Renin-angiotensin aldosterone profile before and after angiotensin-converting enzyme-inhibitor administration in dogs with angiotensin-converting enzyme gene polymorphism

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Abstract
Background: An angiotensin-converting enzyme (ACE) gene polymorphism occurs in dogs; however, functional importance is not well studied.

Hypothesis: We hypothesized that dogs with the polymorphism would show alternative renin-angiotensin aldosterone system (RAAS) pathway activation and classical RAAS pathway suppression before and after ACE-inhibitor administration, as compared to dogs without the polymorphism that would show this pattern only after ACE-inhibitor administration.

Animals: Twenty-one dogs with mitral valve disease that were genotyped for the ACE gene polymorphism.

Methods: This retrospective study utilized stored samples from 8 ACE gene polymorphism-negative (PN) dogs and 13 ACE gene polymorphism-positive (PP) dogs before and after enalapril administration. Equilibrium analysis was performed to evaluate serum RAAS metabolites and enzyme activities. Results were compared before and after enalapril, and between groups.

Results: The classical RAAS pathway was suppressed and the alternative RAAS pathway was enhanced for both genotypes after administration of enalapril, with no differences before enalapril administration. Aldosterone breakthrough occurred in both PN (38%) and PP (54%) dogs despite angiotensin II suppression. Aldosterone was significantly higher \((P = .02)\) in ACE gene PP dogs (median, 92.17 pM; IQR, 21.85-184.70) compared to ACE gene PN dogs (median, 15.91 pM; IQR, <15.00-33.92) after enalapril.

Conclusions and Clinical Importance: The ACE gene polymorphism did not alter baseline RAAS activity. Aldosterone breakthrough in some dogs suggests non-angiotensin mediated aldosterone production that might be negatively influenced by genotype. These results support the use of aldosterone receptor antagonists with

Abbreviations:
AA2, aldosterone to angiotensin II ratio; ABT, aldosterone breakthrough; ACE, angiotensin-converting enzyme; ACE-S, angiotensin-converting enzyme activity marker; PCR, polymerase chain reaction; PN, polymorphism negative; PP, polymorphism positive; PRA-S, plasma renin activity marker; RAAS, renin-angiotensin aldosterone system.
ACE-inhibitors when RAAS inhibition is indicated for dogs, especially those positive for the ACE gene polymorphism.

**KEYWORDS**
ACE-inhibitor, enalapril, genotype, pharmacogenetic, pharmacogenomic

1 | INTRODUCTION

The classical renin-angiotensin aldosterone system (RAAS) is the neurohormonal cascade initiated by the release of renin that cleaves angiotensin I from angiotensinogen, followed by the conversion of angiotensin I to angiotensin II by the angiotensin-converting enzyme (ACE) and production of angiotensin II metabolites (angiotensin III and angiotensin IV) by aminopeptidases, and ending in the stimulation of aldosterone release from the adrenal glands.\(^1\) Angiotensin II is the major stimulator of aldosterone synthesis and release from the adrenal cortex, although hyperkalemia and adrenocorticotropic stimulating hormone can also induce aldosterone release.\(^2,3\) The RAAS is activated in dogs with advanced heart disease, and contributes to disease progression and clinical signs.\(^1,4-7\) The major end-metabolites of the classical RAAS are angiotensin II and aldosterone, both of which directly or indirectly mediate sodium and water retention, vasoconstriction, and pathological remodeling of cardiac, vascular, and renal tissues.\(^1,4,6-7\) Recent reports describe an alternative RAAS pathway mediated by ACE2, prolyl-carboxy-peptidase 16, prolyl-endopeptidase, and nephrilysin, with production of the metabolites angiotensin 1-9, angiotensin 1-7 and angiotensin 1-5 in people and dogs.\(^8-11\) Additional metabolites within this cascade have also been discovered including angiotensin 2-10, angiotensin 2-7, and angiotensin 3-7.\(^8,10,11\) Whereas the functional importance of some metabolites is not yet known, angiotensin 1-7 has counterbalancing vasodilatory and natriuretic properties.\(^10\)

Angiotensin-converting enzyme-inhibitors prevent the conversion of angiotensin I to angiotensin II, and thereby mitigate the maladaptive effects of these neurohormones through reduced formation of angiotensin II and angiotensin-II-driven aldosterone production.\(^1\) Polymorphisms of the ACE gene are recognized in human beings and dogs, which could influence the therapeutic strategies and outcome of diseases in which the RAAS plays a pathogenic role.\(^12-14\) Directly measured ACE activity is lower in dogs that are homozygous for a single base pair ACE gene polymorphism compared to dogs without the polymorphism; however, ACE activity after administration of ACE-inhibitors is similarly suppressed regardless of genotype.\(^13,14\) The functional effects and clinical importance of these findings are not well understood.

Although the RAAS has historically been challenging to investigate, renin-angiotensin system equilibrium analysis has emerged as a novel technique that allows for comprehensive evaluation of this complex neurohormonal system. Catalyzing enzymes other than ACE (such as ACE2, nephrilysin, chymase, and aminopeptidases) mediate production of RAAS metabolites in this pathway, and recent publications have begun to shed light on blood and tissue activities in dogs.\(^8-11\) This methodology has shown that ACE-inhibitors not only suppress angiotensin II formation, but also increase levels of the beneficial peptide hormone angiotensin 1-7, which is known to be metabolized to angiotensin 1-5 by the N-domain of ACE.\(^15\) We sought to use equilibrium analysis to further investigate the functional effects of the ACE gene polymorphism at 9:11507816:G>A in dogs. Based on previous results showing reduced ACE activity in dogs positive for the ACE gene polymorphism,\(^13,14\) we hypothesized that polymorphism-positive dogs would show activation of the alternative RAAS pathway and suppression of the classical RAAS pathway both before and after administration of enalapril, as compared to polymorphism-negative dogs that would show this pattern only after ACE-inhibitor administration.

2 | METHODS

This study was approved by the Institutional Animal Care and Use Committee at North Carolina State University College of Veterinary Medicine, and client consent was obtained. Dogs diagnosed with myxomatous mitral valve disease were included if they had not received cardiac medications, had at least a grade 4/6 left apical systolic murmur, and cardiac enlargement as determined by a radiographic vertebral heart scale of at least 11.\(^16\) Dogs with other systemic diseases as evident from routine chemistry panel or history were excluded. This study utilized samples stored from dogs meeting inclusion criteria that were previously genotyped for the ACE gene polymorphism at 9:11507816:G>A, 19 of which were previously reported.\(^14\) Biochemistry results, blood pressure, and ACE activity determined by radio-enzymatic activity have been previously reported.\(^14\) Methodology for sample collection is summarized later.

Three milliliters of blood were obtained by peripheral venipuncture at the initial examination. One milliliter of blood was placed into an EDTA tube for genotyping. Two milliliters of blood were placed into a no-additive tube. After centrifugation, the serum was removed and frozen at \(-80^\circ\)C. After the initial evaluation, all dogs were prescribed 0.5 mg/kg of enalapril orally every 12 hours, and repeat blood sampling to obtain serum was performed after 2 weeks of medication. Serum samples were frozen at \(-80^\circ\)C until overnight shipping on dry ice to the laboratory for batch analysis of RAAS metabolites as described later.

2.1 | Genotyping

The previously validated primers (forward, 5’ TCAGCTCCCATGCAAT CCATA 3’; reverse 5’ CCCCTTGCCCTATCTGTAAA 3’) were used to
### TABLE 1
Renin-angiotensin aldosterone system (RAAS) metabolites and ratios, pre- and post-enalapril, for ACE polymorphism negative dogs and ACE polymorphism positive dogs

| Variable                  | PN dogs (n = 8) | Post-enalapril | PP dogs (n = 13) | Post-enalapril | Pre-enalapril versus post-enalapril (P value) | PN versus PP (P value) |
|---------------------------|----------------|----------------|------------------|----------------|-----------------------------------------------|------------------------|
| Ang I (pM)                | 134.2 (70.59-150.6) | 702.0 (432.3-914.9) | 119.2 (50.62-156.60) | 747.20 (353.8-1068.0) | 0.0078                                        | 0.0003                 |
| Ang II (pM)               | 55.14 (31.32-83.18)  | 10.96 (7.86-22.41)  | 47.56 (30.59-89.14)  | 12.64 (3.83-21.43)  | 0.0078                                        | 0.0024                 |
| Ang 1-7 (pM)              | 55.03 (41.30-95.79)  | 172.2 (120.7-277.5) | 34.54 (17.16-87.23) | 127.30 (58.43-248.90) | 0.039                                           | 0.0019                 |
| Ang 1-5 (pM)              | 49.3 (40.33-103.0)   | 5.86 (2.03-17.07)   | 4.90 (3.09-10.65)   | 3.00 (2.50-6.37)    | 0.0078                                        | 0.0005                 |
| Ang III (pM)              | 5.19 (>2.50-9.59)    | >2.50 (>2.50- < 2.50) | >2.50 (>2.50-5.25) | >2.50 (>2.50-5.25) | 0.0625                                          | 0.0039                 |
| Ang IV (pM)               | 8.09 (3.89-12.54)    | <2.00 (<2.00-4.16)  | 7.92 (<2.00-11.56)  | 2.43 (<2.00-4.02)   | 1.56                                            | 0.059                  |
| Aldosterone (pM)          | <15.00 (<15.00-30.38) | 15.91 (<15.00-33.92) | 17.13 (<15.00-77.77) | 92.17 (21.85-184.70) | 1.00                                           | 0.32                   |
| AA2                      | 0.2 (0.18-0.76)      | 1.15 (0.65-4.49)    | 0.56 (0.26-203)     | 6.82 (1.72-21.45)   | 0.02                                           | 0.0002                 |
| PRA-S (pM)                | 173.4 (130.6-212.0)  | 713.6 (436.6-936.8) | 161.8 (83.19-262.6) | 749.9 (35.59-1087.0) | 0.016                                          | 0.0007                 |
| ACE-S                    | 0.31 (0.24-0.76)     | 0.02 (0.013-0.030)  | 0.45 (0.37-0.76)    | 0.020 (0.01-0.03)   | 0.0078                                        | <0.0001               |
| Ang1-5/Ang 1-7           | 1.05 (0.96-1.25)     | 0.05 (0.01-0.06)    | 0.98 (0.75-1.32)    | 0.05 (0.03-0.07)    | 0.0078                                        | 0.0002                 |

Note: Values are shown as median and interquartile range. Statistically significant P values are bolded. Values below the lower limit of quantification are shown as < the lowest reported value for each assay.

Abbreviations: AA2, aldosterone to angiotensin II ratio; ACE-S, angiotensin converting enzyme marker; Ang 1-5, Angiotensin 1-5; Ang 1-5/Ang1-7, angiotensin 1-5:angiotensin 1-7 ratio; Ang 1-7, Angiotensin 1-7; Ang I, Angiotensin I; Ang II, Angiotensin II; Ang III, Angiotensin III; Ang IV, Angiotensin IV; PN, polymorphism negative; PP, polymorphism positive; PRA-S, plasma renin activity marker.

### 2.2 | Equilibrium analysis of RAAS components

The equilibrium concentrations of 6 different RAAS angiotensin peptide metabolites and aldosterone in canine serum samples were measured using liquid chromatography-mass spectrometry analysis performed at a service provider laboratory (Attoquant Diagnostics, Vienna, Austria) using previously validated and described methods.8,10,17 Briefly, serum conditioning for equilibrium analysis was performed at 37°C followed by circulating (stable isotope labeled internal standards for each peptide were included in the ACE polymorphism-positive group) and equilibrium angiotensin metabolite as well as with the deuterated internal standard for aldosterone (aldosterone D4) at a concentration of 150 pM for aldosterone.

Increase in serum aldosterone after enalapril compared to baseline was defined as any increase not due to a direct increase in serum aldosterone but rather a result of aldosterone breakthrough (ABT) was calculated as the increase in aldosterone in the ABT group compared to the pre-enalapril baseline aldosterone concentration. The ratio of ABT to baseline (ABT/Baseline) was calculated as an indicator of adrenal responsiveness to angiotensin II stimulation of aldosterone release. Aldosterone breakthrough after enalapril compared to baseline (pre-enalapril) aldosterone increase in serum aldosterone after enalapril compared to baseline.

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2.3 | Statistical analysis

Statistical analysis was performed using commercially available software (GraphPad Prism 8, San Diego, California). Data values that were below the lower limit of assay quantification were reported as half the lower limit for statistical analysis only.\textsuperscript{19,20} Data sets were divided by polymorphism status as positive (PP) (including heterozygous and homozygous) or negative (PN), as well as by pre- or post-ACE-inhibitor (enalapril) treatment. Data were tested for normality using the D'Agostino and Pearson test and reported as medians and interquartile range because most were not normally distributed. Paired data (pre- and post-enalapril data for each genotype group) were compared using Wilcoxon matched-pairs signed rank test if data were nonparametric or using a 2-way, paired t test if data were parametric. Unpaired data between groups (genotype comparison for pre-enalapril and genotype comparison for post-enalapril) were compared using Mann-Whitney test if nonparametric or 2-way, unpaired t test if parametric. Fisher's exact test was used to evaluate the effect of genotype on ABT. Significance was set at $P < .05$.

3 | RESULTS

Eight PN dogs and 13 PP dogs were included. The mean age of PN dogs was $9.9 \pm 3.8$ (range, 5-16) years, the mean weight was $9.0 \pm 4.5$.
(range, 2.5-18.9) kg, and the breeds were 3 Cavalier King Charles Spaniels, 2 Yorkshire Terriers, and 1 each of American Cocker Spaniel, Shih Tzu, and Toy Poodle. The mean age of PP dogs was 8.6 ± 1.8 (range, 7-14) years, the mean weight was 11.1 ± 5.1 (range, 6.0-22.0) kg, and the breeds were 8 Cavalier King Charles Spaniels, 2 Miniature Poodles and 1 each of mixed breed (Labradoodle), Norfolk Terrier, and Airedale. Polymorphism-positive dogs included 8 dogs that were heterozygous for the polymorphism and 5 dogs that were homozygous for the polymorphism. There was no statistical difference in age ($P = .3$) or weight ($P = .3$) between PN and PP dogs. The mean (SD) time between assessments was 18.9 ± 9.9 days for PN dogs and 14.6 ± 3.0 days for PP dogs ($P = .2$).

### 3.1 | Pre- and post-enalapril comparisons

Polymorphism-negative dogs showed a statistically significant increase in angiotensin I, angiotensin 1-7, PRA-S, and AA2, and a statistically significant decrease in angiotensin II, angiotensin 1-5, ACE-S and Ang 1-5/Ang 1-7 after treatment with enalapril. Three of 8 PN dogs (38%) demonstrated ABT (Table 1, Figure 1).

Polymorphism-positive dogs had a statistically significant increase in angiotensin I, angiotensin 1-7, PRA-S, and AA2, as well as a statistically significant decrease in angiotensin II, angiotensin I-5, angiotensin III, angiotensin IV, ACE-S and Ang 1-5/Ang 1-7 ratio after treatment with enalapril. Seven of 13 PP dogs (54%) demonstrated ABT.

### 3.2 | Genotype comparisons

No significant differences in the RAAS profile and enzyme activities were present between PN and PP dogs before enalapril treatment (Table 1, Figure 1). Post-enalapril group comparisons showed significantly greater aldosterone concentrations and AA2 in PP dogs compared to PN dogs but the number of dogs that exhibited ABT was not different between genotypes (3 PN versus 7 PP; $P = .6$). When only the dogs that exhibited ABT were compared between genotypes, the percentage increase (PP median 658% compared to PN 334%; Figure 2) and absolute increase (PP 155 pM compared to PN 26 pM) in aldosterone was greater for PP dogs compared to PN dogs ($P = .02$).

### 4 | DISCUSSION

This study failed to demonstrate different degrees of ACE activity based on genotype before ACE-inhibitor treatment, in contrast to results of previous studies. Although unexpected, this might be explained by the different methodologies used to quantify ACE activity between studies. Radioenzymatic assay was utilized in previous reports to directly measure the activity of the ACE enzyme. The activity of ACE in the current study was determined indirectly using the turnover of angiotensin I to angiotensin II, measured by equilibrium analysis. Theoretically, this approach could incorporate the contribution of other enzymes to angiotensin II formation and degradation, such as aminopeptidases and chymase that could be altered in PP dogs to maintain a stable angiotensin II level. Alternatively, the affinity of the ACE enzyme for the substrate used in the radioenzymatic assay may be affected by the presence of the ACE gene polymorphism, which could affect results in vitro. Additionally, the overall degree of RAAS activation before enalapril administration was not different between genotypes, suggesting that the functional effects of the polymorphism on angiotensin II production in the whole animal may be counterbalanced by upregulation of other enzymes before administration of ACE-inhibitors.

This study also demonstrated a significantly greater aldosterone concentration and AA2 in ACE gene PP dogs, compared to PN dogs after treatment with the ACE-inhibitor, enalapril. The remainder of the classical and alternative RAAS pathway components were not different based on genotype at baseline and after enalapril treatment. Suppression of the classical RAAS pathway and enhancement of the alternative RAAS pathway with the administration of enalapril was explained by inhibition of the ACE enzyme; however, with the exception of aldosterone and AA2, we did not find differences in the cascade based on genotype. The finding of greater post-enalapril aldosterone and AA2 ratio (the latter supports aldosterone production independent of angiotensin II) in PP dogs was unexpected and potentially clinically important, as it provides a potential explanation for ABT in dogs.

The RAAS profile in both genotype groups showed significant suppression of angiotensin II formation as a result of approximately 2-weeks’ administration of an ACE-inhibitor. This finding, presumed to be secondary to ACE inhibition, was accompanied by expected increases in angiotensin I, PRA-S (enhanced renal renin secretion because of negative feedback of lower angiotensin II), suppression of classical RAAS metabolites (angiotensin III and IV) and enhancement of the alternative RAAS metabolite angiotensin 1-7. The Ang 1-5/Ang
formation of aldosterone through other pathways. These findings are 
consistent with ABT; however, this importantly does not 
appear to be mediated by angiotensin II that was appropriately 
suppressed with enalapril treatment. Aldosterone breakthrough is 
the term used to describe inadequate or temporary suppression of aldoste-
rone, despite the administration of appropriate doses of ACE-inhibi-
tors. The phenomenon of ABT has been shown to occur in both 
people and dogs. Underlying mechanisms of ABT may include 
upward drift of ACE activity in the face of chronic RAAS-suppressive 
treatment or ACE-independent production of either angiotensin II or 
aldosterone. We found no evidence of inadequate ACE suppres-
sion in this study. The finding of high aldosterone in conjunction with 
angiotensin II formation in some dogs in this study suggests that 
ABT is occurring independent of angiotensin II. A multitude of pep-
tides serve as substrates for ACE, and the accumulation of these pep-
tides as a result of ACE-inhibition could be contributing to the 
formation of aldosterone through other pathways. These findings are 
important because they demonstrate the efficacy of enalapril in 
suppressing angiotensin II formation, but also show ABT and indicate 
that multimodal RAAS suppression may be indicated to address modula-
tion of other pathways by ACE-inhibitors. Angiotensin II and aldoste-
rone, directly or indirectly, contribute to sodium and fluid retention, and 
both promote inflammation, cell death, and fibrosis. These results pro-
vide further evidence of ABT and indicate that the combination of 
mineralocorticoid receptor antagonists (eg, spironolactone or eplerenone) 
and inhibition of angiotensin II formation or binding (ACE-inhibitors or 
angiotensin receptor blockers, respectively) is often necessary to pro-
vide comprehensive RAAS inhibition. Additional study is required to 
elucidate other pathways that may be impacted by RAAS inhibitors. 

Additionally, we found that the magnitude of ABT was greater in 
PP dogs compared to PN dogs despite suppression of angiotensin II for-
mation with enalapril in both groups. This was also reflected in signifi-
cantly greater AA2 ratio in PP dogs after enalapril treatment, indicating 
that the high aldosterone was independent of angiotensin II. This finding 
suggests an influence of the ACE gene polymorphism on an, as of yet 
unidentified angiotensin II-independent pathway of aldosterone stimula-
tion. In addition to the many possible substrates for ACE, there is recent 
evidence that numerous non-angiotensin-mediated, nonelectrolyte fac-
tors can control aldosterone secretion from the adrenal cortex. These 
paracrine regulatory factors include bioactive signals released from mast 
cells (eg, serotonin), nerve fibers (eg, catecholamines), chromaffin cells, 
adipocytes (eg, leptin), vascular endothelial cells (eg, endothelin 1), and 
steroidogenic cells (eg, prostaglandin E2). The influence of genotype in 
this setting requires further study to explore the impact of this polymor-
phism on dogs receiving ACE inhibition.

The results of this study provide mechanistic insight into the phe-
nomenon of ABT and hold clinical implications for dogs with advanced 
heart disease. Although ABT occurs in a substantial minority (30%- 
40%) of people and dogs receiving ACE-inhibitors, predicting which 
patients are or will be affected is elusive. Aldosterone break-
through occurring with short-term ACE-inhibitor monotherapy in this 
study was independent of angiotensin II, and the ACE gene polymor-
phism appeared to negatively influence aldosterone concentrations in 
this group of dogs with preclinical mitral valve disease. Dogs positive 
for the ACE gene polymorphism might be at greater risk for adverse 
effects associated with unopposed circulating aldosterone than dogs 
negative for the polymorphism, despite the effectiveness of enalapril 
in suppressing angiotensin II production in both groups. The genetic 
heterogeneity of the ACE gene and the nonuniversal occurrence of 
ABT in dogs could be an explanation for discordant outcome findings 
of previous studies reporting the long-term effects of ACE-inhibitors 
in dogs with heart disease. 

This study is limited by the small study population, which could 
have affected the ability to find differences between groups for some 
parameters. We did not separately analyze dogs that were homozy-
gous from those that were heterozygous for the ACE gene polymor-
phism because of small subject numbers. Future studies with larger 
numbers will be necessary to determine if there are RAAS profile dif-
fferences in dogs with 1 or both abnormal alleles. Genotyping might 
also prove useful for determining subpopulations of dogs that would 
benefit the most from aldosterone antagonizing medications. 

In conclusion, we demonstrated that ABT occurs independent of 
successful angiotensin II suppression with ACE-inhibitor treatment in 
some dogs, and that the ACE gene polymorphism seems to negatively 
influence this suppression. Additionally, the presence of the polymor-
phism appears to not reduce overall RAAS activation in the untreated 
dog, possibly because of upregulation of compensatory enzymes that 
might serve to stabilize angiotensin II levels. These findings not only 
(Figure 1). Promotion of angiotensin 1-7 (predom-
antly through neprilysin conversion of angiotensin I to angio-
tensin 1-7) is believed to mediate ACE-inhibitor benefits of 
vasodilation and natriuresis. Alternate RAAS metabolite production 
did not differ based on genotype.
HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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REFERENCES
1. Ames MK, Atkins CE, Pitt B. The renin-angiotensin-aldosterone system and its suppression. J Vet Intern Med. 2019;33:363-382.
2. Sztechman D, Czarzasta K, Cudnoch-Jedrzejewska A, et al. Aldosterone and mineralocorticoid receptors in regulation of the cardiovascular system and pathological remodelling of the heart and arteries. J Physiol Pharmacol. 2018;69:829-845.
3. Castrop H, Lorenz JN, Hansen PB, et al. Contribution of the basolateral isofom of the Na-K-2Cl cotransporter (NKCC1/BSC2) to renin secretion. Am J Physiol - Ren Physiol. 2005;289:1185-1192.
4. Sisson DD. Neuroendocrine evaluation of cardiac disease. Vet Clin North Am - Small Anim Pract. 2004;34:1105-1126.
5. Fujii Y, Orito K, Muto M, Wakao Y. Modulation of the tissue renin-angiotensin-aldosterone system in dogs with chronic mild regurgitation through the mitral valve. Am J Vet Res. 2007;68:1045-1050.
6. The Cove Study Group. Controlled clinical evaluation of Enalapril in dogs with heart failure: results of the cooperative veterinary Enalapril study group. J Vet Intern Med. 1995;9:243-252.
7. The IMPROVE Study Group. Acute and short-term hemodynamic, echocardiographic, and clinical effects of Enalapril maleate in dogs with naturally acquired heart failure: results of the invasive multicenter PROspective veterinary evaluation of Enalapril study. J Vet Intern Med. 1995;9:234-242.
8. Domenig O, Manzel A, Grobe N, et al. Nepriylin is a mediator of alternative renin-angiotensin-system activation in the murine and human kidney. Sci Rep. 2016;6:1-11.
9. Antlanger M, Bernhofer S, Kovarik JI, et al. Effects of direct renin inhibition versus angiotensin II receptor blockade on angiotensin profiles in non-diabetic chronic kidney disease. Ann Med. 2017;49:525-533.
10. Basu R, Poglitsch M, Yogasundaram H, Thomas J, Rowe BH, Oudit GY. Roles of angiotensin peptides and recombinant human ACE2 in heart failure. J Am Coll Cardiol. 2017;69:805-819.
11. Larouche-Lebel É, Loughran KA, Oyama MA, et al. Plasma and tissue angiotensin-converting enzyme 2 activity and plasma equilibrium concentrations of angiotensin peptides in dogs with heart disease. J Vet Intern Med. 2019;33:1571-1584.
12. Sayed-Tabatabaei FA, Oostra BA, Isaacs A, van Duijn CM, Wittenman JCM. ACE polymorphisms. Circ Res. 2006;98:1123-1133.
13. Meurs KM, Olsen LH, Reimann MJ, et al. Angiotensin-converting enzyme activity in cavalier king Charles spaniels with an ACE gene polymorphism and myxomatous mitral valve disease. Pharmacogenet Genomics. 2018;28:37-40.
14. Meurs KM, Stern JA, Atkins CE, et al. Angiotensin-converting enzyme activity and inhibition in dogs with cardiac disease and an angiotensin-converting enzyme polymorphism. J Renin Angiotensin Aldosterone Syst. 2017;18:1-4.
15. Sharp S, Poglitsch M, Zilla P, Davies NH, Sturrock ED. Pharmacodynamic effects of C-domain specific ACE inhibitors on the renin angiotensin system in myocardial infarcted rats. J Renin Angiotensin Aldosterone Syst. 2015;16:1149-1158.
16. Lamb CR, Wikeley H, Boswood A, Pfeiffer DU. Use of breed-specific ranges for the vertebral heart scale as an aid to the radiographic diagnosis of cardiac disease in dogs. Vet Rec. 2001;148:707-711.
17. Pavo N, Goliash G, Wurm R, et al. Low-and high-renin heart failure phenotypes with clinical implications. Clin Chem. 2018;64:597-608.
18. Bomback AS, Klemmer PJ. The incidence and implications of aldosterone breakthrough. Nat Clin Pract Nephrol. 2007;3:486-492.
19. Keizer RJ, Jansen RS, Rosing H, et al. Incorporation of concentration data below the limit of quantification in population pharmacokinetic analyses. Pharmacol Res Perspect. 2015;3:1-15.
20. Ogden TL, Court M, Way M, et al. Values below detection limit in compositional chemical data. Pharmacogenet Genomics. 2019;764:303-339.
21. Ames MK, Atkins CE, Eriksson A, Hess AM. Aldosterone breakthrough in dogs with naturally occurring myxomatous mitral valve disease. J Vet Cardiol. 2017;19:218-227.
22. Lefebvre H, Duparc C, Naccache A, et al. Paracrine regulation of aldosterone secretion in physiological and pathophysiological conditions. Vitam Horm. 2019;109:303-339.
23. Semis M, Gugiu GB, Bernstein EA, et al. The plethora of angiotensin-converting enzyme-processed peptides in mouse plasma. Anal Chem. 2019;91:6440-6453.
24. Ames MK, Atkins CE, Lantis AC, et al. Evaluation of subacute change in RAAS activity (as indicated by urinary aldosterone:creatinine, after pharmacologic provocation) and the response to ACE inhibition. J Renin Angiotensin Aldosterone Syst. 2016;17:1-12.
25. Kvant C, Haagstrom J, Pedersen HD, et al. Efficacy of Enalapril for prevention of congestive heart failure in dogs with myxomatous valve disease and asymptomatic mitral regurgitation. J Vet Intern Med. 2002;16:80-88.
26. Atkins CE, Keene BW, Brown WA, et al. Results of the veterinary enalapril trial to prove reduction in onset of heart failure in dogs chronically treated with enalapril alone for compensated, naturally occurring mitral valve insufficiency. J Am Vet Med Assoc. 2007;231:1061-1069.

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