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Association between ABCB1 Genotype and Seizure Outcome in Collies with Epilepsy

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Background: Medically refractory seizures are an important problem in both humans and dogs with epilepsy. Altered expression of ABCB1, the gene encoding for p-glycoprotein (PGP), has been proposed to play a role in drug-resistant epilepsy.

Hypothesis: Heterogeneity of the ABCB1 gene is associated with seizure outcome in dogs with epilepsy.

Animals: Twenty-nine Collies with epilepsy being treated with antiepileptic drugs (AEDs).

Methods: Prospective and retrospective cohort study. Dogs were classified as having a good outcome ($\leq$1 seizure/month, no cluster seizures) or a poor outcome (>1 seizure/month, with or without cluster seizures) based on owner-completed questionnaire. Serum AED concentrations were measured, and ABCB1 genotyping was performed on buccal tissue samples. Association analyses were performed for genotype and seizure outcome, number of AEDs administered, serum AED concentrations, and incidence of adverse effects.

Results: Fourteen dogs of 29 (48%) were homozygous for the ABCB1-1Δ mutation (M/M), 11 dogs (38%) were heterozygous (M/N), and 4 dogs (14%) had the wild-type genotype (N/N). Dogs with the M/M genotype were significantly more likely to have fewer seizures and have less AED-related sedation than M/N or N/N dogs (P = .003 and P = .001, respectively). Serum phenobarbital and bromide concentrations did not differ between groups, but the M/N and N/N groups received a larger number of AEDs than the M/M group (P = .014).

Conclusions and Clinical Importance: ABCB1 genotype is associated with seizure outcome in Collies with epilepsy. This cannot be attributed to differences in PGP function, but might be because of intrinsic variations in seizure severity among phenotypes.

Key words: Antiepileptic drug; Canine; Drug resistance; MDR1; P-glycoprotein.

Epilepsy is the most common neurological disorder encountered in small animal practice, with seizures occurring in up to 5% of all dogs in the general population.[1,2] Antiepileptic drugs (AED) remain the cornerstone of treatment for dogs with epilepsy. However, approximately 30% of epileptic dogs are refractory to conventional medical therapy with phenobarbital and potassium bromide, and do not attain satisfactory seizure control despite administration of the drugs at maximally tolerated dosages.[3] A similar incidence of medically resistant epilepsy is seen in humans, with at least 30% of patients continuing to seizure despite appropriate AED therapy.[4] Poor control of seizures is associated with increased morbidity and fatality in both humans and dogs, and accounts for much of the financial burden of epilepsy management.[5,6]

Despite the approval of several new AEDs for use in humans with epilepsy over the past 20 years, the incidence of drug-resistant epilepsy has not changed substantially.[7,8] Individuals with refractory epilepsy are typically resistant to a broad range of AEDs with varied modes of action,[7] which suggests that a relatively nonspecific mechanism is responsible for the poor seizure control obtained in such patients. The 2 prevailing theories on the pathogenesis of drug-resistant epilepsy are the target hypothesis, which proposes that genetic or disease-related alterations in the cellular targets of AEDs result in decreased sensitivity to treatment,[9] and the transporter hypothesis, which postulates that overexpression of P-glycoprotein (PGP) and other drug transport proteins at the blood-brain barrier limits penetration of AEDs into the brain.[10,11] P-glycoprotein is an ATP-dependent transmembrane efflux transporter that is present on the luminal membrane of the capillary endothelial cells of the blood-brain barrier, where it functions to exclude selected xenobiotics from the central nervous system (CNS).[12] It has been hypothesized that differential expression of ABCB1, the gene encoding for PGP, might play a role in an individual's response to AED therapy by altering concentrations of AEDs achieved within the seizure focus in the brain.

Experimental and clinical data support a role for ABCB1 and PGP expression in drug-resistant epilepsy,

Abbreviations:

AED	antiepileptic drug
CNS	central nervous system
M/M	homozygous for ABCB1-1Δ mutation
M/N	heterozygous for ABCB1-1Δ mutation
N/N	homozygous for ABCB1 wild type
PGP	P-glycoprotein

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with both genetic and environmental factors playing a role. Increased levels of ABCB1 mRNA and increased immunohistochemical staining for PGP have been identified in several rodent models of epilepsy,[13–16] as well as in postmortem and surgical biopsy samples of brain tissue obtained from humans with medically resistant epilepsy.[17–24] A recent study evaluating brain tissue from dogs that died shortly after episodes of status epilepticus identified a significant upregulation of PGP expression in specific regions of the cerebrum, suggesting that PGP overexpression might also play a role in the development of drug-resistant epilepsy in dogs.[25]

The ABCB1 gene has been characterized in dogs, and a mutant allele of this gene involving a 4 base pair deletion in exon 4 has been described in Collies and other related breeds.[26] The ABCB1-1Δ mutation, which has a prevalence of >50% in Collies,[27] results in the production of a severely truncated, nonfunctional form of PGP. This, in turn, prevents the normal efflux of substrates from the brain back into the capillary lumen, and results in central nervous system (CNS) accumulation of substrate. Dogs with the ABCB1-1Δ mutation have increased susceptibility to neurological adverse effects of several commonly prescribed drugs, including ivermectin and loperamide.[28]

The Collie breed, with a predisposition to naturally occurring epilepsy as well as a high prevalence of the ABCB1-1Δ mutation, offers a unique opportunity to further investigate the potential role of PGP in the response to AED treatment. We hypothesize that heterogeneity of the ABCB1 gene is associated with seizure outcome in Collies with epilepsy. The specific aim of this study was to determine whether or not epileptic Collies with the ABCB1-1Δ mutation and a lack of functional PGP are less likely to have drug-resistant epilepsy than epileptic dogs of the same breed that do not carry the mutation.

Materials and Methods

Case Selection

Collies with an onset of seizures between 6 months and 5 years of age and a minimum 6-month history of AED administration were recruited for this cohort study. All dogs had a presumptive diagnosis of idiopathic epilepsy, which was made by the primary veterinarian based on examination findings and laboratory analysis. The targeted study enrollment was 30 dogs, which was based on a power analysis assuming a confidence level of 95%, a power of 90%, and the measurable outcome of 50% difference between groups. The study protocol was approved by the Institutional Animal Care and Use Committee at North Carolina State University, and owners were required to provide informed consent before study participation.

Data Collection

Buccal swabs were obtained from each dog and shipped to the Veterinary Clinical Pharmacology Laboratory at Washington State University for ABCB1 genotyping according to a previously published technique.[29] Results of serum AED concentrations were obtained from the primary veterinarian when available. If testing had not been performed within the past 6 months, or the dosage of any AED had been adjusted since the last reported result, blood samples were collected and submitted to the Clinical Pharmacology Laboratory at North Carolina State University for determination of serum concentrations of phenobarbital, bromide, or both. Owners were required to complete a standardized questionnaire developed for the study, which gathered information regarding the age of onset of seizures, the type, dosage, and duration of AED administered, the average monthly seizure frequency over the past 6 months, whether there was any history of cluster seizures, and the presence of any drug-related adverse effects.

Dogs were assigned to groups according to the reported seizure frequency over the previous 6 months. Dogs with a good seizure outcome were defined as having an average monthly seizure frequency of ≤ 1 and no history of cluster seizures (2 or more seizures within a 24 hour period), while being maintained on at least 1 AED. Dogs with a poor seizure outcome were defined as having an average monthly seizure frequency >1 or cluster seizures, while being treated with a minimum of 1 AED. Dogs were assigned to groups before any knowledge of genotype results.

Statistics

Data were analyzed for associations between genotype and seizure outcome (good versus poor), number of AEDs administered, serum AED concentrations, and incidence of adverse effects. Fisher's exact test was used for categorical data, and the Kruskal-Wallis test was utilized for continuous data. A significance level of $P < .05$ was established for all analyses.

Results

Twenty-nine dogs were enrolled in the study, including 25 rough coated Collies, 3 smooth coated Collies, and 1 Collie mix. There were 20 males, 17 of which were neutered, and 9 neutered females. Dogs ranged from 1–10 years of age (median, 5.0 years), with a duration of epilepsy of 0.5–8 years (median, 3 years).

Nineteen dogs (66%) were initiated and maintained on 1 AED. Phenobarbital was being administered to 14 dogs, and bromide to 5 dogs. Nine dogs (31%) were being treated with 2 AEDs at the time of the study, including combinations of phenobarbital and bromide (n = 7), phenobarbital and levetiracetam (n = 1), and phenobarbital and zonisamide (n = 1). One dog (3%) was being administered 3 AEDs (zonisamide, felbamate, and levetiracetam).

Eleven of the dogs (38%) were classified as having a poor seizure outcome over the 6-month period before the study, with a monthly seizure frequency of 3.87 (mean, range 1–15). Ten of the 11 dogs had a history of cluster seizures. Among dogs with a poor outcome, 3 (27%) were being treated with 1 AED, 7 (78%) were being treated with 2 AEDs, and 1 (9%) was being treated with 3 AEDs. The remaining 18 dogs (62%) were classified as having a good seizure outcome, with a monthly seizure frequency of 0.29 (mean, range 0–1). Nine of these dogs were reported to be seizure free. Sixteen of the dogs (89%) classified as having a good seizure outcome over the 6-month period before the study were receiving 1 AED and 2 dogs (11%) were

receiving 2 AEDs. Dogs classified as having a poor seizure outcome were being administered significantly more AEDs than dogs with a good seizure outcome ($P = .001$). The daily dose of bromide was higher in the dogs with a poor seizure outcome compared with dogs with a good seizure outcome, but serum bromide concentrations were not significantly different. There was no difference in phenobarbital dose or serum concentrations in dogs based on seizure outcome. Doses and serum concentrations for phenobarbital and bromide in dogs grouped according to seizure outcome are summarized in Table 1.

Fourteen dogs (48%) were homozygous for the ABCB1-1Δ mutation (M/M), 11 dogs (38%) were heterozygous for the mutation (M/N), and 4 dogs (14%) had the wild-type genotype (N/N). The relationship between genotype and seizure outcome is depicted in Figure 1. The M/M group had a significantly better seizure outcome than the M/N or N/N groups ($P = .003$). The M/N and N/N groups had relatively similar rates of good seizure outcome (36 and 25%, respectively). As dogs in both of these groups have at least 1 gene to encode for functional PGP, and it was postulated that these dogs might behave similarly with respect to the variables being evaluated in the study, additional analyses were performed in which the M/N and N/N groups were combined and compared with the M/M group. In this analysis, dogs in the M/M group demonstrated significantly better seizure outcome than the combined group of M/N and N/N dogs (93% versus 33%, $P = .002$).

A repeat analysis of the association between genotype and seizure outcome was performed after excluding the 5 dogs that were on bromide monotherapy, as bromide is not a known PGP substrate. Results were similar to those obtained from the initial analysis. Dogs with the M/M genotype had a significantly better seizure outcome than either the M/N or N/N dogs ($P = .024$), and this difference persisted when the M/N and N/N groups were combined and compared with the M/M group ($P = .013$). Too few dogs were receiving bromide alone to permit comparisons between the subgroup of dogs on bromide monotherapy and the group of dogs on other AED therapy.

Table 1. Phenobarbital and bromide dose and serum concentrations in dogs grouped according to seizure outcome.

	Good Outcome (n = 14)	Poor Outcome (n = 9)	*P*-Value
Phenobarbital dose (mg/kg/d)	3.81 (1.56–9.43)	4.80 (3.24–13.56)	.095
Serum phenobarbital concentration (μg/mL)	17.7 (8.0–29.2) (n = 6)	25.7 (11.4–39.9) (n = 6)	.083
Bromide dose (mg/kg/d)	25.2 (22.9–50.3)	54.4 (33.2–121.0)	.016
Serum bromide concentration (mg/dL)	140.6 (93.0–290.0)	220.8 (110.0–380.0)	.173

Values are expressed as median of range.

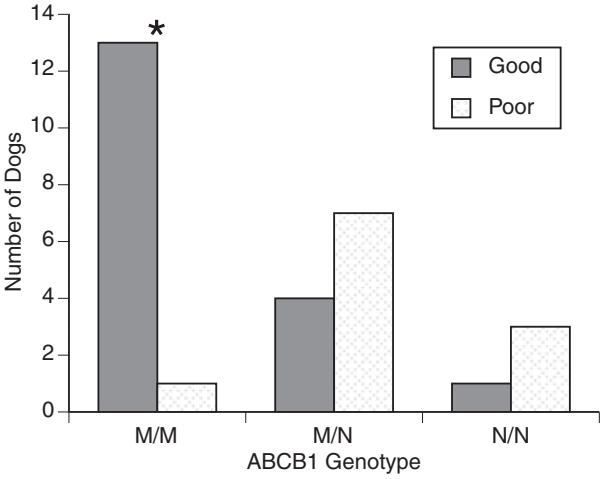

Fig 1. Relationship between ABCB1-1Δ genotype and seizure outcome (good versus poor) among Collies with epilepsy. M/M: homozygous for mutation, M/N: heterozygous for mutation, N/N: wild-type genotype. *denotes a significant difference.

Dogs with the M/N or N/N genotype were being administered significantly more AEDs than M/M dogs ($P = .014$). The difference remained significant when the M/N and N/N groups were combined and compared with dogs of the M/M genotype ($P = .008$). Among the 14 dogs with the M/M genotype, 13 (93%) were being administered 1 AED and 1 dog (7%) was being administered 2 AEDs. In comparison, of the 15 dogs in the combined M/N and N/N groups, 6 (40%) were on 1 AED, 8 (53%) were on 2 AEDs, and 1 (7%) was on 3 AEDs.

Among the 23 dogs receiving phenobarbital, the dose and serum drug concentrations were not significantly different among the genotypes. For the 12 dogs being administered bromide, the dose was significantly higher among the M/N and N/N groups compared with the M/M dogs ($P = .037$), but serum levels did not differ. Phenobarbital and bromide doses and serum concentrations for dogs grouped by genotype are summarized in Table 2.

The incidence of adverse events differed among the genotypes. Adverse effects were reported in 6 of 12 (50%) of the M/M dogs, while 100% of dogs in both the M/N (n = 11) and N/N (n = 4) groups had adverse events ($P = .011$). Furthermore, a significant difference in number of adverse effects was demonstrated among the genotype groups, with M/M dogs having fewer adverse effects than either the M/N or N/N group ($P = .022$). Specific adverse events reported include polyuria/polydipsia, polyphagia, gastrointestinal signs (anorexia, vomiting, diarrhea), behavioral changes and sedation, and are summarized in Table 3. No significant associations were identified between genotype and the adverse effects of polyphagia, gastrointestinal signs, or behavioral changes. The rate of sedation was significantly higher in the groups of dogs with the M/N

Table 2. Phenobarbital and bromide dose and serum concentrations in dogs grouped by ABCB1 genotype.

	M/M (n = 10)	M/N (n = 9)	N/N (n = 4)	*P*-Value
Phenobarbital dose (mg/kg/d)	4.71 (1.56–9.43)	4.42 (3.13–13.56)	4.12 (3.38–6.26)	.918
Serum phenobarbital concentration (μg/mL)	19.1 (8.0–29.2) (n = 5)	18.1 (11.4–39.9) (n = 6)	23.3 (11.6–26.0) (n = 1)	.858
Bromide dose (mg/kg/d)	24.9 (22.9–50.3)	50.5 (33.2–66.7)	121.0 (—)	.018
Serum bromide concentration (mg/dL)	118.8 (93.0–290.0)	220.8 (110.0–328.6)	380.0 (—)	.113

Values are expressed as median of range.

genotype (10 of 11 dogs, 91%) or N/N genotype (4 of 4 dogs, 100%) compared with the dogs with the M/M genotype (3 of 12, 25%) ($P = .001$).

Discussion

This study demonstrated an association between ABCB1 genotype and seizure outcome in epileptic Collies. Dogs with the M/M genotype had a lower seizure frequency and incidence of cluster seizures, were being managed with fewer AEDs, and had a lower incidence of adverse events than dogs with either the M/N or N/N genotype. These findings suggest that epileptic Collies with the M/M genotype are less likely to have drug-resistant epilepsy when compared with epileptic Collies with the M/N or N/N genotype. Furthermore, M/N dogs appeared phenotypically similar to N/N dogs with respect to the parameters evaluated in this study, namely seizure outcome, number of AEDs being administered and incidence of drug-related adverse events. This finding suggests that drug resistance as defined in this study is associated with the presence of at least 1 normal allele.

The International League Against Epilepsy has recently proposed a consensus definition of drug-resistant epilepsy in humans as "a failure of adequate trials of two tolerated and appropriately chosen and used AED schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom."[30] Although similar, precise criteria have not been accepted in veterinary medicine, it is generally agreed that an animal that continues to have frequent or severe seizures or intolerable adverse effects despite appropriate AED therapy is considered resistant to treatment.[31] For both phenobarbital and bromide, established therapeutic serum concentrations should be achieved in order for therapy to be considered appropriate. The present study utilized this general definition of refractory epilepsy, with poor seizure outcome denoted by a frequency of ≥ 1 seizure per month, or the presence of cluster seizures, in a dog on current AED therapy. Although serum concentrations were measured, a specific minimum serum concentration was not required as an inclusion criteria, as the study was based on the premise that dogs with the ABCB1-1Δ mutation might achieve higher AED concentrations in the brain than would be expected based on serum concentrations, and therefore established reference ranges might not be applicable.

Table 3. Incidence of adverse effects grouped by ABCB1 genotype.

	M/M (n)	M/N (n)	N/N (n)	*P*-Value
PU/PD	0% (0/12)	45% (5/11)	0% (0/4)	.012
Polyphagia	25% (3/12)	27% (3/11)	0% (0/4)	.689
Sedation	25% (3/12)	91% (10/11)	100% (4/4)	.001
Behavior changes	8% (1/12)	9% (1/11)	25% (1/4)	.526
Gastrointestinal signs	0% (0/12)	9% (1/11)	0% (0/4)	.556

The present study was not designed to permit an assessment of response to AED treatment. Data on seizure frequency before initiating AED treatment were not required for study inclusion, such that comparisons of seizure frequency before and after treatment are not possible. Accordingly, the seizure outcome evaluated in this study is likely a reflection of response to AED therapy as well as clinical course of disease.

The potential role of ABCB1 polymorphism in drug-resistant epilepsy in dogs has been evaluated in two previous studies. A study involving 50 Australian shepherds with idiopathic epilepsy identified the M/N and M/M genotypes in 22 and 2% of the dogs, respectively, but did not establish an association between seizure control and ABCB1 genotype.[32] However, M/N and M/M dogs were grouped together and compared with N/N dogs, as only 1 dog in the study population was determined to have the M/M genotype. The authors acknowledge that this could have biased an association between the mutation and good seizure control. Interestingly, the 1 Australian Shepherd with the M/M genotype in the previous study was determined to have good seizure control, which is consistent with the findings of the present study.

The 2nd study involved a group of 25 Border Collies with idiopathic epilepsy, that was shown to have a very low (0.04%) incidence of the ABCB1-1Δ mutation.[33] However, the investigators identified a mutation in a noncoding, promoter region of the gene that was associated with resistance to phenobarbital therapy, and hypothesized that regulatory mutations might

affect the expression level of ABCB1 and PGP, which could in turn influence the response to AED therapy. Studies evaluating ABCB1 heterogeneity in humans with epilepsy have yielded conflicting results, and a recent meta-analysis failed to identify an association between genotype and treatment response in humans with epilepsy.[34]

The role of PGP in the transport of AEDs has been extensively evaluated in vitro. The discrepant results obtained from many of these studies can be attributed to differences in the assays utilized, species differences with respect to PGP transport of AEDs, and differences in the concentration of drug evaluated which influences the extent of PGP mediated transport. Nonetheless, there is substantial evidence to suggest that many AEDs, including phenobarbital, are weak substrates for PGP in rodents, humans, and dogs at clinically relevant doses.[35,36] Compared with high affinity PGP substrates such as digoxin and cyclosporine for which many of the in vitro assays are optimized, AEDs would be expected to be relatively weak PGP substrates, so as to allow penetration through the blood-brain barrier under conditions in which PGP is normally expressed. An in vivo study utilizing a rodent model of chronic epilepsy demonstrated that upregulation of PGP is associated with reduced brain levels of phenytoin and seizure control,[37] supporting the notion that PGP overexpression might play a role in the diseased brain but not the resting state.

Most previous investigations into the role of ABCB1 in epilepsy of humans and rodents have concentrated on the regional upregulation of PGP that occurs within a seizure focus.[15,16,20,21] In contrast, Collies with the ABCB1-1Δ mutation lack normal PGP activity throughout the brain, resulting in global brain accumulation of PGP substrates. Collies with the M/M genotype display signs of CNS depression after administration of loperamide, while wild-type Collies do not.[38] Similarly, one would expect a Collie with the M/M genotype to achieve higher concentrations of PGP transported AEDs throughout the brain, and a greater incidence of CNS-related adverse effects. However, the opposite was seen in this study, in that Collies with the M/M genotype had a significantly lower incidence of sedation compared with dogs with the M/N or N/N genotype. This might be attributed to the fact that the M/M dogs were receiving fewer AEDs than the other groups, as the sedative effects of AEDs are often additive. Measurement of brain AED levels would be necessary to evaluate whether differences in CNS AEDs concentrations occur based on ABCB1 genotype, and this was not feasible in the present study. Furthermore, it is not known whether M/M Collies with epilepsy behave similarly to M/M Collies without epilepsy with respect to drug penetration into the CNS. Studies in nonepileptic knockout mice with the ABCB1-1Δ mutation have yielded conflicting results; some studies have failed to demonstrate higher brain concentrations of AEDs in mice homozygous for the mutation compared with mice with the wild-type genotype,[39,40] while another research team has identified significantly higher AED concentrations in the hippocampus of knockout mice compared with wild-type littermates.[14]

Nonetheless, the finding that Collies with the M/M genotype had a better seizure outcome than dogs of the M/N or N/N genotype with fewer reported AED-related adverse effects does not support the transporter hypothesis of PGP-mediated drug resistance. It seems more likely that the association identified in this study between genotype and seizure outcome is an epiphenomenon, with the ABCB1-1Δ mutation being associated with a less robust seizure phenotype that favors drug efficacy. Recently, the intrinsic severity model of epilepsy has been proposed as an explanation for pharmacoresistance, which hypothesizes that there is a continuum in severity of epilepsy reflected in the frequency of seizures in the early phase of disease that determines relative response to medication. It is believed that common biological factors might underlie both severity of epilepsy and drug refractoriness, although currently these factors are poorly understood.[41]

Serum concentrations of phenobarbital and bromide did not differ significantly among Collies based on seizure outcome. These results do not support the premise that individuals with lower serum concentrations have a greater risk of seizures, as has been demonstrated in humans with epilepsy.[42] In addition, dogs classified as having a poor seizure outcome were being treated with a significantly greater number of AEDs than dogs with a good seizure outcome. A lack of association between seizure control and either serum AED concentrations or number of AEDs administered was described in Australian Shepherds with epilepsy.[32] An influential study of humans with epilepsy determined that among individuals with inadequate response to initial AED treatment, only 3% became seizure free when treatment was attempted with a combination of 2 drugs.[43] These findings further support the notion that the clinical course of epilepsy is dependent on intrinsic factors present at the onset of disease, rather than being solely influenced by therapeutic interventions. Indeed, epidemiologic studies on humans indicate that the natural history of epilepsy is complex.[44]

Although not the primary intent, this study provides information on the clinical manifestations of epilepsy in Collies. It is presumed that most dogs in the study population had idiopathic epilepsy, although a complete neurodiagnostic evaluation was not performed. However, it seems less likely that dogs with active underlying neurological disease would be included based on the requirement of a minimum 6-month history of epilepsy and normal examination and laboratory analysis as performed by the primary veterinarian. Breed differences in the clinical course of idiopathic epilepsy have been described, and Collies appear to have a less severe clinical manifestation of epilepsy when compared with other breeds for which data are available. Sixty-two percent (18/39) of the Collies evaluated in this study were classified as having a good seizure outcome, with a history of isolated seizures occurring less than once a month, and 16 (89%)

of these dogs were being managed on a single AED. Furthermore, over half (9/16) of the dogs that were being administered a single AED and were classified as having a good seizure outcome had serum AED concentrations below the accepted therapeutic range for dogs (20 μg/mL for phenobarbital, 100 mg/dL for bromide). In contrast, studies on idiopathic epilepsy in Australian Shepherds[32] and Border Collies[45] have demonstrated both breeds to have a severe clinical course, with cluster seizures, status epilepticus, or both, occurring in 80 and 98% of dogs, respectively.

Some of the study limitations have been previously discussed, namely the fact that treatment outcome was not assessed and that dogs had only a presumptive diagnosis of idiopathic epilepsy. Additional limitations arise from the fact that data on seizure frequency were owner-derived and retrospectively collected, and therefore are subject to inaccuracies and bias. Finally, the study assessed associations between factors, which should not be overinterpreted to imply causation.

In conclusion, the study demonstrated an association between ABCB1 genotype and seizure outcome in Collies with epilepsy, with M/M dogs having a more favorable outcome than dogs with the M/N or N/N genotype. This is not thought to be caused by higher levels of AEDs in the brain of M/M dogs that lack functional PGP, and its significance remains uncertain. The association might be a reflection of intrinsic variations in seizure severity among phenotypes. The Collie breed, with a predisposition to both epilepsy and ABCB1 heterogeneity, offers a unique opportunity to further explore the role of the ABCB-1Δ mutation in drug-resistant epilepsy.

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