AWARENESS OF [illegible] AMONG YEAR 1 & YEAR 2 MEDICAL STUDENTS OF FACULTY OF MEDICINE & HEALTH SCIENCES, UNIVERSITI MALAYSIA SARAWAK

Nurul Shahirah bt Abdul Rashed (24727)
Suganya a/p Saraman (25179)
Cherrymea Denise ak Alexander Anthony (23887)

Elective 1 (2010/2011)
Faculty Of Medicine & Health Sciences
Universiti Malaysia Sarawak

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Nurul Shahirah Binti Abdul Shukor (24767)

Suguna a/p Seraman (25175)

Cherrysca Dante Anak Alexander Anthony (23287)

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Nurul Shahirah Binti Abdul Shukor (24767)
Suguna a/p Seraman (25175)
Cherrysca Dante Anak Alexander Anthony (23287)

Mr. Dominic Dado Sagin

Department of Microbiology

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LRG RAHIM KAJAI 14, TMN TUN DR ISMAIL
60000 KL

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(TANDATANGAN PENYELIA)
(SUPERVISOR'S SIGNATURE)

Dr. Zunika Binti Amit
Senior Lecturer
Department of Basic Medical Sc...
Faculty of Medicine and Health Sciences
Universiti Malaysia Sarawak

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Abstract

The awareness of trimethylaminuria (TMAU) was investigated among year one and year two medical students of the Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak. Out of 200 questionnaires distributed, 141 were completed, of which, 95% of the respondents had awareness on body odour but was unable to identify TMAU. No cases of TMAU was recorded in Sarawak between the years 2000 and 2010 (Sarawak Health Department). This shows that the disease is rare. However, awareness of medical students is important because the disease is caused by a defect in the flavin-containing monooxygease form 3 (FMO_3) enzyme. This enzyme is important in the oxidation of common drugs including tamoxifen, an anti-breast cancer drug. A defect in this enzyme would cause decreased clearance, leading to an accumulation of drugs which may cause fatality to the patient.

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Acknowledgements

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CHAPTER I - INTRODUCTION

Introductory Paragraphs

According to the Office of Rare Diseases (ORD) of the National Institutes of Health (NIH), trimethylaminuria (TMAU) is one of the rare diseases found around the world. TMAU affects less than 200,000 people in the US population. A consortium of European partners, Orpha.net, defines a condition rare when it affects 1 person per 2,000. However, there is a lack of statistical data of patients suffering from TMAU in Sarawak. This may lead to unawareness of natives of Sarawak regarding this fish odour syndrome.

Trimethylamine (TMA) is a volatile tertiary aliphatic amine (boiling point 2.9°C; pK_b 4.13) with a characteristic odour of rotting fish. The enzyme, flavin-containing monooxygenase form 3 (FMO_3) which is needed to break down nitrogen-containing compound derived from the diet is mainly made in liver. TMA is one of the compounds broken down by FMO_3. TMA is derived from the diet either directly from intake of food rich in TMA such as fish, or by consumption of food high in trimethylamine-N-oxide (TMNO), choline and L-carnitine which act as TMA precursors (Bain, Fornasini & Evans 2005). The bacterial spoilage of fish resulting in the reduction of TMNO to TMA causes the characteristic odour of rotting fish. FMO_3 converts TMA into TMNO which is then excreted from the body through urine. According to some researches, FMO_3 is also involved in processing certain types of drugs. The anticancer drug tamoxifen (TAM), the pain medication codeine, the antifungal drug ketoconazole, and certain medications used to treat depression (antidepressants) are broken down by this enzyme. A study on oxidation of TAM by human FMO_1 and FMO_3 to tamoxifen-N-oxide (TNO) and its novel reduction back to TAM by human cytochrome P_{450} and haemoglobin conducted by Priyanka Parte and David Kupfer (2005) showed that TAM is oxidized into TNO by both human FMO_1 and FMO_3. However, FMO_1 is found to be more potent than FMO_3. Meanwhile, another research (Cashman *et al.* 1999) showed that FMO_3 are detoxication catalysts that convert relatively nonpolar compounds to more polar metabolites through the incorporation of one atom of molecular oxygen.

TMAU, or fish odour syndrome is an autosomal recessive human disorder due to defective activity of the FMO_3, as this protein catalyses the NADPH-dependent oxidative metabolism of odorous TMA, derived in the gut from dietary sources, to non-odorous TMNO in the liver. There are two types of TMAU, which result from either due to decrease in the amount or activity of the enzyme FMO_3 or due to substrate overload of FMO_3 enzyme activity. TMAU patients are unable to carry out this reaction and hence, release a fishy body odour, due to the secretion of TMA in their breath and sweat and its excretion in their urine, leading to various psychosocial problems such as schooling problems, clinical depression and suicide attempt. TMAU can be classified into six classes which are primary genetic form, acquired form, childhood forms, transient form associated with menstruation, precursor overload and disease states. Individuals found to be TMAU-positive usually presented with body odour, oral malodour, and chronic halitosis and rarely with vaginal malodour.

Presently, there are not many research conducted regarding the awareness on the population of people who has TMAU. An identification of the awareness of the year one and year two medical students in the Faculty of Medicine and Health Sciences (FMHS) of Universiti Malaysia Sarawak (UNIMAS) on people who have high TMA: TMNO ratio is needed in addition to adding on to the current knowledge. We are conducting this research to find out the statistic of the awareness of the medical students regarding people with a high TMA: TMNO ratio, and thus, suffering from TMAU. If the awareness of this disease remains low, soon to be medical doctors will not be able to diagnose this disease and, thus, may put patients in psychological peril. TMAU may also cause the alteration of the mode of action of certain drugs and may harm undiagnosed patients receiving treatment.

Purpose

The goal of this study is to discover the awareness level of TMAU among medical students in FMHS, UNIMAS.

Significance of Study

To date, there has been no study done on the awareness level of TMAU among medical students or on any other population. TMAU is not as rare as it was once thought. Emerging studies show that TMAU is not only a rare recessive autosomal disorder but a spectrum of phenotypes of transient to mild malodour depending on environmental factors. TMAU exists in the extreme malfunction of FMO_3. The enzyme FMO_3 plays a role in metabolising many nitrogen and phosphorus based compounds, which can be found in many drugs. If the level of awareness of TMAU and the function of FMO_3 enzyme is low within the medical students, this may jeopardise patients when these medical students graduate as doctors; as the effect of FMO_3 on different drugs may have a detrimental effect on patients. If TMAU is under diagnosed, patients may face mental torture and this can lead to legal jeopardy of future medical doctors.

Statement of Problem

TMAU is relatively uncommon in the society. This condition is due to the abnormality in the FMO_3 enzyme. FMO_3 is commonly found in the human liver and converts a wide range of substances to a less toxic form. It is not known the exact number of natives in the population of Sarawak that suffers from this condition. Since FMO_3 is important in metabolizing a number of drugs such as cimetidine and benzydamine, it is imperative to identify the number of natives in the population who is lacking in the activity of enzyme FMO_3.

Therefore, what is the level of awareness of TMAU, and thus the function of FMO_3 enzyme among medical students in FMHS, UNIMAS? The information needed is the total population of year One and year Two medical students.

Research Questions

The objective of this research is:

I. Knowledge of medical students of TMAU

II. Perception of medical students on TMAU

III. Awareness of TMAU among medical students

Scope and Limitations

This research was only conducted on the awareness of TMAU of Year One and Year Two medical students of FMHS. The limiting factor that was encountered during this research is the participants' knowledge on TMAU. We were not able to do a random survey on the whole population of medical students in FMHS due to time and financial constraints. The results obtained from this study are only representative of a small percentage of medical students in FMHS and cannot be used to generalise the medical students of FMHS as a whole.

Assumptions

The population sample chosen for this survey is assumed to represent the medical students of the FMHS, UNIMAS. It is also assumed that the questionnaire is valid and measures the desired conducts as described above. The respondents are assumed to answer truthfully the questions asked to them.

Trimethylaminuria

TMAU is also known as fish odour syndrome. The disease shows an autosomal recessive pattern of inheritance. The first clinical description was by Humbert and colleagues (1970) on a case of a 6 year old girl who have had other few known congenital abnormalities (S.C Mitchell & R.L Smith, 2001). Since then, fish odour syndrome has been encountered several times in clinical settings from 1970 to 1990. The molecular genetics of flavin-monooxygenase enzymes mutation theory had been proposed only in the latter year. According to Mitchell and Smith (2001), the fish odour syndrome had been recorded in some ancient literatures such as Mahabharata (1000 BC), Thai folklore (1250 AD), Shakespeare in *The Tempest* (1600), Arbuthnot in *Nature of Aliments* (1735) and Eisenschiml in his autobiography (1948) (S.C Mitchell & R.L Smith). The history of TMAU is summarised in the **Table 1** and **Table 2**:

Period	Anecdotal Observations
1000 BC	Mahabharata (Indian Epic)
1250 AD	Thai Folklore
1600	Shakespeare, The Tempest
1735	Arbuthnot, Nature of Aliments
1948	Eisenschiml, autobiography

Table 1

Period	Clinical observations
1970	First clinical description; coining of the term " fish odor syndrome, associated with excessive trimethylamine excretion and Turner's syndrome.
1970-1985	Description of isolated and sporadic cases.
1980	Recognition of the "tainted egg" syndrome. Breed of chicken with dysfunctional N-oxidation.
1980-1990	Clinical and biochemical case studies from several countries. characterization of a human genetic polymorphism of trimethylamine N-oxidation.
1990s	Molecular genetics of the flavin-monooxygenase enzyme and recognition of candidate mutations for fish malodor syndrome.

Table 2

(Extracted from *Trimethylaminuria: The Fish Malodor Syndrome,* by S.C. Mitchell and R. L. Smith, The American Society for Pharmacology and Experimental Therapeutics.)

The normal clinical presentations of the disorder are rotting fish odour in bodily secretions such as sweat, urine and breath. This is due to the imbalance between the amount of TMA and TMNO in the body fluids. In summary, TMA is the chemical that leads to the rotting fish odour, whereas, the odourless TMNO is the normal form of TMA excreted by the kidney. FMO_3 is the enzyme that is responsible to catalyse the conversion of rotting fish odor TMA to odourless TMNO. As noted, precursors of TMA are the dietary choline, L- carnitine, lecithin and fish. As a result, any deficiency in the activity of the FMO_3 enzyme in the liver will cause TMA to be excreted excessively in sweat, breath and urine. The excess amount of TMA in body fluids leads to the rotting fish odour (or pungent odour) in the patient. The normal amount of TMA and TMNO to be removed by the kidney are 1 mg and 40 mg, respectively. Refer to the diagram below for the mechanism of trimethylaminuria caused by the lack of FMO_3 enzyme activity in the liver:

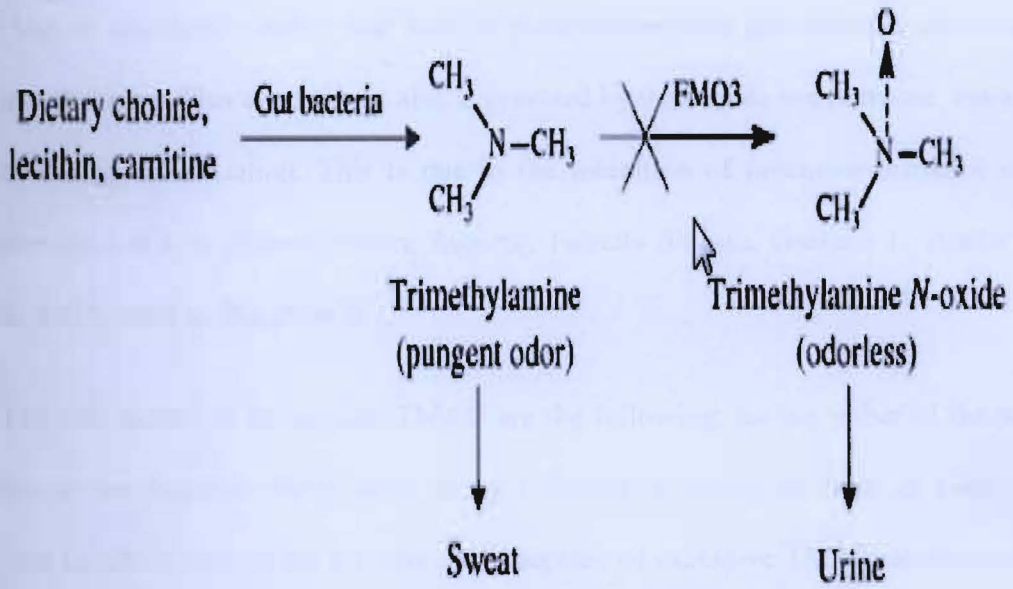

Figure 7.8. Mechanism of trimethylaminuria caused by FMO3 deficiency.

Diagram A

(Extracted from http://www.google.com.my/imglanding?q=trimethylaminuria/)

As shown in **Diagram A**, the FMO_3 deficiency caused no conversion of the pungent odour TMA to odourless TMNO and both of these compounds are normally excreted by the kidney into the urine. This condition is also aggravated by the female sex hormone, estradiol, just before or during menstruation. This is due to the inhibition of precursor estradiol on FMO_3 metabolism on TMA in plasma (Enrica Bignetti, Fiorella Sinesio, Gaetano L. Aiello & Carlo Cannella, 2009); refer to **Diagram B**.

The risk factors of developing TMAU are the following: having either of the parents as the carrier of the mutated FMO_3 gene, being a female, a history of liver or kidney disease without the family history of the disorder, consumption of excessive TMA precursor-containing foods, or the increase in bacteria-producing TMA in the digestive tract. Bignetti, Sinesio, Aiello and Cannella (2009) also thought that stress may trigger the symptoms of TMAuria. The types of FMO_3 mutations influences the variability of the symptoms of TMAuria experienced by the patient. The parameters that are involved in these mutations are the duration of onset and the strength of the rotting fish odour. The sum of different types of FMO_3 mutation are between 40 and 50 types.

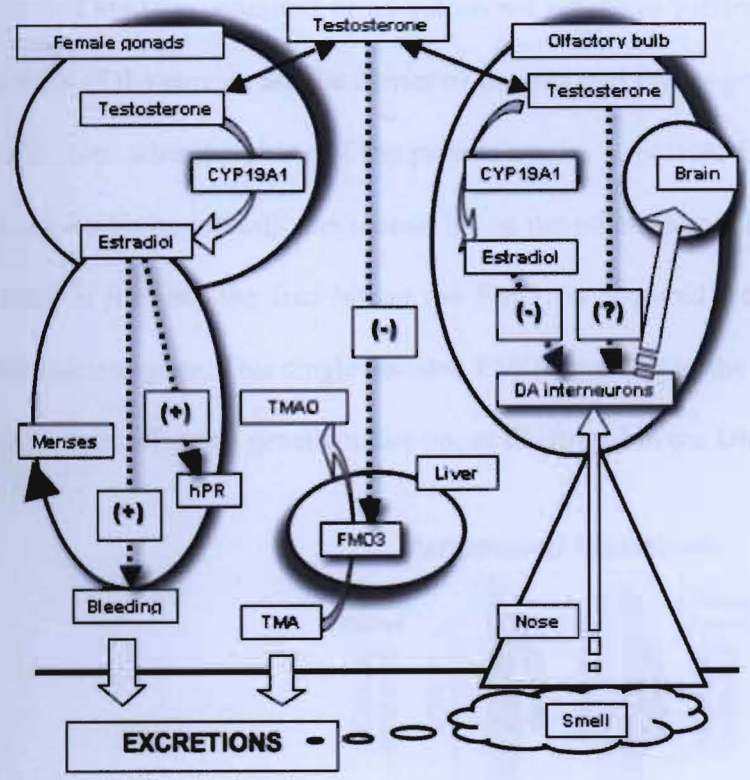

Diagram B

(Extracted from *Nutrients: The Amelioration of Olfactory Acuity up Sexual Maturation* Might Affect Foo Preferences, *Review*, by Enrica Bigne Fiorella Sinesio, Gaetano L. Aiello a Carlo Cannella, 2009.)

Since TMAU is inherited in an autosomal recessive pattern, TMAU is manifested in a patient if both of the parents are the carrier of the mutated FMO_3 gene. TMAU can also manifest itself in a person, whereby either of the parents carries a mutated FMO_3 gene. According to the Knudson's Two-hit hypothesis, the second hit on the other normal FMO_3 on the same locus will happen later in life, but the first hit on the FMO_3 is acquired from either of the parents that carries the mutated gene. This single mutated FMO_3 in either of the parent's chromosome is then passed on to their offspring genetic make-up, as illustrated in the **Diagram C.**

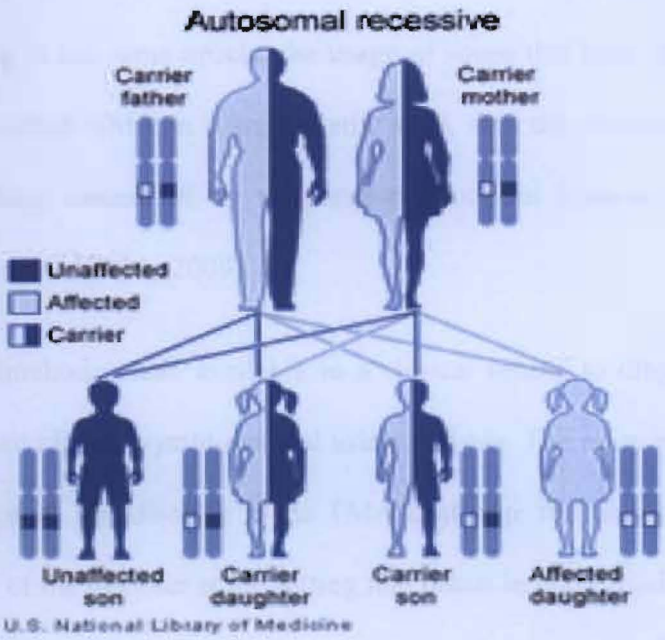

Diagram C Extracted from http://www.search.com/reference/Trimethylaminuria/

Currently, there is no specific treatment for TMAU. However, preventions could be carried out by the patient to minimize the associated symptoms. One of the ways are avoiding foods that are rich in the TMA precursors such as the those rich in choline. The examples of choline-rich foods are eggs, soy products, peas, beans, liver, kidney and peanuts. Other foods that should be avoided are lecithin and lecithin-containing fish oil supplements. In addition, TMNO precursors are obtained from fish, cephalopods and crustaceans. Other preventions include preventing activities that cause excessive sweating such as exercising, stress and emotional upset. Vitamin B_2 supplements can be taken to enhance any residual FMO_3 activities in hepatocytes. According to the same article, the usage of soaps that have pH near to the skin pH (5.5-6.5) can help to retain TMA in a less volatile state, thus the sweat on the skin can be easily removed by washing instead of by evaporation (*National Human Genome Research Institute*, National Institutes of Health, 2009).

There are a few methods made available in a clinical setting to diagnose TMAU. The diagnosis is made based on clinical symptoms and urine analysis. The more recent methods that are being applied to diagnose this disorder is the TMA challenge test and genetic testing. The normal clinical symptom of the disorder is the rotting fish odour in body fluids. Normally, in the screening test of TMAU, the urinalysis sample is to be collected within a 24-hour period after the ingestion of large amounts of choline. For the screening of a carrier of a mutated FMO_3 gene that is presented with mild or no symptoms of TMAU, the preferable diagnostic test is the TMA challenge. The patient is required to ingest a large amount of TMA. The collected urinalysis will show 20% to 30% of the total TMA load ingested. The non-carrier individual will excrete less than 13% of the total dose of TMA load. Genetic testing is preferred to diagnose TMAU due to mutations of the FMO_3 enzyme or a carrier of FMO_3 mutations only, rather than to diagnose

TMAU due to non-genetic causes. Blood analysis will show an elevated level in the plasma of unmetabolised TMA.

Genetics of FMO_3

The gene responsible of the FMO_3 enzyme is located on the long arm of chromosome 1 at the locus of 24.3 (1q24.3) as shown in **Diagram D**.

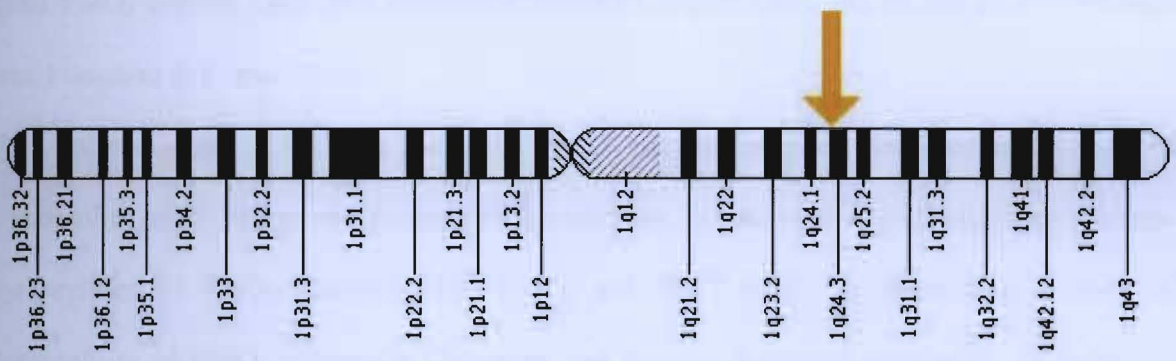

Diagram D
(Extracted from Genetics Home Reference, at http://ghr.nlm.nih.gov/gene/FMO3)

Cashman (1995) reported that human FMOs (EC1.14.13.8) have a length of 532 to 558 amino acids with specific amino acids highly conserved in all species, especially residues 4 to 32, which contains the FAD-binding domain and 186 to 213, which contains the NADPH-binding domain (as cited in Cashman, Beverly, Forrest and Treacy, 1999, p. 169).

FMOs are similar to the cytochrome P450 (CYP) family of mono-oxygenases in terms of function. They too are NADPH-dependent microsomal flavoproteins that are responsible for the oxygenation of nucleophilic nitrogen-, sulphur-, phosphorus- and other heteroatom-containing chemicals, drugs and pesticides. FMO enzymes known substrates are TMA, tertiary amine (S)-nicotine and commonly used drugs such as tricyclic antipsychotics, cimetidine, ranitidine and verapamill. (Treacy, Akerman,Chow, Youil, Bibeau, et al., 1998). Besides detoxicating these

substrates through FMO-mediated metabolism, FMO enzymes have been found to be responsible in the activation of certain xenobiotics.

FMO_3 is responsible for the conversion of TMA to TMNO. TMAU is manifested when less than 65% of TMA is converted (Brunelle, Bi, Lin, Russell, Luy, Berkman and Cashman, 1997) TMA and TMNO are effectively removed from the body by the kidney and subjects with normal FMO_3 activity have been reported to excrete 1 mg of TMA and 40 mg of TMNO daily (Bain, Fornasini & Evans 2005).

Polymorphism of the gene coding the FMO_3 enzyme has been identified as the cause of the variability of the degree of efficiency of this enzyme. Lattard et al. (2003) identified two new polymorphism of FMO_3 namely, His^{132}-FMO_3 and Pro^{360}-FMO_3 in conducting a study of polymorphism of FMO_3 enzyme in Caucasian and African-American population. Both His^{132}-FMO_3 and Pro^{360}-FMO_3 variants were able to metabolize the substrates examined. Compared to wild-type FMO_3, His^{132}-FMO_3 was less catalytically efficient. The His^{132}-FMO_3 variant altered the catalytic efficiency of FMO_3 moderately (decrease of 30%, 60% and 6% with methimazole, trimethylamine and 10-(N,*N*-dimethylaminopentyl)-2-(trifluoromethyl)-phenothiazine, respectively). The Pro^{360}-FMO_3 variant was more catalytically efficient than wild-type FMO_3. Pro^{360}-FMO_3 oxygenated methimazole, trimethylamine and 10-(N,*N*-dimethylaminopentyl)-2-(trifluoromethyl)phenothiazine, respectively, 3-, 5- and 2-fold more efficiently than wild-type FMO_3. Based on the functional activity of the variant FMO_3 enzymes, it is likely that population differences exist for compounds primarily metabolized by FMO_3. A more recent research done by Mao et al. (2009) has also identified three coding region variations: g.15167G>A (E158K), g.18281G>A (V257M) and g.21443A>G (E308G) in 13 ethnic populations from Europe, East Asia and sub-Saharan Africa. Thus, variations in the coding region dictate the degree of

efficiency of the FMO_3 enzyme. Both the research mentioned above illustrates that different polymorphisms exist for different ethnic populations across the world. Unfortunately, our research will not be able to identify the exact polymorphism of the FMO_3 enzyme.

A variable extent of decreased FMO_3 enzyme activity as a functional impact of the three variants *in vitro* has been characterised often in a substrate dependant manner. A transient or mild form of TMAuria can be triggered to an exposure of high TMA and hormonal changes related to menstruation in participants homozygous for both K158 and G308.

Pharmacokinetics of FMO_3

FMO is similar to cytochrome P450 (CYP) in location, co-factor requirement and activity aspects, that is, it uses oxygen and NADPH to convert N,N'-dimethylaniline to N – oxide. The human flavin-containing monooxygenase form 3 (FMO_3) is involved in the oxygenation of nucleophilic heteroatom-containing drugs, xenobiotics, and endogenous materials. FMO_3 is the major form in adult human liver within the six forms of FMO that have been identified. Severe defect in FMO_3 enzyme leads to a disease known as trimethylaminuria.

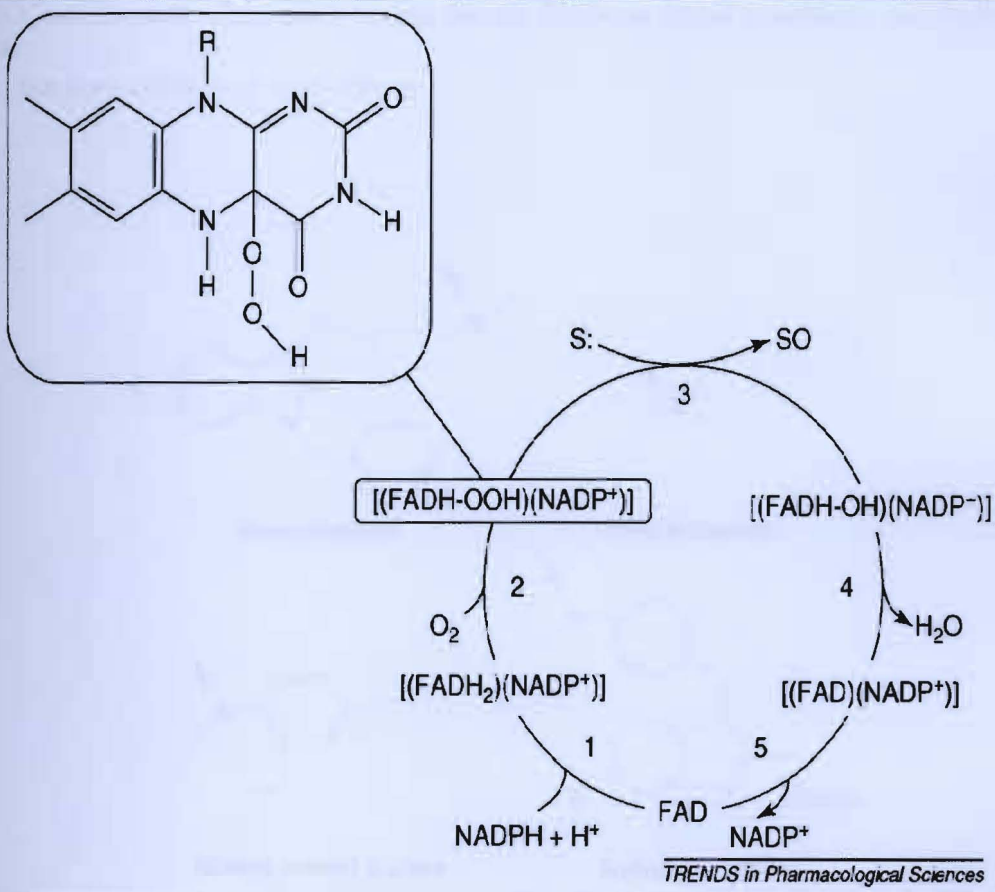

Diagram E. The catalytic cycle of mammalian FMOs. Evidence for the cycle is based on spectrophotometric and kinetic studies (reviewed in Refs [5,6]). The cofactor NADPH binds and reduces the prosthetic group FAD to FADH2, via hydride ion transfer (step 1). Molecular oxygen then binds and is reduced, forming 4a-hydroperoxyflavin (4a-HPF or FADH-OOH) (step 2), which is stabilized by NADP+. Steps 1 and 2 are fast. The 4a-HPF, the structure of which is shown, is a s intermediate and is considered to be the form in which FMO is present in the cell. Oxygenation of substrate S to SO occurs via nucleophilic attack on the distal O atom of 4a-HPF, leaving FAD as the 4a-hydroxyflavin (FADH-OH) (step 3). The rate-limiting step is the release of water from the 4a-hydroxyflavin to reform FAD (step 4) and consequently the value of kcat should be independent of the structure of S. The final step is the release of NADP+ (step 5).

(Extracted from Short Communication: Effect of Genetic Variants of the Human Flavin-Containing Monooxygenase 3 on *N*- and *S*-Oxygenation Activities)