SNP Communication

Three Novel Single Nucleotide Polymorphisms of the FMO3 Gene in a Japanese Population

Makiko Shimizu, Haruka Fujita, Takahiko Aoyama and Hiroshi Yamazaki

Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, Tokyo, Japan

Summary: We sequenced all exons and exon-intron junctions of the flavin-containing monoxygenase 3 (FMO3) gene from 2 Japanese individuals and their family members, who were case subjects that showed low FMO3 metabolic capacity among a population of self-reported trimethylaminuria Japanese volunteers. We found two novel single nucleotide polymorphisms (SNPs) (21254 C > A and 24006 A > G) causing amino acid substitutions, Thr201Lys in exon 5 and Met260Val in exon 6, respectively. The Thr201Lys and Met260Val also presented together with known SNPs (Glu156Lys-Glu159Gly and Val207Met, respectively) in the same alleles of the FMO3 gene to form novel haplotypes. A SNP (30398 C > T) in the FMO3 gene causing a stop codon at Arg259 in exon 9 was also discovered.

These sequences are as follows:

1) SNP, 060116Shimizu001; GENE NAME, FMO3; ACCESION NUMBER, AL021026; LENGTH, 25 base; 5'-GTGATATTGCGCACTAGAAGACTCAGCCG-3'.

2) SNP, 060116Shimizu002; GENE NAME, FMO3; ACCESION NUMBER, AL021026; LENGTH, 25 base; 5'-TAC(G/A)TGAAAGCAGAGAT-3'.

3) SNP, 060116Shimizu003; GENE NAME, FMO3; ACCESION NUMBER, AL021026; LENGTH, 25 base; 5'-CCCATGCAGACAATGAGTGTCGGA-3'.

Key words: flavin-containing monoxygenase; trimethylaminuria; fish-odor syndrome; genetic polymorphism; Japanese

Introduction

The flavin-containing monoxygenase (FMO, EC 1.14.13.8) is an NADPH-dependent enzyme that catalyzes the oxygenation of a wide variety of nucophile compounds containing sulfur, nitrogen or phosphorus atoms. To date, eleven FMO genes have been identified in humans (FMO1 to FMO11p). FMO3 is considered a prominent functional form expressed in adult human livers. The reduced capacity of FMO3 to N-oxidize trimethylamine (TMA) is believed to cause a metabolic disorder known as fish-odor syndrome or trimethylaminuria in which patients excrete higher amounts of TMA in the urine, sweat and breath. Single nucleotide polymorphisms (SNPs) of the FMO3 gene were shown to cause fish-like odor syndrome predominantly in Caucasians. In our SNP communication, there are some FMO3 SNPs in Japanese, like Cys195Stop, Asp198Glu or Arg200Cys, which are not characterized yet. We recently reported that low FMO3 metabolic capacity associated with liver damage could be another causal factor for mild trimethylaminuria in Japanese, independent of the FMO3 genotype present. In order to identify novel SNPs and/or haplotypes of the FMO3 gene found in Japanese individuals suffering from trimethylaminuria, we sequenced the entire coding region of the FMO3 gene using genomic DNA. We found three novel SNPs causing Thr201Lys in exon 5, Met260Val in exon 6, and Arg500Stop in exon 9 from 2 Japanese probands, who showed low FMO3 metabolic capacity, and their family members.

As of January 16, 2006, these polymorphisms did not appear either in the FMO3 Allelic Variant Database (http://human-fmo3.biochem.ucl.ac.uk/Human_FMO3/), the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/) or the JSNP Database (http://snp.ims.u-tokyo.ac.jp/).

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To whom correspondence should be addressed: Prof. Hiroshi Yamazaki, Ph.D., Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, 3-3165, Higashi-tamagawa Gakuen, Machida, Tokyo 194-8543, Japan. Tel. +81-42-721-1406, Fax. +81-42-721-1406, E-mail address, hyamazak@ac.shoyaku.ac.jp
Materials and Methods

The ethics committees of Showa Pharmaceutical University approved this study. Volunteer subjects for sequencing the FMO3 gene and for screening of urinary TMA N-oxide levels included 35 Japanese individuals. Informed consent was obtained from each subject or parent. The sequence of the complete human FMO3 gene described in the GenBank (accession number AL021026) was used as a reference. The PCR products were directly sequenced on both strands using an ABI PRISM 3730xl DNA analyzer (Applied Biosystems, Foster City, CA, USA) with the sequencing primers.9 Individuals showing impaired FMO3 metabolic capacity, defined as the ratio of TMA N-oxide to total TMA in their urine samples, lower than 90% were considered to constitute abnormal TMA metabolism and possibly suffering from trimethylaminuria.9

Results and Discussion

We found the following three novel SNPs:

1) SNP, 060116Shimizu001; GENE NAME, FMO3; ACCESSION NUMBER, AL021026; LENGTH, 25 base; 5'-GTGATATTGCACAC/GAGACTCAGCCG-3'.

2) SNP, 060116Shimizu002; GENE NAME, FMO3; ACCESSION NUMBER, AL021026; LENGTH, 25 base; 5'-TACCA/TGAAGCAGAAGTGAATGCAAGAT-3'.

3) SNP, 060116Shimizu003; GENE NAME, FMO3; ACCESSION NUMBER, AL021026; LENGTH, 25 base; 5'-CCCATGCAACAC/TGAGTTGTGGCGGA-3'.

We found a SNP from C to A at the position of 21254 in exon 5 (Fig. 1A). The SNP caused an amino acid substitution from Thr to Lys at the residue 201 (Thr201Lys). A family study for the novel 21254 mutation in exon 5 revealed that Thr201Lys presented together with known SNPs, namely 20883 G>A in exon4 (Glu208Gly), and 27159 A>G in exon 7 (Glu308Gly), in the same allele of the FMO3 gene (Fig. 1B). This novel allele also had other SNPs, 5907 A>G in intron 2, 21082 G>A and 21115 G>A in intron 4, 27091 C>T in exon 7 (silent), and 27454 T>C and a 29232 T insertion in intron 7.

In addition, we found another haplotype containing 24006 A>G in exon 6 that resulted in Met260Val (Fig. 2A). A SNP 30398 C>T in the FMO3 gene causing a stop codon at Arg500 in exon 9 was discovered (Fig. 2B). The family study for the novel 24006 mutation in exon 6 indicated that Met260Val presented together with known SNP, 23997 G>A in exon 6 (Val257Met), in the same allele of the FMO3 gene (Fig. 2C).

These novel SNPs are expected to alter the catalytic properties of the FMO3, because these are located with amino acid substitutions in relatively conserved exons 5 and 6 of the FMO3 gene and the probands showed less
than 40% of FMO3 metabolic capacity. The allele frequencies of the three haplotypes (Glu158Lys-Thr
201Lys-Glu208Gly, Val257Met-Met260Val, and Arg508Stop) were approximately 1% because we found each one
allele among 70 alleles sequenced, except for the family members harboring the novel alleles found from the
proband.

The present study is the first report for significant genetic polymorphisms of the FMO3 causing amino
acid substitutions in Japanese trimethylaminuria patients, although further studies are needed to be
directed to clarify the effects of these heterozygous mutations on the FMO3 phenotype and whether these
SNPs and/or haplotypes are specific for Japanese suffering from trimethylaminuria. The findings of the
present study provide fundamental information for the
importance of future investigation of the human FMO3
gene associated with trimethylaminuria (fish-like odor
syndrome).

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