*Original Article*

# Determination of Multidrug Resistance (MDR1) Gene and Its Mutations in Dogs by Using Polymerase Chain Reaction

**Sariya Asawakarn[1] Valaiporn Ruangchaiprakarn[2] Naparee Srisowanna[2]**
**Leelawat Wongwan[3] Anusorn Kanuengthong[1] Gunnaporn Suriyaphol[1*]**

***Abstract***

The objective of this study is to establish a polymerase chain reaction (PCR) laboratory for detection of multidrug resistance1 (*MDR1)* mutation in Thailand. Blood samples were collected from 67 dogs, namely 16 Collies, 9 Shetland Sheepdogs, 5 Border Collies, 6 Shih tzus, 13 German Shepherds and 18 mixed breeds. Genomic DNA was extracted and PCR was performed to amplify 341 and 577 bp fragments to detect homozygous wild-type (+/+) and homozygous mutant (-/-), respectively. For the heterozygous mutant (+/-), both PCR fragments would be presented. An analysis of DNA sequences encompassing the 4-base pair deletion in the coding region of *MDR1* gene was performed to confirm different *MDR1* genotypes. Nine Collies (56.2%) were heterozygous for the *MDR1* mutation (carrier) and 7 dogs (43.8%) were homozygous for the mutant allele (affected). None of the studied Collies were homozygous for the normal allele (normal). One Shetland Sheepdog (11.1%) was heterozygous mutant. All the other breeds were homozygous normal. This research can be used for further studies to establish the PCR-based diagnostic test in suspected dogs.

**Keywords:** diagnosis, dog, *MDR1* gene, mutation, PCR

[1]*Biochemistry Unit, Department of Veterinary Physiology, Faculty of Veterinary Science, Chulalongkorn University, Henri Dunant Rd, Pathumwan, Bangkok 10330, Thailand*
[2]*Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University, Henri Dunant Rd, Pathumwan, Bangkok 10330, Thailand*
[3]*Charoensuk Animal Hospital, 167 Soi Charoensuk Ekamai Road Klongton, Wattana, Bangkok, 10110, Thailand*
***Corresponding author: E-mail:** Gunnaporn.V@chula.ac.th*

# บทคัดย่อ

## การศึกษาการกลายพันธุ์ของยีน *MDR1* ในสุนัขโดยวิธีปฏิกิริยาลูกโซ่พอลิเมอเรส

*ศริยา อัศวกาญจน์[1] วลัยพร เรืองชัยปราการ[2] ณพารี ศรีโสวรรณา[2] ลีลวัฒน์ วงษ์แหวน[3] อนุสรณ์ คนึงทอง[1] กรรณาภรณ์ สุริยผล[1*]*

จุดประสงค์ในการศึกษาครั้งนี้เพื่อพัฒนาเทคนิคการวินิจฉัยความผิดปกติของยีน multidrug resistance1(*MDR1*) ในสุนัขด้วยวิธีปฏิกิริยาลูกโซ่พอลิเมอเรสในประเทศไทย โดยทำการเก็บตัวอย่างเลือดจากสุนัขพันธุ์คอลลี่จำนวน 16 ตัวอย่าง เช็ทแลนด์ชีพด็อก 9 ตัวอย่าง บอร์เดอร์คอลลี่ 5 ตัวอย่าง ชิสุ 6 ตัวอย่าง เยอรมันเชฟเฟิร์ด 13 ตัวอย่างและพันธุ์ผสม 18 ตัวอย่าง นำมาสกัดจีโนมิกดีเอ็นเอและทำการเพิ่มจำนวนดีเอ็นเอด้วยวิธีปฏิกิริยาลูกโซ่พอลิเมอเรส ได้ชิ้นส่วนดีเอ็นเอขนาด 341 และ 577 คู่เบสซึ่งใช้แสดงยีน *MDR1* ปกติ (+/+) และ ยีน *MDR1* ที่มีการกลายพันธุ์แบบ homozygous (-/-) ตามลำดับ ถ้าพบชิ้นส่วนดีเอ็นเอทั้ง 2 ชิ้นขนาด 341 และ 577 คู่เบส จะแสดงถึงการกลายพันธุ์แบบ heterozygous (+/-) อย่างไรก็ดีต้องทำการหาลำดับคู่เบสคร่อมตำแหน่งที่หายไปเพื่อยืนยันผลของจีโนไทป์ ผลการศึกษาพบว่าสุนัขพันธุ์คอลลี่จำนวน 9 ตัวมีการกลายพันธุ์แบบ heterozygous (56.2%) และ 7 ตัว มีการกลายพันธุ์แบบ homozygous (ร้อยละ 43.8) ไม่พบสุนัขที่เป็น homozygous แบบปกติพบ สุนัขพันธุ์เช็ทแลนด์ชีพด็อก 1 ตัวอย่างที่มีลักษณะการกลายพันธุ์แบบ heterozygous (ร้อยละ 11.1) ในสุนัขพันธุ์อื่นๆ ไม่พบการกลายพันธุ์ของยีน *MDR1* เลย การศึกษาครั้งนี้สามารถใช้เป็นต้นแบบสำหรับการวินิจฉัยกลายพันธุ์ของยีน *MDR1*ในสุนัขในอนาคต

**คำสำคัญ:** การกลายพันธุ์ การวินิจฉัยโรค ปฏิกิริยาลูกโซ่พอลิเมอเรส ยีน *MDR1* สุนัข

*[1]หน่วยชีวเคมี ภาควิชาสรีรวิทยา คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย 39 ถนนอังรีดูนังต์ ปทุมวัน กทม. 10330*

*[2]โรงพยาบาลสัตว์เล็ก คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย 39 ถนนอังรีดูนังต์ ปทุมวัน กทม. 10330*

*[3]โรงพยาบาลสัตว์เจริญสุข 167 ซ.เอกมัย5 (ทองหล่อ10) แขวงคลองตันเหนือ เขตวัฒนา กทม. 10110*

***ผู้รับผิดชอบบทความ E-mail:** Gunnaporn.V@chula.ac.th*

## *Introduction*

A P-glycoprotein (P-gp), also known as a multidrug resistance 1 (MDR1) protein or an adenosine triphosphate (ATP)-blinding cassette sub-family B member 1 (ABCB1), is a 170 kDa membrane protein, encoded by the *MDR1*gene (Dean et al., 2001). A major role of *MDR1* protein is an ATP-dependent efflux pump of xenobiotics and cellular metabolites in several tissues, including tumor cells, apical border of intestinal epithelial cells, biliary canalicular cells, renal proximal tubular epithelial cells, peripheral blood mononuclear cells, brain capillary endothelial cells (blood-brain barrier), spinal cord, placenta and testes (Thiebaut et al., 1987; Cordon-Cardo et al., 1990; Chaudhary et al, 1992). Therefore, it plays an important role in drug transportation and protective barrier against a wide variety of substrates, avoiding drug entry into the central nervous system (Linardi and Natalini, 2006). P-gp substrates include drugs commonly used in veterinary medicine such as ivermectin, digoxin, loperamide, vinblastine, cyclosporine A, paclitaxel, verapamil, doxorubicin, dexamethasone (Geyer et al., 2005).

The *MDR1* gene has already been identified in mice, rats, rodents, dogs, ruminants, monkeys and human (Linardi and Natalini, 2006). In dogs, *MDR1* gene is localized on chromosome 14 and consists of 28 exons. *MDR1* mutation locates on exon 4 as an exonic 4-bp deletion (AGAT) at nucleotide positions 294-297 of the *MDR1* open reading frame. The homozygous mutation results in a nonsense frame shift at amino acid position 75 followed by a premature stop codon, producing a nonfunctional P-gp protein (Mealey et al., 2001; Roulet et al., 2003). That mutation leads to the high intracellular accumulation of P-gp substrate drugs. Affected breeds with MDR1 mutation included Collies, Border Collies, Shetland Sheepdogs, Old English sheepdogs, McNabs, English Shepherds, Australian Shepherds, Miniature Australian Shepherds, and also found in Longhaired Whippets, Silken Windhounds, White German Shepherds and mixed breeds (Neff et al., 2004; Dowling, 2006; Baars et al., 2008; Mealey and Meurs, 2008; Gramer et al., 2010). Collies with homozygous *MDR1* gene mutation treated with ivermectin showed serious neurological signs such as hypersalivation, ataxia, blindness, tremor, respiratory distress and even death due to the greater penetration of ivermectin through the blood-brain barrier or reduced elimination from the brain (Seward, 1983; Pulliam et al., 1985; Paul et al., 1987; Vaughn et al., 1989; Hopper et al., 2002). Homozygous mutant dogs may easily show adverse effects from ivermectin and other P-glycoprotein substrate drugs

**Table 1** Primer sequences and length of the amplified PCR products

| Primers (P′) | Primer sequences | Amplicon (bp) |
|---|---|---|
| 1. forward wt P′* | 5′- GCTGGTTTTTGGAAACATGACAGA-3′ | 341 |
| 2. reverse wt P′ | 5′- TCCTGAAACTTCCTGGGATCT-3′ | |
| 3. forward mut P′† | 5′- GGTTTTTGGAAACATGACAGC-3′ | 577 |
| 4. reverse mut P′ | 5′- AGAGCCCAACCTGTGACAAT-3′ | |
| 5. forward seq P′‡ | 5′- TTTAGGTTGGACCAGGATGG-3′ | 426 |
| 6. reverse seq P′ | 5′- CCCTTTTCCCCCAGAAATAA-3′ | |

*wt P′ denotes a primer used to amplify wild-type *MDR1* fragments.
†mut P′ denotes a primer used to amplify mutant *MDR1* fragments.
‡seq P′ denotes a primer used to amplify *MDR1* fragments for DNA sequencing.

at dosages that are not harmful to normal dogs whereas heterozygous dogs may express toxicity at increased doses such as ivermectin dose for treating demodecosis (Hugnet et al., 1996; Dowling, 2006).

Some P-gp substrate drugs were documented to cause neurotoxicity in dogs with homozygous MDR1 mutation such as ivermectin, doramectin, loperamide, vincristine, vinblastine and doxorubicin (Mealey et al., 2001; Mealey et al., 2003; Yas-Natan et al., 2003; Sartor et al., 2004). Hence, the genotypes of dogs in affected breeds should be determined before treatment. The test for detecting the *MDR1* gene mutation is an important clinical diagnosis that helps veterinarians to use suitable drugs in safely to increase efficacy of treatment of several diseases in predisposed dogs, and improve the selection procedure in dog breeding. This study describes the genetic analysis method of *MDR1* mutation by PCR-based technique.

## *Materials and Methods*

***Animals and sample collection:*** EDTA-treated blood samples were collected from 16 Collies, 9 Shetland Sheepdogs, 5 Border Collies, 6 Shih tzus, 13 German Shepherd, and 18 mixed breeds. The Collies, Shetland sheepdog and Border collie were collected from breeding farms. The other breed samples were from a non-selected population in the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University. The study was conducted under Animal Use Protocol number 1031036, authorized through the Chulalongkorn University Animal Care and Use Committee (CU-ACUC).

***PCR-based technique detecting the MDR1 mutant gene:*** To achieve allelic discrimination between wild-type and mutant alleles, two physically separated PCR reactions containing the primers to detect wild-type alleles (forward and reverse wt P′) and mutant alleles (forward and reverse mut P′) were performed. The primer setting was based on the published DNA sequence for the canine Pgp-encoding gene MDR1 (ENSCAFG00000001835), using Primer 3 (version 0.4.0) free software (http://frodo.wi.mit.edu/primer3/) and checked for specificity using the UCSC In-Silico PCR (http://genome.ucsc.edu/). Details of the primers used for the amplification of MDR1 are provided in Table 1. Locations of the primers on the chromosome are shown in Fig 1. All reactions were carried out in the presence of 100 ng of template DNA in 25 µl reaction mixtures, containing 0.5 µM of each primer, 0.2 mM of each dNTP, 1.5 mM of $MgCl_2$, and 1 unit of Taq DNA Polymerase (Invitrogen, CA, USA). Reactions were performed in a GeneAmp PCR System 9700 thermocycler (Applied Biosystem, CA, USA), according to the following thermocycling conditions: 1 cycle of $94^0$C for 2 min; 25 cycles of $94^0$C for 15 sec, $63^0$C for 30 sec, and $68^0$C for 45 sec; and a final extension of $68^0$C for 5 min. After the amplification reaction, samples were held at -$10^0$C until analysis. The PCR products were separated by 1% agarose gel electrophoresis. Analysis and documentation of the ethidium-bromide stained gels were performed in a Gel Doc XR imager (Bio-Rad Laboratories, CA, USA).

In order to detect mutation, PCR amplification was performed in the presence of 300 ng of template DNA in 50 µl reaction mixture, containing 0.2 µM of each primer (forward and reverse seq P′), 0.2 mM of each dNTP, 2 mM of $MgSO_4$, and 1 unit of Platinum Taq DNA Polymerase High Fidelity (Invitrogen, CA, USA). Gene-specific primers encompassed the site of mutation. Details of the primers used for the amplification of MDR1 are provided in Table 1. Reactions were performed according to the following thermocycling conditions: 1 cycle of $94^0$C for 2 min; 40 cycles of $94^0$C for 15 sec, $50^0$C for 30 sec, and $68^0$C for 45 sec. After the amplification reaction, samples were held at -$10^0$C until analysis. The PCR products were separated by 1% agarose gel electrophoresis. Analysis and documentation of the ethidium-bromide stained gels was performed.

***Gel extraction and Sequencing:*** Template genomic DNA was extracted from approximate 300 mg of agrose gel band, using a gel extraction kit, according to the manufacturer's instructions (HiYield Gel/PCR DNA Extraction Kit, RBC Bioscience, Taipei, Taiwan). Verification of the amplified PCR-fragments was carried out by automated DNA sequencing, using 200 ng DNA and 10 µM of primer per reaction. Sequencing analysis of the MDR1 gene was used to identify whether 67 dogs carried the different MDR1 genotypes by a Sequence Scanner program (version 1.0) (Life Technologies, NY, USA).

**Table 2** Genotype results of the dogs used for validation of the allele-specific PCR

| Breed (n) | Genotype | Positive samples |
|---|---|---|
| Collie (16) | -/-* | 7 |
| | +/-† | 9 |
| Shetland Sheepdog (9) | +/- | 1 |
| | +/+‡ | 8 |
| Border Collie (5) | +/+ | 5 |
| Shih tzu (6) | +/+ | 6 |
| German Shepherd (13) | +/+ | 13 |
| Mixed breed (19) | +/+ | 19 |

*: homozygous mutation, †: heterozygous mutation,
‡: homozygous wild-type

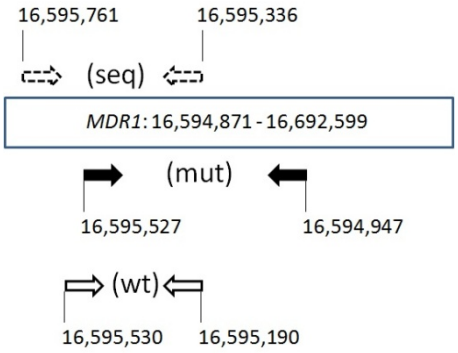


**Figure 1** Location of primers on the chromosome 14. Numbers indicated chromosome locations of the 3'ends of each primer. White, black and dashed leftward and rightward arrows indicated primers for amplifying wild-type DNA fragments (wt), mutant DNA fragments (mut) and for sequencing (seq), respectively.

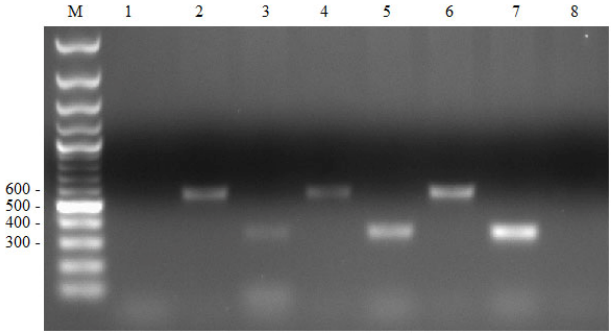


**Figure 2** Detection of homozygous (-/-) and heterozygous (+/-) *MDR1* mutation. Genomic DNA was isolated from whole blood and subjected to PCR and electrophoresesed in 1% agarose gel. Representative cases are shown. *MDR1* fragments with homozygous wild-type (+/+) and homozygous mutant alleles (-/-) with lengths of 341- and 577-bp, respectively, are demonstrated. In the case of heterozygous MDR1 mutation (+/-) fragments of both sizes are visible. Lanes 1 and 2 represent bands from a homozygous *MDR1* mutated Collie; Lanes 3 and 4 represent bands from a heterozygous *MDR1* mutated Collie; Lanes 5 and 6 represent bands from a heterozygous *MDR1* mutated Shetland Sheepdog; Lanes 7 and 8 represent bands from a homozygous wild-type Shetland Sheepdog; M represents 100 bp Plus DNA ladder marker.

## *Results*

The MDR1 genes from 67 samples were amplified by PCR technique. A band from wt primer set and mut primer set indicates the presence of the homozygous wild-type (+/+) and homozygous mutation (-/-), respectively, and 2 visible bands represent the heterozygous mutation (+/-) (Fig 2). The accuracy of the results was further verified by DNA sequencing of a 426-bp PCR product. MDR1 gene analysis of the samples was compared with MDR1 gene sequence from GenBank/Ensembl databases. The chromatograms showed characteristics of each genotype (Fig 3). The results of the sequencing are shown in Table 2. History taking about family tree of Collie dogs was performed. P1 Collie dog is a male parent. P2, P3, P4 and P5 Collie dogs are female parents with unknown genotype. The genetic analysis of P1 and his puppies revealed potential MDR1 genotypes of the female parent dogs (Fig 4).

## *Discussion*

The multidrug resistance 1 (*MDR1*) gene mutation is 4 bp deletion of *MDR1* gene. The study of multidrug resistance 1 (*MDR1*) gene mutation in dogs by PCR-based technique in this report was performed and the chromatograms clearly demonstrated different genotypes (Figs 2 and 3), which were confirmed with the dog histories. From 6 dog breeds tested in the present study, the *MDR1* (-/-) genotype was detected only in Collies (56.2%) whereas the heterozygous mutant (+/-) was observed in Collies (43.8%) and Shetland Sheepdogs (11.1%). High frequency of *MDR1* mutation was observed in Collies because most families share the same male parent dog (P1) which has the *MDR1* (-/-) genotype. Previous studies revealed much higher percentage of Collies carrying the MDR1 mutation either homozygous (-/-) or heterozygous (+/-) genotypes than that of normal Collies (+/+) as shown in Australia, France, Germany, the UK, the Northwestern USA and the USA (Mealey et al., 2002; Hugnet et al., 2004; Neff et al., 2004; Geyer et al., 2005; Mealey et al., 2005; Mealey and Meurs, 2008; Tappin et al., 2008; Gramer et al., 2010). For Shetland Sheepdogs and Border Collies, the low percentage was found to be the *MDR1* (-/-) genotype. Some heterozygous mutations were observed in these breeds as well as in mixed breeds, but most of the dogs had normal alleles (Neff et al., 2004; Geyer et al., 2005; Kawabata et al., 2005; Mealey and Meurs, 2008; Tappin et al., 2008; Gramer et al., 2010).

Several PCR-based techniques were developed, attempting to diagnose the canine *MDR1* gene mutation after the etiology of the ivermectin sensitivity as the 4-based in *MDR1* gene deletion was demonstrated (Mealey et al., 2001). In the early stages, the differentiation of only a 4 base gap by PCR was demonstrated, including using 6.5% polyacrylamide gel electrophoresis to detect differentiation of 138 and 134 bp fragments (Geyer et al., 2005), using ethidium bromide-stained 12% polyacrylamide gel electrophoresis to identify 69 and 65 bp fragments (Roulet et al., 2003) or using 5% agarose gel electrophoresis to separate 60 and 56 bp fragments of wild-type (+/+) and mutated alleles (-/-), respectively (Kawabata et al., 2005). However, it was difficult to differentiate 2 PCR products with only a 4 bp length difference. The multiplex PCR was demonstrated to distinguish MDR1 genotypes by 2 amplicons with the sizes at 300 and 500 bp in 2 PCR experiments with different annealing temperatures. The first set of experiments was to detect a wild-type allele and the second one was to detect a mutant allele (Baars et al., 2005). However, following Baars' method, 2 annealing temperatures were required to detect wild-type and mutant alleles. Hence, 2 rounds of PCR experiments or a gradient thermal cycler are required. Recently MDR1 mutation has been detected

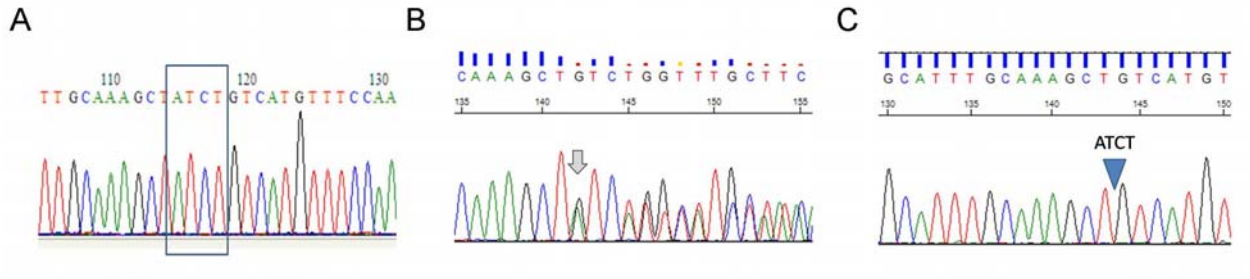


**Figure 3** Chromatograms of reversed canine *MDR1*gene with homozygous wild-type (A), heterozygous mutant (B) and homozygous mutant (C). A rectangular demonstrates 4 bases that are skipped in *MDR1*-mutant. An arrow shows a location where *MDR1*(-) and *MDR1*(+) alleles are overlapped. An inverted triangle shows a 4-bp deleted site.

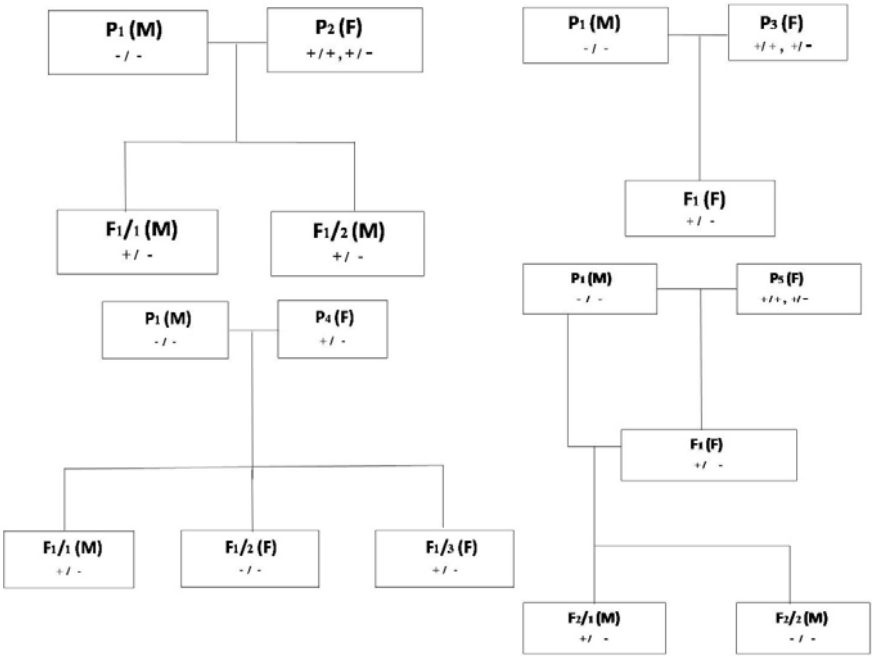


**Figure 4** Family tree and MDR1 genotype of some studied Collies that are closed genetic relationship. (+/+: homozygous wild-type, +/-: heterozygous mutant, -/-: homozygous mutant, M: Male, F: female)

by a fluorogenic 5′ nuclease TaqMan method (Klintzsch et al., 2010). This method requires a real-time PCR machine and expensive TaqMan probes in order to be useful for the routine diagnostics claimed by the authors. In this study, a single round of PCR was performed and different *MDR1* genotypes were determined readily from distinct PCR product sizes on 1% agarose gel electrophoresis. However, the DNA sequencing should be performed to verify the results, preventing misinterpretation from the absence of amplified products.

The PCR-based diagnostic test of *MDR1* gene mutation in this study are important not only for veterinarians to select the safety drug in animal especially ivermectin but also for breeders to control the spread of the *MDR1* gene mutation within dog population since our study can be used for *MDR1* gene mutation prediction of the dogs in the family with unknown genotypes (as in P4 dog) (Fig 4). The PCR-based technique in this study simplifies *MDR1* genotyping. The method was sensitive in discriminating 4-base deletion and is of value for future research as a guideline for setting up the PCR-based diagnostic test in suspected dogs and for studying epidemiology, inheritance patterns or further drug incompatibilities.

## *Acknowledgements*

This work was supported by the Faculty of Veterinary Science, Chulalongkorn University Fund (Grant number RG 16/2553, 2009-2010).

## *References*

Baars, C., Leeb, T., von Klopmann, T., Tipold, A. and Potschka, H. 2008. Allele-specific polymerase chain reaction diagnostic test for the functional MDR1 polymorphism in dogs. Vet J. 177: 394-397.

Chaudhary, P.M., Mechetner, E.B. and Roninson, I.B. 1992. Expression and activity of the multidrug resistance P-glycoprotein in human peripheral blood lymphocytes. Blood. 80: 2735-2739.

Cordon-Cardo, C., O'Brien, J.P., Boccia, J., Casals, D., Bertino, J.R. and Melamed, M.R. 1990. Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. J Histochem Cytochem. 38: 1277-1287.

Dean, M., Rzhetsky, A. and Allikmets, R. 2001. The human ATP-binding cassette (ABC) transporter superfamily. Genome Res. 11: 1156-1166.

Dowling, P. 2006. Pharmacogenetics: It's not just about ivermectin in collies. Can Vet J. 47: 1165-1168.

Geyer, J., Doring, B., Godoy, J.R., Leidolf, R., Moritz, A. and Petzinger, E. 2005. Frequency of the nt230 (del4) MDR1 mutation in Collies and related dog breeds in Germany. J Vet Pharmacol Ther. 28: 545-551.

Gramer, I., Leidolf, R., Doring, B., Klintzsch, S., Kramer, E.V., Yalcin, E., Petzinger, E. and Geyer, J. 2010. Breed distribution of the nt230 (del4) MDR1 mutation in dogs. Vet J. 189: 67-71.

Hopper, K., Aldrich, J. and Haskins, S.C. 2002. Ivermectin toxicity in 17 collies. J Vet Intern Med. 16: 89-94.

Hugnet, C., Bentjen, S.A. and Mealey, K.L. 2004. Frequency of the mutant MDR1 allele associated with multidrug sensitivity in a sample of Collies from France. J Vet Pharmacol Ther. 27: 227–229.

Hugnet, C., Cadore, J.L., Buronfosse, F., Pineau, X., Mathet, T. and Berny, P.J. 1996. Loperamide poisoning in the dog. Vet Hum Toxicol. 38: 31-33.

Kawabata, A., Momoi, Y., Inoue-Murayama, M. and Iwasaki, T. 2005. Canine mdr1 gene mutation in Japan. J Vet Med Sci. 67: 1103-1107.

Klintzsch, S., Meerkamp, K., Döring, B. and Geyer, J. 2010. Detection of the nt230[del4] MDR1 mutation in dogs by a fluorogenic 5' nuclease TaqMan allelic discrimination method. Vet J. 185: 272-277.

Linardi, R.L. and Natalini, C.C. 2006. Multi-drug resistance(MDR1) gene and P-glycoprotein influence on pharmacokinetic and pharmacodynamic of therapeutic drugs. Cienc Rural. 36: 336-341.

Mealey, K.L. and Meurs, K.M. 2008. Breed distribution of the ABCB1-1Delta (multidrug sensitivity) polymorphism among dogs undergoing ABCB1 genotyping. J Am Vet Med Assoc. 233: 921-924.

Mealey, K.L., Bentjen, S.A. and Waiting, D.K. 2002. Frequency of the mutant MDR1 allele associated with ivermectin sensitivity in a sample population of Collies from the Northwestern United States. Am J Vet Res. 63: 479-481.

Mealey, K.L., Bentjen, S.A., Gay, J.M. and Cantor, G.H. 2001. Ivermectin sensitivity in collies is associated with a deletion mutation of the mdr1 gene. Pharmacogenetic. 11: 727-733.

Mealey, K.L., Northrup, N.C. and Bentjen, S.A. 2003. Increased toxicity of P-glycoprotein-substrate chemotherapeutic agents in a dog with the MDR1 deletion mutation associated with ivermectin sensitivity. J Am Vet Med Assoc. 223: 1453–1455.

Mealey, K.L., Munyard, K.A. and Bentjen, S.A. 2005. Frequency of the mutant MDR1allele associated with multidrug sensitivity in a sample of herding breed dogs living in Australia. Vet Parasitol. 10: 193-196.

Neff, M.W., Robertson, K.R., Wong, A.K., Safra, N., Broman, K.W., Slatkin, M., Mealey, K.L. and Pedersen, N.C. 2004. Breed distribution and history of canine mdr1-1d, a pharmacogenetic mutation that marks the emergence of breeds from the collie lineage. Proc Natl Acad Sci USA. 101: 11725-11730.

Paul, A.J., Tranquilli, W.J., Seward, R.L., Todd, K.S. Jr. and DiPietro, J.A. 1987. Clinical observations in collies given ivermectin orally. Am J Vet Res. 48: 684-685.

Pulliam, J.D., Seward, R.L., Henry, R.T. and Steinberg, S.A. 1985. Investigating ivermectin toxicity in Collies. Vet Med. 80: 33-40.

Roulet, A., Puel, O., Gesta, S., Lepage, J.F., Drag, M., Soll, M., Alvinerie, M. and Pineau, T. 2003 MDR1-deficient genotype in Collie dogs hypersensitive to the P-glycoprotein substrate ivermectin. Eur J Pharmacol. 460: 85-91.

Sartor, L.L., Bentjen, S.A., Trepanier, L. and Mealey, K.L. 2004. Loperamide toxicity in a collie with the MDR1 mutation associated with ivermectin sensitivity. J Vet Intern Med. 18: 117-118.

Seward, R.L. 1983. Reactions in dogs given ivermectin. J Am Vet Med Assoc. 183: 493.

Tappin, S.W., Goodfellow, M.R., Peters, I.R., Day, M.J., Hall, E.J., Bentjen, S.A. and Mealey, K.L. 2008. Frequency of the mutant MDR1 allele associated with multidrug sensitivity in dogs in the United Kingdom. In: BSAVA Congress, Scientific Proceedings: Veterinary Programme. 83/49.

Thiebaut, F., Tsuruo, T., Hamada, H., Gottesman, M.M., Pastan, I. and Willingham, M.C. 1987. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. Proc Natl Acad Sci USA. 84: 7735-7738.

Vaughn, D.M., Simpson, S.T., Blagburn, B.L., Whitmer, W.L., Heddens-Mysinger, R. and Hendrix, C.M. 1989. Determination of homovanillic acid, 5-hydroxyindoleacetic acid and pressure in the cerebrospinal fluid of collie dogs following administration of ivermectin. Vet Res Commun. 13: 47-55.

Yas-Natan, E., Shamir, M., Kleinbart, S. and Aroch, I. 2003. Doramectin toxicity in a collie. Vet Rec. 153: 718-720.