Cytochromes P450 (P450) are a gene superfamily consisting of a large number of heme proteins, metabolizing numerous xenobiotics and endogenous substrates. In human P450s, 57 functional genes and 58 pseudogenes have been identified [7]. Although some P450s (especially CYP1–3) are important enzymes for drug metabolism, other P450s play physiological roles by metabolizing endogenous substrates [6]. For example, CYP17A1 participates in synthesis of sex steroids and thus is important for reproduction [8]. CYP7A1, CYP27A1 and CYP51A1 are involved in biosynthesis and metabolism of cholesterol and bile acids [5]. CYP7A1 is a potential therapeutic target for cholesterol lowering because CYP7A1 is a major determinant of plasma cholesterol levels, and drugs, such as bile acid-binding resin cholestyramine, is known to increase CYP7A1 activity [10]. A mutation of CYP7A1 results in no enzyme activity and increased plasma cholesterol level [9]. Because CYP51 has been found not only in mammals, but also in fungi, plants and prokaryotes, some antifungal drugs, such as imidazoles and triazoles, have been developed targeting CYP51 [4]. There are also orphan P450s, including CYP20A1, which have not been fully characterized [2].

Cynomolgus macaques are frequently used in biomedical research due to their evolutionary closeness to humans. We previously conducted expressed sequence tags (EST)-sequencing in the liver samples of three cynomolgus macaques [15], from which we subsequently identified and characterized a number of P450s that are mainly important for drug metabolism [12–14]. However, CYP7A1, CYP17A1, CYP20A1, CYP27A1 and CYP51A1 cDNAs, which were also isolated by the EST-sequencing, have not been characterized. In this study, therefore, these P450s were analyzed by sequence analysis, phylogenetic analysis and tissue expression patterns.

For analysis of cynomolgus P450, brain, lung, heart, liver, kidney, adrenal gland, small intestine (jejunum), testis, ovary and uterus were collected from six purpose-bred cynomolgus macaques (three males and three females from Indochina, 4–5 years of age and 3–5 kg), and total RNA was extracted from these tissues as previously described [12]. Pooled samples of these six animals were used to measure mRNA expression. The study was reviewed and approved by the local animal ethics committee. Sequencing of the P450 cDNA was performed using ABI Prism BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, U.S.A.), and the sequence data were analyzed using DNASIS Pro (Hitachi Software, Tokyo, Japan), as described previously [12]. A homology search was performed using BLAST (National Center for Biotechnology Information). The phylogenetic tree was created using the neighbor joining method as described previously [12].
Human and rhesus macaque genome data were analyzed using BLAT (UCSC Genome Bioinformatics). The cynomolagus cDNAs reported in this paper have been deposited to GenBank under accession numbers DQ074791 (CYP7A1), DQ074802 (CYP17A1), KJ922552 (CYP20A1), DQ074803 (CYP27A1) and DQ074804 (CYP51A1).

Sequencing and sequence analysis revealed that cynomolagus CYP7A1, CYP17A1, CYP20A1, CYP27A1 and CYP51A1 identified by EST-sequencing had higher amino acid sequence identities (94–99%) to the human orthologs as compared with those of dogs and rats which are also used in biomedical research (Table 1). By comparing to human CYP7A1 cDNA, cynomolagus CYP7A1 cDNA lacked the 5′ end of the sequence, so that the first 186 amino acid residues were missing. A full-length CYP7A1 cDNA (NM_001193803) was found in GenBank for rhesus macaques, closely related to cynomolgus macaques, raising the possibility that cynomolgus macaques might also express a full-length CYP7A1 transcript. By comparing to human CYP20A1 gene sequence using BLAT, cynomolagus CYP20A1 cDNA lacked the sequence of exon 10, so that this cDNA did not contain a complete open reading frame (ORF) as described previously [14] using SYBR Green PCR Master Mix (Applied Biosystems). The primers used were mfCYP7A1 (5qrt1) 5′-AGTCAGCTTGGAAGGCAATC-3′ and mfCYP7A1 (3qrt1) 5′-TTGAGGGAGGCACTGGAA-3′ for CYP7A1, mfCYP17A1 (5qrt1) 5′-ACCATCCGAGAGGTGCTTC-3′ and mfCYP17A1 (3qrt1) 5′-CCTTGCACTGGAGAATCCAAGTG-3′ for CYP17A1, mfCYP20A1 (5qrt1) 5′-CTTTATGCCCTTGGTGTGGT-3′ and mfCYP20A1 (3qrt1) 5′-TGTGCCTGAGAATCCAAGTG-3′ for CYP20A1, mfCYP27A1 (5qrt1) 5′-GGTGTCTGGCTACCTGGCACT-3′ and mfCYP27A1 (3qrt1) 5′-CATGTCAGCGTTGGATG-3′ for CYP27A1, and mfCYP51A1 (5qrt1) 5′-GCTGCCTTTGCCTAGTTTCA-3′ and mfCYP51A1 (3qrt1) 5′-CGCCCATCCTTGTATGTAGC-3′ for CYP51A1. The primers were used at a final concentration of 200 nM. The raw data were normalized with the 18S ribosomal RNA level to determine the relative expression level.

To measure expression of cynomolagus CYP7A1, CYP17A1, CYP20A1, CYP27A1 and CYP51A1 mRNAs, quantitative polymerase chain reaction (qPCR) analysis was performed with brain, lung, heart, liver, kidney, adrenal gland, jejunum, testis, ovary and uterus RNAs as described previously [14] using SYBR Green PCR Master Mix (Applied Biosystems). The primers used were mfCYP7A1 (5qrt1) 5′-AGTCAGCTTTGGAAGGCAATC-3′ and mfCYP7A1 (3qrt1) 5′-TTGAGGGAGGCACTGGAA-3′ for CYP7A1, mfCYP17A1 (5qrt1) 5′-ACCATCCGAGAGGTGCTTC-3′ and mfCYP17A1 (3qrt1) 5′-CCTTGCACTGGAGAATCCAAGTG-3′ for CYP17A1, mfCYP20A1 (5qrt1) 5′-CTTTATGCCCTTGGTGTGGT-3′ and mfCYP20A1 (3qrt1) 5′-TGTGCCTGAGAATCCAAGTG-3′ for CYP20A1, mfCYP27A1 (5qrt1) 5′-GGTGTCTGGCTACCTGGCACT-3′ and mfCYP27A1 (3qrt1) 5′-CATGTCAGCGTTGGATG-3′ for CYP27A1, and mfCYP51A1 (5qrt1) 5′-GCTGCCTTTGGAAGGCAATC-3′ and mfCYP51A1 (3qrt1) 5′-CGCCCATCCTTGTATGTAGC-3′ for CYP51A1. The primers were used at a final concentration of 200 nM. The raw data were normalized with the 18S ribosomal RNA level to determine the relative expression level.

qPCR analysis showed that among the 10 tissue types analyzed, cynomolagus CYP7A1 and CYP17A1 mRNAs were preferentially expressed in liver and adrenal gland, respectively (Fig. 2). Cynomolagus CYP27A1 and CYP51A1

|    | CYP7A1 (%) | CYP17A1 (%) | CYP20A1 (%) | CYP27A1 (%) | CYP51A1 (%) |
|----|------------|------------|------------|------------|------------|
| Human | 95         | 94         | 97         | 98         | 99         |
| Dog  | 82         | 80         | 94         | 80         | 97         |
| Rat  | 82         | 68         | 80         | 72         | 94         |

Amino acid sequence identities were determined using BLAST.

Table 1. Sequence identities of cynomolgus P450 cDNAs to the human, dog and rat orthologs in amino acid sequences

Fig. 1. Phylogeny of cynomolgus P450s. A phylogenetic tree was created using the neighbor joining method. CYP7A1, CYP17A1, CYP20A1, CYP27A1 and CYP51A1 amino acid sequences were from humans (h), cynomolgus macaques (mf), dogs (d) and rats (r). Human CYP39A1 was used as outgroup. For the distance measurement, the scale bar indicates 0.1 amino acid substitutions per site.
mRNAs were expressed in all the 10 tissue types analyzed, but were most abundantly expressed in liver and testis, respectively (Fig. 2). Cynomolgus CYP20A1 mRNA showed a ubiquitous expression pattern with the most abundant expression in kidney, followed by adrenal gland, testis and lung (Fig. 2). Therefore, cynomolgus CYP7A1, CYP17A1, CYP20A1, CYP27A1 and CYP51A1 mRNAs showed distinct tissue expression patterns.

CYP7A1, CYP27A1 and CYP51A1 are involved in biosynthesis and metabolism of cholesterol and bile acids. CYP7A1, cholesterol 7α-hydroxylase, catalyzes the major rate-limiting step of the classical pathway of bile acids [5]. Human CYP7A1 is considered to be liver-specific [5], coinciding well with a predominant expression of cynomolgus CYP7A1 (Fig. 2). CYP27A1, sterol 27-hydroxylase, catalyzes the oxidation of cholesterol to 27-hydroxycholesterol in the bile acid biosynthesis and participates in other processes of cholesterol homeostasis [5]. CYP27A1 is also involved in metabolism of vitamin D as vitamin D 25-hydroxylase. Human CYP27A1 is expressed in various tissues [9], including liver [1], similar to cynomolgus CYP27A1 (Fig. 2).

CYP51A1, the only P450 involved in cholesterol biosynthesis, catalyzes the oxidative 14α-demethylation of lanosterol [5]. Although human CYP51 is expressed in various tissues [5], the abundant expression is found in testis [3], similar to cynomolgus CYP51A1 (Fig. 2). CYP51 shows stage-specific expression patterns during spermatogenesis, which requires extensive changes of membrane structure.
Because cholesterol is involved in modulation of membrane properties [3], CYP51 might play essential roles in spermatogenesis. High sequence identity and similar tissue expression patterns indicate the potential functional similarity of cynomolgus CYP7A1, CYP27A1 and CYP51A1 to the human orthologs in biosynthesis and metabolism of cholesterol and bile acids.

CYP17A1 is involved in steroid synthesis by catalyzing steroid 17α-hydroxylation and 17,20-lyase activity toward production of sex steroids and thus is important for reproduction [8]. Human CYP17A1 is expressed abundantly in adrenal gland [8], similar to cynomolgus CYP17A1 (Fig. 2). In contrast, mouse and rat CYP17 expression appears to be absent in adrenal gland [8]. Moreover, substrate preference of CYP17A1 is somewhat different between rats and humans [8]. Cynomolgus CYP17A1 showed high sequence identity and similar tissue expression pattern to human CYP17A1, suggesting that cynomolgus CYP17A1 might have more similar function to human CYP17A1 as compared with rodent CYP17A1.

CYP20A1 is an orphan isoform in humans, and the function has not been characterized [2]. Human CYP20A1 mRNA is expressed in brain and liver [11], and cynomolgus CYP20A1 was also expressed in these tissues (Fig. 2). Cynomolgus CYP20A1 cDNA identified in this study lacked exon 10 sequence due to alternative splicing. Similarly, other than the full-length CYP20A1 transcript, two alternatively-spliced transcripts are expressed in rats, lacking either exon 2 or exons 2/3 [11]. Such alternatively-spliced transcripts have also been identified in various P450s, including cynomolgus CYP2C93, which lacked exon 2 sequence due to the mutation at the splicing acceptor site of intron 1 [16]. Hence, the full-length CYP2C93 transcript with a complete ORF appears to be expressed in rhesus macaques, but not in cynomolgus macaques, indicating potential differences between the two lineages in a CYP2C93-dependent drug metabolism. It is of great interest to investigate to see if full-length CYP20A1 transcript is expressed in cynomolgus macaques.

In conclusion, cynomolgus CYP7A1, CYP17A1, CYP20A1, CYP27A1 and CYP51A1 were characterized by sequence analysis, phylogenetic analysis and tissue expression pattern. Each of these cynomolgus P450s had a high sequence identity to the human ortholog and was most closely related to the human ortholog as compared with the dog or rat ortholog. Moreover, each of these cynomolgus P450 mRNAs showed a tissue expression pattern generally similar to the human ortholog in the 10 tissue types analyzed. These results suggest the molecular similarities of CYP7A1, CYP17A1, CYP20A1, CYP27A1 and CYP51A1 between cynomolgus macaques and humans. Further investigation of drug-metabolizing capability would help understand the function of these cynomolgus P450s.

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