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*Abstract*

# FMO3 misfolding might indirectly block the unfolded protein response via PERK in hepatocytes †

**Simona Alibrandi[1,2,3*], Luigi Donato[1, 2], Concetta Scimone[1,2], Fabiana Nicita[1,2], Domenico Mordà[1], Carmela Rinaldi[1], Rosalia D'Angelo[1] and Antonina Sidoti[1]**

[1] Department of Biomedical and Dental Sciences and Morphofunctional Imaging, Division of Medical Biotechnologies and Preventive Medicine, University of Messina, Messina 98125, Italy; ldonato@unime.it (L.D.); cscimone@unime.it (C.S.); salibrandi@unime.it (S.A.); domenico.morda@studenti.unime.it (D.M.); crinaldi@unime.it (C.R.); rdangelo@unime.it (R.D.); asidoti@unime.it (A.S.)

[2] Department of Biomolecular strategies, genetics and cutting-edge therapies, I.E.ME.S.T., Palermo 90139, Italy

[3] Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Messina 98125 Messina, Italy

* Correspondence: simona.alibrandi@unime.it ; Tel.: +39-0902213136

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**Abstract:** Trimethylaminuria (TMAU) is a metabolic syndrome characterized by the accumulation and the excretion of trimethylamine (TMA), synthesized by gut microbiota, which is excreted through sweat, breath, urine and other body fluids, determining an unpleasant rotten fish odor in affected patients. The primary form (TMAU1) is determined by homozygous causative mutations in the FMO3 gene that could impair enzyme function. Frequently, TMAU1 affected patients do not carry causative mutations in homozygous condition. Therefore, we hypothesized that compound heterozygosity and haplotype variants might also cause FMO3 misfolding playing a significant role in FMO3 activity reduction or alteration. FMO3 misfolding might determine hepatocytes ER stress that in this case could be amplified by the TMAO levels reduction. In fact, it is known that TMAO binds the luminal domain of protein kinase R-like endoplasmic reticulum kinase (PERK), activating the unfolded protein response and consequently reducing endoplasmic reticulum stress.

To confirm our hypothesis, we performed a mutational analysis of *FMO3* gene in 26 patients by Sanger sequencing. Then, a proteomic in silico analysis, using different platforms and software, was carried out with the final aim of revealing how these variant combinations could influence the enzyme folding, also simulating its dynamic behaviour with the TMA substrate. Results revealed the presence of 17 variants distributed in 26 different haplotypes which might lead to possible impairments of FMO3 activity, probably reducing the interaction time between the enzyme catalytic site and TMA or losing the wild-type binding site.

Since little is still known about the role that the combination of multiple variants could exert on the enzyme activity, our analysis could represent a starting point to unveil new scenarios about the genetic form of TMAU.

**Keywords:** TMAU; molecular docking; Sanger sequencing

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