【Original Article】

Allele and genotype frequencies of *CYP3A5* in the young Japanese population

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(Received: 28/August/2010)
(Accepted: 11/February/2011)

Summary

The goal of this study was to determine the frequencies of allele of *CYP3A5* in Japanese young subjects. Genotyping of allele *CYP3A5* (**1,*2, *3, *4, *5*, and **6*) was carried out in 235 young Japanese subjects by polymerase chain reaction (PCR) and DNA sequence. The frequencies of *CYP3A5*1, *2, *3, *4, *5* and **6* variant alleles in young Japanese are found at frequencies of 0.157, 0, 0.843, 0, 0 and 0, respectively. It provides evidence that the allele frequencies for *CYP3A5* in young Japanese are same from those for adult Japanese population. Our result should contribute to a better understanding of the molecular basis of age differences in drug response, which may help to improve individualization of drug therapy in the Japanese population.
(Med Biol **155:** 237-240 2011)

Keyword : *CYP3A5*, SNP, Japanese

Introduction

Cytochrome P450 enzymes (CYP) play an important role in the metabolism of many medicines. Differences in the activities of these enzymes are thought to be responsible for individual variability in drug response and rich effects. Among CYP enzymes, *CYP3A* and family enzymes metabolize 60% of pharmaceutical medicines. Four *CYP3A* genes have been identified in humans: *CYP3A4, CYP3A5, CYP3A7* and *CYP3A43*.[1,2)]

Overlapping substrate specificities between *CYP3A4* and *CYP3A5* have previously made it difficult to separate the metabolism of these medicines by two enzymes. There is evidence of polymorphic expression of *CYP3A5*. Recently, genetic polymorphisms have been found in *CYP3A5* genes. The single nucleotide polymorphisms (SNPs) in *CYP3A5*3* and *CYP3A5*6* cause alternative splicing and protein truncation, resulting in an absence of *CYP3A5* from the tissues of some people [3)]. Smoking and alcohol consumption are possible to cause the mutation of *CYP3A5* in the younger population.

Therefore, SNPs inducing changes in the function of the *CYP3A5* are thought to be potential cause for variation in *CYP3A* drug metabolism in human. The SNPs of *CYP3A5* have been reported on the gene of adults whereas no reports on the gene in young men have been published.

In this study, we analyzed DNA sequenc of all exons of *CYP3A5* of 235 young Japanese subjects.

Materials and methods

DNA Samples

Mouth mucous cells were obtained from 235 unrelated healthy Japanese volunteers of Meijo University students living in Nagoya. The persons there who were administered with anticancer, steroidal and antiepileptic drugs and persons with experience of smoking or drinking were excluded. The cell sample was collected from toothbrush rubbing in the mouth. The mucous cells were collected by centrifugation by 800 × g for 1 min. DNA was isolated from these cells using DNA

isolation kit according to the manufacturer's instructions (QIAGEN, Tokyo, Japan). This study was approved by the ethics committees of the faculty of pharmacy of Meijo Univ. Written informed consent was obtained from all participating students.

PCR amplication and Sequence Analysis

Each exon (from exon 1 to 9) of *CYP3A5* gene was amplified by polymerase chain reaction (PCR). The primer sets used for PCR amplification and sequencing are shown in Table 1. The PCR reactions were carried out in 25 μl of solution consisting of 2.5 μl of 10×Taq buffer, 0.1 μM each primer, 0.25 μM dNTPs, 25 ng of genomic DNA, and 2.5 U of Taq polymerase (TaKaRa, Shiga, Japan). PCR was performed at 96℃ for 5 min, followed by 32 cycles of 96℃ for 35 sec, 62℃ for 50 sec, and 72℃ for 80 sec, then 72℃ for 7 min. PCR products were directly sequenced on both stands using the ABI BigDye Terminator cycle sequencing kit Ver. 3.0 (Applied Biosystems, Foster City, CA, USA) with the same PCR primers. PCR products were analyzed on an ABI PRISM 3100 genetic Analyzer (Applied Biosystems). The sequences of all SNPs detected were confirmed independently twice. Electropherograms were analyzed independently by two researchers.

Statistical Analysis

Data were compiled according to the genotype and allele frequencies estimated by the observed numbers of each specific allele. The frequency of each allele in our subjects is given together with the 95% confidence interval (CI). Differences in allele frequenciy between Japanese and Caucasian populations[4,5] were measured using Fisher's exact test. A *P* value below 0.05 was considered statistically significant throughout the population comparisons.

Results

As shown in Table 2, in 235 young Japanese subjects the frequencies of the *CYP3A5*1,*2, *3, *4, *5,* and **6* alleles were 15.7, 0, 84.3, 0, 0, and 0%, respectively. Allele *3 was more frequent then the other variant alleles. The genotype frequencies of *CYP3A5 *1/*1, *1/*3,* and **3/*3* were 15.7%, 37.2%, 47.1%, respectively. These results are in good agreement with the expected genotype distributions, calculated using the Hardy-Weinberg equation.

Discussion

The result in our present study is the first to document the distribution of *CYP3A5* allele variants and genotypes of *CYP3A5* in the young Japanese population. In this study, the variant *CYP3A5*5* allele was not identified among 235 Japanese. It was reported that *CYP3A5*1, *2, *3, *4, *5* and **6* variant alleles in adult Japanese are found at frequencies of 0.256, 0, 0.74, 0, 0.04 and 0, respectively[7]. This provides evidence that the allele frequencies for *CYP3A5* in young Japanese

Table.1 Primer sequences for isozyme specific *CYP3A5* amplication

Name	Sequence
*CYP3A5*2*	(F) 5'-AAATACTTCACGAATACTATGATCA -3'
	(R) 5'-CAGGGACATAATTGATTATCTTTG -3'
*CYP3A5*3*	(F) 5'- GCATTTAGTCCTTGTGAGCACTTG -3'
	(R) 5'- CATACCCCTAGTTGTACGACACACA -3'
*CYP3A5*4, *6*	(F) 5'- GCAGATAGTTCTGAAAGTCTGT -3'
	(R) 5'- GTGTTGACAGCTAAAGTGTG -3'
CYP3A5 5*	(F) 5'-CGCCCCACATACACTCAGAA-3'
	(R) 5'-AGACCATTTTTAGGAAGCTCG-3'

The primer sequences, written from 5' to 3' end , for the *CYP3A5* of Exon are shown.

Table.2 Allelic frequencies of *CYP3A5* in young Japanese subjects.

CYP3A5 genotype		Frequency（%）
*CYP3A5*1*		15.7%
*CYP3A5*2*		0.0%
*CYP3A5*3*	(intron3)	84.3%
**1/*3*		
**3/*3*		
*CYP3A5*4*	(exon7)	0.0%
*CYP3A5*5*	(exon5)	0.0%
CYP3A5*6	(exon7)	0.0%

experimental in the presents experiment are the same as those for the adult Japanese population. These results suggest that *CYP3A5*5* genotype in absent or at least very rare in the young Japanese population. Routine genotyping for these variant alleles may not be necessary to determine the effects of the variants on the catalytic activity of *CYP3A5* in this population. It was reported that *CYP3A5*1, *2, *3, *4* and **5* variant alleles in Chinese subjects are found at frequencies of 0.28, 0, 0.7, 0.01 and 0.01, respectively[6]. These results indicated that the frequencies for *CYP3A5* allele in Chinese are not so different from these in Japanese. However, only 9.2% of Caucasians have been found to carry *CYP3A5*1* wild-type allele[5]. The frequency of *CYP3A5*1* is 2.8-3.0 times higher in Japanese and Chinese than in Caucasians[5]. The *CYP3A5*2* allele accounts for 5.2% of the *CYP3A5* alleles in Caucasians [7]. However, this allele was not found in any of the Japanese subjects in the present study. This finding is consistent with a recent study of Chinese subjects, in which the *CYP3A5*2* allele was found to be unique to Caucasians. These results suggest that *CYP3A5*2* is either very rare in Asian populations or specific to Caucasians.

In conclusion, the allelic variants of *CYP3A5* are shown to exist in young Japanese people. But, the frequencies were not different from those in Japanese adult population. This finding provides further evidence for age heterogeneity in drug-metabolizing activity. Our result should contribute to a better understanding of the molecular basis of age differences in drug response, which may help to improve individualization of drug therapy in the Japanese population.

Acknowledgements

We are grateful to Dr. K. Inagaki of Meijo University for his cooperation to this study. The present study was supported in part by the Frontier Project from the Japan Society.

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若い日本人における *CYP3A5* の対立遺伝子型と遺伝子変異頻度

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要　旨

日本人の若い世代を対象に薬物代謝酵素である *CYP3A5* の SNPs 解析を行った。235 名の遺伝子を対象に *CYP3A5* の **1,*2,*3,*4,*5,*6* を PCR と DNA シーケンスにて調べた。

その結果、*CYP3A5* の **1,*2,*3,*4,*5,*6* の発現頻度は、15.7,0,84.3,0,0,0％であった。また、対立遺伝子型である *CYP3A5 *1/*3*，**3/*3*，は、それぞれ 37.2，47.1％であった。日本人における若い世代と成人における遺伝子変異箇所および頻度はほぼ同様の結果であった。

キーワード：*CYP3A5*、SNP、Japanese

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