NOTE *Pharmacology*

Development of a genotyping tool for a functionally relevant *CYP2C19* allele (Phe100Asn, Ala103Val and Ile112Leu) in cynomolgus macaques

Yasuhiro UNO[1)]* and Hiroshi YAMAZAKI[2)]

[1)]*Pharmacokinetics and Bioanalysis Center, Shin Nippon Biomedical Laboratories, Ltd., Kainan, Wakayama 642–0017, Japan*
[2)]*Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, Machida, Tokyo 194–8543, Japan*

(Received 9 July 2015/Accepted 7 August 2015/Published online in J-STAGE 21 August 2015)

ABSTRACT. In cynomolgus macaques, which are widely used in drug metabolism studies, CYP2C19 (formerly known as CYP2C75) is abundantly expressed in liver, metabolizes human CYP2C substrates and is thus an important drug-metabolizing enzyme. One of the cynomolgus *CYP2C19* alleles (p.Phe100Asn, p.Ala103Val and p.Ile112Leu) results in substantially reduced metabolic activity and thus is an important allele in drug metabolism studies. For this allele, a genotyping tool was developed using allele-specific TaqMan probe. Genotyping 40 Cambodian cynomolgus macaques using this tool found 1 homozygote, 17 heterozygotes and 22 wild type animals, and the result was confirmed by direct-sequencing. Interestingly, this allele frequency was similar to that of Chinese cynomolgus macaques. The genotyping tool established is useful for drug metabolism studies using cynomolgus macaques.
KEY WORDS: CYP2C19, cytochrome P450, genotyping, monkey, polymorphisms

doi: 10.1292/jvms.15-0416; *J. Vet. Med. Sci.* 78(1): 147–148, 2016

Cytochrome P450 (P450 or CYP) is a family of the important drug-metabolizing enzymes consisting of a large number of subfamilies [3]. In humans, CYP2C enzymes (CYP2C8, CYP2C9 and CYP2C19) metabolize ~20% of prescribed drugs, including clinically important drugs, such as tolbutamide, phenytoin and warfarin [1]. Cynomolgus macaques (*Macaca fascicularis*) are frequently used in drug metabolism research due to their evolutionary closeness to humans. In cynomolgus macaques, CYP2C8, CYP2C9 and CYP2C19 (formerly known as CYP2C20, CYP2C43 and CYP2C75, respectively) are expressed in liver and metabolize a number of human CYP2C substrates [6]. Moreover, cynomolgus CYP2C76 that is not orthologous to any human CYP2C, is partly responsible for the differences in drug metabolism to those of humans [4, 5, 7].

Cynomolgus CYP2C19 has an amino acid sequence highly homologous (92–93%) to human CYP2C9 and CYP2C19. Cynomolgus CYP2C19 is polymorphic due to a number of genetic variants including the allele (p.Phe100Asn, p.Ala103Val and p.Ile112Leu), which results in the reduced catalytic activity of warfarin metabolism *in vitro* and *in vivo* [8, 9]. This allele has been found in Chinese cynomolgus macaques with relatively high frequency, but not in Indonesian cynomolgus macaques [8], and thus is an important allele to be considered in drug metabolism studies using cynomolgus macaques. Despite its importance, a feasible genotyping tool has not been developed, unlike an essential genotype of human drug-metabolizing enzyme, such as *UGT1A1*6* (c.211G>A) [10]. Therefore, in this study, a genotyping tool was developed using allele-specific TaqMan probe.

Because the allele has three linked mutations (c.298TT>AA, c.308C>T and c.334ATC>CTT), any of these three mutations can be used to genotype this allele. In this study, the tool was developed to genotype c.298TT>AA. The oligonucleotides were designed using Primer Express software (Applied Biosystems, Foster City, CA, U.S.A.), including TaqMan probes mmCYP2C19_c298mut_mgbFW1 5′-FAM-AGAGGACATA-ATCCATTGG-MGB-3′ and mmCYP2C19_c298wt_mgbFW1 5′-VIC-AGAGGACATTTTCCATTGG-MGB-3′, and primers mmCYP2C19_c298 (5abi1) 5′- ATGGTGGTGCTGCATGGA-TA-3′ and mmCYP2C19_c298 (3abi1) 5′-ATGAAAAGCTCT-GCTAGTCTGTTTTC-3′. Real-time PCR was performed using the ABI Prism 7500 sequence detection system (Applied Biosystems) according to the manufacturer's instructions. PCR was carried out in a total volume of 25 μl using TaqMan Genotyping Master Mix (Applied Biosystems) with 5 *n*g of each genome sample. The probes and primers were used at a final concentration of 250 nM and 900 nM, respectively. Thermal cycler conditions were 10 min at 95°C, followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. Data were captured and analyzed using SDS v1.3 software (Applied Biosystems) to determine the genotype of each sample.

The accuracy of the tool developed was assessed by genotyping 40 Cambodian cynomolgus macaques (20 males and 20 females, 4–5 years of age, weighing 3–5 kg). The genome samples were prepared from whole blood samples of these animals using DNeasy kit (Qiagen, Valencia, CA, U.S.A.) according to the manufacturer's instructions. The study was reviewed and approved by the Institutional Animal Care and Use Committee of Shin Nippon Biomedical Laboratories, Ltd. Using the tool developed, 1, 17 and 22 animals were genotyped

*CORRESPONDENCE TO: UNO, Y., Pharmacokinetics and Bioanalysis Center, Shin Nippon Biomedical Laboratories, Ltd., 16–1 Minami Akasaka, Kainan, Wakayama 642–0017, Japan.
e-mail: uno-yasuhiro@snbl.co.jp

Table 1. The frequency of the *CYP2C19* allele (p.Phe100Asn, p.Ala103Val and p.Ile112Leu) in cynomolgus macaques

Variant	Number of animals		
	Cambodia	China [a)]	Indonesia [a)]
Wild type	22	23	35
Heterozygote	17	10	3
Homozygote	1	5	0

Forty Cambodian cynomolgus macaques were genotyped using the newly developed tool as described in text. The results were confirmed by direct-sequencing method. a) These data were from our previous study [8].

as the homozygous, heterozygous and wild-type, respectively (Table 1). Allelic discrimination and amplification curves for homozygote, heterozygote and wild type are shown in Fig. 1 and Fig. S1, respectively. For the same animals, genotyping was performed by the direct-sequencing method previously described [8], which showed the same results. Therefore, the newly developed tool can be used to genotype the *CYP2C19* allele (p.Phe100Asn, p.Ala103Val and p.Ile112Leu).

Interestingly, the frequency of the allele was similar between Cambodian and Chinese cynomolgus macaques (Table 1). This might be accounted for by the fact that breeding of cynomolgus macaques started using the founder animals from Indochina [11]. Moreover, this allele has been found in Indonesian cynomolgus macaques and Chinese rhesus macaques (*Macaca mulatta*) with very low frequency [8], indicating the difference of the allele frequency between the groups of cynomolgus macaques or between cynomolgus macaques and rhesus macaques. Therefore, one should pay attention to the origin and lineage of the animals to be used in drug metabolism studies.

Cynomolgus macaques are bred in various locations, such as Mauritius and Philippines, but those other than Cambodian, Chinese and Indonesian animals have not been genotyped for this allele, and thus, this allele might be prevalent in the animals of the other origins. Moreover, CYP2C19 is an important enzyme involved in the metabolism of various human CYP2C substrates [2, 5, 6]. Therefore, when differences in CYP2C-dependent drug metabolism among the animals or between cynomolgus macaques and humans are critical, the animals can be genotyped using the tool developed in this study to assess the involvement of the *CYP2C19* allele (p.Phe100Asn, p.Ala103Val and p.Ile112Leu).

ACKNOWLEDGMENTS. We sincerely thank Mr. Masahiro Utoh for his support of this work and Mr. Lance Bell for his advice on English writing.

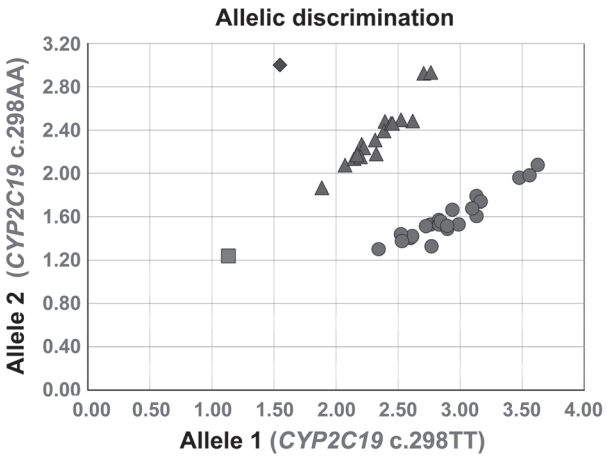

Fig. 1. CYP2C19 genetic variants detected using TaqMan allelic discrimination. Forty Cambodian cynomolgus macaques were genotyped as described in text. Based on the fluorescent signals (FAM, VIC) viewed using the sequence detection software, the samples were categorized into wild type (●), heterozygotes (▲) or homozygote (♦). No template control is also shown (■).

REFERENCES

1. Goldstein, J. A. 2001. Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. *Br. J. Clin. Pharmacol.* **52**: 349–355. [Medline] [CrossRef]
2. Hosoi, Y., Uno, Y., Murayama, N., Fujino, H., Shukuya, M., Iwasaki, K., Shimizu, M., Utoh, M. and Yamazaki, H. 2012. Monkey liver cytochrome P450 2C19 is involved in *R*- and *S*-warfarin 7-hydroxylation. *Biochem. Pharmacol.* **84**: 1691–1695. [Medline] [CrossRef]
3. Nelson, D. R., Zeldin, D. C., Hoffman, S. M., Maltais, L. J., Wain, H. M. and Nebert, D. W. 2004. Comparison of cytochrome P450 (*CYP*) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. *Pharmacogenetics* **14**: 1–18. [Medline] [CrossRef]
4. Uno, Y., Fujino, H., Iwasaki, K. and Utoh, M. 2010. Macaque CYP2C76 encodes cytochrome P450 enzyme not orthologous to any human isozymes. *Curr. Drug Metab.* **11**: 142–152. [Medline] [CrossRef]
5. Uno, Y., Fujino, H., Kito, G., Kamataki, T. and Nagata, R. 2006. CYP2C76, a novel cytochrome P450 in cynomolgus monkey, is a major CYP2C in liver, metabolizing tolbutamide and testosterone. *Mol. Pharmacol.* **70**: 477–486. [Medline] [CrossRef]
6. Uno, Y., Iwasaki, K., Yamazaki, H. and Nelson, D. R. 2011. Macaque cytochromes P450: nomenclature, transcript, gene, genomic structure, and function. *Drug Metab. Rev.* **43**: 346–361. [Medline] [CrossRef]
7. Uno, Y., Kumano, T., Kito, G., Nagata, R., Kamataki, T. and Fujino, H. 2007. CYP2C76-mediated species difference in drug metabolism: A comparison of pitavastatin metabolism between monkeys and humans. *Xenobiotica* **37**: 30–43. [Medline] [CrossRef]
8. Uno, Y., Matsushita, A., Shukuya, M., Matsumoto, Y., Murayama, N. and Yamazaki, H. 2014. *CYP2C19* polymorphisms account for inter-individual variability of drug metabolism in cynomolgus macaques. *Biochem. Pharmacol.* **91**: 242–248. [Medline] [CrossRef]
9. Utoh, M., Yoshikawa, T., Hayashi, Y., Shimizu, M., Iwasaki, K., Uno, Y. and Yamazaki, H. 2015. Slow *R*-warfarin 7-hydroxylation mediated by P450 2C19 genetic variants in cynomolgus monkeys *in vivo*. *Biochem. Pharmacol.* **95**: 110–114. [Medline] [CrossRef]
10. Wong, F. L., Wang, M. K., Boo, N. Y., Hamidah, N. H. and Ainoon, B. O. 2007. Rapid detection of the UGT1A1 single nucleotide polymorphism G211A using real-time PCR with Taqman minor groove binder probes. *J. Clin. Lab. Anal.* **21**: 167–172. [Medline] [CrossRef]
11. Zhiyong, F. and Yanling, S. 2002. Chinese primate status and primate captive breeding for biomedical research in China. International perspectives: the future of nonhuman primate resources 36–45.