general, within a family, similar patterns of *FMO* intron/exon organization exist. This supports the notion that *FMO* genes resulted in gene duplication. Approximately

TABLE 1 Tissue expression of FMOs[a]

	FMO1	FMO2	FMO3	FMO4	FMO5
Fetal brain	56.4	17.6	5.6	14.6	21.0
Adult brain	3.1	140.9	10.7	19.6	56.5
Fetal liver	945.7	93.1	445.6	488.3	4406.8
Adult liver	96.0	988.7	23,088.6	4881.7	26,539.5
Adult kidney	6198.2	4682.7	530.9	2509.9	1628.3
Adult lung	595.7	115,895.5	2223.9	738.1	2274.9
Adult small intestine	522.9	928.7	74.2	403.0	2586.3

[a]Data normalized to HPRT and expressed as copies per ng RNA.

4 Mb telomeric of the original *FMO* gene cluster is another cluster of five *FMO* genes (i.e., *FMO* 7P, 8P, 9P, 10P, and 11P) that shows the characteristics of *FMO* pseudogenes (17). Presumably, there were selective pressures to develop enzymes with new and advantageous function. It may have been that ancestral FMO was important in processing natural products because many such materials are detoxicated by FMO. Later, as humans were exposed to less of these materials, it may be that other, more region-specific issues placed selective pressure for molecular evolution. For example, certain FMOs may have evolved to detoxicate specific toxins. The prevalence of abnormal *FMO3* in individuals from the tropics and its role in TMA metabolism may be an example of evolutionary pressure to decrease metabolism of TMA so that TMA could be used as some primitive volatile insecticide (18, 20, 40).

GENETIC VARIATION

In some cases, single nucleotide polymorphisms (SNPs) of FMO are associated with dramatic functional differences in enzyme activity. For example, decreases in FMO activities are observed for 158/308 double mutants, and in other cases an increase in enzyme activity is seen for 360P variants (41). Although SNPs have been observed for all five functional human *FMOs*, observation of *FMO3* SNPs was spurred after detection of abnormal human TMA metabolism. Because human FMO3 is solely responsible for TMA *N*-oxygenation in vivo, and the majority of FMO-mediated adult hepatic metabolism, most of the published information about *FMO* SNPs has attempted to relate human *FMO3* genotype to phenotype (42, 43). Some *FMO3* mutations have been associated with trimethylaminuria, a rare metabolic disorder caused by the inability to *N*-oxygenate dietary-derived TMA. The best understood form of trimethylaminuria is the primary genetic form associated with dysfunctional enzyme arising from genetic mutations of *FMO3*.

Of significant relevance, however, are recent genetic studies showing that polymorphisms at the *FMO3* gene locus may play a role in variation of the *N*-oxygenation of certain amines, including drugs, dietary agents, and other xenobiotics (44–47). In healthy humans, approximately 13% of benzydamine is converted to its N-oxide in an FMO3-mediated process. In individuals with severe trimethylaminuria, a 2.3- to 4.2-fold decrease in the average percentage of benzydamine N-oxide formation was observed. This confirmed the assumption that severe FMO3 deficiency is associated with decreased FMO3-mediated drug metabolism (43). Common *FMO3* SNPs differ widely across ethnic groups, and it is possible that interethnic variation contributes to therapeutic drug variability for drugs metabolized by FMO3. In addition to common SNPs, other very rare variants have been described that can significantly affect enzyme activity (48, 49). In addition, an acquired form possibly arising from hepatitis (50), a transient form associated with menstruation (51), childhood forms (35), and substrate or precursor overload or other impaired disease states can lead to trimethylaminuria. A rare FMO3 360P SNP only observed in individuals of African origin has been associated with an extensive metabolizer phenotype, and although too few people have been characterized with this genotype, it is possible that it could contribute to greater FMO-mediated metabolism and possibly greater drug clearance (41). Another polymorphism affording very great FMO3 functional activity could arise from gene duplication. This has not been observed for FMO3, but SNPs in the promoter region have been associated with an eightfold increase in promoter activity (52). Other SNPs caused a near complete loss of promoter function (52). It is notable that loss of function promoter haplotype was observed in DNA samples from individuals of African or Caucasian descent, whereas the gain of function promoter haplotype was associated with Hispanics. The prevalence of the loss of function SNPs (i.e., a calculated 3%–7% of the population examined) located near the transcription start site may impact the ability of an individual to detoxicate drugs metabolized primarily by FMO3. It is possible that individuals with the loss of function promoter haplotype could also have decreased TMA *N*-oxygenation and be susceptible to trimethylaminuria. Further studies examining potentially affected populations should be examined. SNPs of the promoter region of *FMO* and other monooxygenases tend to be rarer than other SNPs in exons, but mutations can abolish binding of transcription factors and proteins that bind to enhancers, and FMO SNPs of this type should be examined further.

Transcriptional Regulation

Transcriptional regulation of FMO involving receptors that bind ligands and interact with DNA has not been as widely studied as other forms of regulation. However, the effects of hormones on FMO activity have been described. Estradiol plays a role in determining FMO activity in rat lung and kidney (53, 54). Rat FMO is positively regulated by testosterone and repressed by estradiol (55). Cortisol regulates hepatic FMO activity in female mice via diurnal secretion (56). *FMO2* mRNA

and protein expression peaks in the lung of pregnant rabbits at days 15 and 28–31, which correlates with progesterone and corticosterone plasma concentrations (8). Up to a 20-fold variation of FMO activity has been observed in the corpora lutea of the pig during estrous (57). The mechanisms controlling the expression of FMO have not been fully characterized, and much work along these lines is needed. One report suggested that *FMO1* is regulated by HNF1α and HNF4α (58). For human *FMO1*, an upstream SNP was identified that lies within the conserved core binding sequence for the yin yang (YY1) transcription factor. Transient expression assays showed this SNP could account for two- to threefold loss of *FMO1* promoter activity, and genotype analysis showed individuals of Caucasian, African, and Hispanic descent had 11%, 13%, and 30% frequency, respectively (59). In cultured rat hepatocytes, after administration of lipopolysaccharides and proinflammatory cytokines, mRNA levels of FMO1 decreased via a cGMP-independent destabilizing effect of nitric oxide (NO) rather than decreased transcription. It is possible that NO acts directly in a cGMP-independent mechanism, decreasing the half-life of FMO1 mRNA and decreasing FMO function in endotoxemia (60).

Posttranscriptional Regulation

Posttranscriptional regulation of FMO has likewise not received a great deal of attention. For example, not much is known about enhancement of FMO mRNA stability or selective stimulation of translation of FMO mRNA transcripts. FMOs have a consensus sequence for *N*-glycosylation and mass spectrometry studies have shown that FMO1 is selectively *N*-glycosylated at Asn 120 (61). The highly conserved nature of this site suggested that this region could be important in a structural or functional role in enzyme action, but cDNA expression of FMOs in bacterial expression systems showed that enzyme activity did not require *N*-glycosylation. Fusion of peptides or the N-terminal side of FMO with maltose-binding fusion proteins has resulted in an enzyme with greater stability than the nonfusion protein (62). Coupling a poly-His sequence at the C terminus allows for elaboration of a stable and a readily purified enzyme that has great specific activity (41). In terms of posttranslated modification, NO appears to modify FMO3. The mechanism is unclear but NO directly suppresses FMO activity in vitro, and dithiothreitol or ascorbate restores FMO3 activity (63).

INTERINDIVIDUAL DIFFERENCES OF FMO

In human drug metabolism the role of FMO is not as widely documented as that of CYP. However, FMO3 attains expression levels approaching 60% of the major CYP3A in adult human liver (64). In fetal liver, FMO1 attains expression levels of approximately 32% of CYP3A7 based on relative activity measurements (65). Because FMO is not induced, interindividual differences are due to genetics. Significant variation in the frequencies of single and multiple site alleles, haplotypes,

and genotypes of *FMO* have been observed in DNA from healthy individuals. Based on the *Homo sapiens* chromosome 1 working draft sequence from locus link of Genebank, *FMO1*, *FMO2*, *FMO3*, *FMO4*, and *FMO5* has 34, 57, 40, 30, and 40 genomic DNA variants (42). The *FMO* genomic variants translated into 2, 9, 19, 1, and 2 coding region variants for *FMO1*, *FMO2*, *FMO3*, *FMO4*, and *FMO5*, respectively. Below, we outline the in vitro and in vivo functional consequences of *FMO* SNPs.

Interindividual Differences of FMO: In Vitro Studies

Because FMO3 is a prominent enzyme in adult human liver, study of microsomal functional activity is a routine method to examine preclinical candidate metabolism by FMO3 (Figure 1). This picture has been confounded by the observation that FMO5 is the major FMO form present in adult human liver (66). However, FMO5 appears to have a narrow substrate specificity and low activity. For FMO substrates, intersample metabolism variability in the presence of adult liver microsomes is likely due largely to FMO3, although for certain substrates a role of FMO5 cannot be ruled out. The considerable FMO functional variability observed in adult liver microsomes may have a contribution from FMO5 as well. There are a number of ways to ascertain the contribution of FMO3 to the metabolism of a chemical or drug in adult liver preparations. Correlation analysis of the oxygenation of the substrate with immunoreactivity with well-characterized microsomes is typically the first approach (67). Verification of FMO activity with recombinant enzyme is a second useful step (67). Heat inactivation studies or use of the test compound as an alternate substrate competitive inhibitor in the presence of known substrates has also been successfully employed. Another approach is use of inhibitor phenotyping FMO3 studies using highly selective substrates such as TMA (19), benzydamine (68, 69), (*S*)-nicotine (70), cimetidine (71), itopride (72), clozapine (73), and ranitidine (44). Prototypical substrates of FMO3 such as nicotine and cimetidine, for example, have shown a 6.6-fold variation in (*S*)-nicotine

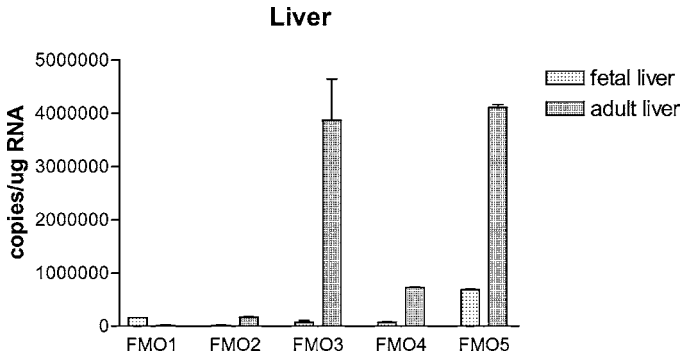

Figure 1 Copies of FMO1–5 in human liver.

N-1′-oxygenation activity (70) or a 2.4-fold variation in cimetidine *S*-oxygenation activity (71). It is important to use microsomes where immunoreactivity and selective functional FMO substrate oxygenation activity is highly correlated (74). Also, FMO functional activity variability owing to thermal inactivation of microsomes (i.e., from mishandling of the tissue, improper preparation of the microsomes, or improper storage of the microsomes) must be distinguished from that of variability owing to genetic variation. Postmortem inactivation of human FMO3 probably accounts for the majority of the lack of congruence where selective functional FMO3 activity is not correlated with FMO3 immunoreactivity. In view of the variability of FMO3 owing to tissue preparation or thermal inactivation or owing to genetic polymorphisms, it is important to use well-characterized microsomes (i.e., microsomes with high correlation coefficients for FMO functional activity and immunoreactivity). In the future, genotyping FMO from microsomes may become important.

Interindividual Variability of Human FMO3: In Vivo Studies

To verify a role of FMO in drug or chemical metabolism, in vivo studies are necessary. A few selective probes are available. In some cases [i.e., (*S*)-nicotine and cimetidine], stereoselective oxygenation has been used advantageously to study FMO3 (71, 74). Human FMO3 exclusively forms *trans* (*S*)-nicotine *N*-1′-oxide (71) and after administration of (*S*)-nicotine to humans, only *trans* (*S*)-nicotine *N*-1′-oxide was observed. The amount of *trans* (*S*)-nicotine *N*-1′-oxide in the urine of smokers, smokers infused with (*S*)-nicotine-d_2, or smokers administered (*S*)-nicotine via the dermal route varied 3.3-fold, 11.3-fold, and 7.1-fold, respectively (74). The results showed significant interindividual variability in (*S*)-nicotine metabolism in humans (74), which paralleled observations made about nicotine *N*-1′-oxygenation in vitro. However, the relatively minor amount of *trans* (*S*)-nicotine *N*-1′-oxide formed in vivo owing to the relatively high K_m of (*S*)-nicotine for human FMO3 represents a short-coming for using (*S*)-nicotine as a probe substrate of FMO3 in human smokers (74).

Because TMA is readily available from a large number of dietary precursors and because it is exclusively *N*-oxygenated by FMO3, it also provides a selective means to phenotype human FMO3 (20) in vivo and study trimethylaminuria. In individuals with trimethylaminuria, TMA accumulates in the urine, sweat, and breath, and it results in an abnormal odor syndrome. Other forms of trimethylaminuria exist, but it is the common allelic variation and more rare mutations that are responsible for the mild or more severe forms, respectively, of this condition. Severe trimethylaminuria occurs for individuals that have unmetabolized urinary TMA greater than 40%. Summaries of some of the common mutations responsible for the more severe forms of trimethylaminuria have been reported (20, 42, 75).

Cimetidine *S*-oxygenation provides a probe of FMO3 but not as selective as that observed for (*S*)-nicotine *N*-1′-oxygenation (71). Interindividual variability for

cimetidine *S*-oxygenation is less than that for nicotine based on pharmacokinetic parameters (i.e., AUC, $t_{1/2}$, and CL in the range of 1.7- to 2.6-fold), and this could be due to *FMO* genetic variability. Although other explanations are possible for in vivo cimetidine *S*-oxygenation variability, the similarity in the stereoselectivity of cimetidine *S*-oxide formed in human liver microsomes and that observed in vivo suggests it is a useful stereoselective probe of human FMO3 functional activity (76). Below, we summarize the functional variability for FMOs 1–5.

FMO1 FUNCTIONAL ACTIVITY AND SNPS

Human FMO1 apparently contains a more restricted substrate-binding domain than animal FMO1 (77) and accepts substrates that may be distinct in terms of size or shape than that based on small animal FMO1 studies. FMO1 is a prominent form of FMO in adult human kidney (Figure 2). FMO1 is also present in esophagus and nasal mucosa (8). It is unlikely that FMO1 contributes extensively to amine *N*- and other chemical oxygenation unless the amine or chemical is present at relatively elevated substrate concentrations or has a very low K_m value. There are examples that suggest human FMO1 accepts larger N- and S-containing nucleophiles than human FMO3 (78–80), but additional studies need to be done. For example, the side-chain tertiary amine nitrogen of imipramine, orphenadrine, and chlorpromazine are *N*-oxygenated by human FMO1 (77). Disulfiram, an alcohol deterrent agent, may be a possible tissue-selective FMO1 substrate (81). Reduction of disulfiram to *N*,*N*-diethyldithiocarbamate and metabolic *S*-methylation produces a prominent *S*-methyl metabolite that is efficiently *S*-oxygenated by FMO1 (81). Although the contribution of human kidney microsomal FMO1-mediated *S*-oxygenation of the *S*-methyl metabolite is 2- to 3-fold greater than CYP, the clinical significance is not clear because the human kidney has at least 14-fold less metabolic capacity than the human liver.

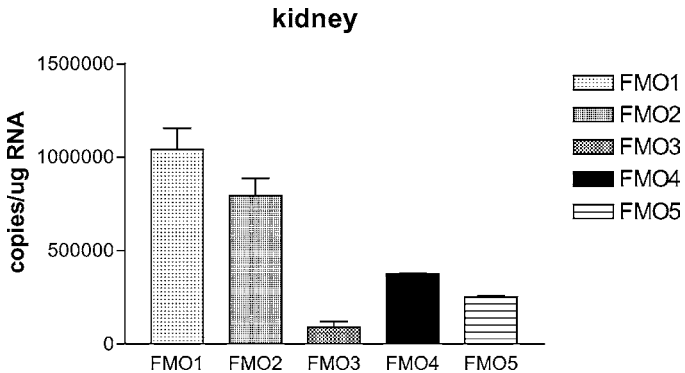

Figure 2 Copies of FMO1–5 in human kidney.

FMO1 shows significant interindividual differences in expression of functional activity. (35). Initially, two SNPs were detected in the human *FMO1* gene. However, the observed SNPs did not result in amino acid changes (42). Seven SNPs were then described for *FMO1* that were identified in DNA from African American (82) but not Caucasian individuals (59). cDNA-expression of FMO1 coding variants showed substrate-dependent kinetics (83). R502X showed wild-type *para*-tolylsulfide *S*-oxygenation activity, but the mutant abrogated methimazole *S*-oxygenation. Previous studies showed that truncated pig FMO1 (23 amino acids removed) possessed 7% of the tertiary amine *N*-oxygenation activity of wild-type FMO1 (84). H97Q and I303T FMO1 variants increased *para*-tolylsulfide *S*-oxygenation but slightly decreased *S*-oxygenation of methimazole. The I303V variant showed wild-type activity for both substrates (83). Many of the *FMO1* variants currently described in the literature are synonymous or encode amino acid changes for a very similar amino acid. Based on the functional activity of *FMO1* SNPs, it is likely FMO1 activity would be largely unaffected by the amino acid substitutions thus far observed. Because of the lack of highly selective functional substrates and the smaller contribution of FMO1 in kidney and other nonhepatic tissues, the role of FMO1 variants in abnormal chemical or drug metabolism may be challenging to detect in vivo. However, FMO1-mediated pesticide metabolism may represent an example where FMO1 plays a larger role than previously thought (85). Because FMO1 is the major form in fetal human liver, there may be instances of altered metabolism in the fetus owing to *FMO1* variants.

FMO2 FUNCTIONAL ACTIVITY AND SNPS

Although FMO2 is a prominent FMO expressed at high levels in nonhuman primates and other mammals, it is not a prominent functionally active enzyme in human lung (86). The reason is because Caucasians and Asians examined to date are homozygous for a Q472X truncation mutation that results in a nonactive protein (Figure 3) (87, 88). The Q472X variant does not incorporate FAD and is likely removed because of incorrect folding. Approximately 26% of African Americans, 7% of Puerto Ricans, and 2% of Mexicans (89) possess one normal allele and express a full-length enzymatically active functional protein. Additional SNPs affecting expression of FMO2 have been reported (88), but it is not likely that these affect the functional activity of the enzyme if a full-length protein were expressed (90). cDNA-expressed full-length rabbit, monkey, and human FMO2 have been examined for functional activity (87–89), and in general, sulfur-containing chemicals appear to be somewhat better substrates for FMO2 compared with N-containing chemicals (14). FMO2 wild-type enzyme showed efficient activity for *S*-oxygenation of sulfur-containing chemicals, such as thioureas and thioamides, and because the sulfenic acids of thioureas are chemically reactive, humans with full-length FMO2 may be more susceptible to toxicity of certain thiourea-containing chemicals (92). On the other hand, thioether-containing

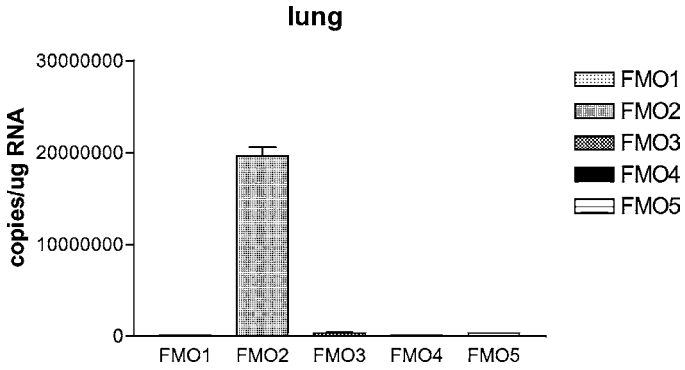

Figure 3 Copies of FMO1–5 in human lung.

pesticides should be detoxicated by *S*-oxygenation via full-length FMO2, and individuals with wild-type FMO2 may be more protected from the toxic properties of thioether-containing pesticides (92).

FMO3 FUNCTIONAL ACTIVITY AND COMMON SNPS

Full-length FMO3 mRNA is strongly detected in adult human liver, but only 3.6% to 4.3% of the amount of hepatic FMO3 is detected in adult kidney and lung, respectively, from human (39, 93). In view of the prevalence of FMO5 in adult and fetal liver, it is not clear which FMO form dominates FMO functional activity. It may be that the role of FMO3 versus FMO5 will depend on the chemical structure of the substrate. In general, human FMO3 prefers to oxygenate nucleophilic heteroatom–containing substrates that are slightly smaller that those accepted for human FMO1. Based on limited information, FMO5 substrate specificity is poorly defined, although it is apparently distinct from FMO3 (i.e., unlike FMO3, FMO5 can accept nucleophiles with neighboring carboxylic acids, see below).

The relative allelic frequencies of common *FMO3* polymorphisms have been determined from the DNA of healthy male and female Caucasians, Hispanics, Asians, and African Americans (16, 42, 45). Significant differences among ethnic groups for *FMO3* allelic frequencies have been observed. Previously, a list of the prominent SNPs, amino acid changes, and predicted functional activity of variant *FMO3*s has been reported (42, 43). Apparent pharmacogenetic effects of *FMO3* SNPs on drug metabolism have been observed. For example, extent of benzydamine *N*-oxygenation was correlated to *FMO3* genotype in a cohort of individuals with trimethylaminuria (95). Prototypical chemicals have been examined as in vitro substrates for *FMO3* variants (41), and the oxygenation of methimazole, TMA, and the probe substrate 10-(*N*,*N*-dimethylaminopentyl)-2-(trifluoromethyl) phenothiazine (5-DPT) were all significantly decreased. The catalytic efficiency

of oxygenation for both substrates decreased in the following order of the common polymorphisms: WT > *FMO3* K158 > *FMO3* G308 > *FMO3* K158/G308. On the other hand, the *FMO3* 360P variant increased the catalytic efficiency for these same substrates 3- to 4.5-fold (41). The effect of the *FMO3* 360P variant was to increase the V_{max} of oxygenation. Based on the mechanism of the enzyme, the *FMO3* 360P amino acid change may be to increase the rate of dehydration of the flavin pseudobase or facilitate desorption of $NADP^+$, both thought to be the rate-limiting steps in FMO catalysis.

Rare *FMO3* Mutations

One of the best studied relationships between *FMO3* SNPs and enzyme function is the association between *FMO3* variants and TMA metabolism (20, 75, 96). Determination of TMA/TMA N-oxide ratios in urine provides a direct means of phenotyping human FMO3. Although multiple mechanisms could contribute to trimethylaminuria, the primary genetic form is best understood and accounts for the vast majority of the reported cases (20). The incidence of trimethylaminuria has been reported to be between 0.1% and 1% in British Caucasians (96). A larger prevalence of trimethylaminuria is observed in the tropics (40). A study of North American individuals self-reporting trimethylaminuria showed that approximately 27% of the individuals had the syndrome (20, 97). As described above, the *FMO3* K158/G308 double mutant shows only 7% of the catalytic efficiency for TMA *N*-oxygenation compared with WT enzyme, for example, and the prediction is that homozygous individuals with this double mutant may have mild trimethylaminuria and possibly other impaired sulfide and/or amine metabolism (41, 98). Because the prevalence of the FMO3 K158/G308 double mutant reaches as much as 20% in some European populations, the prediction is that approximately 2%–5% of the otherwise normal general population probably possesses the homozygous variant allele. Based on in vitro phenotyping studies of various FMO3 substrates, other FMO3-mediated metabolism may be impaired (99).

On the basis of studies done primarily with British, Australian, and American trimethylaminuria cohorts, a phenotype-genotype relationship has emerged. Several rare mutations (i.e., P153L, M66I, E305X) cause severe trimethylaminuria (20, 100, 101). Human FMO3 cDNA-expression of the rare *FMO3* mutations showed that these *FMO3* mutations caused minimal or no TMA *N*-oxygenation functional activity. Additional trimethylaminuria-causing mutations have been identified in individuals from North America (i.e., E32K, A52T, E314X and R387L, G475D, M66I, R492W, A52T, G148X, M82T, N61S, M434F, etc.) (42, 99, 100), Europe (101, 102), and Japan (103). Additional rare mutations continue to be reported (75, 104), including deletion mutations that result in a frame shift and cause premature termination of the *FMO3* gene immediately after codon 65 (105). In summary, an extensive literature now supports the direct causative relationship between mutant *FMO3* and abnormal TMA *N*-oxygenation and trimethylaminuria. Much still needs to be explained regarding this condition. For example, the

episodic nature of trimethylaminuria to some but not all women during menstruation is unexplained (106, 107). Although some antidotes such as antibiotics (20) or copper chlorophillin (108) are available, avoidance of large quantities of choline-containing foods and TMA precursors appears to be helpful.

FMO4 AND FMO5 FUNCTIONAL ACTIVITY AND SNPS

Human FMO4 is present in adult liver and kidney to approximately the same extent (Figures 1 and 2). About tenfold less FMO4 is present in human fetal liver and small intestine compared with adult liver (39). FMO4 is unstable and cDNA expression is problematic and affords poorly active enzyme (109). For this reason it has been difficult to establish extensive FMO4 substrate specificity relations. In light of the prominence of FMO5 in adult human liver and small intestine (Figures 1 and 4), examination of FMO5 has taken on new significance. FMO5 does not oxygenate typical FMO3 substrates, such as methimazole, ranitidine, or cimetidine, and is apparently an atypical FMO (15, 36, 110). However, an increasing number of compounds are reported to be oxygenated by FMO5, including compounds with functionality not typically associated with FMO activity (i.e., thioethers with proximal carboxylic acids) (111), and distinct substrate specificity is likely associated with FMO5. Currently, however, FMO3 is the enzyme associated with the vast majority of human FMO-mediated hepatic metabolism. As more information is discovered about FMO4 and FMO5, revision of the role in drug and chemical metabolism is likely.

Human FMO4 and FMO5 are polymorphic, and a few SNPs have been reported (42). Amino acid changes predicted from the coding region of variants of FMO4 and FMO5 have been reported (42). The FMO4 V323A variant is likely to possess similar functional activity as wild-type enzyme. For FMO5, the S351P variant is likely to alter the functional activity because of possible disruption of protein

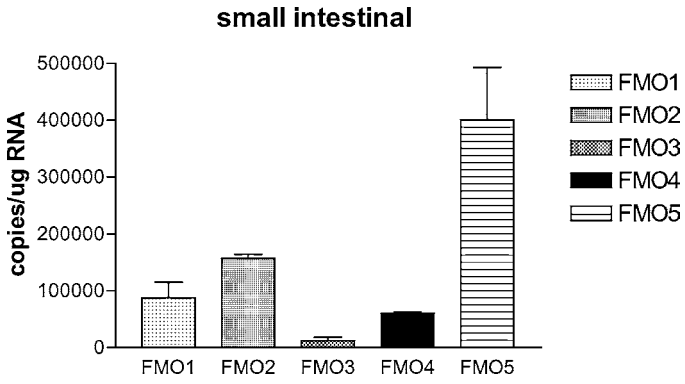

Figure 4 Copies of FMO1–5 in human small intestine.

structure. Because FMO4 and FMO5 are difficult to assay for functional activity because of the paucity of substrates, predicting changes that arise from cDNA-expressed variants is challenging. In addition, neither cDNA-expressed FMO4 nor FMO5 has been observed to possess great levels of functional activity. It is not clear whether this arises from proteolytic instability or some other mechanism leading to enzyme inactivation (109).

SPLICING VARIANTS

FMO functional diversity is determined by expression of five FMO genes (9–11), but there are also a large number of *FMO* splice variants detected in human tissues (i.e., adult and fetal liver, kidney, and fetal and adult brain) (109). Most *FMO* splice variants either caused a frameshift or lacked essential functional sites and were not capable of encoding a functional enzyme. A common inframe deletion variant encoding deletion of 63 amino acids was identified for *FMO1*, *FMO3*, *FMO4,* and *FMO5*. cDNA-expressed FMO1, FMO3, and FMO4 deletion variants were not capable of catalyzing oxygenation of prototypical substrates (109). Alternative splicing might result from other SNPs, but this remains to be established, and although *FMO* genes have previously been reported to produce single gene products, this is definitely not the case.

For *FMO1*, an alternate splice variant was detected in all tissues examined that skipped exon 3 (i.e., a 189-bp fragment). Skipping of exon 7 or 8 leads to a frameshift variant, but skipping exon 3 did not modify the reading frame but led to inactive protein (109). Overall, however, full-length *FMO1* was the predominant species present in adult kidney, fetal liver, and fetal brain, but not in adult brain and adult liver (64, 65, 109).

For *FMO2*, except for lung and kidney, relatively low levels of *FMO2* are present, and nested PCR was required to observe splice variants (109). Two splice variants were observed, one from skipping exons 4 and 5 (i.e., a 306-bp fragment) and the other from skipping exon 2 (i.e., a 138-bp fragment).

Like *FMO1*, normally spliced human *FMO3* is the dominant species in all tissues examined, but splicing variants are apparent for *FMO3* (109). Nested PCR amplification showed that two splice variants [i.e., skipping exon 3 (i.e., a 189-bp fragment) that conserved the reading frame and skipping exon 7 (i.e., a 356-bp fragment) leading to a frameshift] were observed in all tissues examined (i.e., adult liver, fetal liver, and adult kidney). *FMO3* splice variants were only slightly detectable in the presence of adult human or fetal brain tissues. cDNA expression of exon 3–deleted FMO3 resulted in enzyme not competent to catalyze *N*- or *S*-oxygenation of standard substrates (109). Thus, loss of 63 amino acids leads to modification of protein structure and loss of FMO3 functional activity.

The most complex splice variant scenario for the *FMO* family members examined was observed for *FMO4*. In contrast to other FMOs, full-length FMO4 was not always the most prominent transcript present. For *FMO4*, exon 4 corresponds

to exon 3 of other *FMOs*. cDNA expression of exon 4–deleted *FMO4* did not afford a functionally active enzyme. In brain, splice variants (i.e., exon 4 or exon 7 or exons 6 and 7 skipping variants) were present. Full-length *FMO4* was detected in kidney and liver but was only present as trace material in the brain. Two-step PCR amplification showed that a very complex pattern of *FMO4* transcripts were present in the tissues examined (109).

FMO5 splice variants were readily detected in adult kidney, fetal liver, and adult liver. However, *FMO5* splice variants were nondetectable from fetal or adult human brain with direct PCR. Nested PCR showed predominantly normally spliced *FMO5* but also a number of poorly expressed splice variants, including skipping exon 3, skipping exon 6, and skipping exon 7, which were present in all the tissues examined. Additional splice variants (i.e., skipping of exons 3–5 and exons 6 and 7) were also observed. Although a few small-animal *FMO* splice variants have been reported previously (38), the complexity of the human situation is impressive (109). Splice variants are probably relatively important for *FMO4* and *FMO5* and less important for the other FMOs. Changes in splice donor or acceptor sites are predicted not to make a significant contribution to *FMO3* expression variability owing to the fewer number of *FMO3* splice variants observed.

CATALYTIC MECHANISM AND PROTEIN STRUCTURE

Structural aspects and catalytic mechanism of FMO have been reviewed elsewhere (14, 112), and therefore, this section is less detailed. The catalytic mechanism is presented in the context of the chemistry the enzyme does and the products produced. The first step is reduction of the FAD by NADPH. The next step is formation of a C4a-hydroperoxyflavin by addition of molecular oxygen to the reduced FAD. FMO is distinct from other monooxygenases in that the enzyme forms a relatively stable hydroperoxy flavin intermediate (113, 114). The protein environment of FMO apparently protects the hydroperoxy flavin from decomposing, conserving NADPH and affording an efficient two-electron oxygenating agent for nucleophiles with the appropriate size and shape. The active site also minimizes uncoupling or formation of reactive oxygen species. Somehow, the enzyme also evolved a mechanism to retard acceptance of biological nucleophiles, such as glutathione or cysteine (3, 115). After the hydroperoxy flavoenzyme oxygenates the substrate, the oxygenated substrate departs and the hydroxy flavin then eliminates the elements of water to return the FAD to the state of oxidized flavin. Ultimately, $NADP^+$ is desorbed from the enzyme, but it is not clear whether $NADP^+$ desorption or loss of water is the rate-limiting step (116, 117). In contrast to CYP, substrate binding occurs after the enzyme reactive oxygen species is prepared. Thus, most of the lifetime of the enzyme is spent as a peroxyflavin species, ready to oxygenate appropriate substrates. Barring steric constraints, nucleophiles that are readily oxygenated by peroxides or peracids are also oxygenated by FMO (5). In contrast to CYP that generally works by a series of one-electron steps, FMO operates

by two-electron oxygenation. Accordingly, it is relatively straightforward to predict initial FMO oxygenation products. For example, nucleophilic tertiary amines, such as sulfides, thiones, and phosphines, are converted to N-, S-, and P-oxides, respectively, by FMO. Primary and secondary amines are converted into hydroxylamines. Because hydroxylamines are more nucleophilic than primary amines, hydroxylamines are then subsequently *N*-oxygenated. Thus, primary hydroxylamines are converted into oximes and secondary hydroxylamines are converted into nitrones (4). Many FMO oxygenation products are stable and excreted unchanged. However, exceptions are known and N-oxide or S-oxide metabolites sometimes undergo rearrangements or elimination to give products that are only remotely related to the parent compound. Some oxime or nitrone metabolites are quite unstable and hydrolyze to give products not readily identifiable with the parent compound (118).

In addition to FMO forming a relatively stable hydroperoxy flavin, another remarkable property is that relatively reactive metabolites formed by FMO do not inactivate the enzyme (1, 25). Rather, a number of cases are known whereby reactive metabolites are formed by FMO, leave the product binding domain, and inhibit or covalently modify proximal proteins, including CYP (119, 120). Because CYP is a microsomal protein that is also dependent on NADPH for catalytic activity, additional studies are required to distinguish direct inactivation of CYP from indirect FMO-mediated mechanisms (67). An important point is the resilience of FMO to inactivation by electrophilic reactive materials and the potential indirect mechanism of CYP inactivation. The recalcitrance of FMO to inhibition has supported the general idea that FMO may have evolved to detoxicate nucleophilic nitrogen- and sulfur-containing compounds present in the primordial biota (121).

ROLE OF FMO IN DRUG DEVELOPMENT

Compared with FMO, many more examples of CYP-mediated metabolism have been reported. Because FMO is more thermally labile than CYP in the absence of NADPH, the paucity of reported FMO-mediated oxygenations may be due to the way incubations have been done in the past (i.e., initiation with NADPH). Another possibility is the similarity in the types of metabolic products produced by FMO and CYP or possibly the use of inadequate bioanalytical methods. Recent evidence suggests, however, that a significant contribution of FMO to the metabolism of a drug may be advantageous (31, 43). Decreasing the dominance of CYP in the metabolism of a drug by increasing the involvement of FMO may lead to fewer adverse drug-drug interactions. For example, because FMO is not readily induced or inhibited by environmental chemicals or drugs, the contribution of FMO to the metabolism and detoxication of a chemical or drug may be an attractive feature. Lack of FMO induction may cause fewer adverse drug-drug interactions for small molecules primarily metabolized by FMO because variability

will be less. Interindividual variability of FMO is likely due to genetic variation, but as outlined above, this is becoming better understood. There are only a few true competitive inhibitors of FMO reported (122, 123) (i.e., indole-3-carbinol and *N*,*N*-dimethylamino stillbene carboxylates), and most of the apparent FMO inhibition is due to alternate substrate competitive inhibition where a good nucleophile competes with the drug for FMO oxygenation. The majority of drugs are not likely to serve as FMO substrates and hence undergo alternate substrate competitive inhibition. Less inhibition of FMO should be observed and this should lead to a decrease in the number of adverse drug-drug interactions. The general proposal to decrease the number of potential drug-drug interactions is to develop drug candidates that are metabolized by multiple enzyme systems (including FMO) that decrease the reliance on the one-enzyme system. Incorporation of functional groups known to be oxygenated by FMO (i.e., tertiary amines, sulfides) into drug development candidates may decrease the dependence on CYP metabolism and decrease the number of adverse drug-drug interactions. Of course, any discussion of FMO-mediated oxygenation should also consider retroreduction of metabolite and substrate reoxygenation. Such a process represents an example of futile metabolic cycling. For example, S-oxides are polar and tend to traverse cell membranes less efficiently than their parent sulfides. Sulfide *S*-oxide oxidation-reduction cycling inside the cell would tend to provide a reservoir of drug (i.e., S-oxide metabolite and parent drug). Such a dynamic equilibrium might be important in drug targeting and drug accumulation. There are also examples of FMO-related amine metabolites that have been developed as prodrugs (124). Building advantageous metabolic features into drug candidates at an early stage in the drug design and development paradigm may increase the number of drug-like materials that enhance the hit rate in the clinical setting.

OTHER FACTORS INFLUENCING FMO

Diet

Natural regulation and normal function of the gastrointestinal tract requires direct administration of dietary proteins, fats, and carbohydrates. Total parenteral nutrition (TPN) provides nutrition by direct infusion into the systemic circulation. However, bypassing the intestine and processes associated with absorption can cause additional pathophysiological changes to occur (125). Administration of TPN produces effects on drug and xenobiotic metabolizing enzyme systems. Continuous infusion of TPN to rats decreased CYP content 25% and produced a 40%–55% decrease in CYP functional activity (126), compared with control animals fed an identical diet by the enteral route of administration. Infusion of TPN via the jugular vein significantly decreased CYP 3A1 and CYP 2C11, although CYP 2A1 and CYP 2C6 were unchanged compared with control animals receiving the same diet by the enteral route of administration (127). In another study, a significant increase of FMO and CYP2E1 functional activity was observed in

hepatic microsomes obtained from animals treated with TPN + choline for five days (128). Hepatic FMO4 immunoreactivity was increased in rats treated with TPN + choline compared with rats receiving a control diet. The in vivo activity of FMO was determined by analysis of urinary TMA and TMA N-oxide concentrations. Changes observed in vitro also were manifested in vivo because TPN + choline significantly modulated urinary concentrations of TMA, TMA N-oxide, and some biogenic amines (128). Little is known, however, about a role of TPN and FMO in human drug and chemical metabolism.

Age

Selective loss of certain genes in the population as a function of age could indicate a role for those genes in aging or diseases such as cancer. On the other hand, an increase in the frequency of a gene as the population ages might indicate the gene was associated with protective properties. A time course study of *FMO* genes in human brain tissues revealed that *FMO1* showed a distinct profile compared with other *FMOs* (39). Expression of FMO isoforms in brain was examined in 60 samples. Based on their age, the samples were separated into five groups as shown in Figures 5–9. When 25 and 75 percentile values were analyzed for each group, individual variation within each group was less than fivefold variation. The scatter plots of data from all 60 samples are presented in Figures 5–9. It is possible that the overall picture may be slightly altered because the house-keeping gene that the *FMO* data analysis was based on (i.e., hypoxanthine phosphoribosyl transferase, *HPRT*) has a slight decrease with age (Figure 10).

Expression of FMO1 in brain is much lower than expression in other tissues, and it represents less than 1% of FMO1 in kidney. For FMO1, a significant difference was observed between prenatal 17–21 week samples and the three postnatal groups

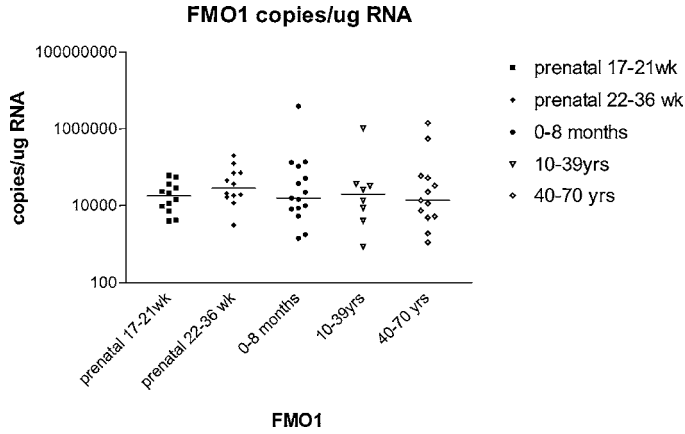

Figure 5 Copies of FMO1 in human brain as a function of age.

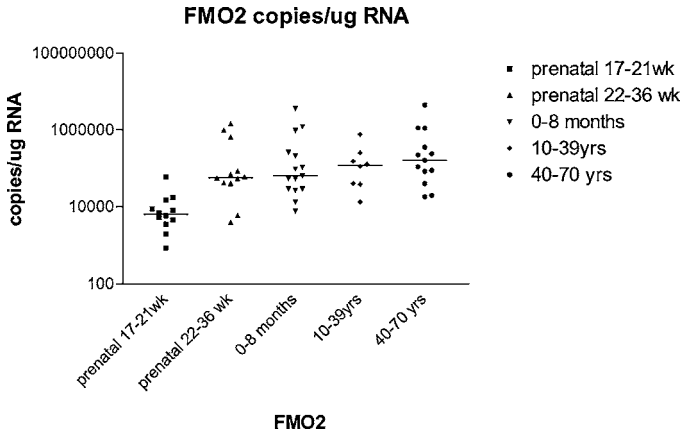

Figure 6 Copies of FMO2 in human brain as a function of age.

(0–8 months, 10–39 years, and 40–70 years groups). This result indicated that expression of *FMO1* in brain was downregulated during or shortly after birth. This regulatory pattern is similar to the downregulation of *FMO1* in liver previously reported (52). Expression of FMO2, FMO3, FMO4, and FMO5 in brain is also among the lowest in adult tissues examined, and they represented less than 1% of the gene present in adult lung, liver, kidney, and liver, respectively. No significant difference was observed between the different age groups through prenatal and postnatal samples for these four genes. The prevalence of the *FMO* gene throughout the age periods examined suggested it may represent an important detoxication catalyst retained for cell homeostasis.

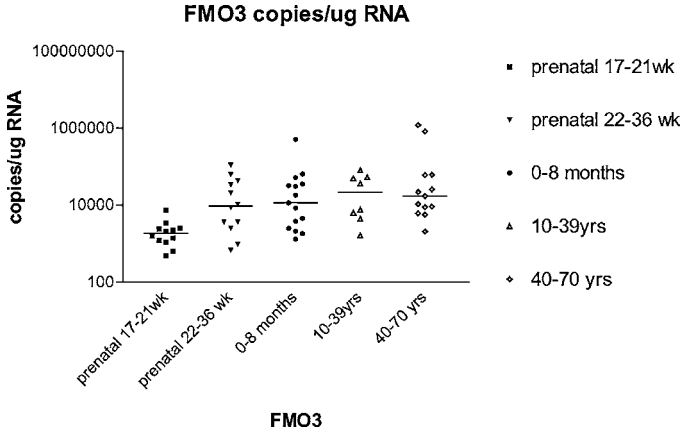

Figure 7 Copies of FMO3 in human brain as a function of age.

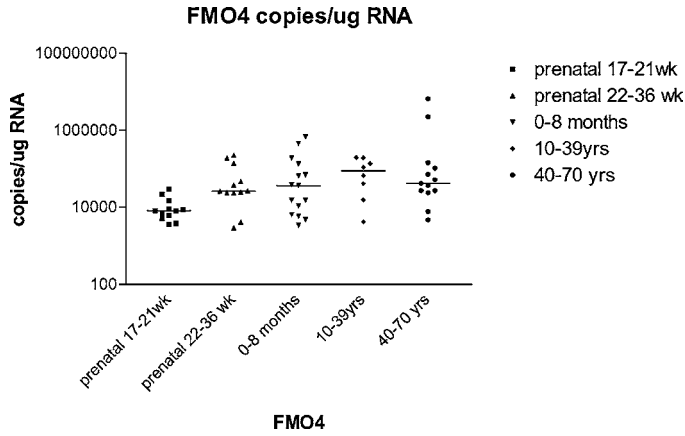

Figure 8 Copies of FMO4 in human brain as a function of age.

Gender

The effect of gender on human FMO function has not been extensively investigated. Based on individuals self-reporting trimethylaminuria, females represent a much larger percentage of individuals self-reporting (20). However, in another cohort of African Americans, males had a higher percentage of abnormal TMA *N*-oxygenation (20). Women appear to have greater FMO3 functional variability because some, but not all, have transient trimethylaminuria during menstruation (106, 108). On the other hand, healthy women do not differ from pregnant women with regard to nicotine *N*-1′-oxygenation (a marker product of FMO3) (129). There

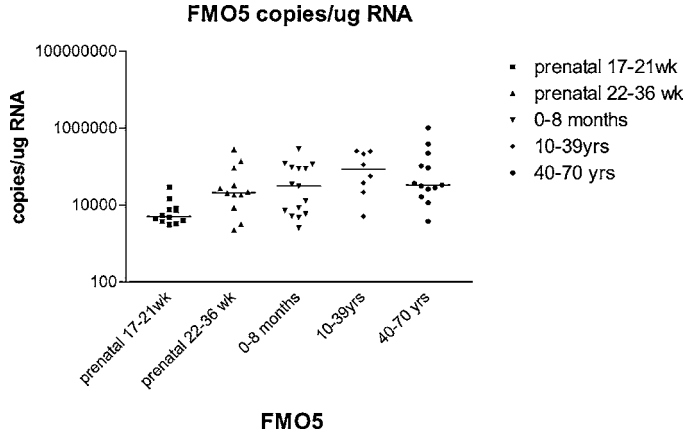

Figure 9 Copies of FMO5 in human brain as a function of age.

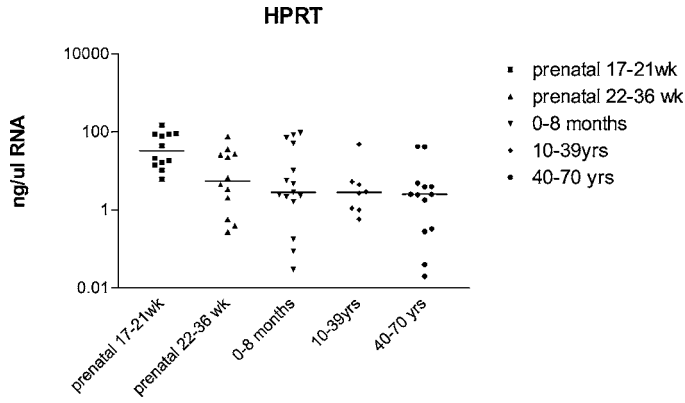

Figure 10 Copies of HPRT in human brain as a function of age.

is some suggestion that *FMO3* SNP frequency is different for healthy adult males and females, but this may simply be due to sampling errors (45). In summary, additional work needs to be done to understand the influence of gender on FMO functional activity.

CLINICAL SIGNIFICANCE: RELATIONSHIP OF FMO TO DISEASE

Hypertension

In mammalian systems, a key role of long-term blood pressure has been established and a close correlation between renal perfusion pressure, urine flow, and sodium excretion has been described (130). It is possible that hypertension develops when the pressure-natriuresis response is shifted to higher pressures (131). In fish, TMA N-oxide and urine function as organic osmolytes to counterbalance increases in osmotic pressure and peripheral resistance owing to hypersalinity (132). Evolutionary analysis of euryhaline fish adaptation to high-salt environments suggests FMO may play a role in blood pressure regulation (20). In humans, hypertension is a multigenic disease, and if FMO contributes to maintaining blood pressure, it may do so via its role in metabolizing organic osmolytes or agents that play a role in blood pressure control (i.e., biogenic amines). Support for this was obtained from observations of abnormal biogenic amine metabolism in certain individuals with defective *FMO3* (100).

Studies using euryhaline fish as models have indicated the induction of FMO by TMA N-oxide and urea (i.e., as organic osmolytes) during periods of exposure to hypersaline environments (133–135). In humans, diets high in salt content cause increasing peripheral resistance within blood vessels and have been associated with

cardiovascular disease. If TMA N-oxide and other organic osmolytes are not produced to counterbalance increases in osmotic pressure and peripheral resistance, it is possible that individuals deficient in the formation of TMA N-oxide may have a higher prevalence of hypertensive diseases. Because human FMO3 is the primary enzyme system involved in the formation of urinary TMA N-oxide, individuals with diminished enzymatic activities might be susceptible to hypertension or other cardiovascular diseases. In vitro studies have also indicated a role for FMO3 in the metabolism and detoxication of biogenic amines, which may also contribute to this condition (136).

In a study of 104 African Americans, 35 individuals screened for urinary TMA and TMA N-oxide possessed TMA:TMA N-oxide ratios equal to or greater than 10%. Four individuals possessed TMA:TMA N-oxide ratios greater than 20%. Overall, males had a statistically higher incidence (i.e., 46%, 16/35) of TMA:TMA N-oxide ratios $\geq$10% than females (i.e., 29%, 20/69) (20). However, no consistent relationship was observed between urinary TMA content and any specific *FMO3* genotype. The *FMO3* E158K variant was the most common polymorphism identified in this group (10 individuals were heterozygous and 4 were homozygous), followed by FMO3 E308G (identified in 3 heterozygous individuals). The FMO3 D132H SNP was observed in 2 out of 32 chromosomes, but this variant was observed in one subject with normal urinary TMA and in another subject with 13% unmetabolized urinary TMA. No significant differences in urinary TMA were observed between hypertensive females and hypertensive males. Comparison of mean blood pressure, diastolic, and systolic metrics failed to show a significant relationship between TMA:TMA N-oxide ratios in control, uncontrolled, or normal individuals (20). However, when the groups were segregated into controlled, uncontrolled, and normal individuals, a statistically higher number of cases having TMA:TMA N-oxide ratios (i.e., ratios $\geq$ 10%) were observed in uncontrolled female subjects (i.e., 33%, 7/21) compared with controlled females (i.e., 25%, 4(16). Likewise, more cases of elevated urinary TMA (i.e., TMA:TMA N-oxide ratios $\geq$10%) were observed in uncontrolled male patients (i.e., 56%, 5/9) compared with normal males (i.e., 46%, 6/13) and controlled males (i.e., 38%, 5/13). In another study of French Canadians, a significant difference in *FMO3* haplotype frequency was noted among hypertensive and nonhypertensive individuals (137). Individuals with trimethylaminuria have been observed to have adverse reactions to tyramine-containing foods (138, 139) and have shown increased excretion of catecholamines (140). Defective *FMO3* also metabolizes biogenic amines less efficiently than the wild-type enzyme (141) and this may also contribute to this condition.

In summary, a relationship appears to exist between urinary TMA and hypertension, but a genetic basis for a single *FMO3* variant causing hypertension has not been observed in the populations examined to date. A more thorough genetic analysis with larger sample size to evaluate *FMO3* variation and hypertension in different populations is necessary before ruling out a genetic basis for this relationship.

Hemochromatosis

Humans with hereditary hemochromatosis have iron overload from duodenal hyperabsorption. An animal model of hemochromatosis developed with primary iron overload (a mutation in *HFE* that prevents association of *HFE* with β2-microglobulin) in mice showed that RNA expression profiles were consistent with a response to iron overload. Surprisingly, heme oxygenase I (*Hmox I*) was lower in primary iron overload, but CYP oxidoreductase encoded by *Por* was induced. *FMO3*, *Fos,* and *Por* mRNAs were all downregulated in mice injected with Fe-dextran. In contrast, *FMO3*, *Fos*, and *Por* were all upregulated in the liver of primary models of mouse hereditary hemochromatosis. This data suggests that relative deficiency in *Hmox I* or other proteins could have a role in hepatic iron accumulation in *Hfe*-deficient mice and that greater expression of *Por* may be an attempt to compensate for this deficiency (142). Although FMO has not been associated with coordinate induction of hemoproteins, it may be that under certain conditions, coordinate upregulation may occur.

Cancer

Epidemiological evidence suggests that nonsteroidal antiinflammatory agents such as sulindac are effective in chemoprevention of colorectal cancer (143). The mechanism apparently involves inhibition of cyclooxygenase-2 that is often overexpressed in colorectal adenomas and carcinomas (144). For example, celecoxib has been shown to decrease the number of polyps in patients with familial adenomatous polyposis (FAP) (145). Sulindac is a prodrug that contains a racemic S-oxide that is reduced to the sulfide (146). The active sulfide (i.e., sulindac sulfide) is *S*-oxygenated to the sulfoxide and then to the sulfone by FMO3 (147). Polymorphisms of *FMO3* at E158K and E308G loci decrease functional activity of FMO3 and may decrease retrooxygenation of sulindac sulfide to inactive sulfoxide and increase efficacy of sulindac. Among sulindac-treated patients that did not develop FAPs, 33% were homozygous for E158K and 17% were homozygous for E308G variants (148). Additional studies relating *FMO3* genotype to sulindac efficacy are warranted.

Diabetes

The profile of hepatic microsomal CYP expressed in rats and mice is dramatically altered in animal models of type I and II diabetes. For example, in streptozotocin-induced diabetic animals, changes in CYP3, CYP4 (149, 150), 1A2 (151, 152), and CYP2E1 (153–155) were noted. In some cases, rat CYP is decreased after administration of streptozotocin (156). The effect of streptozotocin on rat CYP is gender selective (154, 157). Pretreatment with nicotinamide (149) or other hormones (154, 158, 159) can reverse the induction of CYP and in some cases improve the diabetic condition. In addition to CYP, FMO has been noted to be modulated in

animal models of diabetes. For example, in streptozotocin-induced diabetic rats, FMO1 is increased two- to threefold (153). And in Ob/Ob genetically diabetic mice, CYP and FMO are increased (160, 161). The specific activity of hepatic FMO purified from diabetic rats was 50-fold greater than FMO from untreated animals. The functional activity of FMO in diabetic animals was also increased (153, 162). In humans, it is not clear whether there is a relationship between FMO function and diabetes.

Smoking

Nicotine is extensively metabolized and 4%–7% of nicotine absorbed by smokers is metabolized to nicotine *N*-1′-oxide (163). Although FMO1 is competent to form both nicotine *N*-1′-oxide diastereomers, only the *trans* isomer is formed in humans (70). After administration of nicotine by the intravenous route of administration, transdermal patch, or smoking, only *trans* nicotine *N*-1′-oxide was formed (74). Nicotine *N*-1′-oxide is not further metabolized because infusion of nicotine *N*-1′-oxide into humans resulted in recovery of *N*-1′-oxide with the same relative stereochemistry as the infusate. However, reduction of nicotine *N*-1′-oxide to nicotine by bacteria in the intestine has been observed after rectal administration (164). As described above, *FMO3* is highly polymorphic and individuals with defective *FMO3* are predicted to possess abnormal nicotine *N*-1′-oxide formation. Studies have supported this conclusion. Individuals with defective *FMO3* showed impaired urinary excretion of nicotine *N*-1′-oxide compared with healthy individuals after administration of nicotine (165). Other physiological effects may also modulate FMO3 and lead to changes in nicotine metabolism. FMO3 is altered in some women during menstruation (106, 107), but the menstrual cycle does not have a measurable effect on nicotine pharmacokinetics in healthy women (166). Pregnancy has a marked effect on increasing the clearance of nicotine and especially cotinine, but does not alter nicotine *N*-1′-oxide formation, suggesting that pregnancy does not alter FMO3 (129). Alternate substrate competitive inhibition of FMO3 by methimazole has been shown to decrease urinary nicotine *N*-1′-oxide excretion after nicotine administration via chewing gum (167). Other nicotine minor alkaloids are also *N*-1′-oxygenated by FMO but the stereoselectivity (168) and species-dependent FMO form specificity (169) is complex. Cotinine *N*-1′-oxide is formed by a CYP enzyme system (170) and accounts for only 2%–5% of a dose of nicotine (171), but unlike nicotine *N*-1′-oxide, it can be reduced back to cotinine in vivo (172).

The metabolism of other nicotine analogs has been studied in some detail, and lactam and *N*-1′-oxide metabolites are formed by similar pathways as nicotine. For example, ABT-418, a cognition enhancement agent based on changing the pyridine structure of nicotine to another heteroaromatic group is metabolized similar to nicotine (173). ABT-418 possesses poor bioavailability and has been dropped from clinical development. In contrast, replacement of the *N*-methyl pyrolidine

ring system with heteroaromatic groups, while retaining the pyridine portion of nicotine, resulted in a class of metabolically stable compounds of potential use in smoking cessation and certain CNS diseases (174).

CONCLUSIONS AND FUTURE DIRECTIONS

Description of the role of the human FMO in drug and chemical metabolism has increased in the past few years. This may be a consequence of an increased emphasis of drug metabolism in drug development and the recognition that introduction of functional groups into drug candidates that are metabolized by FMO could help decrease adverse drug-drug interactions. Several drugs have been modified to help improve some aspect of metabolism or bioavailability, and this success may spur other related work. Decreasing the dependence of a drug on one prominent metabolizing enzyme (e.g., CYP) and distributing the metabolism over many enzyme systems (including FMO) may improve the drugability of a candidate. In the future, new drug-like substrates for FMO may be developed to facilitate drug discovery and provide additional tools to study enzyme mechanism as well as aid in drug development. New FMO probes may help explain the increase or decrease in enzyme catalytic efficiency for known FMO variants. Ultimately, X-ray crystallography of FMO will be required to fully explain catalytic function and substrate selectivity. Additional structural biology studies will be required to examine posttranslational events and interaction with cellular components. In the future, considerably more information about the regulation of *FMO* and the role of transcriptional factors will be forthcoming. Combined, this information may suggest a physiological function for FMO. Although considerable insight into human genetic variation and SNPs has been established, additional studies may shed light on the clinical relevance of the *FMO* gene family to chronic diseases.

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