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Health Safety

ORIGINAL PAPER

IDENTIFICATION OF NEW POSSIBLE GENES INVOLVED IN TRIMETHYLAMINURIA

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Received: Dec 4th, 2019
Revised: Dec 9th 2019
Accepted: Dec 10th, 2019.

Abstract

Trimethylaminuria, also known as "fish odor syndrome" is an inherited disease characterized by the accumulation, and consequently, by the excretion of trimethylamine which is the compound responsible for the unpleasant odor. Due to this malodorous condition, patients are devastating from a psychosocial perspective being they marginalized in society. The cause of the syndrome is rooted in the dysfunctional metabolism of trimethylamine that can be caused by a dysbiosis related to gut microbiota or by a deficiency in flavin monooxygenase 3 (FMO3), the essential enzyme for the metabolism of trimethylamine. Nowadays there are reliable diagnostic tests based on genetic analysis of FMO3 gene and on the evaluation of trimethylamine concentration measured in the urine. Although there is no therapy, many simple treatment options exist that may drastically improve the quality of life of these patients. In this paper we provide an approach to identify other genes, related to FMO3, possibly involved in the etiopathogenesis of the trimethylaminuria.

Key Words: TMAU, bioinformatics, analysis, causative genes.

Introduction

Trimethylaminuria is an inherited metabolic syndrome, characterized by the accumulation and the excretion of trimethylamine, a diet compound which is excreted through urine, sweat, breath, and other body secretions, causing an unpleasant rotten fish odor. This pathological condition, as reported in many scientific reviews, is uncommon in the society, and due to the fish odor, affected people are often marginalized. This social impact may represent the first cause of the psychiatric conditions as depression, anxiety, behavior disorders that affect people with trimethylaminuria, as statistical studies have shown. The patients feel shame and embarrassment, fail to maintain relationships,

avoid contact with people who comment on their condition and are obsessive about masking the odor with hygiene products and even smoking. Moreover, the malodorous aspect can have serious and destructive effects also on schooling, personal life, career and relationships, resulting in social isolation, low self-esteem and suicide. The first case of trimethylaminuria was published in 1970 in "The Lancet" by a group of researchers in Colorado, where they have tested this condition in a child who gave off a strong fish odor, by finding in his urine a huge level of trimethylamine. In addition, in one of the Shakespeare's plays and in the epic novel "Mahabharata", are described cases of people that smell of rotten fish. The metabolic and clinical manifestations of trimethylaminuria are generally regarded as benign, as there is no associated organ dysfunction. Some patients may suffer of hypertension. This designation, and the evidence that the condition is often unrecognized by doctors, can have important consequences on the delayed or missed diagnosis [1].

There are different types of trimethylaminuria, but the most common is Type 1 trimethylaminuria, caused by a deficit of the FMO3 enzyme. Consequently, when the pathological condition is suspected or known to occur in a family, the genetic test of the*FMO3* gene, can be helpful in identifying members who have the disorder or any carry causative variant [1]. In particular, most cases of trimethylaminuria appear to be inherited in an autosomal recessive pattern, which means two copies of the gene in each cell are altered. The parents of an individual with an autosomal recessive disorder are both carriers of one copy of the altered gene. Carriers may have mild symptoms of trimethylaminuria or experience temporary episodes of fish-like body odor. In addition, measurement of urine for the ratio of trimethylamine is the standard screening test. The FMO3 enzyme, also known as Flavin-containing monooxygenase 3, is encoded by the *FMO3* gene. The gene FMO3 belongs to the family of *FMO* genes, made up by five genes and multiple pseudogenes, differentially expressed in tissues. *FMO3* is located on the chromosome 1q24.3 and it counts 10 exons. It encodes for a transmembrane protein localized to the endoplasmic reticulum of many tissues, especially in the liver, and it is involved in the metabolism of many compounds. In particular, this enzyme catalyzes the NADPH-dependent oxygenation of various nitrogen-, sulfur-, and phosphorous-containing xenobiotics such as therapeutic drugs, pesticides, and dietary compounds like trimethylamine and tyramine. By the way, the FMO3 enzyme catalyzes the N-oxygenation of trimethylamine in trimethylamine-N-oxide, which is inodorous. Trimethylamine is an organic compound synthesized by gut microbiota after the ingestion of specific choline-rich food, such as red meat, eggs, fishes, legumes. This kind of food contains compounds like lecithin, choline and L-carnitine that represent the substrates of gut microbiota to synthesize trimethylamine. Once synthesized, trimethylamine is absorbed into the bloodstream [2,3] and then it is converted in trimethylamine-N-oxide by the enzyme FMO3, localized in the hepatocytes. The gut microbiota involved in the production of trimethylamine is composed by a group of commensal Gram-positive bacteria that present a large variety of enzyme that are able to convert diet precursors in trimethylamine. Mutations in the *FMO3* gene cause trimethylaminuria and many mutations were reported as causative of trimethylaminuria [4,5]. Nonsense and missense mutations cause the most severe phenotypes. Although FMO3 mutations account for most known cases of trimethylaminuria, some cases are caused by other factors. A fish-like body odor could result from an excess of certain proteins in the diet or from increased bacteria in the digestive system. Type 2 TMAU, indeed, is caused by a dysbiosis of the gut microbiota. Few cases of the disorder have been identified in adults with liver damage caused by hepatitis. This condition is known as acquired trimethylaminuria urinary tract infection, bacterial vaginosis, cervical cancer, advanced kidney disease can be external factors that can distort TMAU diagnosis [6].

A specific form of the disease was diagnosed in children and it is known as "Childhood

trimethylaminuria". It is caused by a transitory reduction of the FMO3 expression in children. Usually, in children is expressed the fetal isoform of FMO, that is FMO1 rather than FMO3.

In addition, other factors can worsen the pathological condition. In particular, female sex hormones as progesterone and estrogen could influence negatively and positively the FMO3 expression, respectively. According to several reports, the condition worsens around puberty. In women, symptoms may worsen just before and during menstrual periods, after taking oral contraceptives, and during menopause [7].

So, in conclusion, the odor depends on various factors, such as diet, hormonal changes, stress level, amount of sweat. There is no known therapy or treatment for the disorder and the ways to reduce the fishy odor may include avoiding foods such as egg yolks, legumes, red meats, fish, beans and other foods that contain choline, carnitine, nitrogen, sulfur and lecithin taking low doses of antibiotics such as neomycin and metronidazole [8] in order to reduce the amount of bacteria in the gut;

using slightly acidic detergent with a pH between 5.5 and 6.5.

Additionally, at least one study [9] has suggested that daily intake of the supplements activated charcoal and copper chlorophyll may improve the quality of life of individuals afflicted with trimethylaminuria, by helping their bodies to oxidize and convert trimethylamine in the odorless N-oxide metabolite. Study participants experienced subjective reduction in odor as well as objective reduction in trimethylamine and increase in trimethylamine-N-oxide concentration measured in their urine. The study found that the 85% of test participants reported complete loss of detectable "fishy" odor, the 10% experienced some reduction in detectable odor and the 5% did not experience any detectable odor reduction. The main purpose of this paper is to identify new strategies to detect other genes related to FMO3 that could act in the severity of the trimethylaminuria.

Methods

We have used a bioinformatic approach to identify new genes probably involved in the etiopathogenesis of trimethylaminuria. In particular, we used the *Cytoscape* platform in order to obtain and characterize molecular network of the *FMO3* gene. *Cytoscape* is an open source software platform for visualize molecular interaction networks and biological pathways and allows to integrate these networks with annotations, gene expression profiles and other state data. *Cytoscape* core distribution provides a basic set of features for data integration, analysis, and visualization. Additional features are available as Apps (formerly called Plugins). Apps are available for network and molecular profiling analyses, new layouts, additional file format support, scripting, and connection with databases. Among the apps, we used *GeneMania*. The *GeneMania Cytoscape* plugin brings fast gene function prediction capabilities to the desktop and it identifies the most related genes to a query gene set using a guilt-by-association approach. The plugin uses a large database of functional interaction networks from multiple organisms and each related gene is traceable to the source network used to make the prediction. Users may add their own interaction networks and expression profile data to complement or override the default data. The plugin follows the look and feel of the *GeneMania* website, but provides more features for power-users. *GeneMania* searches many large, publicly available biological datasets to find related genes. These include protein-protein, protein-DNA and genetic interactions, pathways, gene and protein expression data, protein domains and phenotypic screening profiles. Data is regularly updated.

Network names describe the data source and are either generated from the PubMed entry associated with the data source (first author-last author-year), or simply the name of the data source (BioGRID, PathwayCommons (original data source, Pfam). Significance of detected relations among genes are indicated as follow:

- **Co-expression**: Gene expression data. Two or more genes are linked if their

expression levels are similar across conditions (developmental stages, tissues, exposure to xenobiotics) in a gene expression study. Most of these data are collected from the Gene Expression Omnibus (GEO).We only collected data associated with a publication.

- **Physical Interaction**: Protein-protein interaction data. Two or more genes are linked if the encoded proteins were found to interact in a protein-protein interaction study. These data are collected from primary studies found in protein interaction databases, including BioGRID and PathwayCommons.
- **Genetic interaction**: Genetic interaction data. Two or more genes are functionally associated if the effects of perturbing one gene were found to be modified by perturbations to a second gene. These data are collected from primary studies and BioGRID.
- **Shared protein domains**: Protein domain data. Two or more genes are linked if the encoded proteins have the same domain. These data are collected from domain databases, such as InterPro, SMART and Pfam.
- **Co-localization**: Genes expressed in the same tissue, or proteins found in the same location. Two or more genes are linked if they are both expressed in the same tissue or if their gene products are both identified in the same cellular location at the same time.
- **Pathway**: Pathway data. Two or more gene are linked if the encoded proteins participate in the same reaction within a pathway. These data are collected from various source databases, such as Reactome and BioCyc, via PathwayCommons.
- **Predicted**: Predicted functional relationships between genes, often protein interactions. A major source of predicted data is mapping known functional relationships from another organism via *orthology*. For instance, two or more proteins are predicted to interact if their orthologs are known to interact in another species. In these cases, network names describe the original data source of experimentally measured interactions and which organism the interactions were mapped from.

After selecting these datasets, it is possible to start the analysis and study the network shown.

The pathway analysis is conducted by observing and studying the result panel. The Functions tab of the GeneMania result page displays Gene Ontology (GO) terms enriched among the genes in the network displayed by GeneMania.

We only consider annotations (direct or up-propagated) in GO terms with between 10 and 300 non- “IEA” and non- “RCA” annotations in the organism of interest. The GO categories and Q-values from an FDR corrected hypergeometric test for enrichment are reported, along with coverage ratios for the number of annotated genes in the displayed network versus the number of genes with that annotation in the genome. We estimated Q-values using the Benjamini-Hochberg procedure. Categories are displayed up to a Q-value cutoff of 0.1

Since the functional enrichment is computed on the displayed network, the value selected for number of related genes in the GeneMania advanced option’s panel will influence the results. It may be informative to try other values for this parameter, particularly if the set of functional categories is empty or small for the default value. To only test the query list for enrichment, “0” for the number of returned genes must be selected. As input gene was used the only *FMO3*.

Then, to find out information about the genes, we have used GeneCards and other software such as: NCBI, OMIM, Ensembl. GeneCards is a searchable, integrative database that provides comprehensive, user-friendly information on all annotated and predicted human genes. The knowledgebase automatically integrates gene-centric data from ~150 web sources, including

genomic, transcriptomic, proteomic, genetic, clinical and functional information.

Results

GeneMania results have shown that *FMO3* gene is part of a complex molecular network, in which are involved different genes. These genes belong to different families and they encode different classes of enzymes, as following reported:

- CYP450's family, such as: *CYP2E1, CYP3A7, CYP4F11, CYP2A13, CYP2D6, CYP3A5, CYP2A6, CYP2A7, CYP2C9, CYP2B6, CYP2C8, CYP3A4, CYP1A2*;
- FMO's family, such as: *FMO1, FMO2*;
- ADH's family, such as: *ADH1A, ADH1B, ADH4, ADH6*;
- MAO's family, such as: *MAOB*

In addition, there are other correlations of the *FMO3* gene with two specific metabolic pathways:

- Drugs metabolism and phase I functionalization;
- Biological oxidations;

And with some transcription factors:

- CTTTAAR_UNKOWN;
- GGGAGGRR_V$MAZ_Q6;
- V$HP1SITEFACTOR_Q6

The network results panel shows that:

- 40,29% are genes involved with the *FMO3* in consolidated pathways;
- 37,09% are genes related to *FMO3* because of physical interactions;
- 7,20% are *FMO3*co-expressed genes;
- 5,21% are genes activated by the same transcription factors that active the *FMO3* expression;
- 3,28% are genes encoding for proteins that co-localize with the FMO3 in the same tissues;
- 2,38% are genes encoding for proteins involved in the same metabolic pathways with FMO3 enzyme;
- 0,32% are genes encoding for proteins that share the same domains with the FMO3 enzyme.

Discussion

Focusing the attention on the *MAOB* gene, its relation to the *FMO3* depends on the evidence that they are both co-expressed and also, they share protein domains. *MAOB* is a gene located on chromosome Xp11.3 and belongs to flavin-containing monoamine oxidase family. It counts 18 exons and 1563 nt, as it is reported in OMIM database. It is similar with its paralog MAOA, which is located on the same chromosome. In particular, these genes show identical exon-intron organization. Exon 12 encodes for the covalent FAD-binding site and is the most conserved exon [10]. The *MAOB* gene encodes monoamine oxidase B, while MAOA encodes monoamine oxidase A. They catalyze the oxidative deamination of neuroactive and vasoactive amines, as well as the oxidation of several xenobiotics. MAOA and MAOB are present in the outer mitochondrial membrane in the central nervous system and peripheral tissues. MAOA, the primary type in fibroblasts, preferentially degrades serotonin and norepinephrine. MAOB, the primary type found in platelets and leukocytes, preferentially degrades phenylethylamine, benzylamine and tyramine. It is reported that diseases associated with *MAOB* are related to mental illness, just like behaviour disorders, atypical depressive disorder and the Norrie disease.

We will focus the attention on the tyramine metabolism because, as literature studies have shown, it is also a substrate of the FMO3 enzyme. Tyramine is a natural amine derived from the amino acid tyrosine and it acts as a catecholamine releasing agent. Tyramine occurs widely in plants [11] and animals, and is metabolized and inactivated by various enzymes, including monoamine oxidases. In foods, it is often produced by the decarboxylation of tyrosine during fermentation or catabolism. Foods containing considerable amounts of tyramine include: meats that are spoiled, pickled, aged, smoked, fermented, or marinated (some fish, poultry, and beef), and most pork (except curedham), fermented dairy products such as sour cream, yogurt, and most cheeses (except ricotta, cottage, Monterey Jack,

cream, and Neufchâtel cheeses), fermented plant foods such as alcoholic beverages, soy sauce, soybean condiments, teriyaki sauce, tempeh, miso soup, sauerkraut, chocolate, shrimp paste, broad (fava) beans, green bean pods, Italian flat (Romano) beans, snow peas, edamame, avocados, bananas, raisins, dates, pineapple, eggplants, figs, red plums, raspberries, peanuts, Brazil nuts, coconuts, processed meat, yeast, an array of cacti, and the holiday plant mistletoe. So, food that also contain trimethylamine. Once obtained, tyramine acts as a neuromodulator thanks to the presence of some types of G-protein coupled receptors called TAAR1 [12,13]. The TAAR1 receptor is found in the brain, as well as peripheral tissues, including the kidneys [14]. Tyramine binds to both TAAR1 and TAAR2 as an agonist in Humans.A large dietary intake of tyramine (or a dietary intake of tyramine while taking MAO inhibitors) can cause the tyramine pressor response, which is defined as an increase in systolic blood pressure of 30 mmHg or more. The increased release of norepinephrine (noradrenaline) from neuronal cytosol or storage vesicles is thought to cause the vasoconstriction and increased heart rate and blood pressure of the pressor response.

Conclusions

In conclusion we pointed attention on the *MAOB* gene hypothesizing that mutations in *MAOB* could increase the concentration of tyramine in the blood, and this could worsen the mental disorders and hypertension typical of the trimethylaminuria condition.

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