Slov Vet Res 2007; 44 (1/2): 19-24
UDC 575.117:575.224:631.527.3:636.74

Original Research Paper

MUTATION OF *MDR1* GENE ASSOCIATED WITH MULTIDRUG SENSITIVITY IN AUSTRALIAN SHEPHERDS IN SLOVENIA

Marko Cotman, Jelka Zabavnik*

Institute of Anatomy, Histology and Embryology; Veterinary Faculty, Gerbičeva 60, 1000 Ljubljana, Slovenia

*Corresponding author, E-mail: jelka.zabavnik@vf.uni-lj.si

Summary: The multidrug-resistance 1 (MDR1) transport protein plays an important protective role at blood-tissue barriers by limiting the entry of MDR1 protein substrates to brain, testis, fetus and other tissues. For subpopulation of Collies and related dog breeds increased susceptibility to neurotoxic side effects of several drugs including ivermectin, moxidectin and loperamide was detected. It was demonstrated that in ivermectin susceptible dogs the deletion mutation in *MDR1* gene produces a frame shift resulting in the production of severly truncated non-functional MDR1 protein. In order to evaluate the occurrence and frequency of the mutated *mdr1-1*Δ allele in the population of Australian Shepherds in Slovenia we have screened 10 dogs representing approximately one third of the Slovenian population. The results of our study indicate very high frequency of *mdr1-1*Δ allele (70%) in Australian Shepherds in Slovenia. In 40% of studied population homozygous mutated genotype was determined. Considering the important role of MDR1 protein in drug disposition and blood-brain barrier protection, testing of the MDR1 genotypes and *MDR1* genotype-based breeding programs are recommended for improving the safety of drug therapy with MDR1 protein substrates in Australian Shepherds.

Key words: molecular biology-genetics; P-glycoprotein-genetics; ivermectin-adverse effetcs; genes, MDR-genetics; neurotoxicity syndromes-etiology-genetics; pedigree; dogs-genetics

Introduction

The multidrug-resistance 1 (MDR1) transport protein is a large transmembrane P-glycoprotein (P-gp) encoded by the *MDR1* gene. MDR1 is a member of the ATP-binding cassette (ABC) superfamily of transporters that use the energy derived from ATP hydrolysis to export various molecules including a variety of drugs across cell membranes from the cytosol to the extracellular medium and therefore performing the protective role in cells. MDR1 is expressed in a variety of tissues with excretory function, e.g. small intestine, liver, kidney, and at blood-tissue barriers, such as blood-brain barrier, blood-testis barrier and placenta. This protein is thought to have an important role in removing toxic metabolites from the cells, it limits the absorption of orally administered drugs, promotes drug elimination into bile and urine and protects various tissues, e.g. brain, testis and fetus (1, 2, 3, 4).

Unbalanced level of MDR1 protein expression in the cells can cause disorders in the availability of MDR1 substrates in the cells. The expression of the *MDR1* gene is frequently amplified in multidrug-resistant cells, resulting in a large overproduction of the MDR1 protein. If these cells are exposed to toxic compounds they can develop resistance to several drugs. This is well documented in humans, where the over-expression of human MDR1 protein in tumor cells is causing resistance of these cells to various chemotherapeutic drugs (5). The opposite, lack of MDR1 protein is observed in dogs – because of the mutation in the *MDR1* gene the functional MDR1 protein is not expressed and the protective role is impaired.

More than 20 therapeutic drugs are known substrates of MDR1 protein, one of these is ivermectin that is used extensively in veterinary medicine as parasiticide toxic for nematodes and arthropod

Received: 13 March 2007
Accepted for publication: 22 June 2007

parasites, in dogs it is often used for prevention and therapy of heartworm (*Dirofilaria immitis*). In some Collies and Collie related dog breeds ivermectin treatment causes neurotoxicity (6, 7). In one study 1/200 of the lethal dose of ivermectin for beagles was lethal for Collies (6). Mealey et al. (8) discovered that the affected dogs were homozygous for a 4-bp deletion of the fourth exon of *MDR1* gene. This mutation causes a frameshift causing premature stop codon, presumably resulting in truncated MDR1 protein with loss of function and therefore defective brain-blood barrier.

MDR1 deletion mutation associated with the ivermectin sensitivity has been reported in Collies and related breed dogs in the northwestern United States (9) and in France (10), in herding breeds in Australia (11), Japan (12) and in Germany (13). *MDR1* deletion mutation was observed in many pure-breed dogs: in Australian Shepherds, Collies, English Shepherds, Longhaired Whippets, McNabs, Old English Sheepdogs, Shetland Sheepdogs and Silken Windhounds (14).

Population of Australian Shepherds in Slovenia is relatively small, it comprises only about thirty dogs. In Slovenia it is a relatively new breed, the dogs are imported from different countries. The aim of our study was to determine if *mdr1-1*Δ allele is present in our population of Australian Shepherds.

Material and methods

Animals

13 dogs were included in the study (10 Australian Shepherds, 3 mixed breed dogs). Samples were taken from dogs whose owners were interested in determining the *MDR1* genotype of their dogs or with the consent of the owners to use the samples for research. Owners were informed about the study mainly through announcements made at the meetings of the owners of Australian Shepherds. Samples from mixed breed dogs were taken from a repository at the Laboratory for Molecular Biology and Molecular Genetic at the Veterinary Faculty in Ljubljana.

DNA isolation

Genomic DNA was isolated from blood samples collected in tubes containing anticoagulant (EDTA or acid citrate dextrose solution B) by a standard phenol-chloroform protocol as described by Sambrook et al. (15) or by commercially available Wizard Genomic DNA Purification Kit (Promega).

MDR1 gene amplification

MDR1 gene was amplified by use of primers (5' - GGC TTG ATA GGT TGT ATA TGT TGG TG - 3' and 5' - ATT ATA ACT GGA AAA GTT TTG TTT C - 3') described by Neff et al. (14) in polymerase chain reaction (PCR). The primers bracketed the reported 4 bp deletion in *MDR1*. The PCR consisted of 35 cycles with denaturing (20 seconds at 93°C), primers annealing (20 seconds at 55°C) and primers extension (1 minute at 72°C) in thermocycler (Biometra).

PCR product analyses

PRC products were separated by capillary electrophoresis on the ABI PRISM 310 apparatus to detect the size of the PCR products and analyzed by the programme GeneMapper 3.7.

Results

The allele *mdr1-1*Δ was found in 7 dogs of Australian Shepherd breed. The samples originated from five dogs with one known parent that was shown to be homozygous for the *mdr1-1*Δ allele. All five offspring had *mdr1-1*Δ allele, three were homozygous for this allele, two were heterozygous. In another family the parent was shown to be homozygous for *mdr1-1*Δ allele as well as its single offspring that was tested. The results of genotyping in Australian Shepherds are shown in Table 1 and in Figure 2.

In addition to Australian Shepherds some non-pure breed dogs were tested, one sample was obtained from the dog related to Collie breed. This dog was heterozygous for *MDR1/mdr1-1*Δ. Two other samples obtained from mixed breed dogs were homozygous for the wild type *MDR1* allele.

Discussion

Ivermectin sensitivity connected to *mdr1-1*Δ homozygous genotype in dogs was first described in dogs of Collie breed (16, 17). Initial studies performed to determine the frequencies of *mdr1-1*Δ allele responsible for ivermectin toxicity were done on Collies (9, 10), however, recent studies include also other dog breeds of Collie lineage. The *MDR1* gene frequencies in dogs of Australian Shepherd breed have already been determined in some countries. In

Table 1: Observed allele and genotype frequencies of gene encoding MDR1 protein in studied dogs of the Australian Shepherds breed

		Allele %		*Genotype %*		
Breed	*No. of dogs*	*MDR1*	*mdr1-1Δ*	*mdr1-1Δ/ mdr1-1Δ*	*mdr1-1Δ/ MDR1*	*MDR1/ MDR1*
Australian Shepherd	10	45	55	40	30	30

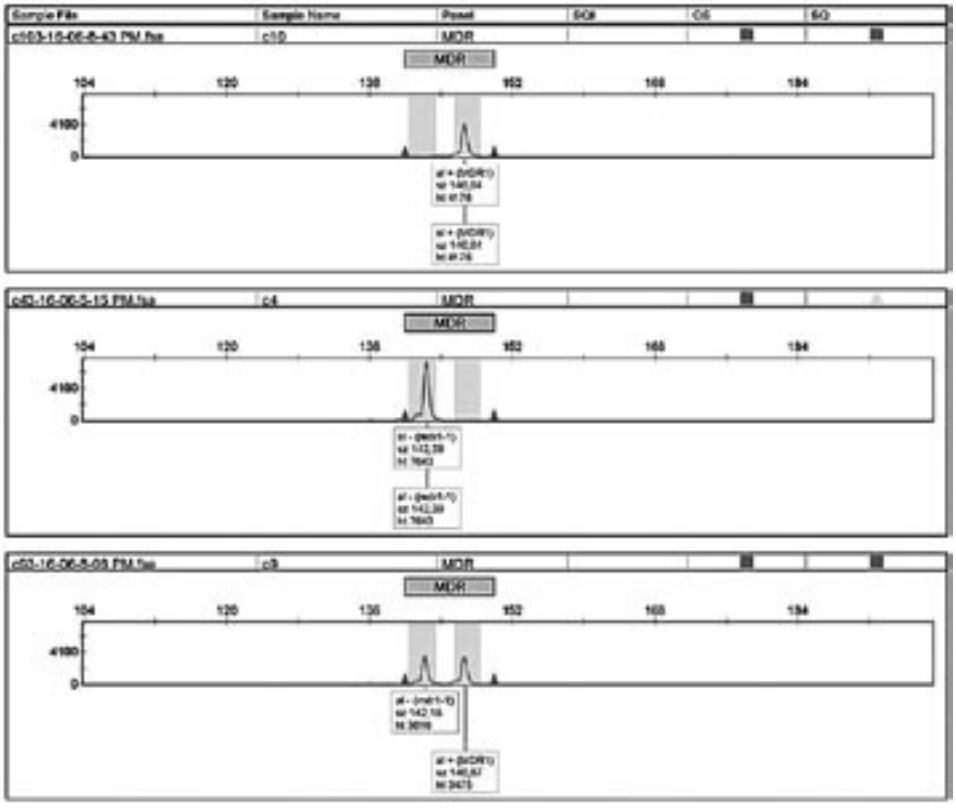

Figure 1: Electropherogram of PCR products after PCR amplification using the primers neighbouring the 4 bp deletion in *MDR1* gene. Top – homozygous wild type genotype *MDR1/MDR1*; middle - homozygous mutated genotype *mdr1-1Δ/mdr1-1Δ*; bottom – heterozygous genotype *mdr1-1Δ/ MDR1*

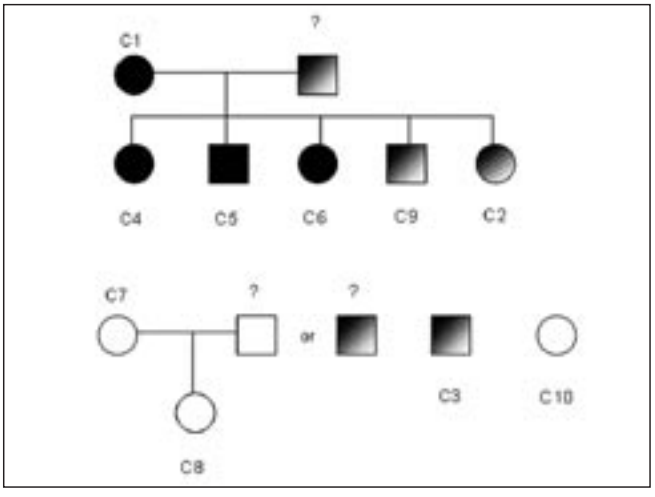

Figure 2: The results of genotyping for the *MDR1* and *mdr1-1Δ* alleles in Australian Shepherds dogs. The samples originated from two families and two non-related dogs. The examined dogs represented approximately one third of all Slovenian dog population of Australian Shepherds breed (*MDR1/MDR1* – white; *mdr1-1Δ/mdr1-1Δ* – black; *mdr1-1Δ/MDR1* – shadowed). Symbols labeled with question-marks represent the dogs that were not tested, the genotypes were only predicted

the United States 178 dogs were examined, obtained *mdr1-1*Δ allele frequency was 16.6 % (14). In Japan 9 Australian Shepherds were tested and the obtained *mdr1-1*Δ allele frequency was 33.3 % (12), in Australia, 17 Australian Shepherds were tested for the same mutation in the *MDR1*, the *mdr1-1*Δ allele frequency was 42.86 % (11). The most extensive study that included 1500 dogs from 7 different dog breeds was performed in Germany. Frequency of the mutated genotype was the highest in Collies (54.6%), followed by Shetland Sheepdog (30.0%) and Australian Shepherd (19.5%). In this study 333 Australian Shepherds were included (13). We have included in our study 10 Australian Shepherds, this is small sample, however, it represents about 30% of Slovenian population of this breed. A very high frequency of the *mdr1-1*Δ allele was detected (55%), 40% of examined animals were homozygous for the *mdr1-1*Δ allele and 30% were carriers of *mdr1-1*Δ allele. Only a minority of studied dogs, 30%, was free of the mutated *mdr1-1*Δ allele, responsible for the multidrug sensitivity (Table 1, Figure 2).

MDR1 gene is displaying the pattern of Mendelian inheritance, the mutation was detected also in some pure breeds related to Collies as well as in non-pure breeds that previously did not have *mdr1-1*Δ allele (11, 12, 13, 14). In our study we have observed one *mdr1-1*Δ allele in one sample obtained from non-pure breed dog that was descendent of Collie. In this dog, most probably the mutated allele was obtained from the Collie parent.

Sensitivity of Collies to ivermectin was initially studied in the United States in the regions where heartworm *Dirofilaria immitis* is endemic and therefore ivermectin was widely used in low preventive doses and in high doses for therapy of heartworm in dogs. Neurotoxicoses were often observed in some Collies after the application of ivermectin in therapeutic doses (7). In Slovenia, heartworm *Dirofilaria immitis* is present in Primorska region and in the neighbouring Italy and Croatia, the preventive application of ivermectin is indicated for the dogs living or visiting these regions.

Ivermectin is the most studied substrate of the transmembrane transporter P-gp encoded by the *MDR1* gene, however, other drugs used in therapy of dogs are also MDR1 protein substrates. MDR1 protein substrates in previously documented interactions with canine *mdr1-1* Δ are antimicrobial agents (erythromycin, grepafloxacin), anticancer agents (doxorubicin, vincristine), immunosuppressants (cyclosporin A, tacrolimus), steroids (dexamethasone, hydrocortisone), gastrointestinal drugs (loperamide, domperidon), cardiac drugs (quinidine, digoxin) (14). Probably there are many more MDR1 protein substrates that can cause neurotoxicoses if the blood-brain barrier is not efficient because of non-functional product of *mdr1-1*Δ allele.

Based on the determined frequency of *mdr1-1*Δ allele in Australian Shepherds in Slovenia we would suggest the verification of the presence of the *mdr1-1*Δ allele before using therapeutic doses of ivermectin or other MDR1 protein substrates. The method used in our study to detect the mutated allele is reliable, fast and affordable. According to the known presence of the *mdr1-1*Δ allele in some dog breeds in countries where the allele frequencies in different dog populations were already determined (9, 10, 11, 12, 13, 14) the same practice would be suggested for dog breeds related to Collie.

In conclusion, the results of this study indicate that very high percentage of Australian Shepherds in Slovenia (70%) contains at least one *mdr1-1*Δ allele responsible for sensitivity to ivermectin and other substrates of P-gp transporter. Considering the important role of *MDR1* protein in drug distribution to the cells and in particular for blood-brain barrier protection special care should be taken when treating Australian Shepherds with drugs that are P-gp substrates. Detection of dogs with mutated *mdr1-1*Δ allele in Slovenia based on MDR1 genotyping are recommended to increase the safety of drug therapy with P-gp substrates and to prepare specific breeding programmes to lower the frequencies of the homozygous *mdr1-1*Δ allele in a dog population.

Acknowledgements

We thank Mrs. Jasna Šporar and dog owners for the blood samples and Mrs. Magdalena Dobravec for her technical assistance. This research was supported by the Slovenian Research Agency in the frame of Research Programme P4 0053.

References

1. Juliano RL, Ling VA. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 1976; 455: 152-62.

2. Schinkel AH, Wagenaar E, Mol CAAM, van Deemter L. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. J Clin Invest 1996; 97: 2517-24.

3. Dean M, Rzhetsky A, Allikmets R. The human ATP-binding cassette (ABC) transporter superfamily. Genome Res 2001; 11: 1156-66.

4. Fromm MF. Importance of P-glycoprotein at blood –tissue barriers. Trends Pharmacol Sci 2004; 25: 423-9.

5. Gottesman M, Fojo T, Bates SE. Multidrug resistance in cancer: role of ATP-dependent transporters. Nature Rev 2002; 2: 48-58.

6. Pulliam JD, Seward RL, Henry RT, Steinberg SA. Investigating ivermectin toxicity in collies. Vet Med 1985; 7: 33-40.

7. Paul AJ, Tranquilli WJ, Seward RL, Todd KS, DiPietro JA. Clinical observations in collies given ivermectin orally. Am J Vet Res 1987; 48: 685-8.

8. Mealey KL, Bentjen SA, Gay JM, Cantor GH. Ivermectin sensitivity in collies is associated with a deletion mutation of the mdr1 gene. Pharmacogen 2001; 11: 727-33.

9. Mealey KL, Bentjen SA, Waiting DK. 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 2002; 63: 479-81.

10. Hugnet C, Bentjen SA, Mealey KL. Frequency of the mutant MDR1 allele associated with multidrug sensitivity in a sample of collies from France. J Vet Pharmacol Ther 2004; 27: 227-9.

11. Mealey KL, Munyard KA, Bentjen SA. Frequency of the mutant MDR1 allele associated with multidrug sensitivity in a sample of herding breed dogs living in Australia. Vet Parasitol 2005; 10:193-6.

12. Kawabata A, Momoi Y, Inoue-Murayama M, Iwasaki T. Canine mdr1 gene mutation in Japan. J Vet Med Sci 2005; 67:1103-7.

13. Geyer J, Doring B, Godoy JR, Leidolf R, Moritz A, Petzinger E. Frequency of the nt230 (del4) MDR1 mutation in Collies and related dog breeds in Germany. J Vet Pharmacol Ther 2005; 28 :545-51.

14. Neff MW, Robertson KR, Wong AK, et al. Breed distribution and history of canine mdr1-1, a pharmacogenetic mutation that marks the emergence of breeds from collie lineage. Proc Natl Acad Sci USA 2004; 101: 11725-30.

15. Sambrook J, Russell DW. Molecular cloning: a laboratory manual. Cold Spring Harbor : Cold Spring Harbor Laboratory Press, 2001: 6.4 – 6.12.

16. Preston JM.Adverse reactions to unapproved applications. Vet Res 1983; 112: 286.

17. Seward RL. Reactions in dogs given ivermectin. J Am Vet Med Assoc 1983; 183: 493.

MUTACIJA GENA MDR1, POVEZANA S PREOBČUTLJIVOSTJO NA RAZLIČNA ZDRAVILA PRI AVSTRALSKIH OVČARJIH V SLOVENIJI

M. Cotman, J. Zabavnik

Povzetek: Transportni protein MDR1 (multidrug-resistance 1 protein – protein za odpornost proti različnim drogam) igra pomembno zaščitno vlogo v različnih krvno-tkivnih pregradah in s tem omejuje dostop MDR1-substratov do možganov, testisov, zarodka in drugih tkiv. Pri deležu populacije škotskih ovčarjev in njim sorodnih pasmah je bila opažena povečana pojavnost nevrotoksikoz ob obdelovanju z različnimi zdravili – z ivermektinom, moksidektinom in loperamidom. Pri psih s povečano občutljivostjo na ivermektin je bila ugotovljena delecijska mutacija gena (alel *mdr1-1*Δ). Zaradi mutacije pride do zamika bralnega okvira gena, kar povzroči proizvodnjo zelo skrajšanega nedejavnega proteina MDR1. Z raziskavo smo želeli ugotoviti pojavnost in frekvenco mutiranega alela *mdr1-1*Δ v populaciji avstralskih ovčarjev v Sloveniji. Preiskali smo 10 psov pasme avstralski ovčar, kar predstavlja približno eno tretjino populacije te pasme v Sloveniji. Rezultati naše študije kažejo zelo visoko frekvenco alela *mdr1-1*Δ (70%) v slovenski populaciji avstralskih ovčarjev. V 40 % proučevanih avstralskih ovčarjev se je mutirani alel pojavil v homozigotni obliki. Glede na pomembno vlogo proteina MDR1 pri razporejanju drog v organizmu in pri zaščiti v krvno-možganski pregradi priporočamo ugotavljanje genotipov *MDR1* in uvedbo rejskega programa za usmerjeno izbiranje genotipov *MDR1*, kar bi povečalo varnost ob obdelovanju avstralskih ovčarjev z zdravili, ki so substrat proteina MDR1.

Ključne besede: molekularna biologija-genetika; P-glikoprotein-genetika; ivermektin-škodljivi učinki; gen MDR-genetika, nevrotoksični sindromi-etiologija-genetika; rodovnik; psi-genetika