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Genetic polymorphism of canine cytochrome P450 2D25 gene

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*S u m m a r y. Human CYP2D6 is involved in the metabolism of a number of drugs including neuroleptics, tricyclic antidepressants, β-adrenoreceptors antagonists and antiarrhythmic drugs. The corresponding canine CYP2D15 have shown similar catalytic activities. In an attempt to characterize the polymorphism of canine CYP2D15, we have cloned a segment of CYP2D15 gene from exon 5 to end of exon 7 using PCR. Sequencing and alignment of the DNA fragment produced by PCR, revealed two polymorphic sites: 1) A834T (Ile250→Phe) (CYP2D15*1) located in exon 5, and 2) A1005G (Ile307→Val) (CYP2D15*2) located in exon 6. These polymorphic sites occurred with frequency of 0.725 and 0.843, respectively. The approach described here could be used as a tool for quick assessment of canine CYP2D15 polymorphism.*

INTRODUCTION

The human polymorphic isoenzyme CYP2D6 has a major role in the oxidative metabolism of a variety of widely used drugs, such as neuroleptics, tricyclic antidepressants, β-adrenoreceptors antagonists and antiarrhythmic drugs (1,2). The CYP2D6 polymorphism is characterized by two discrete phenotypes: individuals with a normal CYP2D6 activity (extensive metabolizers) and individuals lacking this activity (poor metabolizers. The corresponding ortholog of human CYP2D6, canine CYP2D15, has been isolated and characterized (3). Purified CYP2D15 or heterologous expressed in COS cells have shown similar catalytic activities to those attributed to CYP2D6 (disipramine 2-hydroxylation, metoprolol α-hydroxylation, dextramethorphan O-demethylation, bunitrolol, and imipramine hydroxylation and N-demethylation) (3-5). It was also found to metabolize the COX-2 inhibitor, celecoxib, with different velocities suggesting that polymorphism may affect the catalytic activity (6). Furthermore, *in vitro* expression of mutated and non-mutated enzyme isoforms have shown that the mutated CYP2D15 protein metabolized drugs with different velocity that non-mutated protein (7). The only canine CYP that has been genotyped is the CYP2E1 gene (8).

In this study, we describe a easy method of identification of polymorphism of canine CYP2D15 gene using PCR-RFLP and we determine the frequencies of two polymorphic sites found in a number of samples.

MATERIALS AND METHODS

DNA isolation.- DNA was isolated from peripheral whole blood samples, collected on EDTA, of 51 dogs obtained from the local animal hospital using a DNA isolation kit for whole blood samples (Gentra, Minneapolis, MN, USA).

PCR conditions.- PCR reaction was performed for each DNA sample using primer pair designed from start of Exon 5 and the end of Exon 7. The forward and reverse primers were

5'-GCCCTGAACTCCATCCCCGTG-3' (CYP2D15ex5-F) and 5'-CTTGGGGATGAGGAAGCCCTG-3' (CYP2D15Aex7-B),

respectively. The PCR mix contained 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 μM each primer, and 0.5 units Platinum *Taq* DNA polymerase (Invitrogen) in a total volume of 30 μl. PCR, performed on a MJ Research PTC-200 Peltier Thermal Cycler, consisted of an initial 3-min denaturation at 95 °C followed by 35 cycles of melting (95 °C, 0.5 min), annealing (60 °C, 1 min), and extension (72°C 1,5 min) with a final extension step (72 °C, 10 min). The PCR product

was purified (QIAquick PCR purification Kit, Qiagen) and sequenced (MWG, Germany).

Restriction Fragment Length Polymorphism (RFLP) conditions.- To determine the polymorphism of CYP2D15, 10 µl of the amplified DNA (859 bp) was digested with 1 unit of each re-striction enzymes, separately, incubating at 37 °C for 3 h. Thereafter, the resulting DNA fragments subjected in 2% ethidium bromide-stained agarose electrophoresis at 100 V for 90 min.

RESULTS

Development of a genotyping method for canine CYP2D15 gene

To develop a genotyping method for canine CYP2D15 we have designed primers based on CYP2D15 cDNA with accession number D17397 (3). Alignment of this sequence with human CYP2D6 protein from CYP2D6 gene locus (M33388) (9) revealed the possible starts and ends of exons and introns for canine CYP2D15 gene. Based on this approach, we have designed the forward primer (pair bases 761-782) that is annealed at the beginning of exon 5 and backward primer (pair bases 1248-1268) annealed to the end of exon 7, was used in this study. Pair bases were numbered according to canine CYP2D15 (D17397). PCR using these primers revealed a 859 bp DNA fragment that contains: exon 5 177 bp, intron 5 152 bp (human 189 bp), exon 6 142 bp, exon 6 200 bp (human 206 bp) and exon 7 188 bp. Exon (5-7) sizes are identical to the human CYP2D6 exons. DNA isolated from 51 dogs was used to perform PCR and thereafter the PCR products were digested with *StuI* and *DrdI* restriction enzymes. *StuI* digested when DNA is mutated DNA at A834T into 789 bp and 70 bp fragments. When DNA is not mutated *StuI* do not cut PCR products. When *DrdI* was used, DNA is digested when it is mutated at A1005G into 393, 238 and 228 bp fragments (Figure 1). When it is not mutated gave 621 and 238 bp fragments.

Ethidium bromide-stained 2% agarose gel showing the actual fragmentation patterns resulting from *DrdI* and *StuI* digestions of the PCR products amplified from the genomic DNA from dogs 11 and 14. The uncut PCR product is shown in lane 1 (859 bp). PCR product was digested with *DrdI*, showed as homozygotic G1005 (lane 2) (393 bp, 238 bp, 228 bp) or heterozygotic A/G1005 (621 bp is not digested) (lane 3). When PCR product was digested with *StuI*, showed as homozygotic T834 (789 bp) (lane 4) or heterozygotic A/T834 (859 bp, 789 bp). The leftmost lane contains 100-bp DNA ladder.

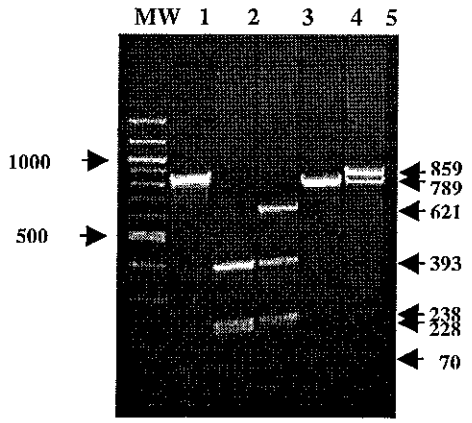

Figure 1. PCR-based restriction enzymes test of CYP2D15 DNA

Allele frequency determination

Analysis of restriction fragment patterns have shown that the Phe250 allele (T834) occurred at a frequency of 0.725 (37/51) (homozygotes for the SNP) but none homozygote was found for A834. Heterozygotes were occurred at a frequency of 0.275 (14/51). For the other polymorphic site (A1005G), the Val307 allele (G1005) occurred at a frequency of 0.843 (43/51) (homozygotes for the SNP) and none homozygote for A1005 was found. Heterozygotes were occurred at a frequency of 0.157 (8/51) (Table 1).

CONCLUSIONS

These preliminary results suggest that the CYP2D15 gene is polymorphic and the method described here can be used as a tool of CYP2D15 genotyping in dogs.

Table 1
Canine CYP2D15 genotyping

SNP/Allele	*Restriction enzyme*	*Band patterns*	*Frequency*
A834T (CYP2D15*1)	*StuI*	A: 859	0
		T: 789, 70	0.725
		A/T: 859, 789, 70	0.275
A1005G (CYP2D15*2)	*DrdI*	A: 621, 238	0
		G: 393, 238, 228	0.843
		A/G: 621, 393, 238, 228	0.157

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