Presence of the *ABCB1* (*MDR1*) deletion mutation causing ivermectin hypersensitivity in certain dog breeds in Belgium

Aanwezigheid van de ABCB1 (MDR1) deletiemutatie verantwoordelijk voor ivermectine overgevoeligheid bij enkele hondenrassen in België

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ABSTRACT

Hypersensitivity to ivermectin and certain other drugs in Collies and related breeds is caused by a 4-base pair deletion mutation in the *ABCB1* gene, better known as the *MDR1* gene, encoding P-glycoprotein. There is no information available, however, regarding the presence of this mutation in dogs in Belgium. In this study, the *ABCB1* genotype was assessed in 92 dogs of breeds suspected to possess the deletion mutation. The results indicated that the mutation was present in the Australian Shepherd, Collie, Shetland Sheepdog and Swiss White Shepherd, but was not detected in the Bearded Collies, Border Collies and German Shepherds of this study, which is in accordance with the findings in similar breed populations of other countries. In Belgium it is therefore important to take the *ABCB1* genotype of the breeds involved into account, in order to use drugs in a safe and efficient manner and to improve the selection procedure in dog breeding.

SAMENVATTING

De overgevoeligheid van Collies en aanverwante rassen voor ivermectine en bepaalde andere geneesmiddelen wordt veroorzaakt door een deletiemutatie van 4 basenparen in het *ABCB1*-gen, beter bekend als het *MDR1*-gen, dat codeert voor P-glycoproteïne. Er is echter geen informatie beschikbaar omtrent de aanwezigheid van deze deletiemutatie bij honden in België. In deze studie werd het *ABCB1*-genotype bepaald bij 92 honden van verschillende rassen waarvan bekend is dat ze de mutatie kunnen bezitten. De deletiemutatie werd gevonden bij de Australische Herder, Collie, Sheltie en Zwitserse Witte Herder, maar was afwezig bij de Bearded Collies, Border Collies en de Duitse Herders van deze studie, wat overeenkomt met de resultaten van studies van soortgelijke populaties in andere landen. Voor een veilig en efficiënt gebruik van geneesmiddelen en bij de selectie in de hondenfokkerij is het daarom ook in België van belang rekening te houden met het *ABCB1*-genotype van de betrokken hondenrassen.

INTRODUCTION

Ivermectin sensitivity in Collies and related breeds is a well described phenomenon caused by a 4-base pair deletion mutation in exon 4 of the *ATP-binding cassette, sub-family B (MDR/TAP), member 1* (*ABCB1*) gene, better known as the *multidrug resistance 1* (*MDR1*) gene (Mealey *et al.*, 2001). This gene encodes P-glycoprotein, which is a transmembrane protein pump involved in the transportation of several drugs. It is expressed in various tissues, such as the intestines, kidney, liver, testis, placenta, eye and nerves, and it has a strong influence on drug absorption and excretion (Cordon-Cardo *et al.*, 1990; Fromm, 2000). P-glycoprotein also constitutes an important part of the blood-brain barrier, in which it regulates the permeability for certain drugs (Jonker *et al.*, 1999; Ose *et al.*, 2008).

The deletion mutation in *ABCB1* causes a shift of the reading frame, creating a premature stop codon. This results in the synthesis of an incomplete and non-functional protein that consists of only the first 10% of the total amino acid sequence (Mealey *et al.*, 2001). The lack of normal P-glycoprotein affects the integrity of the blood-brain barrier, causing an increase of drug concentration (e.g. ivermectin) in the brain, which ex-

Table 1. *ABCB1* genotype in several dog breeds in Belgium.

Breed	wt/wt	wt/del	del/del	Total
Australian Shepherd	2	3	0	5
Bearded Collie	9	0	0	9
Border Collie	35	0	0	35
Collie	1	4	2	7
German Shepherd	27	0	0	27
Shetland Sheepdog	1	3	0	4
Swiss White Shepherd	4	1	0	5

wt/wt: homozygous *ABCB1* wildtype; wt/del: heterozygous *ABCB1* deletion mutation; del/del: homozygous *ABCB1* deletion mutation.

plains the accompanying neurotoxic symptoms. When the same drug dose is administered to different dogs, the intensity of these symptoms depends on the genotype of the animal involved. If a dog possesses 1 wild-type and 1 mutant allele, still a certain amount of complete P-glycoprotein will be synthesized, but this is not the case when both alleles contain the deletion mutation in *ABCB1* (Mealey and Meurs, 2008).

It has been shown that various classes of drugs, such as antiparasitics, chemotherapeutics, immunosuppressants, steroid hormones, opioids, anti-emetics and others, are transported by P-glycoprotein in several mammals (Mealey, 2004; Zhou, 2008). This number will probably increase, because numerous drugs have not yet been evaluated as a potential substrate for P-glycoprotein transport. Not only for ivermectin and other macrocyclic lactones like doramectin and moxidectin (Mealey *et al.*, 2001; Nelson *et al.*, 2003; Roulet *et al.*, 2003; Geyer *et al.*, 2005a, 2007), but also for loperamide, acepromazine, digoxin, vincristine, doxorubicin and dexamethasone a dose-dependent increased sensitivity has been associated with the deletion mutation in the *ABCB1* gene (Mealey *et al.*, 2003, 2007, 2008; Sartor *et al.*, 2004; Henik *et al.*, 2006; Martinez *et al.*, 2008). When administering these drugs, it is therefore important to take the *ABCB1* genotype of the animal into account, but there is no information available regarding the prevalence of this deletion mutation in dogs in Belgium. The aim of this study was therefore to determine its presence in a number of dog breeds known to carry the mutation in populations in other countries.

MATERIALS AND METHODS

Samples

Blood samples from the dog breeds summarized in Table 1 (presented for consultation at the Department of Medicine and Clinical Biology of Small Animals in Merelbeke for various reasons) were collected in EDTA blood tubes and stored at -20°C until usage.

Genotyping

The first part of the genotyping procedure was conducted with 2 equivalent methods. The first method used proteinase K to isolate genomic DNA from 200 µl of blood (Cler *et al.*, 2006), after which PCR was used to amplify a 148 base pair region of *ABCB1* in which the ATAG deletion mutation is located. The PCRs were performed using the FastStart Taq DNA Polymerase Kit (Roche) with the 5'-GGCTTGATAGGTTGTATATGTTGGTG-3' forward and 5'-ATTATAACTGGAAAAGTTTTGTTT-3' reverse primer pair (Mealey *et al.*, 2005). The composition of the PCR mix was as described in the manufacturer's protocol. In the second method, the PCR was directly conducted on the blood samples, without a previous DNA isolation step. For this purpose, 1 µl of blood and 5 µl of the KAPA Blood PCR Mix B (KAPA Biosystems) were used in a 10 µl reaction, as described in the instructions manual. The PCR program itself was the same for both methods. It started with a denaturation step of 5 minutes at 95°C, followed by 35 cycles of 30 seconds at 95°C, 30 seconds at 58°C and 1 minute at 72°C, after which a final elongation step was conducted at 72°C for 1 minute. To verify each reaction, 2 µl of the PCR product was loaded onto a 2% agarose gel. Next, the remaining volume of PCR product was purified with a combination of 4U of exonuclease I and 2U of antarctic phosphatase (Promega), by subsequently incubating the mixture for 30 minutes at 37°C and 15 minutes at 80°C. These enzymes break down primers and nucleotides that have not been used during the PCR reaction, which would otherwise interfere with the subsequent sequencing reaction. This last reaction was conducted on an Applied Biosystems 3730xl DNA Analyser with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and the forward primer, according to the instructions manual.

RESULTS AND DISCUSSION

The deletion mutation in the *ABCB1* gene responsible for ivermectin hypersensitivity was originally identified in the Collie, but has meanwhile also been detected in several related breeds such as the Australian Shepherd, Old English Sheepdog, Border Collie and Shetland Sheepdog, as well as in sighthounds (Mealey *et al.*, 2001; Neff *et al.*, 2004; Geyer *et al.*, 2005b). The mutation in all these breeds has been traced back to a single ancestor living in the 19th century in Great Britain (Neff *et al.*, 2004). The results from our study in dogs in Belgium are summarized in Table 1, and the 3 possible *ABCB1* genotype sequence out-

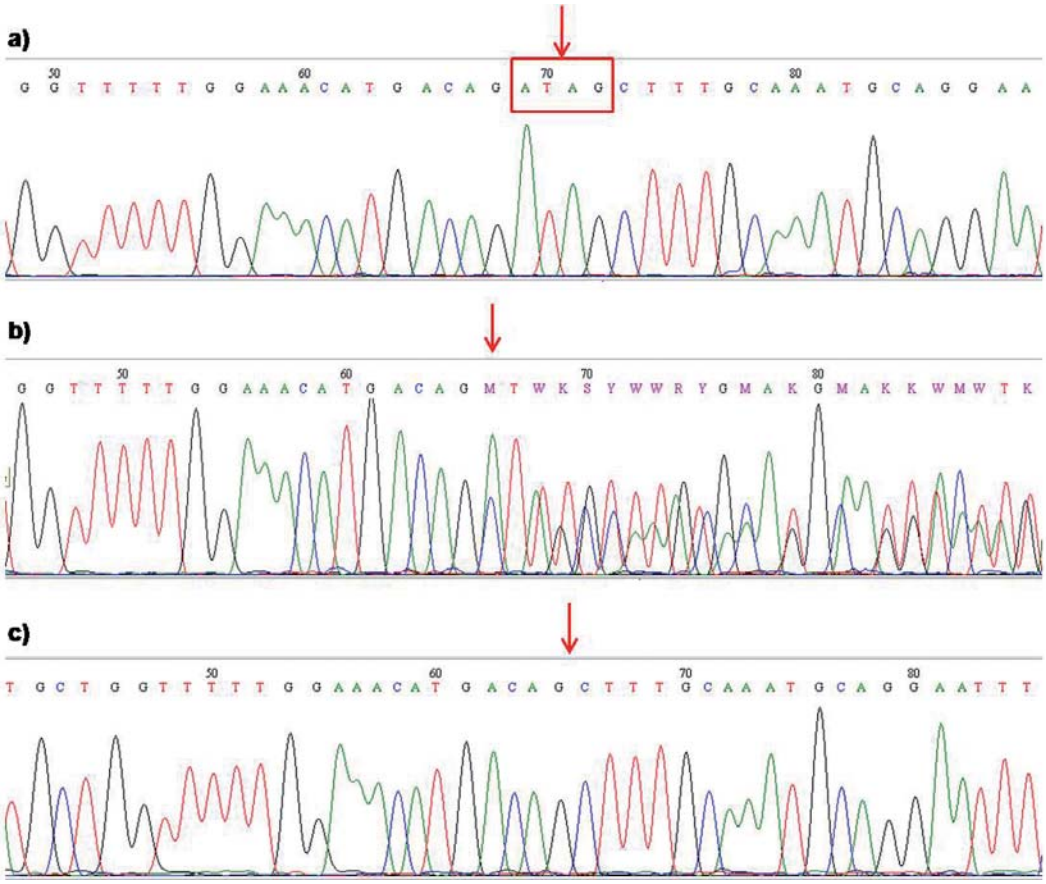

Figure 1. DNA sequence output of the region comprising the *ABCB1* deletion mutation.
The red arrow indicates the location of the ATAG deletion mutation in the *ABCB1* gene. The DNA sequence actually consists of 2 sequences, but they can only be distinguished if there is a difference between them.
a) Wildtype: neither of the 2 alleles contains the mutation, and therefore their sequence is identical.
b) Heterozygous: 1 complete allele and 1 allele carrying the deletion mutation, which causes distortion (double peaks) of the sequence output from this point onward.
c) Homozygous mutation: both alleles possess the deletion mutation and therefore their sequence is identical.

puts are shown in Figure 1. Despite the limited number of dogs analyzed in this study, the mutation was detected in the Collie, Australian Shepherd, Shetland Sheepdog and Swiss White Shepherd. For these first 3 breeds, this is in accordance with the results of studies using both smaller and larger dog populations in Australia, Germany, France, Japan and the USA, which have shown that the mutant allele occurs frequently in these breeds (Hugnet *et al.*, 2004; Neff *et al.*, 2004; Geyer *et al.*, 2005b; Kawabata *et al.*, 2005. Mealey *et al.*, 2005; Mealey and Meurs, 2008). The 9 Bearded Collies and 35 Border Collies evaluated in the current study did not possess the deletion mutation (Table 1), which, for the Bearded Collies, is in accordance with previous findings (Geyer *et al.*, 2005b). Two independent reports from Germany and the USA (both conducted on more than 300 dogs) showed that less than 2% of the Border Collies possessed the mutation in the *ABCB1* gene (Geyer *et al.*, 2005b; Mealey and Meurs, 2008). Because of the low prevalence in this breed, well-considered selection could possibly eliminate the mutant allele from the population without reducing the genetic variation of the entire population and without an increased risk of unintentional selection for possible accompanying negative characteristics.

Elimination by selection, however, is much more difficult in breeds in which the deletion mutation is present more frequently. For instance, it has been shown in several large Collie populations that more than 75% of the dogs possess at least 1 mutant allele (Neff *et al.*, 2004; Geyer *et al.*, 2005b; Mealey and Meurs, 2008). In these kinds of populations, the allele frequency of the mutant allele can be decreased without affecting the genetic variation of the entire breed, preferably by using breeding dogs that are heterozygous for the *ABCB1* mutation, instead of ones that are homozygous for it. The wildtype *ABCB1* allele of heterozygous dogs still guarantees the synthesis of a certain amount of normal and functional P-glycoprotein, which explains the reduced number and intensity of side effects compared to homozygous mutant dogs.

Recently, the deletion mutation in the *ABCB1* gene was also found in the Swiss White Shepherd (Geyer *et al.*, 2007) and the German Shepherd (Mealey and Meurs, 2008), but in the present study the mutation was only detected in the first of these two (Table 1). Geyer *et al.* (2007) showed that the mutation in the Swiss White Shepherd is of the same origin as the mutation in the Collie-related breeds and sighthounds.

Based on the relationship with the Swiss White Shepherd and the fact that several German Shepherds carrying the mutant allele had a white coat (or one of their (grand)parents did), it can be expected that the mutation found in the German Shepherd has the same origin as that found in all these other breeds, although this has not yet been verified (Mealey and Meurs, 2008). Because the German Shepherd and the Swiss White Shepherd are usually not associated with the deletion mutation in *ABCB1* and are not regarded as breeds at risk when problem drugs are used, it is essential to genotype a larger number of German Shepherds and related breeds to assess the potential importance for the Belgian population.

Information regarding the *ABCB1* genotype of potentially affected dog breeds is important not only for breeding purposes, but also for the choice and use of drugs. However, the potential presence and intensity of neurotoxic side effects is determined not only by the genotype of the animal, but also by the dose of the drug (Mealey, 2008). It has been reported that certain drugs, like macrocyclic lactones for heartworm prevention, only cause problems when a higher dose is administered. In addition, it is likely that hypersensitivity reactions to an increasing number of drugs will be associated with the deletion mutation in the *ABCB1* gene as more drugs are identified as P-glycoprotein substrates. Besides having an important function in the permeability of the blood-brain barrier, P-glycoprotein also directly limits the absorption and increases the excretion of drugs, thus controlling their blood concentration. The lack of functional P-glycoprotein resulting from the deletion mutation in the *ABCB1* gene therefore causes increased plasma drug levels and thus increased chances of adverse drug reactions (Hugnet *et al.*, 2004; Geyer *et al.*, 2005a).

Despite the limited sample size for some breeds in this study, the results clearly show that the deletion mutation in the *ABCB1* gene is present in several dog breeds in Belgium. However, to evaluate the relative risk when using problem drugs, larger sample sizes of all breeds at risk would be necessary. It would also be interesting for future studies to look for common ancestors in pedigrees or to identify breeding lines with high prevalence. Before treatment with problem drugs, it therefore remains of vital importance either to genotype all breeds at risk, in order to avoid their use, if possible, or (in some cases) to adjust the dose of the problem drug.

In conclusion, the results from this study highlight the importance of the *ABCB1* genotype, both for sensible dog breeding and for efficient and safe drug use.

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