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Citation	Cullen, J. M., Willson, C. J., Minch, J. D., Kimbrough, C. L., & Mealey, K. L. (2014). Lack of association of ABCB4 insertion mutation with gallbladder mucoceles in dogs. <i>Journal of Veterinary Diagnostic Investigation</i> , 26(3), 434-436. doi:10.1177/1040638714532099
DOI	10.1177/1040638714532099
Publisher	Sage Publications
Version	Version of Record
Terms of Use	http://cdss.library.oregonstate.edu/sa-termsfuse

Lack of association of *ABCB4* insertion mutation with gallbladder mucoceles in dogs

Journal of Veterinary Diagnostic Investigation
 2014, Vol. 26(3) 434–436
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 DOI: 10.1177/1040638714532099
jvdi.sagepub.com

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Abstract. The etiology of canine gallbladder mucocele (GBM) has not yet been identified. However, several studies have linked GBM in dogs to particular breeds (Shetland Sheepdogs are commonly implicated), concurrent endocrine disease (hyperadrenocorticism and/or hypothyroidism), and a mutation in the canine *ABCB4* gene (*ABCB4* 1583_1584G), particularly in Shetland Sheepdogs. The current study assessed *ABCB4* 1583_1584G, in a wider sample of dogs with GBM compared with age and breed-matched controls. *ABCB4* 1583_1584G was identified in 4 of 8 Shetland Sheepdogs and 13 of 28 other breeds with GBM. *ABCB4* 1583_1584G was also detected in 9 of 12 Shetland Sheepdogs and 23 of 37 other breeds that did not have GBM. No statistically significant association existed between *ABCB4* 1583_1584G and the presence of GBM for all dogs combined or for Shetland Sheepdogs alone. In contrast to previously reported findings, the current study did not identify a strong association between *ABCB4* 1583_1584G and GBM in Shetland Sheepdogs or other breeds.

Key words: *ABCB4* gene; canine; gall bladder; mucocele.

Gallbladder mucocele (GBM) has been reported with increased frequency in dogs since 2004 (19 articles listed in PubMed) compared to the period from 1994 to 2003 (4 articles listed in PubMed). While the etiology of GBM has not yet been identified, a previous study has linked GBM in dogs to specific endocrine diseases,⁷ while another has identified an association with a mutation in the canine *ABCB4* (adenosine triphosphate-binding cassette, subfamily B, member 4) gene.⁶ A strong association between hypercortisolism and/or hypothyroidism and GBM has been identified. Specifically, the odds of a dog having a GBM were 29 times and 3 times, respectively, that of dogs without those endocrinopathies.⁷ An insertion mutation in the canine *ABCB4* gene (*ABCB4* 1583_1584G) has also been associated with GBM in a small number of dogs, primarily Shetland Sheepdogs.² Each of the factors that have been associated with GBM in dogs (hypercortisolism, hypothyroidism, and *ABCB4* mutation) can be linked to abnormal bile composition and/or flow. In human beings, hypercortisolism has been shown to decrease bile flow and alter bile composition resulting in excessive cholesterol and a relative deficiency of phospholipids and cholic acid.⁴ Hypothyroidism in rats has been shown to decrease both bile flow and bile salt excretion by 50%.¹⁰ Functional *ABCB4* mutations in human beings cause abnormalities in bile flow and composition that result in a variety of hepatobiliary diseases,^{2,3,5} but GBM is not among them. Whereas hepatobiliary disease-causing *ABCB4* mutations are well characterized in human beings, the authors are aware of only 1 study involving *ABCB4* dysfunction and hepatobiliary disease in dogs.⁶ Thus, the objective of the current study was to determine if *ABCB4* 1583_1584G, the mutation previously

associated with GBM in Shetland Sheepdogs is present in a wider sample of dogs with GBM compared with age and breed-matched controls without GBM.

Cases were selected by searching the electronic database at North Carolina State University (Raleigh, North Carolina) for dogs with a diagnosis of GBM.⁸ The search yielded 46 dogs (either biopsy or necropsy cases) including 8 Shetland Sheepdogs, spanning the years 2004–2010. The next search was for breed and age-matched dogs without a diagnosis of GBM at necropsy. Age matching was performed as closely as possible and generally resulted in dogs of the same breed with an age within 2 years of those with GBM. Additional dogs, beyond those necessary for breed and age matching, were also used for Shetland Sheepdogs ($n = 12$) and other breeds ($n = 48$) to analyze the presence of gene mutations in dogs without GBM, for a total of 60 unaffected dogs. Additional dogs were included because it was anticipated that the quality or quantity of DNA obtained from some formalin-fixed, paraffin-embedded samples might not be adequate for sequencing. The final number of sequenced DNA samples included 36 dogs with GBM and 49 control dogs.

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Table 1. Presence of *ABCB4* 1583_1584G or wild-type *ABCB4* gene sequence in a sample of dogs affected or unaffected by gallbladder mucocele (GBM).

	Shetland Sheepdog		Other breeds	
	GBM affected	GBM unaffected	GBM affected	GBM unaffected
<i>ABCB4</i> wild type	4	3	15	14
<i>ABCB4</i> 1583_1584G	4	9	13	23
Total	8	12	28	37

Formalin-fixed, paraffin-embedded liver tissue samples were processed using a commercial RNA extraction kit^a until step C3. The interphase from this step (containing DNA and protein) was then subjected to DNA extraction using a commercial kit.^b Exon 12 of canine *ABCB4* was amplified with the following primers: 5'-GAGATACATCAGGAGCTCCTCC-3' and 5'-CAGGTGTTTCGGGTTGACTG-3'. Purified PCR amplicons were then sequenced.^c

Proc FREQ in SAS^d was used to perform the statistical analysis of the *ABCB4* insertion mutation and GBM data. Due to the small number of counts in each of the cells in the frequency tables, Fisher exact test was used to determine whether an association existed between *ABCB4* 1583_1584G and GBM. The odds ratio was also calculated to determine if an association existed between dogs with the *ABCB4* mutation and GBM. The analysis was performed for all dogs, and then separately for the Shetland Sheepdogs because previous studies have indicated that this breed is predisposed to GBM. All tests were performed at the 5% level of statistical significance unless otherwise noted.

Gallbladder mucocele was diagnosed on the basis of appropriate gross appearance (pigmented, tenacious, and rubbery contents) at the time of surgery or by evaluation by pathologists and histologic confirmation (proliferation of gall bladder mucosa with prominent projections of epithelium into the mucus) at biopsy or postmortem for all cases.⁹ The gallbladder was ruptured or had mural necrosis and/or infarction in 12 of 36 (33.3%) cases. Fibrosis of the gallbladder was observed in 2 of 36 (5.6%) cases. Gallbladder epithelial hyperplasia and cholecystitis were present in 4 of 36 (11.1%) and 1 of 36 (2.8%) cases, respectively, with a GBM. For the comparison group, only postmortem specimens were evaluated, and the absence of GBM was confirmed by histologic review and examination of gall bladder contents.

Liver samples were analyzed from 36 dogs with GBM and 49 dogs without GBM (Supplemental Tables 1, 2). Eight Shetland Sheepdogs (age range: 4–12 years, median age: 10) and 28 dogs of other breeds (age range: 5–14 years, median age: 10) with GBM were included while control dogs consisted of 12 Shetland Sheepdogs (age range: 3–13 years, median age: 10) and 37 dogs (age range: 1–19 years, median age: 10) of similar breeds to those with GBM. Shetland Sheepdogs comprised 22% of GBM cases (8 of 36). Other breeds with more than 1 dog with a GBM included the

Cocker Spaniel (5), Beagle (2), Bichon Frise (2), Chihuahua (2), Dachshund (2), Labrador Retriever (2), Miniature Pinscher (2), and Miniature Schnauzer (2).

The presence of *ABCB4* 1583_1584G or the wild-type canine *ABCB4* sequence was determined for all 85 dogs using DNA extracted from formalin-fixed, paraffin-embedded liver biopsies (Table 1). The overall genotype distribution was 42.4% for the homozygous *ABCB4* wild-type allele and 57.6% for *ABCB4* 1583_1584G heterozygotes. *ABCB4* 1583_1584 was identified in 4 of 8 Shetland Sheepdogs with GBM and 9 of 12 Shetland Sheepdogs without GBM. For other breeds, *ABCB4* 1583_1584 was identified in 13 of 28 dogs with GBM and 23 of 37 dogs without GBM.

The statistical analysis used to determine if an association existed between the mutation and GBM data showed no statistically significant differences for all dogs combined or for Shetland Sheepdogs. The Fisher exact test *p*-values were greater than 0.05, and the 95% confidence limits for the odds ratios included 1 (odds ratios of 1 indicate no association).

The canine *ABCB4* insertion mutation *ABCB4* 1583_1584G has previously been reported to be associated with GBM in dogs, particularly Shetland Sheepdogs.² Results of the current study, which are discordant from the previous study, are that *ABCB4* 1583_1584G is not associated with GBM.

The discordance of results between the original study and the current one may be due to several factors, including the potential for false-negative “control” dogs due to the fact that GBM is a disease of older animals, the lack of a gold standard method for diagnosing GBM, and the potential for a greater degree of relatedness among Shetland Sheepdogs in the original study. Mutations causing dysfunction of *ABCB4* in human patients result in a variety of hepatobiliary diseases. The unaffected population of dogs in the original study included only dogs with no evidence of hepatobiliary disease based on normal serum biochemical panels. In contrast, several of the control dogs in the current study had histologic findings of periportal or bridging fibrosis, cholangitis, and/or biliary hyperplasia. These are hepatic abnormalities frequently observed in human patients with *ABCB4* mutations. It is conceivable that hepatobiliary disease in some of these dogs may have eventually progressed to GBM. Additionally, GBM tends to occur in older animals (mean: 9–10 years of age).^{1,6-8} Because 13 of 49 of the “unaffected”

dogs were less than 7 years of age, they might also represent false negatives thereby contributing to the discordant results of the 2 studies.

Lack of a gold standard method for diagnosing GBM, particularly developing GBM, may also be a contributing factor. Inclusion criteria for affected dogs in the original study consist of surgical diagnosis, ultrasonographic diagnosis, or necropsy/histologic diagnosis.⁹ In contrast, inclusion criteria for dogs in the current study consisted strictly of a necropsy diagnosis of GBM with histologic confirmation. Limitations of each of these criteria for diagnosing GBM have been discussed previously.⁶ Whether or not each of these diagnostic methods perform comparably in accurately diagnosing GBM has not been investigated. Thus, the affected population in both studies might include false positives and the unaffected population might contain false negatives. Another source of false negatives in the current study may be that dogs with *ABCB4* 1583_1584G could have eventually developed GBM had they not died of other causes first.

Lastly, it is possible that there was a higher degree of relatedness, particularly among Shetland Sheepdogs, in the original study compared to the current one. Because affected dogs were solicited through the American Shetland Sheepdog Association in that study, it is reasonable to suspect that word-of-mouth recruitment occurred among owners of related dogs. In fact, a sibling pair of affected Shetland Sheepdogs was identified in the initial study. In the current study, owners and breeders were not solicited. Rather, the patient population was selected from submissions to a particular hospital, which may be less likely to include related dogs.

Hyperadrenocorticism and hypothyroidism have been reported to be significantly associated with the diagnosis of GBM in dogs.⁷ Both hyperadrenocorticism and hypothyroidism are considered common endocrinopathies in dogs whereas GBM occurs much less commonly, suggesting that these are relative, not absolute, risk factors for GBM formation. The findings in the current study do not rule out the possibility that *ABCB4* 1583_1584G may also be one of many relative risk factors for GBM in dogs.

In conclusion, the present study brings into question the association of *ABCB4* 1583_1584G with GBM in dogs. However, it does not rule out the possibility that the etiology of GBM in dogs is multifactorial and that *ABCB4* dysfunction may be one of many contributing factors. What this study does make clear is that clinical use of a genetic test for *ABCB4* 1583_1584G is premature and would not yet provide useful diagnostic information for veterinarians. It would also not provide dog breeders with information necessary to make informed breeding decisions.

Sources and manufacturers

- a. Ambion RiboPure, Life Technologies, Grand Island, NY.
- b. DNeasy blood and tissue kit, Qiagen Inc., Valencia, CA.
- c. ABI 3730 sequencer, Life Technologies, Grand Island, NY.
- d. FREQ Procedure of SAS/STAT, SAS Institute Inc., Cary, NC.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Aguirre AL, Center SA, Randolph JF, et al.: 2007, Gallbladder disease in Shetland Sheepdogs: 38 cases (1995–2005). *J Am Vet Med Assoc* 231:79–88.
2. Delaunay JL, Durand-Schneider AM, Delautier D, et al.: 2009, A missense mutation in *ABCB4* gene involved in progressive familial intrahepatic cholestasis type 3 leads to a folding defect that can be rescued by low temperature. *Hepatology* 49:1218–1227.
3. Elferink RO, Groen AK: 2002, Genetic defects in hepatobiliary transport. *Biochim Biophys Acta* 1586:129–145.
4. German SV, Lemesko ZA, Vaïnšteïn Tla, Petrova EA: 1989, Zhelchevydelitel'naia sistema pri sindrome endogennogo giperkortitsizma [Bile excretion system in endogenous hypercorticism syndrome]. *Sov Med* (3):15–19. In Russian. Abstract in English.
5. Gonzales E, Davit-Spraul A, Baussan C, et al.: 2009, Liver diseases related to *MDR3* (*ABCB4*) gene deficiency. *Front Biosci (Landmark Ed)* 14:4242–4256.
6. Mealey KL, Minch JD, White SN, et al.: 2010, An insertion mutation in *ABCB4* is associated with gallbladder mucocele formation in dogs. *Comp Hepatol* 9:6.
7. Mesich ML, Mayhew PD, Paek M, et al.: 2009, Gall bladder mucoceles and their association with endocrinopathies in dogs: a retrospective case-control study. *J Small Anim Pract* 50:630–635.
8. Pike FS, Berg J, King NW, et al.: 2004, Gallbladder mucocele in dogs: 30 cases (2000–2002). *J Am Vet Med Assoc* 224:1615–1622.
9. van den Ingh T, Cullen JM, Twedt DC, et al.: 2006, Morphological classification of biliary disorders of the canine and feline liver. *In: WSAVA standards for clinical and histological diagnosis and canine and feline liver diseases*, ed. Rothuizen J, Bunch SE, Charles JA. et al., pp. 61–76. Saunders, New York, NY.
10. Van Steenberg W, Fevery J, De Vos R, et al.: 1989, Thyroid hormones and the hepatic handling of bilirubin. I. Effects of hypothyroidism and hyperthyroidism on the hepatic transport of bilirubin mono- and diconjugates in the Wistar rat. *Hepatology* 9:314–321.