Non-P450 Drug-metabolizing Enzyme Flavin-containing Monooxygenase: Polymorphisms and Interactions

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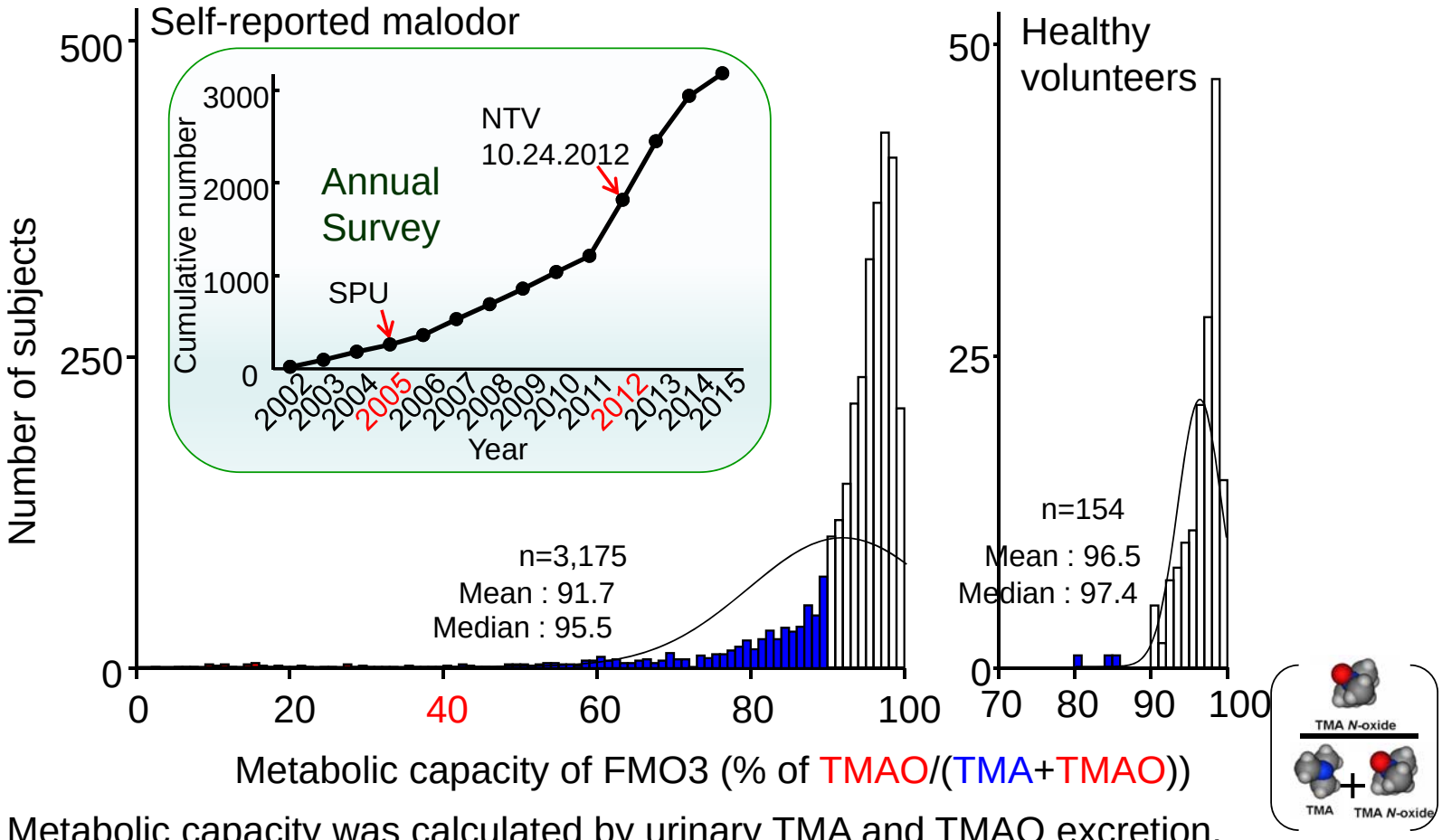
Flavin Containing Monooxygenase and Trimethylaminuria

- The flavin-containing monooxygenase (FMO) is an NADPH-dependent enzyme that catalyzes the oxygenation of *N*- and *S*-containing substances.
- Genetic polymorphisms of *FMO3*, the enzyme catalyzing for trimethylamine *N*-oxygenation, contribute the inherited disorder trimethylaminuria.
- Trimethylaminuria, also known as fish-like odor syndrome, is a metabolic disorder characterized by excretion of dietary-derived trimethylamine (TMA).
- Unpleasant malodor from urine, sweat or breath caused by excess TMA may lead to social problems.

TMA TMA *N*-oxide

Possible Trimethylaminuria in Japanese: Urinary Trimethylamine Excretion by Daily Food Intake



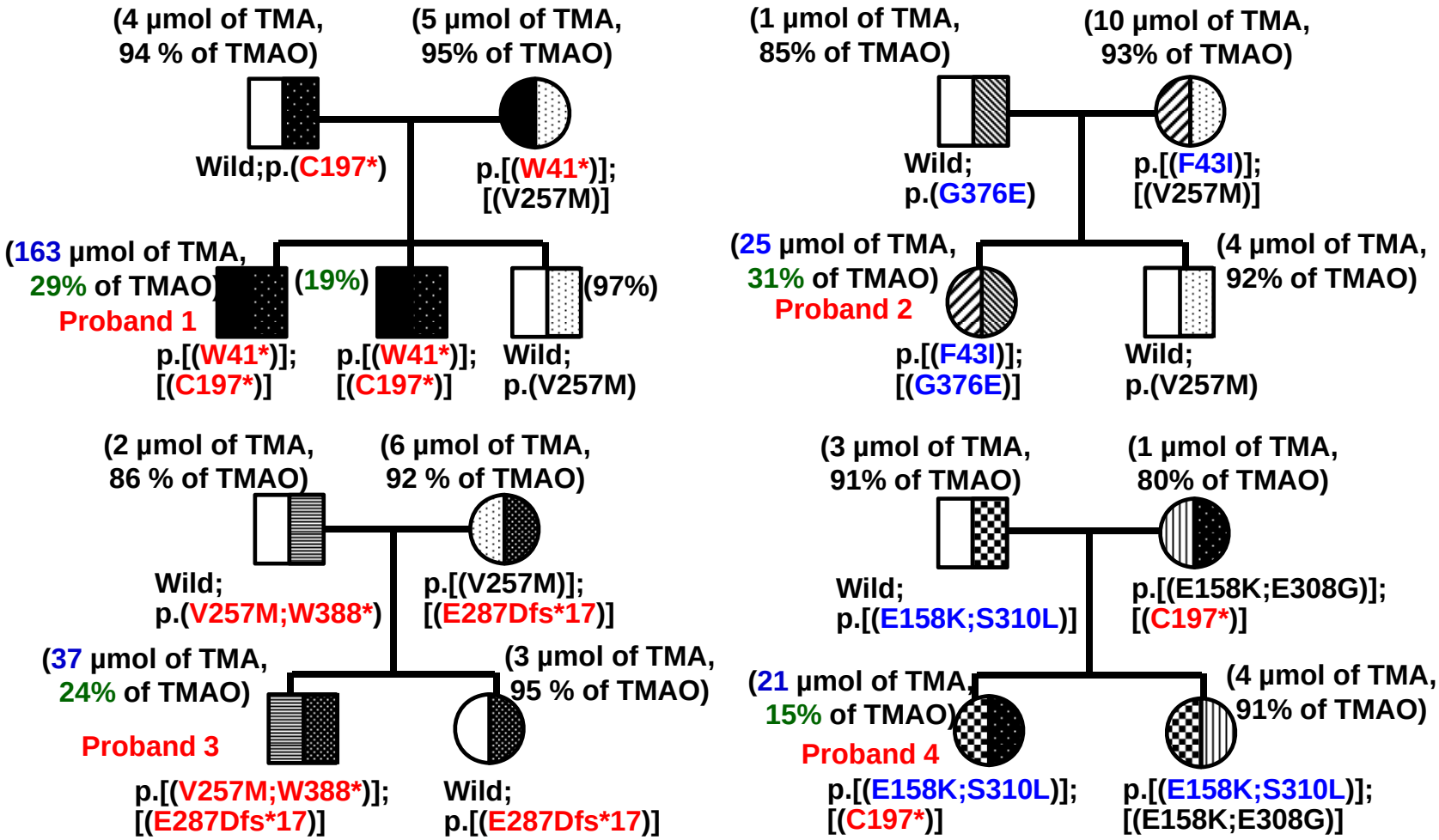
Metabolic capacity of FMO3 (% of TMAO/(TMA+TMAO))

Metabolic capacity was calculated by urinary TMA and TMAO excretion.

Approximately 1.5% of subjects showed less than 40% of FMO3 metabolic capacity in urine tests.

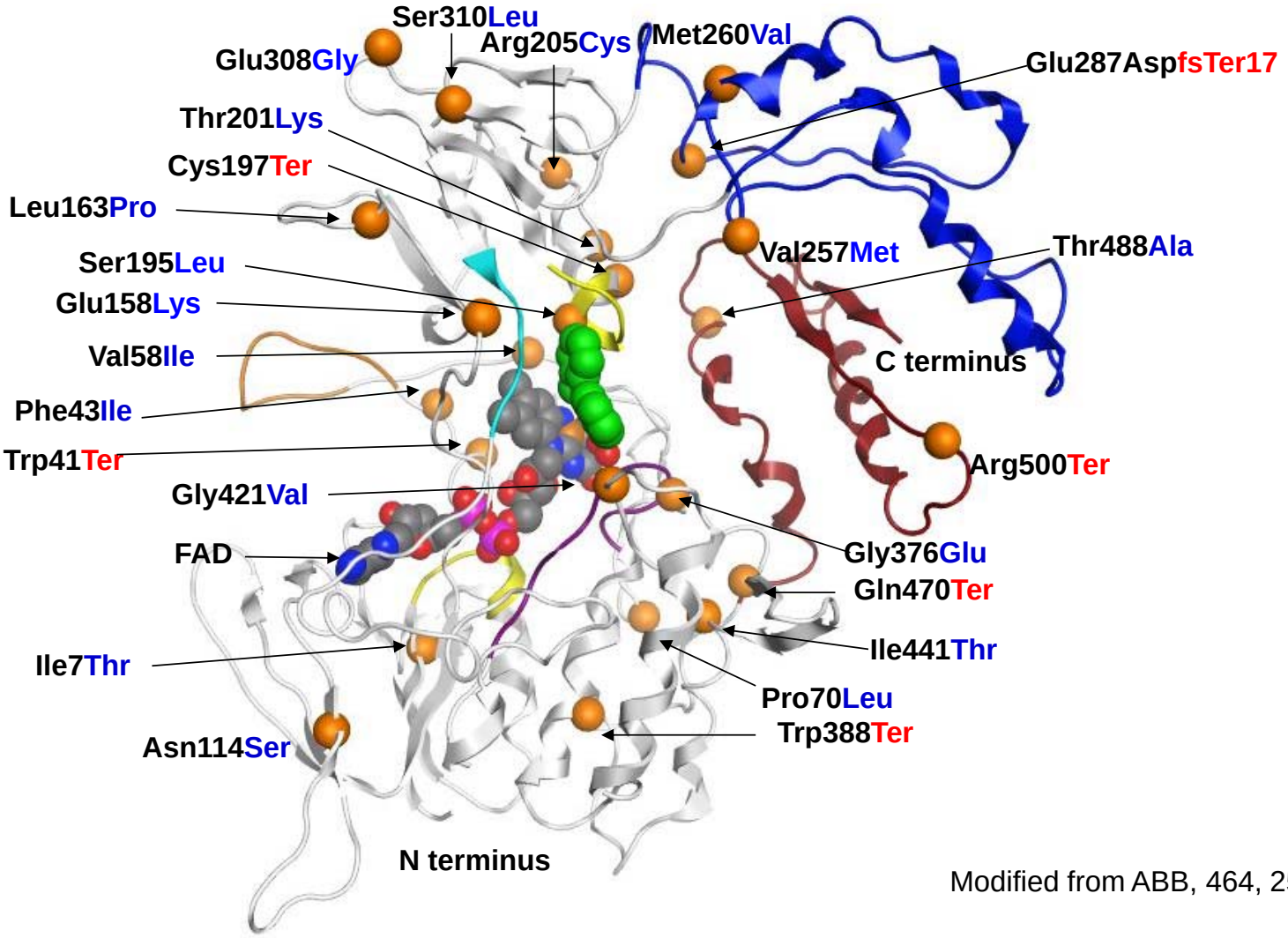
Yamazaki and Shimizu, Curr Drug Metab, 8, 487-491 (2007) and updated

Representative Pedigree Analyses in *FMO3* Gene in Japanese



Shimizu et al., Mol Genet Metab Rep, 5, 89-93 (2015).

Amino Acid Substitutions of FMO3 Found in Japanese



Modified from ABB, 464, 251 (2007)

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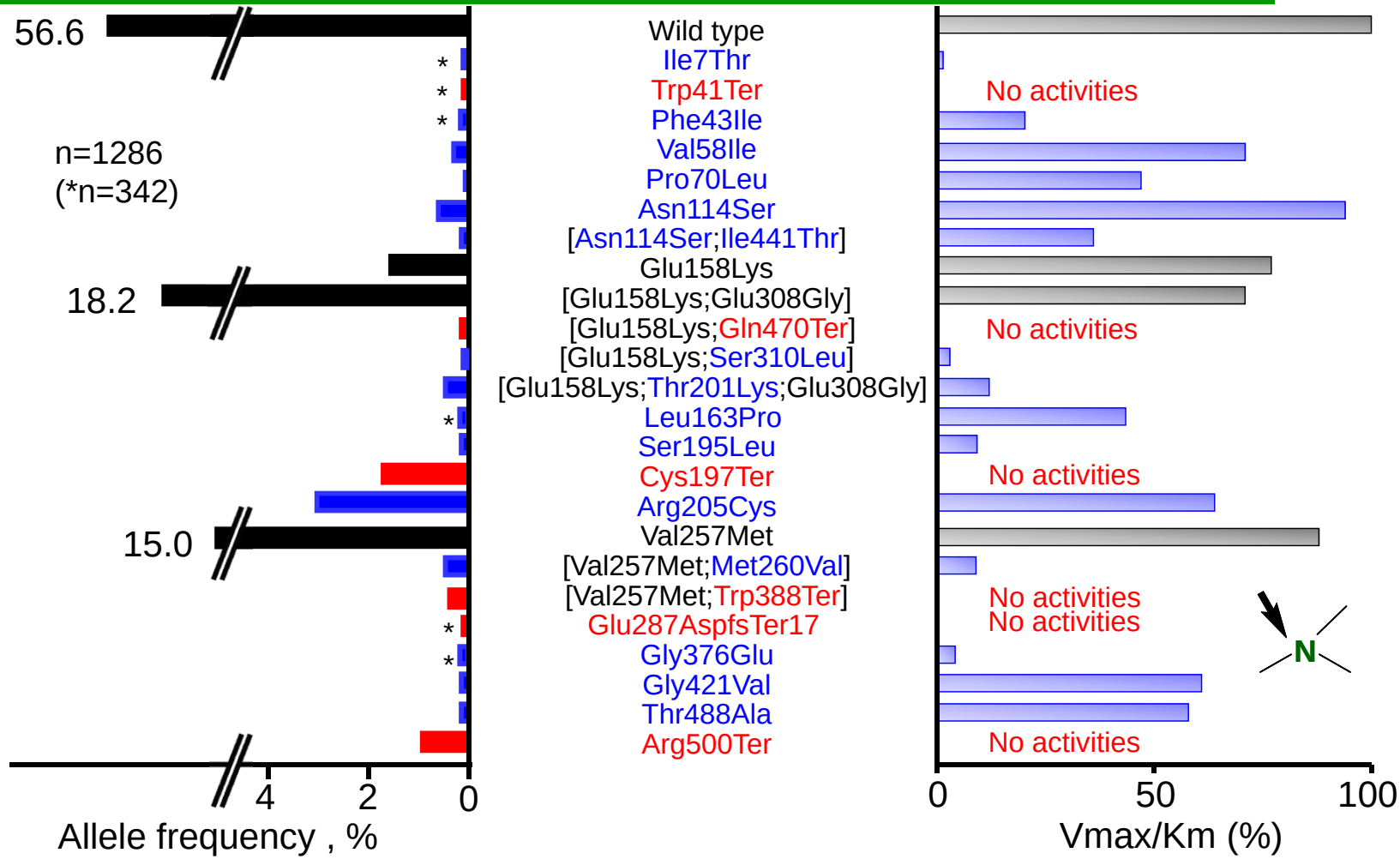
Allele Frequency of *FMO3* and Trimethylamine *N*-Oxygenation Activities of Recombinant FMO3



Yamazaki and Shimizu, Curr Drug Metab, 8, 487-491 (2007) and updated

Wild-type (532 amino acids) and variant FMO3 proteins were expressed in *E. coli* membranes.

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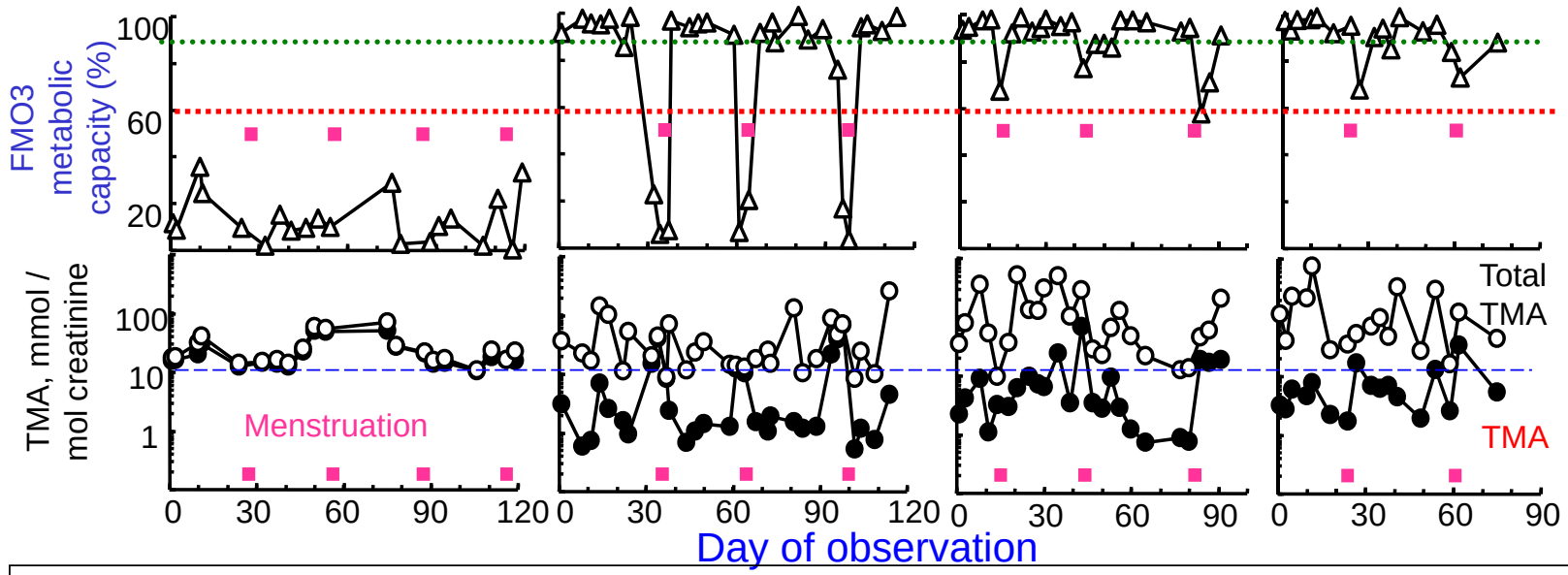
Transient Trimethylaminuria Related to Menstruation

Self-reported subjects suffering from malodor　　Control subjects

(A) p.[(R500*)];[(R500*)] *FMO3*　(B) p.[(E158K;E308G)]; [(E158K;E308G)] *FMO3*　(C) wild;p[(E158K;E308G)] *FMO3*　(D) wild;wild *FMO3*



- Abnormal FMO3 metabolic capacity is caused by menstruation even in wild-type, heterozygote, particularly in homozygote of this mild genetic variants.
- Homozygous for Arg500Ter showed decreased capacity during observation.
- This would further suggest that sex hormones play a role in the variable regulation of FMO3 to cause intra-individual variations.

Shimizu et al., BMC Med Genet, 8, 2 (2007)

Effects of Methimazole on Sulindac Sulfide *S*-Oxygenation Activities in Liver Microsomes and FMO3

Enzyme	Sulindac sulfide *S*-oxygenation		Methimazole
	V_{max},	K_m	K_i
Liver microsomes genotyped for p.[(Glu158Lys;Glu308Gly)] *FMO3*			
	nmol/min/mg protein	μM	μM
Wild homozygotes (n=3)	3.5 (3.0, 3.6, 4.0)	53 (50, 58, 59)	22 (18, 24, 25)
Heterozygotes (n=2)	2.9 (2.7, 3.0)	45 (35, 54)	23 (23, 24)
Mutant homozygote	1.9	50	12
Recombinatly expressed FMO3 in *E. coli* membranes			
	min^{-1}	μM	μM
Wild-type FMO3	230 ± 54	54 ± 20	22 ± 5
158Lys;308Gly FMO3	160 ± 43	56 ± 25	11 ± 2
205Cys FMO3	99 ± 10	36 ± 11	8 ± 1

Genetic polymorphism in the human *FMO3* gene might lead to unexpected changes of catalytic efficiency and drug interactions.

Yamazaki and Shimizu, Biochem Pharmacol, 85, 1588-1893 (2013); Shimizu et. al., DMPK, 30, 70-74 (2015).

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Conclusion

- Individuals homozygous or heterozygous for any of the missense, duplication, and nonsense *FMO3* variants may possess abnormal trimethylamine *N*-oxygenation.
- Expressed FMO3 would cause intra-individual variations, especially in childhood and adult women.
- Genetic polymorphism in the human *FMO3* gene might lead to some changes of catalytic efficiency and drug interactions for *N*- or *S*-oxygenations of xenobiotics and endogenous substances under daily intake.

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