

Short Communication

Investigation of parallel and simultaneous selection for collie eye anomaly and ivermectin toxicosis

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COLLIE eye anomaly (CEA) is an inherited canine ocular disorder causing regional choroidal hypoplasia and coloboma of the optic disk or adjacent areas, and is associated with a 7.8-kilo base pair (bp) deletion in the canine *NHEJ1* gene (Parker and others 2007). A 4-bp deletion in the canine *MDR1/ABCB1* gene (*MDR1* gene defect) causes neurotoxicosis after administration of P-glycoprotein substrates such as ivermectin (Gramer and others 2011). These two mutations are common in collie-related and sighthound breeds, suggesting a possible parallel selection of the two defects, although these canine genes are located on different chromosomes: *NHEJ1* on chromosome 37 and *MDR1* on chromosome 14 (Parker and others 2007, Gramer and others 2011).

Recently, a very high frequency of the CEA mutation has been reported in the Hokkaido inu, a traditional Japanese breed; this is the first identification of a CEA-predisposed breed not classified as collies or sighthounds (Mizukami and others 2012a). A native Japanese breed, the shiba inu, has been previously localised near collies in multibreed cluster analyses (Parker and others 2007), suggesting that collies and such native Asian breeds share more genetic traits than one may realise. However, prevalence of the *MDR1* gene defect in the

Hokkaido inu is unknown. Furthermore, epidemiologic surveys targeting potential breeds likely to possess these two mutations have not been performed. This study investigated allele frequencies of these multiple dog breeds and checked whether a parallel and simultaneous selection for these two mutations has occurred.

In this study, 1331 DNA samples were collected from nine dog breeds including two collie-related breeds (collie and border collie), one sighthound (Saluki), two non-collie European breeds (toy poodle and miniature dachshund), three Japanese breeds (Hokkaido, shiba, and Ryukyu inu), and the Korean Jindo, via veterinary hospitals, a volunteer breeders association, and preservation associations. Although some of these samples belonged to the same kennels, nearly all of them were unrelated to at least the grandparent level. These breeds were chosen for the following reasons. Collie-related breeds are known to commonly carry both mutations (Parker and others 2007, Gramer and others 2011). The saluki is a sighthound that has not been demonstrated to carry the mutations. The toy poodle and miniature dachshund possibly carry the CEA mutation, because sporadic cases of CEA-like ophthalmological signs have occurred in these breeds (Parker and others 2007). Japanese breeds are suspected to carry the mutations because the Hokkaido inu has very high frequency of the CEA mutation (Mizukami and others 2012a). The Korean Jindo is also suspected to carry the mutations, because this breed has similar appearance and inherited traits to the Japanese breeds (Yamato and others 1999). The number of dogs in each breed is shown in Table 1. The genotypes of the two mutations were determined using real-time PCR methods reported previously (Chang and others 2010, Mizukami and others 2012b).

The results of this study are shown in Table 1. Collies had very high frequency of both mutations. Border collies had high frequency of the CEA mutation, but very low frequency of the *MDR1* gene defect. Hokkaido inus did not show the *MDR1* gene defect but displayed very high frequency of the CEA mutation. Ryukyu inus did not show the CEA mutation, but 6 of 33 dogs carried the *MDR1* gene defect. The other breeds did not carry either mutation. It should be noted that the sample numbers of native breeds (e.g. Ryukyu inu and Korean Jindo) are low enough to allow for the possibility of misrepresenting the frequency, or entirely missing the presence, of these mutant alleles. However, due to the rarity of these breeds even in Japan and Korea, these numbers are likely adequate to evaluate the frequencies of these alleles.

The results of the present study demonstrated that the CEA and *MDR1* gene defects do not always coexist in canine breeds even if they are predisposed toward one of these defects. This suggests that there is no parallel and simultaneous selection for the two defects, although definitive statistical analysis could not be performed because of the small number of breeds possessing one or both of the mutations. Collies had markedly high frequencies of both mutations as previously reported (Parker and others 2007, Gramer and others 2011). Border collies also had high frequency of the CEA mutation but very low frequency of the *MDR1* gene defect, as first demonstrated in the same population previously (Mizukami and others 2012b). The saluki has long been considered an 'ancient' breed; however, a recent genetic study demonstrated that ancient breeds do not resemble early domestic dogs more closely but have rather avoided recent admixture with other breeds, likely facilitated by geographical and cultural isolation (Larson and others 2012). Therefore, the reason why the saluki had neither of the mutations might be that these mutations have not been introduced because of isolation in the course of establishment of this breed. The toy poodle and miniature dachshund had neither of the mutations, although sporadic cases having ophthalmological lesions similar to those in CEA have been found in these breeds (Parker and others 2007). These lesions are observed in non-CEA disorders including merle syndrome (Parker and others 2007); therefore, disorders such as merle syndrome might cause similar ocular lesions in these two breeds.

The results demonstrated negligible relationship between the CEA and *MDR1* mutations in the Japanese and Korean breeds.

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TABLE 1: The number of dogs with each genotype (carrier and affected) and allele frequency for the collie eye anomaly and *MDR1* mutations in various dog breeds

Breed	Number	Collie eye anomaly			<i>MDR1</i> gene defect		
		Carrier	Affected	Frequency	Carrier	Affected	Frequency
Collie	9	0	9	1	3	6	0.833
Border collie	437	128	9	0.167	2	0	0.002*
Saluki	29	0	0	0	0	0	0
Toy poodle	229	0	0	0	0	0	0
Miniature Dachshund	232	0	0	0	0	0	0
Hokkaido inu	18	12	6	0.667†	0	0	0
Ryukyu inu	33	0	0	0	6	0	0.091
Shiba inu	208	0	0	0	0	0	0
Jindo	136	0	0	0	0	0	0

*These data were reported previously and updated in this study (Mizukami and others 2012b)

†These data were reported previously (Mizukami and others 2012a)

Hokkaido inus had very high frequency of the CEA mutation as previously reported (Mizukami and others 2012a), but no *MDR1* gene defect, in this study. However, the pattern seen in the Hokkaido inu breed was not observed in other Japanese and Korean breeds. Neither mutation was found in the Japanese shiba inu and Korean Jindo dogs. However, in the Ryukyu inu breed, 6 of 33 dogs had the *MDR1* gene defect, although none carried the CEA mutation.

The *MDR1* gene defect has also been found in Old English sheepdogs, German shepherd dogs and Wällers as well as collie-related dogs and sighthounds (Gramer and others 2011). There are several possible origins of the *MDR1* gene defect. All the dogs carrying this mutation are thought to be descendants of a dog that lived in Great Britain before genetic isolation of pure breeds by registered breeding (Neff and others 2004). However, the ancestors of Ryukyu and Hokkaido inus are believed to have been brought to the Japanese archipelago from the Asian Continent by ancient people 10 to 12 thousand years ago (Tanabe 2006). These two breeds have been isolated in southern (Okinawa, alias Ryukyu) and northern (Hokkaido) islands, respectively. In view of the postulated migration route and the isolated habitats of these dogs, the origin of the CEA and *MDR1* mutations might exist in more primitive dogs than once thought. Further studies are needed to verify this hypothesis.

In conclusion, no parallel and simultaneous selection was found between the CEA and *MDR1* mutations in various dog breeds. These two mutations existed independently in breeds that are considered distinctly different: the collie and Japanese breeds, which supports the earlier data (Parker and others 2007) demonstrating that these breeds are genetically close to one another. It is possible that these mutations originated in ancient dogs and resultant phenotypes had neutral or only weak effects on survival and selection. Veterinarians should be aware that predispositions toward these two defects differ considerably depending on breed.

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Correction notice This article has been corrected since it was published Online First. In the title, 'An investigation' has been amended to 'Investigation' to match house style.

References

- CHANG, H. -S., MIZUKAMI, K., YABUKI, A., HOSSAIN, M. A., RAHMAN, M. M., UDDIN, M. M., ARAI, T. & YAMATO, O. (2010) A novel rapid genotyping technique for Collie eye anomaly: SYBR Green-based real-time polymerase chain reaction method applicable to blood and saliva specimens on Flinders Technology Associates filter paper. *Journal of Veterinary Diagnostic Investigation* **22**, 708–715
- GRAMER, I., LEIDOLF, R., DÖRING, B., KLINTZSCH, S., KRÄMER, E. M., YALCIN, E., PETZINGER, E. & GEYER, J. (2011) Breed distribution of the nt230(del4) *MDR1* mutation in dogs. *The Veterinary Journal* **189**, 67–71
- LARSON, G., KARLSSON, E. K., PERRI, A., WEBSTER, M. T., HO, S. Y., PETERS, J., STAHL, P. W., PIPER, P. J., LINGAAS, E., FREDHOLM, M., COMSTOCK, K. E., MODIANO, J. F., SCHELLING, C., AGOULNIK, A. I., LEEGWATER, P. A., DOBNEY, K., VIGNE, J. D., VILÀ, C., ANDERSSON, L. & LINDBLAD-TOH, K. (2012) Rethinking dog domestication by integrating genetics, archeology, and biogeography. *Proceedings of the National Academy of Sciences of the United States of America* **109**, 8878–8883
- MIZUKAMI, K., CHANG, H. -S., OTA, M., YABUKI, A., HOSSAIN, M. A., RAHMAN, M. M., UDDIN, M. M. & YAMATO, O. (2012a) Collie eye anomaly in Hokkaido dogs: case study. *Veterinary Ophthalmology* **15**, 128–132
- MIZUKAMI, K., CHANG, H. -S., YABUKI, A., KAWAMICHI, T., HOSSAIN, M. A., RAHMAN, M. M., UDDIN, M. M. & YAMATO, O. (2012b) Rapid genotyping assays for the 4-base pair deletion of canine *MDR1/ABCB1* gene and low frequency of the mutant allele in Border Collie dogs. *Journal of Veterinary Diagnostic Investigation* **24**, 127–134
- NEFF, M. W., ROBERTSON, K. R., WONG, A. K., SAFRA, N., BROMAN, K. W., SLATKIN, M., MEALEY, K. L. & PEDERSEN, N. C. (2004) Breed distribution and history of canine *mdr1-1Delta*, a pharmacogenetic mutation that marks the emergence of breeds from the collie lineage. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 11725–11730
- PARKER, H. G., KUKEKOVA, A. V., AKEY, D. T., GOLDSTEIN, O., KIRKNESS, E. F., BAYSAC, K. C., MOSHER, D. S., AGUIRRE, G. D., ACLAND, G. M. & OSTRANDER, E. A. (2007) Breed relationships facilitate fine-mapping studies: a 7.8-kb deletion cosegregates with Collie eye anomaly across multiple dog breeds. *Genome Research* **17**, 1562–1571
- TANABE, Y. (2006) Phylogenetic studies of dogs with emphasis on Japanese and Asian breed. *Proceedings of the Japan Academy, Series B* **82**, 375–387
- YAMATO, O., LEE, K. -W., CHANG, H. -S., TAJIMA, M. & MAEDE, Y. (1999) Relation between erythrocyte reduced glutathione and glutamate concentrations in Korean Jindo dogs with erythrocytes possessing hereditary high activity of Na-K-ATPase and a high concentration of potassium. *Journal of Veterinary Medical Science* **61**, 1179–1182



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