SNP Communication

**CYP3A4*18: It is not Rare Allele in Japanese Population**

Takehito Yamamoto1, Nobue Nagafuchi1, Takeshi Ozeki1, Takahiro Kubota1, Hiroshi Ishikawa2, Seishi Ogawa3, Yasuhiko Yamada1, Hisamaru Hirai3 and Tatsuji Iga1

1Department of Pharmacy, University of Tokyo Hospital, Tokyo, Japan
2Research and Development, Center for Molecular Biology and Cytogenetics, SRL Inc., Tokyo, Japan
3Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

**Summary:** We sequenced all 13 exons of the CYP3A4 gene derived from 48 Japanese subjects. One subject possess the 20070 T>C mutation in the exon 10 (result in leu293Pro substitution, namely CYP3A4*18), as heterozygote. Thus, we investigated the frequency of CYP3A4*18 in 118 Japanese population using polymerase chain reaction-restriction fragment length polymorphism with Msp I and determined that the frequency of the CYP3A4*18 allele was 1.3%.

**Key words:** CYP3A4; CYP3A4*18; allele frequency; Japanese; PCR-RELP

Cytochrome P450 (CYP) 3A4 is a major CYP isoenzyme in the adult human liver and intestine and is known to metabolize a large variety of xenobiotic and endogenous compounds.1,2) The activity of CYP3A4 in the general population is highly variable, and this variability affects the efficacy and safety of drugs metabolized by this enzyme.2,3) One of the causes for this drug metabolism variation is genetic polymorphisms. To clarify the genetic polymorphisms of the CYP3A4 gene, we screened sequences of all exons in 48 Japanese and found a subject who possesses the CYP3A4*18 (L293P) allele4) heterozygously. The CYP3A4*18 allele results in increased metabolic activity towards testosterone and chlorpyrifos in vitro, and this mutant allele is thought to be responsible for the inter-individual variability of CYP3A4 activity.4) Thus, we investigated and report here the frequency of the CYP3A4*18 allele in Japanese.

A PCR-restriction fragment length polymorphism (RFLP)-based genotyping for CYP3A4*18 was developed. Whole blood samples were obtained from 118 healthy Japanese subjects recruited from employees of SRL Inc. (Tokyo, Japan). The protocol of the present study was approved by the local institutional review board. Written informed consent was obtained from all subjects. Genomic DNA was isolated using a DNA Extractor WB Kit (Wako Pure Chemical Industries, Osaka, Japan). PCR primers used for amplifying the full length of exon 10 and exon-intron boundary are the same as those reported by Sata et al.5) PCR was carried out in 25 µL of solution consisting of 1× PCR buffer II, 0.2 mM of each dNTP, 0.8 µM of each primer, 1.5 mM of magnesium chloride, 0.2 units of AmpliTaq DNA polymerase (Applied Biosystems Japan, Tokyo, Japan), and 50 ng of genomic DNA as a template. The conditions for PCR amplification were as follows: initial denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec, and extension at 70°C for 1 min, and final extension at 72°C for 10 min. A 20070T>C change in exon 10 of the CYP3A4 gene creates a recognition site of Msp I (CCGG). Five µL of the PCR product was digested for 2 hr at 37°C with 3 units of Msp I before electrophoresis using 2% agarose gel. The wild type (20070T) allele gave a single 431 bp band, while the mutant type (20070C) allele gave 285 and 146 bp bands (Fig. 1).

![Fig. 1](image-url)

Three of the 118 subjects carried the CYP3A4*18 allele as a heterozygote, and the frequency of the CYP3A4*18 allele was determined to be 1.3% (95% confidential interval (CI): 0.45–3.85%). The genotype distribution was $^{*1}/^{*1}$, 97.5% (95% CI: 92.9–99.2%), $^{*1}/^{*18}$, 2.5% (95% CI: 0.85–7.2%) and $^{*18}/^{*18}$, 0% (95% CI: 0–2.5%), and these values are consistent with the Hardy-Weinberg law. Although the CYP3A4*18 allele has been found in the cell lines established from Japanese individuals,6) the frequency of the CYP3A4*18 allele in Japanese population has not been determined. To the best of our knowledge, the present study is the first study in which the frequency of the
CYP3A4*18 allele in a Japanese population was determined. As a preliminary study, we directly sequenced all 13 exons of the CYP3A4 gene derived from 48 Japanese subjects and found no missense mutation except for the CYP3A4*18. These results suggest that frequencies of mutations in the exonic region of the CYP3A4 gene are very low in Japanese, and it is unlikely that mutations in the exonic region have a great impact on CYP3A4 activity. Further study of the promoter regions of the genes that encode CYP3A4 and transcription factors such as pregnenone X receptor (PXR) is needed to elucidate the genetic components of the interindividual variation of CYP3A4 activity.

**References**


