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Allele-specific polymerase chain reaction diagnostic test for the functional *MDR1* polymorphism in dogs

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Abstract

The major multidrug transporter P-glycoprotein (Pgp) contributes to the barrier function of several tissues and organs, including the brain. In a subpopulation of Collies and seven further dog breeds, a 4 base pair deletion has been described in the Pgp-encoding *MDR1* gene. This deletion results in the absence of a functional form of Pgp and loss of its protective function. Severe intoxication with the Pgp substrate ivermectin has been attributed to the genetically determined lack of Pgp. An allele-specific polymerase chain reaction (PCR)-based screening method has been developed to detect the mutant allele and to determine if a dog is homozygous or heterozygous for the mutation. Based on this validation, the allele-specific PCR proved to be a robust, reproducible and specific tool, allowing rapid determination of the *MDR1* genotype of dogs of at risk breeds using blood samples or buccal swabs.

Keywords: *MDR1*; P-glycoprotein; Multidrug transporter; Ivermectin; Genetic mutation

Introduction

The major multidrug transporter P-glycoprotein (Pgp) protects tissues from accumulation of xenobiotics, including drugs (Fromm, 2003). There is increasing awareness that the transport function of Pgp has a significant impact on the distribution of several drugs and the body's response to these drugs (Fromm, 2003; Löscher and Potschka, 2005). The substrate spectrum of Pgp is broad and includes antiparasitic drugs, antibiotics, anticancer drugs, glucocorticoids, analgesics, cardiac glycosides, antidiarrheal agents and antiepileptic drugs.

The influence of Pgp on drug disposition has been demonstrated in Collies and other herding dog breeds (Mealey et al., 2001; Nelson et al., 2003; Roulet et al., 2003). Severe intoxication in response to treatment with the antiparasitic drug ivermectin and other avermectins has been reported in a subpopulation of these breeds. It has been assumed that susceptibility to ivermectin is related to a genetic deficiency of Pgp, leading to increased permeability of the blood–brain barrier (Schinkel, 1999). This allows ivermectin to enter the brain and exert effects on γ-amino butyric acid (GABA)-gated chloride channels.

Mealey et al. (2001) identified a 4 base pair (bp) deletion in the Pgp-encoding *MDR1* gene of a subpopulation of Collies, which was later also detected in ivermectin-sensitive Australian Shepherd dogs (Nelson et al., 2003). The deletion results in a frame shift and a preliminary stop codon, which prematurely terminates Pgp synthesis (Mealey et al., 2001). Recently, Neff et al. (2004) detected the mutant allele in subpopulations of six additional breeds, including the English Shepherd, Longhaired Whippet, McNab, Old English Sheepdog/Bobtail, Shetland Sheepdog/Sheltie and Silken Windhound.

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In these breeds, clinicians should be cautious with the use of drugs known to be Pgp substrates. However, treatment with such a drug may be preferred or even urgently needed in some clinical cases. Here, genetic analysis may reveal the *MDR1* genotype of the patient, which would allow the choice of drug to be tailored to the individual patient. Pgp substrates could be used in dogs with wild type *MDR1* alleles, whereas the drugs could be avoided in dogs with the deletion mutation.

In the present study, we developed and validated an allele-specific polymerase chain reaction (PCR) as an alternative rapid and cost-effective screening tool to available methods (Mealey et al., 2001, 2002; Nelson et al., 2003; Hugnet et al., 2004; Neff et al., 2004) for identification of the *MDR1* genotype in breeds of dogs at risk. Sequencing analysis as the most reliable approach for genotyping was used to validate the new method.

Materials and methods

Animals

Blood samples or buccal swabs were taken from 55 dogs, comprising at risk breeds (Collie, Australian Shepherd, Shetland Sheepdog/Sheltie, Border Collie, Bearded Collie), other pure breeds (Pon, Magyar Viszla, Labrador Retriever, Golden Retriever, German Shepherd, Dachshund, Schnauzer, Husky) and mixed breed dogs. These dogs were healthy animals from private owners or breeders, or were patients with different diagnoses at the Department of Small Animal Medicine and Surgery, University of Veterinary Medicine, Hannover. Dogs were from a non-selected population and to our knowledge had not been tested previously for the presence of mutant *MDR1*.

Allele-specific polymerase chain reaction

DNA was extracted from blood samples or buccal swabs using the Qiagen DNEasy Tissue Kit (Qiagen). Approximately 100–200 ng DNA were used for PCR amplification.

Primers were selected on the basis of published RNA sequences for the Pgp-encoding gene *MDR1* (GenBank AF045016 and AF269224 for the wild type allele and AJ419568 for the mutant allele; Table 1). Primers were designed to allow detection of the wild type *MDR1* allele by PCR1 (primers PgpA, PgpB and PgpD) and detection of the mutant allele by PCR2 (primers PgpA, PgpC and PgpD) (Fig. 1). Primer B only matches the wild type allele and, therefore, the 326 bp amplicon is only generated with primers B and D in PCR1 when at least one wild type allele is present. Primer C is specific for the mutant allele and, therefore, the 326 bp amplicon in PCR1 is only generated with primers C and D when at least one mutant allele is present. Independent of the presence or absence of the 4 bp deletion, an additional control amplicon is obtained with primer pair

Table 1
Nucleotide sequences of Pgp specific primers

Primer	Nucleotide sequence (5′ → 3′)	Position
PgpA	CAT GAA ACT GTG CTA ATT TCC	Intron 4
PgpB	TTG GAA ACA TGA C**AG ATA** GC	Exon 5 wild type, bp 281–300[a]
PgpC	GTT TTT GGA AAC ATG ACA GC	Exon 5 with 4 bp deletion, bp 216–236[b]
PgpD	AAC TTC CTG GGA TCT TTC TG	Intron 5

[a] GenBank AF045016. The 4 bp sequence deleted in mutant dogs is emboldened.
[b] GenBank AJ419568.

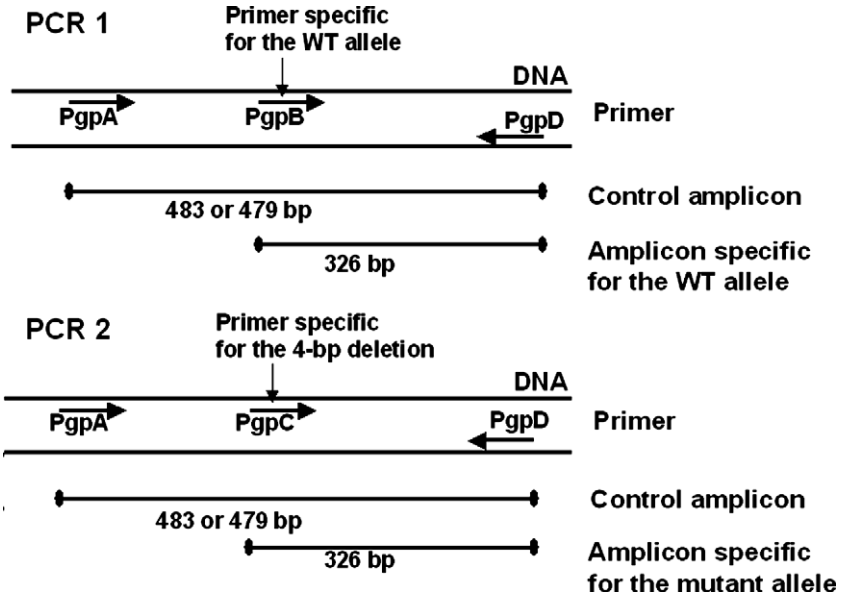

Fig. 1. Design of the allele-specific PCR. The assembly of the two forward primers and one reverse primer are shown schematically for PCR1 and PCR2. PCR1 was designed to detect the wild type *MDR1* allele and PCR2 to detect the mutant *MDR1* allele. The size of the generated amplicons is indicated.