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Genetic Polymorphisms of Cytochrome P450 2D14 (CYP2D14) Gene in Japanese Black Cattle and Holstein-Friesian Cattle

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Abstract

Two types of cytochrome P450 2D14 (CYP2D14) gene deletions (GD1 and GD2) in Japanese Black cattle has been reported by us. In this study, we determined the frequency of cytochrome P450 2D14 (CYP2D14) genetic polymorphisms in 48 Japanese Black (JB) and 48 Holstein-Friesian (HF) cattle using polymerase chain reaction (PCR)-direct sequencing. Two types of CYP2D14 gene deletion variants, GD1 and GD2, were observed 58% in JB cattle, but only 8% of HF cattle. Genotype frequencies of CYP2D14 gene (105 C>A) also were assessed, but the AA substitution was not detected. The results of CYP2D14 gene variation study in cattle show that CYP2D14 GD allele may be an important mutation relevant to an enzyme defect in JB cattle because of high frequency of gene deletion variants.

Keywords: Cytochrome P450 2D14; Genetic polymorphism; Japanese Black cattle; Holstein-Friesian cattle

Introduction

As a member of the human cytochrome P450 superfamily, CYP450 2D6 (CYP2D6) is involved in the endogenous biotransformation and in the metabolism of many drugs, including antipsychotics, antidepressants, β-blockers, antiarrhythmics, and opiates [1]. Approximately 40% of hepatic human phase I metabolism processes in the human liver involve CYP2C9, CYP2C19, and CYP2D6 are associated with [2]. Various medications include substrates of CYP2D6, even though CYP2D6 accounts for only a small percentage of total hepatic CYPs (2-3%) [3].

More than 80 allelic variants and subvariants of the CYP2D6 have been reported, relating to the metabolism of 20–25% of over-the-counter and prescribed medicines [2,4-12]. Substrates of CYP2D6 are lipophilic bases with a protonatable atom [9]. The main non-functional (null) mutations of CYP2D6 are known as CYP2D6*3, *4, *5 and *6 [4,13,14]. The null mutation results in the gene product having less or no function; therefore CYP2D6*3, *4, *5 and *6 are important mutations in human CYP2D6. In particular, CYP2D6 (CYP2D6*5) is associated with altered or delayed drug metabolism [12,14], and CYP2D6 polymorphisms can lead to adverse drug reactions and altered drug responses [2,15].

Tsuneoka et al. [16] cloned, mapped, and characterized CYP2D14 cDNA from the cattle liver, revealing 80% and 68% similarity to human CYP2D6 and rat CYP2D1, respectively. The CYP2D14 gene is located on bovine chromosome 5 [17]. Although some of sequence differences in the cattle CYP2D14 were indicated in the National Center for Biotechnology Information (NCBI), little has been reported on genetic polymorphisms of the CYP2D14 gene in cattle.

Metoclopramide (4-amino-5-chloro-N-(2-(diethylamino)ethyl)-2-methoxy-benzamide, CAS: 364-62-5) is widely used as a gastrointestinal prokinetic agent for humans, cattle, swine, cats, and dogs in Japan. The CYP2D6 polymorphism (CYP2D6*4/*4 or CYP2D6*4/*5) reportedly results in delayed metabolism of metoclopramide in human and metoclopramide-induced acute dystonic reactions may occur in patients as a result of delayed metoclopramide metabolism [18]. The clinical consequence of the CYP2D6 polymorphism can be either occurrence of adverse drug reactions and altered drug response [12,19]. As for cattle CYP2D14 enzyme, the relationship between the CYP2D14 gene deletion and pharmacological effects in Japanese Black (JB) cattle has recently been reported by us [20].

On the other hand, it is known that CYP2D6 polymorphisms differ markedly in frequency among different ethnic and racial in human [21,22]. In cattle, there may be possibility that difference of CYP2D14 polymorphisms among cattle breeds; however, differences in frequency of CYP2D14 polymorphisms among the breeds has not been reported.

The aim of the present study was to investigate frequency of the CYP2D14 genetic polymorphisms, specifically those related null alleles reported in human (CYP2D6*3, *4, *5, and *6), in JB and Holstein-Friesian (HF) cattle. CYP2D14 genotype was analyzed by means of polymerase chain reaction (PCR) amplification and direct DNA sequencing for the CYP2D14 gene.

Materials and Methods

Chemicals and reagents

The Fast Start High Fidelity PCR System, dNTpack (Roche Applied Science Inc., Penzberg, Germany) was used as PCR reagent. As DNA molecule standards, Lambda-HindIII Digest (Takara Bio Inc., Shiga, Japan) and 200 bp DNA ladder (Takara Bio Inc., Shiga, Japan) were purchased for electrophoresis of PCR products amplified by PCR primer pairs. BigDye terminator ver.3.1 Cycle sequencing kit (Applied Biosystems Inc., CA, USA) and BigDye Xterminator purification kit

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(Applied Biosystems Inc., CA, USA) were used for direct sequencing of PCR products by an ABI 3130 genetic analyzer (AB Sciex Pte. Ltd., CA, USA).

DNA samples and animals

To prepare genomic DNA, blood and sperm were obtained from 48 healthy adult JB cattle and 48 healthy adult HF cattle. Genomic DNAs were prepared from blood and sperm as described in Abe et al. [23]. Because JB cattle are likely to have different genetic background depending on their locale, samples of DNA were gathered from JB cattle in three regions of Japan (Miyazaki, Iwate and Hokkaido prefectures). Samples of DNA were collected from HF in Iwate prefecture. Genotyping for CYP2D14 alleles was carried out by DNA sequencing after PCR of the DNA samples.

Polymerase chain reaction (PCR) and sequencing analysis

PCR primers and PCR protocol for amplification of cattle genomic DNA were used according to previous PCR method for amplification of JB cattle genomic DNA [20].

For the gel electrophoresis of the PCR product, an 8 μLof each PCR product was pre-mixed with 2 μL of 6x loading buffer (Takara Bio Inc., Shiga, Japan). A 5 μL of the PCR sample and 2 μL of the DNA molecule standard were loaded into well in 1% agarose gel (Agarose H14; Takara, Bio Inc., Shiga, Japan). The agarose gel was set in an electrophoresis apparatus (ViewaBlue Stain, Kanto Chemical Co. INC., Tokyo, Japan) and fill with 1 × TAE buffer until the gel was covered. The output of electrophoresis apparatus was set the output level to 100 V. After 30 min, the finished gel was immersed in an ethidium bromide solution (0.5 μg /mL) for 15 min to stain the DNA. Specific primers for sequencing and sequencing analysis to determine frequency of the CYP2D14 genetic polymorphisms in JB and HF cattle was performed according to the previous method [20].

Results

Figure 1 shows the typical agarose gel electrophoresis results of PCR products from exon 1 to exon 4 and from exon 5 to exon 9 of the CYP2D14 gene. The PCR products obtained with the primer pair (L:5'-GCCCAGAAAGATAAACCTAAAATGT-3' and R: 5'-ACTCTCCTTGACCCCTCTCTGTACT-3') and another primer pair (L:5'-GTGGGAGTACTTCACTGCAAGG-3' and R: 5'-AGACACTGGTTTATTGACCATCAG-3') were approximately 2.5 kbp from exon 1 to exon 4 and approximately 2.0 kbp from exon 5 to exon 9, respectively. The trial primer pair (Figure 1) was not used for this study because of its low specificity.

The allele frequencies mainly determined by sequencing in exon 1 and exon 4 of the CYP2D14 gene after PCR amplification. Two types of gene deletion alleles, GD1 and GD2, were identified among the CYP2D14 gene polymorphisms [20]. For the GD1 allele, DNA sequencing could not be performed in either exon 1 or exon 4 of the CYP2D14 gene. For the GD2 allele, DNA sequencing could not be performed in only exon 1.

The GD1 and GD2 polymorphisms were found in 14.6 and 43.8% of the 48 analyzed JB cattle, respectively, with 58.3% of JB cattle carrying either the GD1 or GD2 variant. In contrast, only 2.1 and 6.3% of HF cattle exhibited the GD1 and GD2 variant, respectively. Therefore, deletion of the CYP2D14 gene in cattle (which is suspected to be similar to CYP2D6*5 in human) varied considerably depending on the cattle species.

All DNA samples of the JB and HF cattle used in this study were able to be amplified and sequenced by using a primer set for sequencing of the CYP2E1 gene. Therefore, sufficient DNA was extracted from the blood or sperm of JB and HF cattle. The CYP2D14 gene deletions found in the DNA sequencing study were similarly observed when sequencing tests were performed with a double volume of the same

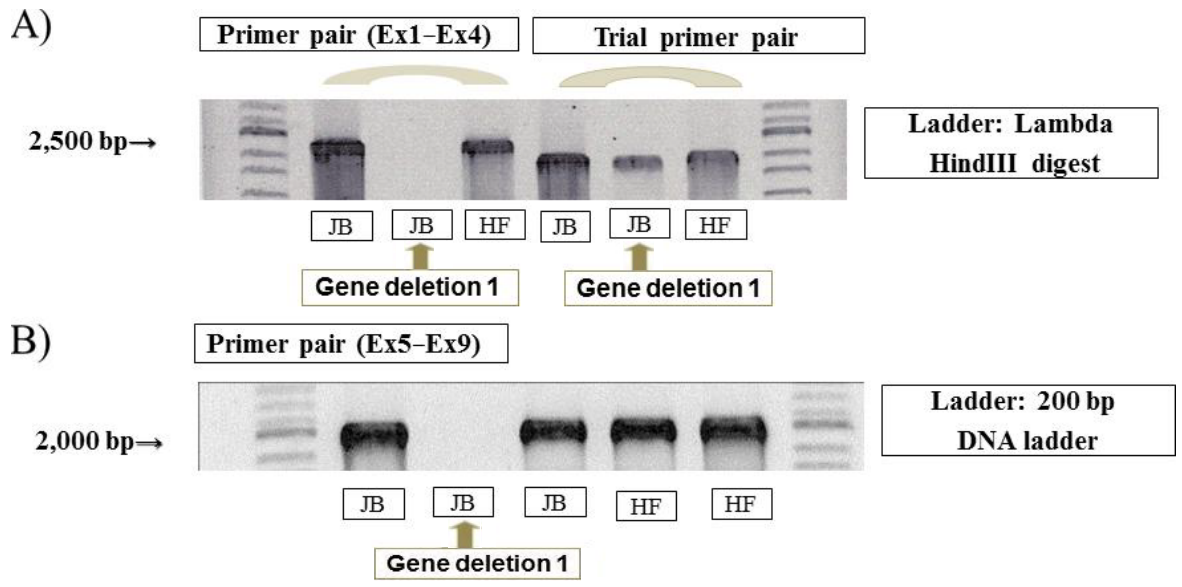

Figure 1: A) Typical agarose gel electrophoresis of PCR products amplified from exon 1 (Ex1) to exon 4 (Ex4) by the primer pair (L:5'-GCCCAGAAAGATAAACCTAAAATGT-3' and R: 5'-ACTCTCCTTGACCCCTCTCTGTACT-3') and a trial primer pair (L: 5'-CGTCAAGTCCAACAGCTTGA-3' and R: 5'-AACACAATCTCCCCGACAAG-3') in order to detect alleles of CYP2D14 gene related to human null alleles (CYP2D6*4, *5, *6), B) Typical agarose gel electrophoresis of PCR products amplified from exon 5 (Ex5) to exon 9 (Ex9) by the primer pair (L:5'-GTGGGAGTACTTCACTGCAAGG-3' and R: 5'-AGACACTGGTTTATTGACCATCAG-3') in order to detect alleles of CYP2D14 gene related to human null allele (CYP2D6*3).

PCR samples, and when the PCR-sequencing test was repeated three times with the same method.

Figure 2 summarizes the frequencies of the alleles related to human CYP2D6*4, which were obtained by sequencing exon 1 and exon 4 of the PCR-amplified CYP2D14 gene. Over 10 alleles related to CYP2D6*4 have been reported in human. As this study was focused primarily on the human CYP2D6*4 allele (100 C>T and 1846 G>A), the 105C>A and 1790 G>C substitutions in JB and HF cattle were determined. The allele frequencies of 105 C/C, C/A, and A/A, except for GD1 and GD2, were 80, 10 and 10% in 20 JB cattle, compared to 82, 18 and 0% in 44 HF cattle, respectively (Figure 2). The allele frequencies of 1790 G/G, G/C, and C/C, except for GD1 and GD2, were 80, 15 and 5% in 20 JB cattle, compared to 61, 25 and 14% in 20 HF cattle, respectively (Figure 2). The polymorphism from exon 5 to exon 9 of CYP2D14 related to human CYP2D6*3 was not observed in cattle. Similarly, SNPs in the part of CYP2D14 that is near the corresponding human Cyp2D6*6 were not detected in JB and HF cattle.

Discussion

Although the CYP450 family members present in different animals and their roles in veterinary drug interactions have been reviewed [24], little is known about the CYP450 types in cattle. This paper represents the first report on percentages of the CYP2D14 deletion alleles and other CYP2D14 polymorphisms in JB and HF cattle.

Approximately 25% of all human medicines on the market are metabolized by CYP2D6 enzyme [4]. The CYP2D6 was the first CYP P450 for which a classical pharmacogenetic polymorphism become known, and is the most intensely study polymorphic CYP450 in human. There is known more than 80 variants of CYP2D6 and some of them is reported as important variants (e.g., CYP2D*2, CYP2D6*3,CYP2D6*4,CYP2D6*5,CYP2D6*6 and CYP2D6*10, CYP2D6*17, CYP2D6*41 and so on). Some of them caused serious functional change of CYP2D6 enzyme that include a fully functional allele, a reduced functional allele and a null allele. Especially, CYP2D6*3, *4, *5 and *6 alleles is well recognized as important allele because of relation to a CYP2D6 enzyme deficit [19]. There is a considerable variability in the CYP2D6 allele distribution among different ethnic groups, resulting in variable percentages of ultrarapid, extensive, intermediate, and poor metabolizers in a given population. Phenotyping studies in the United State and Europe suggested that approximately 7% of Caucasians are poor metabolizers and deficient in CYP2D6 expression. Proportions in subjects of African and Asian heritage are 1% and 3%, respectively [15,25]. The most frequent null allele is CYP2D6*4, with an allele frequency of 20-25% in Caucasians, where it is responsible for 70-90% of all poor metabolizers [14]. The frequency of the gene deletion allele (CYP2D6*5) is similar in other ethnic groups (4-7%).

Not only the cDNA but also the AA sequences of CYP2D subfamily members show high similarity between human CTP2D6 and cattle CYP2D14 [16], and CYP2D14 is an important phase I drug-metabolizing enzyme for veterinary medicine in cattle. In the present study, we scrutinized null alleles and gene deletion allele as targets for CYP2D14, corresponding to the typical human null alleles CYP2D6*4 (100 C>T and 1846 G>A) [4,9] and CYP2D6*5 (gene deletion allele). We identified the CYP2D14 gene deletion allele in cattle, as well as the proportions of the CYP2D14 105 (C>A) and CYP2D14 1790 (G>C) variants. The CYP2D14 gene deletion variants were observed two types (GD1 and GD2). The GD1 allele frequency in JB cattle (14.6%) was approximately 7 times higher than that in HF cattle (2.1%). The frequency of the GD2 allele was also higher in JB cattle (43.8% vs. 6.3%). As CYP2D7 has been reported to be a pseudogene of human CYP2D6 [26,27], the GD2 allele in cattle may be similar to the human pseudogene.

Frequencies of variants with the CYP2D14 105 (A/A) and 1790 (C/C) alleles, except for GD1 and GD2, were 10 and 5% in 20 JB and 14 and 0% in 44 HF cattle, respectively. In human, there is a transitional C>T mutation at nucleotide 100 in the CYP2D6*4 and CYP2D6*10 sequences, which results in an AA substitution of proline to serine. However, this AA substitution was not detected in cattle. The CYP2D14 1790 (G>C) allele was situated on an intron between exon 3 to exon 4 of CYP2D14. No allele around exon 5, which is similar to CYP2D6*3 of human (2549 (deletion of A) allele), was detected in cattle. We detected no SNPs in the part of CYP2D14 that corresponds to human

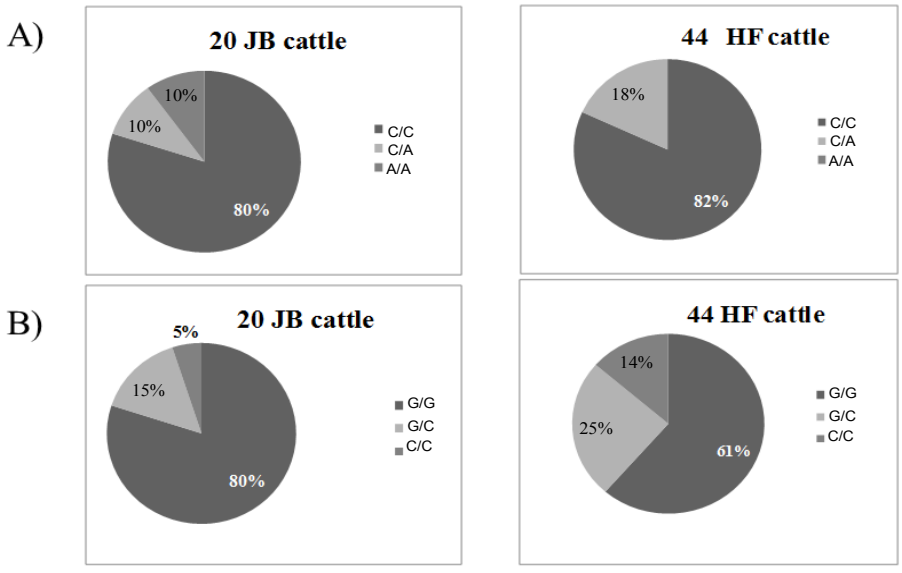

Figure 2: Ratio of polymorphisms at position 105 (A) and 1790 (B) from beginning of CYP2D14 gene in 20 JB and 44 HF cattle except for GD1 and GD2 variants.

Cyp2D6*6. These results indicate that CYP2D14 gene deletion allele may be the most important mutation relevant to an enzyme defect in JB cattle, because the allele frequencies were very high.

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