The role of FMO3 in metabolic diseases

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Abstract

Flavin containing monooxygenase 3 (FMO3) is a member of the flavin monooxygenase family, which can oxidize the precursor Trimethylamine (TMA) provided from food to produce Trimethylamine N-oxide (TMAO). The autosomal recessive inherited disease caused by partial functional loss of Fmo3 gene, which leads to excessive excretion of TMA in body fluids and emits fishy odor, is called Fish Odor Syndrome or Trimethylaminuria. This disease has been documented for 3,000 years ago and was first reported in the case report in 1970. FMO3 mainly exists in the liver and can participate in the TMA-TMAO metabolic balance in intestinal microorganisms, liver, and kidneys, closely related to insulin resistance, diabetes, cholesterol metabolism, and cardiovascular disease. Due to its wide range of catalytic substrates and low susceptibility to metabolite accumulation, its role in drug metabolism, new drug development, and discovery of new drug targets are increasingly valued. This review will summarize the research progress on the metabolic process and localization of FMO3, congenital genetic defects, metabolic diseases, and its related possible mechanisms.

Keywords: metabolic diseases; FMO3; TMA; TMAO
Flavin containing monoxygenase 3 (FMO3) can participate in metabolic cycle through Trimethylamine (TMA)-Trimethylamine N-oxide (TMAO) pathway in intestinal microbiome, liver, and kidney. FMO3 gene deficiency leads to autosomal recessive disease with fishy odor, and its single nucleotide polymorphism is associated with metabolic diseases. FMO3 is involved in intestinal microbiome, glycolipid homeostasis, cholesterol-bile acid metabolism, metabolic cardiovascular diseases. It may be related to factors such as nuclear factor E2 related factor 2 (Nrf2), nuclear receptor family, and forkhead box O1 (FoxO1).

Partly loss of Fmo3 gene function leads to excessive excretion of fishy TMA in body fluids. That autosomal recessive genetic disorder is called Fish Odor Syndrome or Trimethylaminuria. The earliest description in ancient texts dates back 3,000 years to an Indian woman who was rejected by her tribe due to her bad smell. Shakespeare created the character "Poor John", which reeked of fish, in the tragicallyomedy The Tempest in 1611 C.E. Some suicides of concubines in Thailand about 750 years ago have also been linked to fish odor. The unusual condition was first reported by J. A. Humbert's case report published in the Lancet in 1970, and the cause was found to be FMO3 the next 20 years. FMO3 can participate in gut microbiota metabolism, glycolipid metabolism, cholesterol transport, and chronic cardiovascular diseases, and is closely related to metabolic diseases.

**Background**

FMO3 is a member of the flavin monoxygenase family. Intestinal bacteria convert choline or carnitine in food into the precursor TMA, while FMO3 in the liver mediates further oxidation of TMA to produce TMAO [1]. The autosomal recessive inherited disease caused by partial functional loss of Fmo3 gene, which leads to excessive excretion of TMA in body fluids and emits fishy odor, is called Fish Odor Syndrome or Trimethylaminuria (TMAU). This disease has been recorded as a rare disease since ancient times and was first reported in 1970 C.E. [2]. In recent years, studies have shown that single nucleotide polymorphisms in the Fmo3 gene lead to a decrease in FMO3 enzyme activity, resulting in TMA-TMAO metabolic disorders associated with metabolic diseases such as non-alcoholic fatty liver disease and chronic cardiovascular disease [3-7]. Research has shown that FMO3 can be associated with pathological and physiological processes such as glucose metabolism, cholesterol metabolism, and gut microbiota metabolism by participating in the TMA-TMAO metabolism of gut microbiota-liver-kidney, thereby affecting cardiovascular and cerebrovascular complications in metabolic diseases. This paper will provide a review of the research progress on FMO3 affecting metabolic diseases. It has important pathophysiological significance for a deeper understanding of the TMA-TMAO metabolism of gut microbiota-liver-kidney and can provide new drug targets and research directions for the treatment of metabolic diseases.

**Metabolic process and localization of FMO3**

FMOs are significant phase 1 metabolic enzymes, ranking second only to cytochrome P450 (CYP450). These enzymes possess the ability to facilitate oxidation reactions of exogenous and certain endogenous compounds that contain nucleophilic heteroatoms, including nitrogen, sulfur, phosphorus, and selenium. Consequently, FMOs convert these substances into metabolites that exhibit high polarity, low toxicity, and ease of excretion. Therefore, it has a wide range of drug application prospects [8]. Table 1 displays the substrates, inhibitors, and activators. Among the drugs under development, the main metabolic enzymes of CDK4/6 inhibitor, Ribocilb, and dipeptideleptide IV inhibitor, teneligliptin, are FMO3 [9, 10]. There are not many reports on classical inhibitors of FMO3, mainly including indole methanol, indole-3-carbinol, trigonelline, and methylthioimidazole, as shown in Table 1 [11-13]. FMO3 is a member of the flavin monoxygenase family, which catalyzes the oxidation of TMA to produce TMAO in the form of a catalytic reaction:

\[ \text{TMA} + \text{NADPH} + \ H^+ + \text{O}_2 = \text{TMAO} + \text{NADP}^+ + \ H_2O. \]

FMO3 requires FAD as a cofactor, NADPH as a cofactor, and molecular oxygen as a substrate to complete the catalytic process. The specific mechanism is shown in Figure 1 [1]. FMO3 is predominantly found in the liver and exhibits high expression levels in the skin, with a certain degree of expression in the kidneys [14, 15]. Protein-rich diets, encompassing fish, eggs, and chicken, comprise a multitude of TMA amine complexes. Certain intestinal microbiota possesses the capacity to generate enzymes that catalyze the breakdown of TMA precursors, namely TMAO, choline, phosphatidylethanolamine, carnitine, γ-buty1 betaine, betaine, croton betaine, and glycero1 phosphatidylethanol, thereby converting them into TMA, shown in Figure 2 [16]. These precursors can be acquired through dietary intake or synthesized indirectly. TMA is transported to the liver through the portal circulation and undergoes enzymatic oxidation by the FMO family, specifically FMO3, resulting in the production of odorless TMAO. TMAO, along with unmetabolized TMA, is released into the bloodstream from the liver and is primarily eliminated through the kidneys, with secondary elimination occurring through respiration, sweat, and other glands [17]. The mechanism is visually depicted in Figure 1, 2.

The onset of FMO3 expression occurs at birth in individuals and remains stable throughout adulthood, regulated by tissue-specific hormones. FMO3 is predominantly localized in the livers of adult female mice, exhibiting significantly lower expression levels in males. The abundance of FMO3 mRNA in liver tissue samples obtained from females is approximately 80-fold higher compared to that observed in liver samples from males [15]. FMO3 exhibits significant expression levels in the hepatic tissues and actively engages in the metabolic pathway of TMA-FMO3-TMAO within the liver, thereby exerting an influence on glucose and lipid metabolism throughout the organism. By mitigating endoplasmic reticulum stress induced by lipids, FMO3 facilitates the regulation of hepatic glucose metabolism [18]. FMO3 exhibits significant expression in the kidneys, and its genetic variation influences the synthesis of TMAO [19]. Investigations have demonstrated elevated FMO3 activities and TMAO concentrations in mice afflicted with renal insufficiency, culminating in the onset of uremia. Consequently, FMO3 potentially assumes a pivotal function in the etiology and progression of kidney diseases [20].

In summary, FMO3 is mainly expressed in the liver and can oxidize TMA from food sources into TMAO, participating in the TMA-TMAO metabolism of gut microbiota-liver-kidney. It has a wide range of catalytic substrates and is less prone to metabolite accumulation, making it a potential target for studying metabolic diseases.

**Deletion and mutations of Fmo3 gene**

Certain individuals with loss-of-function mutations in the Fmo3 gene exhibit a deficiency in the Fmo3 gene on the first pair of chromosomes at positions 23-25 on the long arm (1q23-q25). This deficiency leads to insufficient enzyme activity of FMO3, resulting in the inadequate metabolism of fishy TMA. Consequently, excessive excretion of TMA occurs in urine, breath, sweat, and glandular secretions, thereby producing a fishy odor. This condition, known as Fish Odor Syndrome or TMAU, follows an autosomal recessive inheritance pattern [21]. The presence of a distinct odor associated with this condition can impose a considerable psychological burden on patients. Furthermore, the diagnosis and treatment of this condition reside at the confluence of biochemistry and psychology, rendering the research on TMAU highly intricate and yielding substantial psychosocial implications. There have also been reports of enzyme activity reduction caused by...
*Pmo3* single nucleotide polymorphism in addition to the rare *Pmo3* mutation in patients with TMAU. It is worth noting that different *Pmo3* activities in humans can result from different *Pmo3* variations. The genotype carriers of Glu158Lys and Glu308Gly polymorphisms have a higher risk of hypertension related ischemic stroke [3]. There are studies indicating an association between rs2266782 (E158R) polymorphism and the risk of chronic heart diseases, which is associated with plasma TMAO concentration [4]. A plasma TMAO concentration increase indicates an increased risk of cardiovascular death or composite outcomes of heart transplantation [5]. The coding region variations of rs2266782G/A (E158K), rs2266780A/G (E308G), and rs1736657G/A (V257M) are associated with susceptibility to chronic kidney diseases [6]. High levels of circulating TMAO in patients with non-alcoholic fatty liver disease may be associated with *Pmo3* specific genotypes – 2177G>C [7]. Although the essential relationship between *Pmo3* activity regulatory factors and metabolic disease is very complex and unclear, existing data suggests that *Pmo3* activity plays an important role in the pathology of metabolic diseases [17].

**Pathophysiology of FMO3 regulating metabolic diseases**

The prevalence of metabolic diseases is steadily rising and has garnered significant attention among researchers. FMO3 and its catalytic product, TMAO, have emerged as promising serum markers and therapeutic targets for metabolic diseases [22]. It is primarily due to their involvement in multiple aspects of metabolic diseases, such as their association with gut microbiome metabolism, glycolipid metabolism, cholesterol-bile acid metabolism, and metabolic cardiovascular diseases [23–28]. The subsequent sections will provide detailed explanations for these four aspects of pathophysiological mechanism, as shown in Figure 2.

**Gut microbiome metabolism**

Among the five functional FMOs present in humans (FMO 1–5), it is only FMO3 that effectively facilitates the conversion of TMA to TMAO. Consequently, FMO3 serves as an exemplary illustration of proteins involved in the metabolic interaction between the host gut microbiome and exhibits a significant association with the gut microbiome [29]. TMA is produced via the metabolic activity of gut microbiota from dietary constituents. Following absorption, TMA undergoes swift hepatic conversion into TMAO. The extent of TMA absorption is contingent upon the nature and quantity of dietary precursors, as well as the composition of the gut microbiome. Multiple studies have demonstrated that the consumption of probiotics has the capacity to modulate the composition of the intestinal microbiota. Specifically, probiotics have been found to decrease the abundance of bacteria responsible for the conversion of choline and carnitine precursor compounds into TMA, while concurrently increasing the presence of bacteria involved in the clearance of TMA. Consequently, this microbial shift leads to a reduction in the production of TMAO. Furthermore, probiotics have been observed to induce alterations in

The genetic material of bacteria, rendering them incapable of converting choline and carnitine substances into TMA [30, 31]. Research conducted on mice has demonstrated the essential role of gut bacteria in the conversion of dietary compounds into TMA [32]. The administration of broad-spectrum antibiotics significantly inhibits the production of both TMA and TMAO, with TMAO concentrations returning to baseline levels following one month of discontinuation of antibiotic treatment [33]. In addition, FMO3 can also affect the progression of metabolic diseases together with gut microbiota [34]. In mice induced by diabetes, *Lactobacillus plantarum* counteracts the expression of FMO3 and ICAM through the c-Jun NH2 terminal kinase pathway, which is closely linked to diabetes enteropathy [35]. Inhibiting intestinal microbiota or FMO3 activity can block the production of TMAO in mice, thereby inhibiting the formation of abdominal aortic aneurysm [36]. *Akkermansia muciniphila* and *Lactobacillus plantarum* can reduce liver FMO3 expression, thereby preventing the occurrence of hyperglycemia and hyperlipidemia in mice with insulin resistance [35, 37]. Therefore, regulating the types and proportions of gut microbiota may be an important factor in improving the regulation of metabolic diseases by FMO3.

**Glycolipid metabolism**

FMO3 is associated with metabolic syndrome related to glycolipid metabolism. Overexpression of FMO3 increases lipid generation and gluconeogenesis, while *Pmo3* gene knockout has the opposite effect [38]. In the context of metabolic syndrome, the elevated levels of TMAO, a metabolite of FMO3, have positively correlated with increasing body mass index, visceral obesity index, and fatty liver index [39]. Inhibiting FMO3 to reduce TMAO can lower the levels of protein kinase R-like endoplasmic reticulum kinase (PERK) and FoxO1 in the liver, key factors in metabolic syndrome [40]. The main pathological and physiological changes of metabolic syndrome include insulin resistance and glycolipid metabolism disorders, and *Pmo3* knockout has been found to prevent the occurrence of hyperglycemia and hyperlipidemia in mice with insulin resistance [34]. From the perspective of lipid metabolism, the metabolite of FMO3, TMAO, is a risk factor for metabolic associated fatty liver diseases [41]. FMO3 can also regulate obesity and browning in white adipose tissues by reducing endoplasmic reticulum stress in liver cells treated with lipid [18, 42]. Mice with *Pmo3* gene inactivation or deletion under a high-fat diet are immune to obesity and exhibit high gene expression related to beige adipocytes [42]. Liver specific knockout of *Pmo3* in low-density lipoprotein (LDL) receptor knockout mice leads to a decrease in circulating TMAO levels, while *Pmo3* overexpression increases lipid levels in liver and blood plasma [38]. Therefore, FMO3 can affect liver cell lipid metabolism through its metabolite, TMAO, thereby affecting blood lipids and lipid browning. In addition, FMO3 is also associated with insulin resistance. Mice treated with a high-fat diet and added with TMAO, a metabolite of FMO3, will show more significant glucose tolerance damage, causing interruption of the liver insulin signaling pathway [17, 23]. TMAO can

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<th>FMO3 substrates</th>
<th>FMO3 inhibitors</th>
<th>FMO3 activators</th>
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<td>(1) Endogenous biomolecules</td>
<td>Indoly carbilin, indole-3-carbilon, trigonelline, methylthioimidazole</td>
<td>Chlorpromazine, imipramine</td>
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<tr>
<td>Epinephrine, norepinephrine, phenethylamine, Trimethylyamine, tyramine</td>
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<tr>
<td>(2) Exogenous substances</td>
<td>Amphetamine and its hydroxylamine intermediate, benzydamine, cimetidine, clozapine, N-deacetyl ketonazol, vadimezan (DMXAA), ethionamide, itopride, methamphetamine and its hydroxylamine intermediate, methimazole, nicotine – only the (S)-(−)nicotine enantiomer, olopatadine, phenothiazines – 2-(trifluoromethyl) analogs, rantidine, sulindac sulfide, tamoxifen, thiozamide, xenamoline</td>
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![Table 1 FMO3 substrates, inhibitors, and activators](https://www.tmrjournals.com/tmr)
Figure 1 Catalytic mechanism of FMO3. FMO3 requires FAD as a cofactor, NADPH as a co-substrate, and molecular oxygen as a co-substrate to complete the catalytic process. (1) The cofactor NADPH binds to FAD and rapidly reduces it to FADH₂. (2) The reduced form FADH₂, which receives two electrons (H₂), rapidly binds to molecular oxygen (O₂) to form a stable C4 site flavin peroxide intermediate (FADH-OOH), waiting to bind to an appropriate nucleophilic substrate (RSH). (3) Through nucleophilic attack of the substrate on FADH-OOH, an oxygen atom is transferred to the substrate to form an oxidation product (RSOH). (4) Another oxygen atom forms water (H₂O). (5) FADH-OOH is reduced to FAD, and NADP⁺ is released to wait for the next catalytic cycle.

Figure 2 The pathophysiological role and related molecular mechanisms of FMO3 in regulating metabolic diseases. Choline or carnitine are found in protein-rich foods such as fish, chicken, eggs, and hamburgers. Gut microbiota in intestine convert these foods into TMA and transport them to the liver through ATP-binding cassette sub-family G member 5/8 (ABCG5/8) and Niemann-Pick C1-like 1 (NPC1L1). FMO3 in liver oxidizes them into harmful TMAO, and then transports them to the whole-body tissues and organs through circulation with final excretion in kidneys. FMO3 can regulate TMA-TMAO to affect changes in blood lipids through transintestinal cholesterol excretion (TICE) pathway, affect lipid metabolism through its interaction with nuclear receptors and CYP family, regulate the metabolic core of PPARs, FoxO1 and PERK to affect insulin resistance, and affect cardiovascular diseases through the Nrf2-Kelch-like ECH-associated protein-1 (Keap1) anti-oxidation. Cholesterol-bile acid metabolism is also affected by FMO3 via liver X receptor (LXR) and farnesol X receptor (FXR). The deficiency of Fmo3 gene function leads to excessive excretion in body fluids with fishy odors. This autosomal recessive genetic disease is called Fish Odor Syndrome or TMAU, and its single nucleotide polymorphism is also closely related to metabolic diseases. TMA, Trimethylamine; TMAO, Trimethylamine N-oxide; FMO3, flavin containing monooxygenase 3; TICE, transintestinal cholesterol excretion; TG, triacylglycerol; CE, cholesterol ester; LXR, liver X receptor; ER, estrogen receptor; PPAR, peroxisome proliferators activated receptor; HDL, high-density lipoprotein; ApoA, apolipoprotein; LDL, low-density lipoprotein; VLDL, very low density lipoprotein; FoxO1, forkhead box O1; PERK, protein kinase R-like endoplasmic reticulum kinase.
also potentially induce insulin resistance through the elevation of serum inflammatory cytokine chemokine CCL2 [43]. Furthermore, it has been observed that the destruction of islet cells by streptozotocin leads to an increase of FM03 in the liver [35]. Fmo3 knockdown in mice with insulin resistance also inhibits the metabolic factor, FoxO1, independent of TMAO [17]. Thus, FM03 can affect liver lipid metabolism and pancreatic islet dysfunction through its metabolite, TMAO, via metabolic factors, PPAR and FoxO1, thereby affecting insulin resistance, dyslipidemia, obesity, diabetes, metabolic associated fatty liver disease, and other disease processes related to metabolic syndrome.

Cholesterol-bile acid metabolism
FM03 serves as the primary regulator of cholesterol balance and is increasingly recognized for its involvement in cholesterol metabolism [44, 45]. Hypercholesterolemia can aggravate the development of atherosclerosis, and aortic lesion size is positively correlated with increased TMAO levels and Fmo3 mRNA expression [46]. Dietary TMAO can reduce mRNA expression of NPC1L1 and ABCG5/8, and inhibit intestinal cholesterol absorption, can also impair the non-bile output pathway for cholesterol inflow via the intestinal, contributing to the formation of gallstones [26, 47, 48]. In addition, dietary supplementation with TMAO, a product of FM03, inhibits bile acid synthesis by down-regulating CYP7A1 and CYP27A1 [26]. Fmo3 knockdown mice fed with cholesterol altered lipid secretion in bile duct, hindered intestinal cholesterol absorption, and inhibited oysterols and cholesterol esters produced in the liver [17]. FM03 expression is induced by dietary bile acids, and its mechanism involves a bile acid-activated nuclear receptor, FXR [25, 49]. FM03 inhibits the non-biliary pathway that transfers cholesterol to 30% of total cholesterol loss in the body, resulting in systemic cholesterol balance recombination, while LXR activation increases total cholesterol loss to 60% [44, 50]. Thus, FM03 activity and the TMA/FM03/TMAO pathway appear to be major determinants for LXR and FXR regulating cholesterol-bile acid metabolism [48].

Chronic cardiovascular disease and kidney diseases
TMAO, a metabolite of FM03, is associated with atherosclerosis and cardiovascular disease risk [24, 27, 51–53]. The formation of aortic atherosclerosis is observed in male ApoE knockout mice fed with TMAO [54]. Mice fed with a Western diet have higher plasma TMAO concentrations and show cardiac dysfunction and fibrosis [55]. TMAO also has the potential to induce endothelial damage [56]. In addition, TMAO has been identified as a potential biomarker for the risk of major adverse cardiovascular events, including myocardial infarction, stroke, and death [57]. These effects may be attributed to excess cholesterol accumulation in macrophages and the long-term hypertensive effects of angiotensin II [58]. Besides, FM03 may form the TMA/FM03/TMAO pathway to regulate cardiovascular events. Systemic TMAO levels and the likelihood of thrombosis are significantly reduced in Fmo3 knockout mice, and Fmo3 knockdown can prevent atherosclerosis in mice with insulin resistance [59, 60]. Many drugs work through that mechanism. For example, *Alisma orientalis* inhibits FM03 and prevents TMA from being converted into harmful TMAO in humans, thus preventing vascular inflammation for therapeutic atherosclerotic purposes [61]. Intestinal flora metabolites increase the risk of cardiac complications such as cerebral thrombosis, atherosclerosis, and heart failure after metabolic surgery through the TMA/FM03/TMAO pathway [62]. In addition, inhibiting FM03 activity and blocking TMAO production could also inhibit the formation of abdominal aortic aneurysm in mice [36].

Through the four sections above, we can draw a conclusion that FM03 affects TMA-TMAO levels by affecting intestinal microbial metabolism. It can affect diabetes, obesity, gallstones, and other related diseases by regulating cholesterol-bile acid metabolism and insulin sensitivity, thus regulating cardiovascular and renal functions, and participating in cardiovascular complications, as shown in Figure 2.

Possible molecular biological mechanisms

**Nrf2-mediated oxidative stress**

Nrf2 plays a crucial role as a transcription factor in the regulation of cellular oxidative stress response and serves as a pivotal regulator in maintaining intracellular redox homeostasis. Previous research has posited that the variability of FM03 expression in hepatic tissues exists, and recent investigations have demonstrated that not all hepatotoxic drugs inducing oxidative stress can modify the expression of the Nrf2 gene. The precise mechanisms by which FM03 influences bile stasis models remain incompletely elucidated, and the protein transcription regulation of FM03 during acetaminophen-induced hepatic injury in Nrf2 knockout mice may not be mediated by Nrf2 [63]. The regulation of the human Nrf2 gene is mediated by the transcription factor Nrf2, which is involved in oxidative stress response. In HepG2 cells, the expression of the constitutive Fmo3 gene is suppressed, rendering this cell line a suitable model for investigating the regulatory mechanisms of Fmo3 gene. Notably, the upregulation of Nrf2 in HepG2 cells resulted in enhanced expression of the target gene Nrf2 while having no discernible effect on the expression of the Nf2 gene. The upregulation of FM03 in Hepa-1 cells could potentially be facilitated through the Nrf2/antioxidant response pathway. However, it has been established that the modulation of Nrf2 activation or the induction of oxidative stress by pharmaceutical agents does not have any impact on the expression levels of FM03 mRNA [64]. The co-transfection of Nrf2 and KEAP1 expression vectors, along with the construction of luciferase reporter genes containing different lengths of Fmo3 promoters, did not yield a significant impact on the activity of the Fmo3 gene [65]. Consequently, the in vitro findings suggest that the transcriptional regulation of FM03 may not be mediated through the Nrf2/Keap1 regulatory pathway. The verification of FM03’s involvement in the regulation of oxidative stress response via Nrf2 is still pending.

**Metabolic nuclear receptors**

The nuclear receptor superfamily is a collection of transcription factor families that are activated by various ligands, including steroid hormones, vitamin D, ecdysone, 9-cis and all trans retinoic acid, thyroid hormones, fatty acids, oxidative steroids, prostaglandin J2, leukotriene B4, Farnitol metabolites, among others. These ligands play a crucial role in regulating cell growth and differentiation by facilitating communication between signaling molecules and transcriptional responses. In humans, the nuclear receptor family comprises 48 members, such as peroxisome proliferators activated receptor (PPAR), FXR, LXR, and retinoid X receptor (RXR). Metabolic nuclear receptors, which are closely associated with the onset and progression of diabetes, fatty liver, and other diseases, have been found to have a significant correlation with FM03. Several studies propose the potential involvement of FM03 in the modulation of nuclear receptors. Enhanced expression of FM03 has been observed to promote adipogenesis and gluconeogenesis, whereas the absence of the Fmo3 gene in mice leads to an opposing effect. This effect is mediated by the PPARa pathway, specifically through the Kruppel like factor 15 (KLF15) pathway, rather than directly through the TMA-TMAO pathway [38]. Furthermore, scholarly research has demonstrated that the expression of FM03 is stimulated by dietary bile acids through the activation of the nuclear receptor, FXR, which is itself activated by bile acids [25]. It has been discovered that hepatic FM03 expression is under the regulation of hepatic FXR, and the magnitude of the bile acid reservoir can impact the FXR-mediated regulation of FM03. Additionally, hepatic bile acid synthase enzymes CYP7A1 and CYP27A1 may modulate LXR and PPARa signaling pathways, thereby diminishing hepatic inflammation, fostering hepatic lipid production, and promoting gluconeogenesis [48]. The transduction of FXR signals is closely associated with the production of TMAO. Investigations have revealed that the regulation of FMO3 responsible for the synthesis of atherogenic TMAO, is governed by FXR. Following treatment with FXR ligands, the expression of FM03 was induced, leading to an increase in TMAO levels [24, 26]. In mouse
liver cells, the impact of various steroid hormones, including dexamethasone, 5α-dihydrotestosterone, thyroid hormone, and progesterone, on the accumulation of FMO3 mRNA is negligible. However, the presence of 17β-estradiol, which can interact with estrogen receptor (ER) α, leads to the inhibition of FMO3 mRNA accumulation. This inhibitory effect can be counteracted by the administration of the ER inhibitor, ICI 164.384 [66]. Therefore, the relationship between FMO3 and metabolic nuclear receptor (especially FXR, LXR, PPAR, and ER) is an important research direction in the future.

FoxO1, core for metabolic control
FoxO1 is a transcription factor that exerts significant influence over gluconeogenesis and glycogenolysis via insulin signaling, while also serving as a key determinant in adipogenesis [67, 68]. According to recent research, it has been discovered that TMAO exhibits binding affinity to PERK at concentrations that are relevant in terms of physiological conditions in mice with FMO3 mutations. This binding selectively triggers the activation of the unfolded protein response pathway mediated by PERK, consequently leading to the induction of the transcription factor, FoxO1, in a manner that is dependent on PERK. This activation of FoxO1 is considered a crucial factor in the development of metabolic diseases. Furthermore, it has been observed that the regulation of gut microbiota or the inhibition of TMAO synthase can effectively reduce the activation of PERK and the levels of FoxO1 in the liver, indicating that TMAO and PERK may be the core of studying the pathogenesis of metabolic syndrome mediated by FMO3 [40]. In vitro, insulin inhibits FMO3, while it is increased in male mice with obesity and insulin resistance, as well as in humans with obesity and insulin resistance. Inhibiting FMO3 in insulin resistant mice can effectively inhibit FoxO1, which serves as the central node of metabolic control, and consequently prevents the onset of hyperglycemia, hyperlipidemia, and atherosclerosis [69]. Hence, FoxO1 may be considered a crucial factor in the development of metabolic diseases mediated by FMO3.

Bioinformatics analysis
The investigation into FMO3 is currently in its preliminary phases, and we employed the STRING (Version 11.0, https://string-db.org/) and the GeneMANIA Cytoscape App (Cytoscape 3.0, http://genemania.org/) in conjunction with bioinformatics software to analyze the co-expression or interaction proteins associated with FMO3 [70, 71]. The outcomes of this analysis are depicted in Figure 3. STRING protein interaction analysis showed that FMO3 was correlated with dimethylglycine dehydrogenase (DMGDH), hydroxy acid oxidase 2 (HAO2), NRIH4 (namely bile acid-associated nuclear receptor, FXR), CYP family (CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP3A4, and CYP2D6), and 2,4-dienoyl-CoA reductase 1 (DECR1) (Figure 3A, Supplementary Table S1) [46, 72–76]. GeneMANIA protein interaction analysis showed that FMO3 was associated with the whole FMO family, some members of the CYP family (CYP4F11, CYP2E1, and CYP7A1), diacylglycerol kinase-epsilon (DGKE), keratin 4 (KRT4), casein alpha S1 (CSN1S1), mannose lectin lectin (MBL2), alcohol dehydrogenase 1C (ADH1C), ATPase, Cu+ transporting β polyepitide (ATP7B), solute carrier organic anion transporter family, member 1B3 (SLC1B3), DNA methyltransferase 1 (DMC1), guanylate cyclase soluble subunit α-3 (GUCY1A3), GC-box (GC), C6 alkyl side chains (C6), and proteoglycan 4 (PRG4) (Figure 3B, Supplementary Table S2). These results are consistent with some reported preliminary studies, but lack of in-depth mechanism studies. Therefore, the identified interacting proteins from these analysis results are anticipated to serve as a significant avenue for future research on the mechanisms related to FMO3.

Thus, FMO3 regulates oxidative stress response through Nrf2, bile acid metabolism through metabolic nuclear receptors, and insulin resistance through FoxO1, as shown in Figure 2. These discovered mechanisms may be the core of studying the involvement of FMO3 in metabolic diseases. The relevant bioinformatics analysis also helps to provide direction for future research.

Conclusions
In summary, FMO3 plays a crucial role in the metabolic cycle involving the TMA-TMAO pathway in the gut microbiota, liver, and kidney. Mutations in the FMO3 gene can result in autosomal recessive inherited disorders characterized by the production of fishy odors. Its single nucleotide polymorphism is associated with metabolic diseases. FMO3 can participate in intestinal microbial metabolism, metabolic syndrome related to glycolipid metabolism, cholesterol-bile acid metabolism, and metabolic cardiovascular diseases, which may be related to Nrf2-mediated oxidative stress, metabolic nuclear receptors, and metabolic core factor, FoxO1. Bioinformatics analysis showed that proteins that interact with FMO3 may also be the direction of future research. The main metabolic enzyme of the drugs now partially under development is FMO3, which has reliable regulators from gut microbes and food sources, and it can oxidize a variety of substances into metabolites that are easy to be excreted, so FMO3 has a wide range of drug application prospects for metabolic diseases.
References


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