Research Article

High Frequency of a Single Nucleotide Substitution (c.-6-180T>G) of the Canine MDR1/ABCB1 Gene Associated with Phenobarbital-Resistant Idiopathic Epilepsy in Border Collie Dogs

Keijiro Mizukami, Akira Yabuki, Hye-Sook Chang, Mohammad Mejbah Uddin, Mohammad Mahbubur Rahman, Kazuya Kushida, Moeko Kohyama, and Osamu Yamato

Laboratory of Clinical Pathology, Department of Veterinary Medicine, Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan

Correspondence should be addressed to Osamu Yamato; osam@vet.kagoshima-u.ac.jp

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A single nucleotide substitution (c.-6-180T>G) associated with resistance to phenobarbital therapy has been found in the canine MDR1/ABCB1 gene in Border Collies with idiopathic epilepsy. In the present study, a PCR-restriction fragment length polymorphism assay was developed for genotyping this mutation, and a genotyping survey was carried out in a population of 472 Border Collies in Japan to determine the current allele frequency. The survey demonstrated the frequencies of the T/T wild type, T/G heterozygote, and G/G mutant homozygote to be 60.0%, 30.3%, and 9.8%, respectively, indicating that the frequency of the mutant G allele is extremely high (24.9%) in Border Collies. The results suggest that this high mutation frequency of the mutation is likely to cause a high prevalence of phenobarbital-resistant epilepsy in Border Collies.

1. Introduction

Recently, a single nucleotide substitution associated with phenobarbital-resistant idiopathic epilepsy was found in Border Collies [1], which frequently present with severe epileptic seizures that are poorly controlled with antiepileptic drugs [2]. This polymorphic mutation is a substitution of thymine for guanine at intron 1 near the 5' end of the canine MDR1/ABCB1 gene (c.-6-180T>G), where the most important promoter elements are located [3]. The mutation is not directly associated with the pathogenesis of idiopathic epilepsy, but it may create resistance to phenobarbital therapy in epileptic Border Collies [1].

However, the c.-6-180T>G mutation has been shown not to be a fully penetrant polymorphic mutation for phenobarbital nonresponsiveness because phenobarbital-resistant, idiopathically epileptic Border Collies in a previous case control study included a homozygous T/T wild-type dog [1]. On the basis of data from a relatively small cohort of Border Collies, it was hypothesized that the mutation might be related to an upregulation of the gene and an overexpression of P-glycoprotein (P-gp) encoded by the MDR1/ABCB1 gene. Therefore, further studies are required to understand the regulatory effect of the mutation and its potential clinical relevance. For these purposes, an accurate method of genotyping for the mutation should be developed, and the frequency of the mutation should be determined in a large, normal population of dogs.

In the present study, a PCR-restriction fragment length polymorphism (RFLP) assay was developed in order to discriminate the genotypes of the mutation, and a genotyping survey was conducted in Japan by using samples from clinically healthy Border Collies in order to determine the current frequency of the mutant allele.
resulting in 2 fragments (i.e., 313 and 103 bp bands) because there was only 1 restriction site available to the MboI, which was present in all the genotypes and unrelated to the target sequence. In the T/T homozygous wild-type dog, the amplified band was digested into 3 fragments (i.e., 191, 122, and 103 bp bands) because there were 2 restriction sites available: one at the target sequence [\textit{GATC}] and another at the same sequence present in all the genotypes. The amplification product in the T/G heterozygous dog was digested into 4 fragments (i.e., 313, 191, 122, and 103 bp bands). Owing to the inseparability of the 122 and 103 bp bands, these 2 bands externally appeared to be 1 band; therefore, the digested PCR products in the T/T wild-type and T/G heterozygous dogs externally appeared as 2 and 3 fragments, respectively. The genotypes of all the dogs examined were consistent with the results of direct DNA sequencing.

In the survey on 472 Border Collies, 283 (60.0%) were T/T wild-type dogs, 143 (30.3%) were T/G heterozygous dogs, and 46 (9.8%) were G/G homozygous mutant-type dogs. The overall frequency of the mutant G allele was 24.9%.

### 4. Discussion

Pharmacogenetics is a relatively new discipline that investigates how genetic variations are related to drug response, and it is expected to be an important tool for developing personalized medicine [5]. Pharmacogenetic biomarkers relevant to various diseases, drugs, and genes have been found and clinically used to maximize therapeutic efficacy, reduce adverse drug reactions, and determine the most appropriate drug dosage required for efficacious and safe treatment [6]. Information about pharmacogenetics in domestic animals is still not as extensive as in humans; however, in veterinary medicine, there is great potential for advances in the coming years because whole-genome sequencing of many species has been completed [7]. The genetic marker, c.-6-180T>G, associated with phenobarbital-resistant idiopathic epilepsy in dogs may be an important pharmacogenetic biomarker in veterinary medicine in the near future.

In the PCR-RFLP assay developed in the present study, all the genotypic variations of c.-6-180T>G could be easily discriminated by confirming the presence and/or absence of 313 and 191 bp fragments via agarose gel electrophoresis.

**2. Methods and Materials**

Control samples of each genotype, which were determined by direct DNA sequencing, were used to evaluate the accuracy of the genotyping assay. These samples were collected from 4 wild-type (T/T), 9 heterozygous (T/G), and 10 homozygous mutant-type (G/G) dogs. DNA templates were prepared using saliva spotted onto Flinders Technology Associates filter paper (FTA card, Whatman International Ltd., Piscataway, NJ, USA). For the PCR-RFLP assay, a 1.2 mm disc punched out of the FTA card was used as a template after quick washing as reported previously [4]. The PCR test was performed by targeting the sequence around the mutation with forward (5′-GCA GTG GGG TGA GAA CTA GA-3′) and reverse (5′-GGC AAG CCA TGT AAG GTA TG-3′) primers in a 20 μL reaction mixture containing 10 μL of a 2× PCR master mix (GoTaq Hot Start Green Master Mix, Promega Corp., Madison, WI, USA), 12.5 pmol of primers, 1 μL of GC enhancer solution (360 GC Enhancer, Applied Biosystems, Foster City, CA, USA), and the treated disc of the FTA card as a template. After denaturation at 95 °C for 10 min, 45 cycles of amplification were carried out at a denaturing temperature of 95 °C for 30 sec, an annealing temperature of 60 °C for 30 sec, and an extension temperature of 72 °C for 30 sec. Extension during the last cycle was performed at 72 °C for 7 min and 30 sec. The PCR product was digested with a MboI restriction endonuclease (New England Biolabs Inc., Ipswich, MA, USA) at 37 °C for 90 min in a 10 μL reaction mixture containing 8 μL of the PCR product, 5 U of MboI, and 1 μL of 10× restriction enzyme buffer (10× NE buffer, New England Biolabs Inc.) included by the manufacturer. Both the unprocessed and digested PCR products were subjected to electrophoresis in 3% agarose gel (Agarose 21, Nippon Gene Co., Ltd., Tokyo, Japan). The PCR-RFLP assay was designed to digest the wild-type sequence [\textit{GATC}] into 3 fragments and to not digest the mutant sequence [\textit{GAGC}] resulting in 2 fragments.

The genotyping survey was carried out by using DNA templates extracted from saliva samples of 472 Border Collies aged 2 months to 14 years in Japan. These samples were collected between 2006 and 2013 by the Japan Border Collie Health Network, a volunteer breeders’ association with the owners’ informed consent. The PCR-RFLP assay established in this study was used for genotyping.

### 3. Results

As shown in Figure 1, a 416-base pair (bp) DNA band was amplified, in theory, in all the genotypes. In the G/G homozygous mutant-type dog, the amplified band was digested into 2 fragments (i.e., 313 and 103 bp bands) because there was only 1 restriction site available to the MboI, which was present in all the genotypes and unrelated to the target sequence. In the T/T homozygous wild-type dog, the amplified band was digested into 3 fragments (i.e., 191, 122, and 103 bp bands) because there were 2 restriction sites available: one at the target sequence [\textit{GATC}] and another at the same sequence present in all the genotypes. The amplification product in the T/G heterozygous dog was digested into 4 fragments (i.e., 313, 191, 122, and 103 bp bands). Owing to the inseparability of the 122 and 103 bp bands, these 2 bands externally appeared to be 1 band; therefore, the digested PCR products in the T/T wild-type and T/G heterozygous dogs externally appeared as 2 and 3 fragments, respectively. The genotypes of all the dogs examined were consistent with the results of direct DNA sequencing.

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and the interaction between these mutations in the canine MDR1/ABCB1 gene before the pharmacogenetic information related to c.-6-180T>G can be applied to clinical practice.

Conflict of Interests

The authors have no conflict of interests to declare.

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