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Breed is associated with the *ABCB1-1Δ* mutation in Australian dogs

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Abstract

Background

The *ABCB1* gene encodes P-glycoprotein (P-gp), a cellular membrane pump. One functional mutation that leads to expression of a less functional form of P-gp, *ABCB1-1Δ*, has been described in dogs. Individuals with this mutation can have severe adverse reactions to common veterinary pharmaceuticals that are known substrates of this pump. We investigated the detection of this mutation in samples submitted to two Australian diagnostic laboratories.

Methods

A total of 4,842 dogs across 27 breeds were tested for the *ABCB1-1Δ* mutation from buccal swabs or EDTA blood using standard PCR, multiplex PCR, or genotyping chip. Statistical analysis was applied to determine the proportions and odds ratios of the *ABCB1-1Δ* mutation in herding breeds compared to non-herding breeds.

Results

The *ABCB1-1Δ* mutation was detected in nine breeds. The most commonly affected breeds were collies, Australian shepherds, white Swiss shepherds and Shetland sheepdogs. Of 32 dogs in 18 non-herding breeds tested, one cocker spaniel and one labradoodle were positive for the mutation, both heterozygous.

Conclusion

The most frequently affected breeds for *ABCB1-1Δ* mutation are the collie, Australian shepherd, white Swiss shepherd, and Shetland sheepdog. As the mutation is associated with an increased incidence of adverse reactions to commonly used pharmaceuticals, veterinarians need to be aware of the breeds at most risk of carrying this mutation and consider testing these individuals prior to administering these medications.

Keywords: ABCB1; MDR1; mutation; Australia; breed; veterinary oncology.

Introduction

The *ABCB1* gene encodes P-glycoprotein (P-gp), a cellular membrane pump. Located in many tissues with secretory or excretory functions such as the adrenal glands, pancreas, intestines, kidney, and liver,[1] P-gp's function is the transport of intracellular xenobiotics and endogenous cellular metabolites to the external environment of the cell.[2] P-gp is also highly expressed on brain capillary endothelial cells[3] where it restricts entry of potentially toxic compounds into the central nervous system (CNS), and forms part of the blood-brain barrier.[4]

In humans, a number of single nucleotide polymorphisms of *ABCB1* have been reported which have been linked to reduced function of P-gp.[5] In contrast, only one functional mutation in this gene (also known as the canine multidrug resistance gene, or *MDR1*) has been described in dogs. This mutation, a 4-bp deletion, was first reported in 2001.[6] The mutation causes an early frame shift at amino acid 75, generating several stop codons further downstream and prematurely halting P-gp synthesis.[6] The result is a severely truncated P-gp,

less than one tenth its normal length, which is devoid of important elements, including ATP binding sites, substrate binding sites, phosphorylation sites and multiple membrane spanning motifs, required for its efflux action. Currently, the mutation is recognised to be autosomal dominant,[7] and is designated the *ABCB1-1Δ* (or *MDR1-1 Δ*) mutation.

Reduced P-gp function in dogs was first described in the 1980s, where 14 Collies were observed to have severe neurological signs after oral administration of ivermectin.[8] It became apparent that the ivermectin toxicity observed in these dogs was attributable to an inherited *ABCB1-1Δ* mutation and its effect on the integrity of the blood-brain barrier, resulting in accumulation of ivermectin in the CNS. Other known *ABCB1* substrate drugs include vincristine, doxorubicin, loperamide, and acepromazine.[7,9,10] Dogs homozygous or heterozygous for the mutation treated with standard doses of vincristine and doxorubicin are significantly more likely than normal dogs to develop haematological and gastrointestinal toxicity.[7] Loperamide and acepromazine are also associated with clinical effects in *ABCB1* mutant/mutant and *ABCB1* mutant/normal individuals. Like ivermectin, loperamide and acepromazine accumulate in the CNS in dogs with defective P-gp function. Affected dogs are at risk of developing a severe neurotoxicosis with standard doses of loperamide,[9] and will exhibit more profound and prolonged CNS depression when administered standard doses of acepromazine intravenously.[10]

Knowledge of the heritable nature and potential significant adverse effects of the *ABCB1-1Δ* mutation has prompted large scale *ABCB1* genotyping studies to be undertaken around the world. To date, the most common breeds identified with the mutation are the collie,

Australian shepherd, longhaired whippet, and silken windhound.[11,12] Less commonly affected breeds include the German shepherd, old English sheepdog, and border collie.[13] The mutation has also been identified in a number of mixed breed dogs. Geographically, the prevalence of the *ABCB1-1Δ* homozygous mutation has been quite similar amongst the collie breed in the United States of America and Europe, with approximately 30% of collies affected.[11,12] However in other breeds, geographic differences have been shown. In Australian shepherds, the affected proportion in different countries varies from 1.7% to 11.3%, and in longhaired whippets from 0% to 15.7%. In German shepherds, the mutation has been detected in the USA but not in other countries.[11] Therefore, it is not possible to confidently extrapolate from international studies to estimate the likelihood of dogs being affected in Australia.

One study investigating the prevalence of the *ABCB1-1Δ* mutation in dogs in Australia has been performed. This study included 61 dogs of four herding breeds (collie, Australian shepherd, Shetland sheepdog, and border collie).[14] Utilising samples submitted by owners for genetic testing to two Australian diagnostic laboratories, the aim of this study was to determine the breed and *ABCB1-1Δ* gene mutation status in a larger sample size of Australian dogs, including dogs of breeds not included in the previous study.

Methods

Samples were analysed at two different Australian laboratories.

Laboratory 1

Genomic DNA was extracted from buccal swabs using the QIAamp 96 DNA Swab BioRobot kit as per manufacturer-recommended guidelines. Polymerase chain reaction amplification

was performed using the following primers:

Forward: 5' -GGC TTG ATA GGT TGT ATA TGT TGG TG-3' Reverse: 5' -ATT ATA ACT GGA AAA GTT TTG TTT C-3'. Genotyping was performed using the AB3730xl DNA Analyser (Applied Biosystems, Foster City, CA, USA). If a sample produced a 155bp product the dog was negative for the mutation and designated *ABCB1* wildtype/wildtype. If a 151bp product is detected both chromosomes carry the deletion and the dog is homozygous for the mutation, thereby designated *ABCB1* mutant/mutant. If both products are detected the dog is heterozygous for the mutation, designated *ABCB1* wildtype/mutant.

Laboratory 2:

Both buccal swabs and EDTA blood were collected and genomic DNA was extracted using QiaAMP® DNA Mini kit (Qiagen, Hilden, Germany, EU) as per manufacturer-recommended guidelines. The *ABCB1-1Δ* mutation was then assessed using two approaches. Firstly, a widely used approach based on multiplex PCR and single base extension was employed.[15] Secondly, genotyping was carried out using manufacturer-recommended standard protocols on a custom designed Illumina Infinium HD genotyping bead chip (Illumina, San Diego, CA, USA).

Statistical Analysis:

Proportions of wildtype, heterozygote, and homozygote for each breed were determined. For each breed, 95% confidence intervals for the multinomial proportion were generated, using Wilson's method implemented in the 'DescTools' package[16] in R.[17]

The effect of breed on *ABCB1* status was evaluated using multiple binomial logistic regression, using the 'logistf' package[18] in R. The response variable was the subject-level *ABCB1* status (wildtype, heterozygous mutant, or homozygous mutant). Two binary logistic models were generated (wildtype vs. heterozygous, wildtype vs. homozygous). Predictor variables in the model were the breed (categorical, 10 levels) and the testing laboratory (binary). For the breed predictor the reference level was 'Other (non-herding)', such that the breed effect estimates were relative to non-herding breed (coefficient for this breed set to zero).

Model parameters were estimated using the Firth penalized likelihood[19], which was selected due to the sparsity of test positive results in many breeds (Table 1). Uncertainty in the estimates was evaluated by the 95% confidence intervals determined from the profile penalized likelihood[20], which are required for confidence interval accuracy in the sparse case.[21]

Results

A total of 4,842 dogs of 25 different breeds were tested (Table 1). Over half of the submissions were from Australian shepherds (1,433 [29.5%]) and border collies (1,469 [30.3%]). Shetland sheepdogs (773 [15.9%]), white Swiss shepherds (522 [10.8%]), rough collies (436 [9%]), and smooth collies (142 [3%]) accounted for almost 40% of submissions. 18 non-herding breeds represented by fewer than 10 dogs per breed were also included.

The *ABCB1-1Δ* mutation was detected in nine breeds. The observed proportions of wildtype, heterozygous, and homozygous genotype were heterogeneous across breeds (Table 2). The 95% confidence intervals for proportions indicate the range of population proportions that are also consistent with the data, demonstrating that the dataset was a precise description of proportions of genotype in most represented breeds.

Breed was a very strong predictor of P-gp status in the logistic models (Table 3). For breeds positively associated with heterozygosity, odds ratios ranged from 1.68 (95% CI: 0.810-3.216) for Shetland sheepdogs, to 1.78 (95% CI: 0.849-3.484) for white Swiss shepherds. For breeds positively associated with homozygosity, odds ratios ranged from 2.43 (95% CI: 2.047-2.878) for Australian shepherds, to 19.60 (95% CI: 15.53-26.87) for rough collies. No breeds were apparently negatively associated with either hetero - or homozygosity.

It was difficult to make accurate conclusions as to the *ABCB1* status in the smooth collie breed. This was because the smooth collie breed was poorly identifiable in both models (noting the broad confidence limits in table 3), likely related to a combination of relatively small sample size and the similar odds of all outcomes for this breed (Table 2). This resulted in imprecise outcomes for this breed.

There was some evidence that the testing laboratory, expressed as Laboratory 2 versus Laboratory 1, was associated with different odds of positive status, both for heterozygosity (OR: 0.854, 95% CI: 0.712-1.010) and homozygosity (OR: 0.879, 95% CI: 0.838-0.920). This meant that dogs analysed at Laboratory 2 were less likely to be positive when compared to dogs tested at Laboratory 1.

Discussion:

The majority of samples submitted to the two reference laboratories were from herding breed dogs, which is unsurprising given the previously detected frequency of the mutation in these breeds. The *ABCB1-1Δ* mutation was detected in over 70% of collies, over half of the Australian shepherds, and a significant minority of white Swiss shepherds and Shetland sheepdogs. Conversely, of the 18 non-herding breeds analysed the mutation was found in only one cocker spaniel and one labradoodle.

Our results are similar to other large-scale studies published around the world. One study conducted within the United States of America found the mutation in 77% of collies and almost 50% of Australian shepherds.[11] A European study found that 75% of collies and 58% of Australian shepherds were positive for the mutation.[12] When compared to these studies, our results within both collie and Australian shepherd populations were reasonably consistent, as the estimated proportion for rough collies in our study was 73% (95% CI: 69%-77%) and for Australian shepherds was 53% (95% CI: 51%-56%). However, the results did differ when comparing Shetland sheepdogs. One Australian study reported that 43% of Shetland sheepdogs were heterozygous for the mutation and did not report any homozygous mutants.[14] Our study found that 24% of Shetland sheepdogs were heterozygous for this trait (95% CI: 21%-27%) and 4% of this study population were homozygous (95% CI: 3%-5%). Comparing border collies, 4 of 1469 (0.3%; 95% CI 0.1%-0.6%) in our study were positive for the mutation. This is consistent with other international studies, where <1% of border collies were affected. No affected border collies were found in the previous Australian study,

but only seven border collies were assessed and so it is likely that the sample size was insufficient to detect the mutation in this breed. Less likely is that the prevalence has increased in Australian border collies in the time since the previous study. In this study, one cocker spaniel and one labradoodle were positive for the mutation, which has not been previously reported. However, previous studies have also found the mutation in non-herding breeds and mixed breed dogs without obvious herding breed lineage.

Results from our study indicate that the breed most associated with increased odds of being a hetero – or homozygous *ABCB1-1Δ* mutant is the rough collie. Our study demonstrated that a randomly selected rough collie has 7.8 times the odds to be heterozygous (95% CI: 3.749-18.986) and 19.6 times the odds to be homozygous (95% CI: 15.530-26.871) for the *ABCB1-1Δ* mutation, relative to a non-herding breed dog. Referring to Table 3, Australian shepherds, white Swiss shepherds, and Shetland sheepdogs also have a strong breed association with positive *ABCB1-1Δ* mutation status. As this mutation is associated with an increased incidence of adverse reactions to commonly used pharmaceuticals, veterinarians need to be aware of the breeds at most risk of carrying this mutation and consider testing these individuals prior to administering these medications.

The results of this study may have been affected by convenience sampling bias, since the population of tested dogs was not randomly selected and the reasons for sample submission were not known. Therefore, we may have inadvertently selected for a population with an artificially high frequency of the mutation in our study if, for example, samples were submitted from dogs with a known or suspected affected parent or littermate.

In this set, the testing labs do systematically differ (Table 3). Testing Laboratory 2 has a lower odds of a positive result when compared to Testing Laboratory 1. This difference could arise due to a difference in test performance between laboratories if, for example, Laboratory 2's test is less sensitive compared to Laboratory 1, or conversely, if the test Laboratory 2 employs is more specific. This difference could also be due to a chance difference in the absolute number of positive dogs sent to each lab.

Conclusion:

Here, we report that breed is associated with a positive *ABCB1-1Δ* mutation status in Australian dogs. Similar to other studies, the breeds with the highest odds of having the *ABCB1-1Δ* mutation are the collie, Australian shepherd, white Swiss shepherd and Shetland sheepdog. Australian veterinarians should be particularly aware of the risk of the *ABCB1-1Δ* mutation in dogs of these breeds where P-gp substrate drugs are being considered. However, it is prudent to bear in mind that other breeds may be affected and testing may be warranted in specific cases including dogs (or relatives of dogs) with unexpectedly severe adverse reactions to P-gp substrate drugs.

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Conflicts of interest:

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Table 1: Total number of dogs that underwent *ABCB1* genotyping at Laboratory 1 and Laboratory 2

	Laboratory 1	**Laboratory 2**
Australian Shepherd	429	1004
Border Collie	168	1301
Rough collie	169	267
Smooth collie	49	93
German Shepherd	14	0
Shetland Sheepdog	306	467
White Shepherd	17	0
White Swiss Shepherd	49	473

Other (Herding)		
Bearded Collie	4	0
Other (Non - Herding)		
Beagle	1	0
Bernese Mountain Dog	1	0
Borzoi	1	0
Cavalier King Charles Spaniel	2	0
Cocker Spaniel	1	0
French Bulldog	1	0
Greyhound	1	0
Keeshond	1	0
Kelpie	3	0
Kelpie X	2	0
Labradoodle	4	0
Labrador Retriever	1	0
Miniature Schnauzer	1	0
Nova Scotia Duck Tolling Retriever	1	0
Poodle	1	0
Shar Pei	1	0
Skye Terrier	6	0
Staffordshire Bull Terrier	3	0
TOTAL	**1,237**	**3,605**

Table 2: Observed genotype data and calculated proportions, pooled from 2 testing labs. 95% confidence intervals are multinomial and determined from the Wilson method.

PROP.: observed proportion, L 95%: lower 95% confidence limit of proportion, U 95%: upper 95% confidence limit of proportion, *count*: observed count, *n*: total subjects observed per breed.

BREED	OBSERVED PROPORTIONS		
	WILD TYPE	HETEROZYGOUS	HOMOZYGOUS

	PROP.	L 95%	U 95%	PROP.	L 95%	U 95%	PROP.	L 95%	U 95%
	count			*count*			*count*		
Other (Non-herding)	0.938	0.799	0.983	0.0625	0.0173	0.201	0.000	0.000	0.107
n = 32	*30*			*2*			*0*		
Australian Shepherd	0.465	0.440	0.491	0.430	0.405	0.456	0.105	0.0900	0.122
n = 1433	*667*			*616*			*150*		
Bearded Collie	1	0.51	1	0	0	0.49	0	0	0.49
n = 4	*4*			*0*			*0*		
Border Collie	0.997	0.993	0.999	0.00272	0.00106	0.00698	0.000	0.000	0.00261
n = 1469	*1465*			*4*			*0*		
Rough collie	0.271	0.231	0.314	0.445	0.399	0.492	0.284	0.244	0.328
n = 436	*118*			*194*			*124*		
Smooth collie	0.190	0.134	0.263	0.251	0.439	0.602	0.289	0.221	0.368
n = 142	*27*			*74*			*41*		
German Shepherd	1	0.785	1	0	0.000	0.215	0	0.000	0.215
n = 14	*14*			*0*			*0*		
Shetland Sheepdog	0.723	0.691	0.754	0.238	0.209	0.269	0.0388	0.0273	0.0549
n = 773	*559*			*184*			*30*		
White Shepherd	0.941	0.730	0.990	0.0588	0.0105	0.270	0.000	0.000	0.184
n = 17	*16*			*1*			*0*		
White Swiss Shepherd	0.747	0.708	0.783	0.253	0.217	0.292	0.000	0.000	0.00731
n = 522	*390*			*132*			*0*		

Table 3: Parameter estimates from the logistic regression models for genotype as a function of breed and testing lab. Responses for the models are log(Odds): Status, relative to wildtype.

β: coefficient estimate, OR: odds ratio, L 95%: lower 95% confidence limit of odds ratio, U 95%: upper 95% confidence limit of odds ratio. Odds ratios are the effect of breed relative to

the baseline breed 'Other (Non-herding)', and lab relative to the base lab 'Testing Laboratory 2'.

'Breed: Smooth collie' is poorly identifiable from this dataset, reflected in the extreme upper confidence limits.

Model		Status: Heterozygous				Status: Homozygous			
Parameter		β	OR	L 95%	U 95%	β	OR	L 95%	U 95%
Intercept		0.992	-	0.396	1.725	2.019	-	1.863	2.186
Breed	Other (Non-herding)	0*	-	-	-	0*	-	-	-
	Australian Shepherd	1.270	3.563	1.755	7.014	0.890	2.434	2.047	2.878
	Bearded Collie	-0.162	0.851	0.139	11.264	-0.014	0.986	0.634	1.629
	Border Collie	0.027	1.028	0.491	1.944	0.117	1.124	0.946	1.324
	Rough collie	2.060	7.843	3.749	18.986	2.976	19.605	15.530	26.871
	Smooth collie	4.411	82.329	0.020	>100	6.243	514.546	8.365	>100
	German Shepherd	-0.131	0.877	0.280	3.006	-0.003	0.997	0.753	1.334
	Shetland Sheepdog	0.520	1.682	0.810	3.216	0.296	1.344	1.131	1.585
	White Shepherd	-0.013	0.987	0.332	3.170	-0.002	0.998	0.762	1.317
	White Swiss Shepherd	0.579	1.785	0.849	3.484	0.121	1.128	0.945	1.335
Lab	Testing Laboratory 1	0*	-	-	-	0*	-	-	-
	Testing Laboratory 2	-0.158	0.854	0.712	1.010	-0.129	0.879	0.838	0.920

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