Trimethylaminuria ('fish-odour syndrome'): a study of an affected family

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(Received 16 February/30 July 1987; accepted 24 August 1987)

SUMMARY

1. Beginning with a single propositus, who had been previously diagnosed at the age of 10 as suffering from trimethylaminuria (fish-odour syndrome), both her parents and two sisters were investigated biochemically with respect to their ability to N-oxidize trimethylamine (TMA), both when derived from the diet and when administered exogenously.

2. Both the propositus and a second sister were markedly deficient in their ability to N-oxidize TMA, both when derived from the diet and when given as such; furthermore, both siblings readily developed the symptoms of fish-odour syndrome as characterized by a strong objectionable breath and body odour shortly after the oral administration of TMA (300 mg).

3. At this dose level of TMA, neither of the parents nor the third sister showed any evidence of impaired N-oxidation ability nor did they experience any 'fish-odour' symptoms.

4. With an oral challenge of 600 mg of TMA, both the parents showed a clear impairment of N-oxidation capacity which was not seen in six healthy unrelated volunteers. Both parents experienced a fish-odour syndrome at this level of TMA challenge.

5. The family data support the hypothesis that trimethylaminuria is an inborn error in the ability to N-oxidize TMA which is inherited as an autosomal recessive trait. Furthermore, experience with this family suggests that an oral challenge dose with 600 mg of TMA may be used to identify carriers of the condition.

Key words: fish-odour syndrome, impaired N-oxidation, inborn error of metabolism, N-oxidation, trimethylamine.

Abbreviations: TMA, trimethylamine; TMAO, trimethylamine N-oxide.

INTRODUCTION

Trimethylaminuria (fish-odour syndrome) is a metabolic disorder characterized biochemically by the excretion in the urine of relatively large amounts of the volatile tertiary amine, trimethylamine (TMA) [1]. This amine has a strong fish-like odour and it confers upon the breath and sweat of individuals with the disorder, a characteristic objectionable smell: hence the colloquialism 'fish-odour syndrome'. Normally, TMA is readily oxidized in vivo to its N-oxide [2, 3], a metabolite which is odourless and is readily excreted in the urine. TMA is of dietary origin [4]; it is formed by the reduction of the N-oxide which is present at high concentrations in marine fish and it is also formed by the intestinal bacterial degradation of choline present in foods such as egg yolk, liver, kidney, soy beans, peas and meat. The condition of trimethylaminuria has received comparatively little attention as it is believed to be a relatively uncommon metabolic disorder. Indeed, few cases to date appear to have been described in the literature (see Table 1). Although it has been suggested that the condition is an inherited one [5, 6], this remains to be proven, and furthermore if the condition is transmitted in an autosomal recessive manner there is no reliable way to identify individuals who may be carriers of the disorder.

Our own interest in the problem stemmed from recent random population studies on the distribution of the ability to effect the metabolic N-oxidation of TMA which have shown that the condition of impaired N-oxidation may not be rare, as two affected individuals (a 21-year-old male and a 20-year-old female both of healthy disposition) out of a cohort of 169 have been characterized as displaying this condition [7, 8].

Although TMA itself appears to be relatively non-toxic [9], trimethylaminuria cannot be described as benign, since affected patients may show considerable psychological reactions to their condition including anxiety, depression and even attempted suicide. Young people may accumulate educational disadvantages attributable to the consequences of psycho-social isolation [10].
Table 1. Summary of clinical details and methods of diagnosis of published cases of trimethylaminuria ('fish-odour syndrome')

<table>
<thead>
<tr>
<th>Report</th>
<th>No. of cases</th>
<th>Sex</th>
<th>Family history of TMA</th>
<th>Association with other clinical conditions</th>
<th>Method of diagnosis</th>
<th>Treatment</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>F</td>
<td>None</td>
<td>Congenital deformities</td>
<td>Spot urine analysed by g.c.-m.s.; TMA load</td>
<td>Food restriction</td>
<td>[1]</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>3F, 1M</td>
<td>None</td>
<td>None</td>
<td>Spot urine analysed by g.c.</td>
<td>Food restriction was successful in only one case; neomycin sulphate in one case</td>
<td>[20]</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1F, 2M</td>
<td>2 sibs</td>
<td>None</td>
<td>Early morning urine analysed by g.c.-m.s.</td>
<td>Food restriction</td>
<td>[5]</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>M</td>
<td>None</td>
<td>None</td>
<td>Spot urine analysed by g.c.; choline load</td>
<td>Food restriction</td>
<td>[15]</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>M</td>
<td>None</td>
<td>None</td>
<td>Spot urine analysed by g.c.</td>
<td>Food restriction</td>
<td>[10]</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>F</td>
<td>None</td>
<td>None</td>
<td>Early morning urine analysed by g.c.</td>
<td>Food restriction</td>
<td>[6]</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>M</td>
<td>None</td>
<td>Congenital deficiency, pre-term baby</td>
<td>Random urine by g.c.; choline load</td>
<td>Choline restriction</td>
<td>[21]</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>2F, 1M</td>
<td>None</td>
<td>None</td>
<td>Random urine sample analysed by g.c.</td>
<td>Food restriction</td>
<td>[12]</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>M</td>
<td>None</td>
<td>Congenital deformities</td>
<td></td>
<td>Metronidazole</td>
<td>[4]</td>
</tr>
</tbody>
</table>

In the present study an entire family has been studied with respect to trimethylaminuria and the ability to N-oxidize orally administered doses of TMA. Two sisters have been confirmed to display trimethylaminuria and a reduced ability to oxidize TMA, while the findings with oral doses of TMA suggest that this can be used not only to help diagnose what appears to be an autosomal recessive inherited trait but it may also be used to identify carriers.

SUBJECTS AND METHODS

Clinical history

A 21-year-old healthy female with normal intelligence and physical growth had been noted since her early childhood by her mother to have strong body odour and to produce urine that smelt like rotten fish. At the age of 10 she was diagnosed as having trimethylaminuria on the basis of her complaint and the detection of high concentrations of unoxidized TMA in the urine. She has been largely successfully treated by avoidance of fish and food items rich in choline. The patient's history revealed that she is the second child of healthy, unrelated parents. Her two sisters were both apparently healthy but on further questioning it was revealed that her younger sister, who is 18 years old, had also caused concern to her mother as a baby because of her apparent persistent 'urinary smell'. This second sibling was never examined clinically or biochemically. Although the sisters are successful at their present jobs, both had left school at an early age as they were very embarrassed by their bodily smells which had been a permanent source of anxiety and remain so to this day. Neither of the parents nor their eldest daughter suffers from a body odour problem. Neither parent could recall anyone in their own families as suffering from a similar complaint.

Investigations

The following investigations were carried out for each member of the family:

(i) Routine haematology, erythrocyte sedimentation rate, tests of haemostatic function, liver function tests, thyroid function, electrolytes, creatinine and creatinine clearance.

(ii) A 24 h urine collection in a container containing 10 ml of 4 mol/l HCl for estimation of the daily excretion of TMA and its N-oxide.

(iii) An oral trimethylamine loading test where each family member received TMA hydrochloride (485 mg equivalent to 300 mg of TMA base) in a capsule and the subsequent 24 h urine was collected as above. In further TMA loading tests the parents, unaffected daughter 1 and six healthy unrelated volunteers were each given 970 mg of TMA hydrochloride (equivalent to 600 mg of TMA base) in a gelatin capsule and an 8 h urine collection was performed under acidic conditions as above. Control urine samples were collected from all subjects studied during the day preceding challenge with TMA. The volumes of all urine samples were recorded and aliquots were stored at −20°C until analysis.

Ethical approval for these studies was obtained from St Mary's Hospital Ethical Committee. Each participant gave their informed consent for the study.
Estimation of TMA and trimethylamine N-oxide (TMAO) in urine

Five millilitres of urine and 100 µl of 0.2% (v/v) isopropanol (internal standard) were placed in a 15 ml screw-top septum glass vial. Potassium carbonate (2–3 g) and five to ten pellets of KOH were added to the urine, chilled on ice and then the vials were sealed with airtight Teflon-lined septum caps. Vials were vortexed and heated at 90°C for 20–30 min in an aluminium heating block and an aliquot (2 ml) of the head space gas generated was injected directly on to the g.l.c. column using an airtight plastic disposable syringe. The areas of the relevant peaks were calculated by a spectrophysics SP 4270 integrator. Urine samples were analysed in duplicate.

For the determination of TMAO, 2 ml of urine was reduced by titrous sulphate (0.2 ml 15%, w/v, in 23% w/v, aqueous H₂SO₄) in a septum vial at 30°C for 30 min as previously described. Samples of the reduced urine were diluted with water and analysed as above.

The chromatography was performed on a PV 204 (Pye-Unicam, Cambridge, U.K.) using ‘flame ionization’ detection. The temperatures of the injector, detector and column were 130°C, 200°C and 70°C respectively. The column (170 cm x 0.4 cm) was packed with 4% Carbowax 20M and 0.8% KOH and Carbopack B (Supelco Inc., Pennsylvania, U.S.A.) as described by Brewster & Schedewie [12]; the carrier gas (nitrogen) flow rate was 40 ml/min. Using these conditions the retention times of dimethylamine, TMA and isopropylamine were 1.6, 2.1 and 3.5 min respectively. The limit of detection was 1 ng/ml and the assay was employed in the range 1–10 µg/ml where the coefficient of variation was 1–5%.

Expression of results

Results have been expressed in terms of: (a) µmol of TMA and TMAO excreted in the urine in specified times and (b) percentage of the total TMA excreted in the form of the N-oxide, is calculated from the formula:

\[
\frac{\text{Total TMA} - \text{free TMA}}{\text{Total TMA}} \times 100
\]

and (c) in the form of a metabolic ratio for N-oxidation calculated as the ratio of TMA (µmol)/TMAO (µmol) excreted in the 8 h or 24 h urine collections.

RESULTS

Urinary excretion of TMA and TMAO

All the measured haematological parameters and the biochemical indices for renal, thyroid and liver function were within the normal range. Analysis of the urine samples collected for 24 h for both parents and daughter 1 under normal dietary conditions (a diet not containing TMA-rich foods such as fish or eggs) showed the presence of relatively small amounts of TMA together with much larger amounts of the N-oxide. For these three subjects N-oxide excretion accounted for 96.6% (range 95.3–98.3%) of the total TMA excreted. This value is in good agreement with a random population study for 169 unrelated individuals [8] who are extensive metabolizers of TMA in which it was found that the mean value for N-oxide excretion as a percentage of total TMA elimination was 98.8% (range 93.9–99.7%).

The N-oxidation metabolic ratio for the three subjects ranged from 0.02 to 0.05. However, for daughters 2 and 3 the TMA excretion data reflect their impaired ability to effect the metabolic N-oxidation of the amine. Thus both excreted relatively large amounts of unmetabolized TMA under normal dietary conditions. TMAO excretion accounted for only 22.2% and 14.3% of total TMA excretion respectively for the two daughters. The N-oxidation metabolic ratios for these two subjects were 3.51 and 6.02 respectively, two orders of magnitude greater than those observed for the parents and the unaffected daughter 1.

Oral challenge studies

After oral TMA challenge of both parents and daughter 1 with TMA (300 mg) there occurred, not unexpectedly, an increase in the excretion of both TMA and its N-oxide (Table 2). However, at this dose level TMAO excretion, as a percentage of total TMA excretion, remained high at 94.9% (range 92.5–97.6%) and was

<table>
<thead>
<tr>
<th>Subject</th>
<th>Normal dietary conditions</th>
<th>TMA (300 mg) challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TMA (µmol/24 h)</td>
<td>TMAO (µmol/24 h)</td>
</tr>
<tr>
<td>Mother</td>
<td>26</td>
<td>1490</td>
</tr>
<tr>
<td>Father</td>
<td>45</td>
<td>910</td>
</tr>
<tr>
<td>Daughter 1</td>
<td>20</td>
<td>600</td>
</tr>
<tr>
<td>Daughter 2*</td>
<td>485</td>
<td>140</td>
</tr>
<tr>
<td>Daughter 3*</td>
<td>745</td>
<td>125</td>
</tr>
</tbody>
</table>

*Propositi with trimethylaminuria.
similar to that seen under normal dietary conditions. The N-oxidation metabolic ratio ranged from 0.03 to 0.08. These data suggest that in these three subjects the oral dose of 300 mg of TMA did not saturate the N-oxidation process. None of the subjects complained of fish-odour sensation during the course of the challenge apart from the first few minutes after taking the capsule of TMA. In the affected sisters 2 and 3, however, the oral administration of TMA (300 g) was accompanied by the appearance of the classical features of trimethylaminuria: the development of a strong body odour and breath and the appearance in the urine of large amounts of unmetabolized TMA. TMAO excretion accounted for only 18.0 and 9.6% of total TMA excretion for daughters 2 and 3 respectively, while the N-oxidation metabolic ratios were 4.55 and 9.41 respectively. Because the oral administration of 300 mg of TMA had elicited all the characteristic unpleasant features of the fish-odour syndrome, the daughters were not challenged with the higher dose (600 mg) of TMA.

Table 3 shows the urinary excretion data for TMA and its N-oxide after the oral administration of 600 mg of the base to the two parents and daughter 1 and to six healthy volunteers. Even at this challenge level of TMA the six volunteers and unaffected daughter 1 excreted TMA largely in the form of its N-oxide: mean value 92.9% (range 90.5–95.4%), mean N-oxidation metabolic ratio 0.07 (range 0.05–0.11).

By contrast both the mother and father of the affected daughters excreted relatively large amounts of unchanged TMA, while N-oxide excretion accounted for only 78.9 and 77.6% respectively of the total TMA excretion. The N-oxidation metabolic ratios were 0.27 and 0.29 for the mother and father respectively. It is also of interest to note that both parents complained of a ‘fish-odour’ experience at the higher dose level of 600 mg, but there were no complaints from the six unrelated volunteers given the same dose.

Fig. 1 shows the pedigree diagram for the family investigated. Each family member has been characterized with respect to an N-oxidation metabolic ratio after oral challenge with TMA (300 mg) and for the parents and the unaffected daughter 1 at a higher dose of 600 mg.

**DISCUSSION**

Historically, the phenomenon of ‘fish-odour syndrome’ was probably first described over two centuries ago in 1735 by Arbuthnot [13] when he wrote: “the oils with which fishes abound often turn rancid and lie heavy on the stomach and affect the very sweat with a rancid smell which is found to be true in some places where the inhabitants live entirely on fish”. The odour of fish, particularly that of the spoiled marine type, is now known to be mainly due to the presence of the simple aliphatic tertiary base, TMA, which arises from the bacterial degradation of TMAO, present at high concentrations in the tissues of such fish. The first clinical description of a case of fish-odour syndrome attributable to the excretion of unoxidized TMA was the report of Humbert et al. in 1970 [1]. Since then a total of 15 further cases have been reported in the literature (see Table 1). The prevalence of this syndrome is possibly underestimated; it has received relatively little attention and for this reason passes

![Fig. 1.](image_url)

**Table 3. Urinary excretion of TMA and TMAO by parents of the affected family and by healthy unrelated volunteers after oral challenge with 600 mg of TMA.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>TMA (µmol/8 h)</th>
<th>TMAO (µmol/8 h)</th>
<th>Amount of total TMA excreted as TMAO (%)</th>
<th>TMA/TMAO ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td>1445</td>
<td>5410</td>
<td>78.9</td>
<td>0.27</td>
</tr>
<tr>
<td>Father</td>
<td>1530</td>
<td>5290</td>
<td>77.6</td>
<td>0.29</td>
</tr>
<tr>
<td>Daughter 1</td>
<td>425</td>
<td>4230</td>
<td>90.9</td>
<td>0.10</td>
</tr>
<tr>
<td>Volunteer 1</td>
<td>545</td>
<td>5860</td>
<td>91.5</td>
<td>0.09</td>
</tr>
<tr>
<td>Volunteer 2</td>
<td>680</td>
<td>6440</td>
<td>90.5</td>
<td>0.11</td>
</tr>
<tr>
<td>Volunteer 3</td>
<td>345</td>
<td>7115</td>
<td>95.4</td>
<td>0.05</td>
</tr>
<tr>
<td>Volunteer 4</td>
<td>520</td>
<td>6190</td>
<td>92.3</td>
<td>0.08</td>
</tr>
<tr>
<td>Volunteer 5</td>
<td>525</td>
<td>7270</td>
<td>93.3</td>
<td>0.07</td>
</tr>
<tr>
<td>Volunteer 6</td>
<td>345</td>
<td>6055</td>
<td>94.6</td>
<td>0.06</td>
</tr>
</tbody>
</table>
unrecognized in most texts on human metabolic disease or dermatology. It is perhaps not surprising therefore that many doctors are unaware of the existence of this problem. The diagnosis can be complicated by the fact that the condition is episodic, probably reflecting variations in the dietary intake of TMA precursors such as choline, betaine and TMAO.

Trimethylaminuria was described in the original case to be associated with Turner's syndrome and splenomegaly [1]. However, this may have been fortuitous since Calvert [14] found no evidence of trimethylaminuria in a clinically similar case of Noonan's syndrome both with and without loading with TMA. In the two cases reported here both sisters were healthy and successful in their occupations and it seems unlikely therefore that trimethylaminuria is necessarily a feature of a more complex congenital syndrome. However, the possibility of genetic linkage cannot be dismissed and a study of the incidence of trimethylaminuria in Turner's syndrome might be rewarding.

Several earlier investigations have been suggestive of a genetic background to the condition. Thus, Lee et al. [5] have described a case of a brother and sister both exhibiting trimethylaminuria but other family members were apparently not further investigated. Marks et al. [15] studied the family of an affected patient using choline loading, with inconclusive findings. Spellacy et al. [6] studied the parents of a patient also using choline loading and found that under these conditions the father excreted excessive amounts of TMA in the urine. Brewer & Schedewie [12], using an oral challenge of choline (50 mg/kg), showed that the mothers of three trimethylaminuria patients excreted increased amounts of TMA compared with 76 healthy volunteers [12].

We believe that the data presented in this paper provide the clearest evidence available to date for the inherited nature of trimethylaminuria. Furthermore, we believe that the oral challenge test with TMA is superior to the use of choline loading for the confirmation of patients with trimethylaminuria and it offers clear potential for the identification of carriers.

Two daughters have been shown to excrete relatively large amounts of unmetabolized TMA both under normal dietary conditions and challenge with an oral dose of TMA (300 mg). Indeed, the two affected siblings fell 31 and 34 standard deviations respectively below the mean random population value for N-oxide excretion (96.03%) and the probability therefore that the two sisters belong to the same phenotype is infinitesimal. Furthermore, both developed the symptoms of fish-odour syndrome after challenge with TMA (300 mg) both under normal dietary conditions and challenge with TMA (300 mg) both parents readily oxidized the amine and could not be differentiated from randomly selected healthy volunteers in this respect. However, at 600 mg there was clear evidence of metabolic saturation and an increase in the N-oxidation metabolic ratio which was three to four times greater than that seen for six healthy volunteers. This would suggest that at this dose level N-oxidation of TMA has become saturated and the reaction therefore no longer follows first-order kinetics; in this sense therefore both parents may be spoken of as 'affected'. The finding of the two sisters with the same metabolic defect is suggestive that trimethylaminuria is an inherited condition. The N-oxidation metabolic ratios for the two parents at the 600 mg of TMA challenge level have clearly revealed a degree of N-oxidation deficiency compared with healthy volunteers. The N-oxidation metabolic ratio data for the pedigree studied (Fig. 1) are consistent with the hypothesis that trimethylaminuria is an inherited condition transmitted in an autosomal recessive manner and that the two parents in this study are both carriers or heterozygotes. On this hypothesis therefore, daughters 2 and 3 would be considered to be homozygous for the condition and therefore readily display trimethylaminuria or fish-odour syndrome on exposure to low levels of TMA. By contrast, daughter 1 showed no significant change in N-oxidation capacity at the 600 mg of TMA challenge dose and must be considered therefore to be a homozygous extensive N-oxidizer of TMA.

As regards the detection and confirmation of variability in the ability to N-oxidize TMA there are several reasons to consider the use of TMA itself superior to the use of a precursor such as choline. The choline load procedure has the major disadvantage for example that the compound has to await bacterial degradation in the lower intestinal tract in order to generate TMA and the rate and extent to which this occurs may vary markedly with different individuals. People may therefore differ with respect to the amount of TMA to which they are exposed. The administration of a precise dose of TMA in salt form overcomes this objection. Furthermore, the administration of oral doses of TMA of 300 mg and 600 mg is without noticeable effect. The high dose of 600 mg would be contained naturally as the N-oxide in about 150 g of marine fish. Using the TMA loading test both sisters were clearly confirmed in this study as suffering from a deficiency in the ability to N-oxidize the amine. At the higher dose level of 600 mg both parents could also be shown to have a relative deficiency compared with healthy volunteers.

The precise nature of the biochemical defect in trimethylaminuria awaits elucidation. The enzyme responsible for the N-oxidation of TMA is believed to be a microsomal non-cytochrome P-450 enzyme shown by Ziegler & Mitchell [18] to be an NADPH-dependent flavoprotein. It is possible that this enzyme is defective in trimethylaminuria, perhaps as a result of genetic polymorphism at a regulatory or structural gene. Higgins et al. [19] found that the conversion in vitro of TMA to its N-oxide by liver tissue obtained from the trimethy-
laminuric patient described by Humbert et al. [1] was impaired, thus indicating the presence of a defective form or frank absence of the enzyme.

The diagnosis to date has usually depended upon clinical history and measurement of urinary TMA excretion on random urine specimens or after oral challenge with a diet rich in TMA precursors such as eggs and fish, or with choline as such, or TMA itself. The present family study has revealed the usefulness of the TMA challenge test in confirming unequivocally the trimethylaminuric patients as well as indicating the status of the parents as carriers. The use of the other challenge procedures may at times produce equivocal results.

Dietary restriction appears to have been relatively successful in the management of the disorder, although Dankes et al. [20] found that this was useful in only one of the four patients studied. This approach requires the restricted intake of foods rich in choline such as eggs, meat, mayonnaise and marine fish which contains TMAO and the free amine. Antibiotic supplement such as neomycin, presumably by reducing the gut flora degradation of choline and TMAO to TMA, can be helpful [20], while Shelley & Shelley [4] found that a regimen of dietary restriction and metronidazole was effective in suppressing the fish-odour syndrome.

ACKNOWLEDGMENT

We are grateful for the support of the Wellcome Trust (R.A.A.) and the Arabian Gulf University (M.W.). J.R.I. is a Wellcome Trust Senior Lecturer. We also express our thanks to Dr D. J. Atherton for permitting us to study his patient.

REFERENCES