JIMD Reports
DOI 10.1007/8904_2013_238

CASE REPORT

Transient Massive Trimethylaminuria Associated with Food Protein–Induced Enterocolitis Syndrome

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Received: 05 December 2012 / Revised: 03 April 2013 / Accepted: 13 May 2013 / Published online: 3 July 2013

Abstract Trimethylaminuria (TMAU) is an autosomal recessive disease caused by excessive excretion into body fluids and breath of unoxidized trimethylamine (TMA) derived from the enterobacterial metabolism of dietary precursors. The condition is caused by deficiency of flavin-containing monooxygenase 3 (FMO3) which leads to impairment of hepatic TMA oxidation to the odorless trimethylamine *N*-oxide. Secondary TMAU is due to substrate overload in individuals with genetically determined reduced enzyme activity. Food protein–induced enterocolitis syndrome (FPIES) is characterized by recurrent episodes of emesis, diarrhea, dehydration, and lethargy after ingestion of offending foods. Its pathophysiology involves local non-IgE-mediated inflammation of the gastrointestinal tract, which leads to increased intestinal permeability. We report on an 8-month-old male who presented with typical episodes of FPIES associated with intense fish-like body odor. Further investigation in our patient revealed massive urinary TMA excretion during acute FPIES presentation and complete normalization between these episodes. The patient was found to be heterozygous for a novel, paternally inherited nonsense p.Tyr331X mutation and for two maternally inherited common polymorphisms, E158K and E308G, in the *FMO3* gene. We propose that our patient was able to cope with the daily burden of TMA, but when challenged with substrate overload, he failed to oxidize TMA due to limited reserve enzyme capacity. We discuss the pathophysiology of TMAU and FPIES and suggest potential mechanisms for the clinical and biochemical findings. Our report illustrates the complex interplay of genetic and environmental factors in TMAU and sheds light on the pathophysiology of FPIES.

Communicated by: Johannes Zschocke

Competing interests: None declared

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Introduction

Trimethylamine (TMA) is a volatile, fish-smelling compound that is formed via the reduction of trimethylamine-N-oxide (TMAO) and choline (Phillips and Shephard 2011). Choline is found in peas, beans, organ meats, and egg yolks. Different foods contain different amounts of choline: breast milk 160 mg/L of choline (approximately 120 mg/100 g food), formula 175 mg/L (approximately 130 mg/100 g food), banana 9.8 mg/100 g food, rice 2.1 mg/100 g food, oat 32 mg/100 g food (http://www.nal.usda.gov/fnic/foodcomp/Data/Choline/Choln02.pdf) (US Database for the Choline Content of Common Foods 2008). TMAO is found in high concentrations in marine fish. Bacteria in rotting fish reduce TMAO to TMA, producing a fish-like odor. Bacteria in the mammalian gut also reduce TMAO to TMA, which then enters the enterohepatic circulation. In the liver, the hepatic enzyme FMO3 oxidizes TMA back to TMAO, an odorless, water-soluble compound excreted in the urine. Trimethylaminuria (TMAU) is caused by excessive accumulation of the fish-smelling TMA, which is excreted in the urine, sweat, breath as well as other bodily secretions (Mackay et al. 2011).

The superfluous excretion of trimethylamine is the result of a mismatch between enzyme capacity (its ability to form the non-odorous TMAO) and the amount of the substrate (TMA) (Mitchell and Smith 2001). Factors that increase the substrate burden include (1) microbial liberation of TMA from dietary precursor chemicals (bacterial overgrowth, and renal and hepatic diseases), (2) increased TMA absorption (gastrointestinal problems), or (3) simply an enriched diet. Diagnosis is made via measurement of TMA and TMAO in the urine: a TMAO/ (TMA + TMAO) ratio (also known as FMO3 metabolic capacity) (Yamazaki et al. 2004) of <92 % and/ or a urinary concentration of free TMA of 18–20 μmol/mmol creatinine is diagnostic of TMAU (Mayatepek and Kohlmuller 1998; Mitchell and Smith 2001; Cashman et al. 2003).

"Secondary trimethylaminuria" is due to substrate overload in individuals with genetically determined reduced enzyme activity and who might not exhibit any symptoms until they challenged with excessive amounts of TMA or its precursors (Mackay et al. 2011).

Food protein–induced enterocolitis syndrome (FPIES) is a non-IgE-mediated gastrointestinal hypersensitivity to food. It typically presents before 6 months of age, and usually resolves by 3 years of age. It is characterized by repeated episodes of profound vomiting and often diarrhea, beginning 1–5 h after ingestion of the offending food, and leads to lethargy, dehydration, and occasionally to shock. The most common offending foods are milk, soy, and rice (Mehr et al. 2009; Leonard and Nowak-Wegrzyn 2011). The repetitive nature of the condition and its association with lethargy and vomiting may suggest metabolic conditions.

The pathophysiology of FPIES is not clearly elucidated. It likely involves defects in both barrier and immunologic function of the gastrointestinal tract. Ingestion of food allergens causes local inflammation, which may lead to increased intestinal permeability and fluid shifts, and may lead to vomiting and diarrhea (Leonard and Nowak-Wegrzyn 2011). Multiple studies have suggested a role of the pro-inflammatory cytokine, TNF-α (Chung et al. 2002; Caubet et al. 2011). High amounts of TNF-α have been found to be released by antigen-specific T cells in the GI tract. TNF-α works synergistically with IFN-γ to increase intestinal permeability (Heyman et al. 1994). High levels of TNF-α have also been observed in infants with FPIES and associated villous atrophy (Caubet and Nowak-Wegrzyn 2011; Chung et al. 2002). Another cytokine implicated is TGF-β1, which suppresses T cells, protects the epithelial barrier of the gut, enhances binding between epithelial cells and the extracellular matrix, and stimulates the expression of extracellular matrix proteins. Infants less than 3 months old have decreased expression of TGF-β1, and this expression increases with age (Chung et al. 2002). This developmental deficiency of TGF-β1 may allow for increased intestinal permeability and less T cell suppression in young infants (Caubet and Nowak-Wegrzyn 2011).

Here, we describe an infant with repeated episodes of adverse food reactions, consistent with FPIES, associated with a "fishy" odor. He was found to have excessive excretion of TMA during FPIES episodes and to be a heterozygous for the novel nonsense p.Tyr331X mutation and for two common polymorphisms, E158K and E308G, in the *FMO3* gene.

Case Presentation

The proband is a Caucasian male infant who has had recurrent episodes of adverse reactions to food since the age of 4 months. Initially, he developed repeated episodes of profound emesis and lethargy 2 h after eating rice cereal mixed with expressed breast milk. Vomiting was followed by diarrhea that occurred 6–8 h after food ingestion. His symptoms seemed to improve about 8 h after ingestion, and resolved within 24 h. During this episode, his mother noted a strong, "fishy" odor, which had never been noted previously. He had four more similar episodes, at 5 months, 7 months, and 10 months of age, after eating rice cereal, oat, and banana. During each of these episodes, the "fishy" odor was noted, but was not noted between episodes. He was evaluated in the Emergency Department for all the three episodes, and required hospitalization for intravenous hydration during the 10-month episode.

The proband was born full-term at 37 weeks gestation via spontaneous vaginal delivery. His birth weight was 3.29 kg and birth length was 53 cm. The mother was 22 years old at delivery, this was her second pregnancy and it was conceived naturally. She was treated with Procardia and prenatal vitamins. Pregnancy was complicated by premature contractions at 26 weeks gestation. The mother was treated with two injections of steroids for lung maturity at 27 weeks gestation. There were no perinatal or neonatal complications. The patient had normal newborn metabolic and hearing screens, and was discharged home 48 h after delivery. He met his developmental milestones and there has been no history of developmental regression. His past medical history is significant for ptosis status post surgical repair at 6 months of age. He was followed by cardiology for a VSD, which spontaneously closed. His mother is of white Caucasian descent (German/Scottish). She is healthy except for irritable bowel syndrome and Gilbert disease. She has a history of spontaneous abortion at 3 months gestation. His father is 26 years old and is of white Caucasian descent (German). He is healthy except for irritable bowel syndrome.

Food-specific IgE testing, using the Phadia ImmunoCAP system FEIA (Phadia, Uppsala, Sweden), to multiple foods, including rice, banana, oat, beef, chicken, and turkey, were all below level of detection (<0.34 kU/L). CBC, electrolytes, and renal and liver function were all within normal limits. Due to concerns of metabolic disorders, acylcarnitine

Table 1 Urinary excretion of TMA, TMA-N-oxide (TMAO) [(μmol/mmol creatinine] and the percentage of total TMA excreted as TMAO in our patient, patients with trimethylaminuria, and healthy controls

Timing of the testing	TMA (normal <1)	TMAO (normal 15–125)	Percentage of total urine TMA excreted as TMAO (normal >92 %)
Before FPIES	2.3	54.2	95.9
During FPIES	551.91	4423.38	88.9
After FPIES	3.4	63.7	94.9
During acute gastroenteritis	8.8	51.9	85.5
Reported cases of fish-odor syndrome ($n = 4$) (Mayatepek & Kohlmuller 1998)	>18	<2	<10

panel, serum amino acids, urine organic acids, ammonia, lactate/pyruvate, and CK were obtained and were within normal limits. Due to mother's complaints of "fishy odor", a quantitative analysis of urine TMA and TMAO using electrospray ionization tandem mass spectrometry (ESI-MS/MS), as previously described (Johnson 2008), was ordered 3 days before an acute episode of FPIES and revealed normal results. However, during admission for acute FPIES episode, a massive urinary TMA and TMAO excretion with decreased total TMA percentage excreted as TMAO was documented (Table 1). On a follow-up visit after the hospitalization, urine TMA and TMAO levels as well as total TMA percentage excreted as TMAO normalized. During a subsequent hospitalization for gastroenteritis, which was not consistent with FPIES and was not associated with fishy odor, TMA and TMAO levels were normal, but percentage of total urine TMA excreted as TMAO was mildly decreased.

Sequencing of *FMO3*, the gene for trimethylaminuria, revealed paternally inherited nonsense, c.993_994delTA (p.Tyr331Stop) mutation. This truncating mutation has not been previously reported, but its effect on the protein is predicted to be pathogenic. The sequencing also revealed two maternally inherited common polymorphisms, E158K and E308G. Deletion studies of the *FMO3* gene were normal.

Discussion

Primary TMAU is due to an inherited deficiency of the enzyme FMO3, leading to inefficient conversion of TMA to the odorless TMAO in the liver. It is an autosomal recessive condition, and carriers are described as asymptomatic (Mackay et al. 2011). However, carriers can be detected by using an oral challenge of TMA and measurement of TMA and TMAO concentrations in urine. One study specifically examined asymptomatic parents of six patients with TMAU. After an oral challenge with 600 mg of TMA, all obligate carriers showed significant increase in TMA excretion, while healthy volunteers did not show increased TMA excretion until challenged with at least 900 mg of TMA (Al-Waiz et al. 1989). This suggests that the FMO3 enzyme can be overwhelmed by an influx of substrate, and the threshold is lower in heterozygotes, likely due to lower enzyme availability. Functional analysis of several mutations revealed a genotype-phenotype correlation; the greater the effect of the mutation on the FMO3 enzyme activity the more severe the symptoms (Phillips and Shephard 2011). Null mutations predominantly result in more severe and persistent malodor.

Secondary TMAU has been documented, and some have associated genetic variations. Case reports in the literature describe TMAU in patients with viral hepatitis or impaired hepatocellular function, during treatment with therapeutic choline in Alzheimer's patients and even in association with the menstrual cycle (Shimizu et al. 2007; Mackay et al. 2011). There are also case reports of transient childhood TMAU (Mayatepek and Kohlmuller 1998). These children ingested normal childhood diets, some breast fed and some fed choline-containing formula. These children present with typical malodor and demonstrate increased TMA urine excretion and total TMA percentage excreted as TMAO less than 90 %. Without intervention, the malodor resolved and the TMA urinary excretion normalized (Mayatepek and Kohlmuller 1998). It was postulated that the metabolic capacity of FMO3 is overwhelmed in these cases, either because of relative developmental deficiency of FMO3 that normalized with age (Koukouritaki et al. 2002) or an overproduction of substrate (TMA) by gut flora (Mayatepek and Kohlmuller 1998). Interestingly, sequencing of the *FMO3* gene in patients with transient TMAU revealed compound heterozygosity for severe mutations on one chromosome and variant alleles, carrying two amino acid polymorphisms, c.472G > A and c.923A > G (E308G; E158K) on the other chromosome (Zschocke et al. 1999; Zschocke and Mayatepek 2000). It was shown that homozygosity for the allele E158K/E308G can be also associated with symptomatic FMO3 deficiency as in transient childhood TMAU (Zschocke and Mayatepek 2000) or transient TMAU associated with menstruation (Shimizu et al. 2007). This supports the spectrum of phenotypes observed, and the important interplay between genetic and environmental effects in TMAU.

The fish-odor in our patient was only noted during acute episodes consistent with FPIES. At first glance, FPIES and TMAU appear unrelated and, since both conditions are

rare, they are less likely to coexist in the same individual. However, further investigation in our patient revealed massive urinary TMA excretion during acute FPIES presentation and complete normalization between these episodes. Genetic analysis of the *FMO3* gene revealed that he was heterozygous for the novel nonsense p.Tyr331X mutation and for the two above-mentioned common polymorphisms, E158K and E308G.

Our patient showed remarkable increase in TMAO excretion during FPIES with approximately 12.5 % of total trimethylamine excreted as TMA, suggesting a relatively high in vivo residual oxidative activity of FMO3. He was probably able to cope with the daily burden of TMA (from dietary TMAO and choline), but when challenged with a large substrate load, his limited enzyme capacity was overwhelmed. We hypothesize that the mild decrease in FMO3 activity in our patient is linked to relative developmental deficiency of FMO3 (Koukouritaki et al. 2002) and to genetic susceptibility caused by compound heterozygosity for a severe mutation (p.Tyr331X) on one allele and two polymorphisms (E308G; E158K) on the second allele, as was previously described with transient childhood TMAU (Zschocke et al. 1999; Zschocke and Mayatepek 2000). In addition, FPIES-induced increased inflammation in the gastrointestinal tract (Caubet and Nowak-Wegrzyn 2011; Chung et al. 2002; Heyman et al. 1994) may have also played a role in the downregulation of *FMO3*, which has been observed previously in a mouse model of inflammation using *C. rodentium* infection (Zhang et al. 2009).

The patient has exhibited massive TMAU with only mildly decreased urinary TMAO/Total TMA ratio. This suggests that TMAU was caused predominantly by substrate overload rather than severe enzyme deficiency. These levels of urinary trimethylamines are rarely observed even following fish meal or choline loads (Chalmers et al. 2006). We are puzzled over the origin of the substrate as the patient did not take significant amounts of fish, choline, or lecithin. In addition, he has never received supplements such as carnitine or betaine. Other rare causes of secondary TMAU including chronic hepatic disease and renal failure were not present in this patient. Congenital intrahepatic portal-systemic shunt was associated with TMAU but with low TMAO/total TMA ratio. Abnormal overgrowth of small intestinal bacteria can greatly increase TMA production but FPIES is not typically associated with excessive bacterial proliferation. The function of enterocytes tight junctions as a significant barrier to diffusion can be severely altered in FPIES (Caubet and Nowak-Wegrzyn 2011), potentially causing enhanced transepithelial influx of different types of molecules. However, it is unclear whether the increased intestinal permeability contributed to TMAU in this patient. We speculate that the remarkable FPIES-related inflammation can cause local gastrointestinal tissue damage, which subsequently releases significant amounts of choline and lecithin.

To our knowledge, this is the first report of episodic TMAU associated with FPIES, which should be added to the growing list of conditions linked to secondary TMAU. This report illustrates the complex interplay of genetic and environmental factors in TMAU and sheds light on the pathophysiology of TMAU and FPIES. Additional studies and cases are needed to improve our understanding of potential association between these two conditions.

Synopsis

Transient massive trimethylaminuria can be associated with food protein–induced enterocolitis syndrome in genetically susceptible individuals. The findings illustrate the pathogenicity and complex interplay of genetic and environmental factors in trimethylaminuria.

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