Angiotensin-converting enzyme activity in Cavalier King Charles Spaniels with an ACE gene polymorphism and myxomatous mitral valve disease

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Objectives **Myxomatous mitral valve disease (MMVD) is the most common heart disease in the dog. It is particularly common in the Cavalier King Charles Spaniel (CKCS) breed and affected dogs are frequently managed with angiotensin-converting enzyme inhibitors (ACE-I). We have previously identified a canine *ACE* gene polymorphism associated with a decrease in angiotensin-converting enzyme (ACE) activity. The aim of this study was to evaluate for the prevalence of the ACE polymorphism in CKCS with mitral valve disease and to determine whether the presence of the polymorphism is associated with alterations in ACE activity at different stages of cardiac disease.**

Methods **Seventy-three dogs with a diagnosis of mitral valve disease were evaluated and a blood sample was drawn for ACE polymorphism genotyping and ACE activity measurement.**

Results **Forty-three dogs were homozygous for the ACE polymorphism; five were heterozygous and 25 were homozygous wild type. The mean age and the median severity of disease were not different for dogs with the polymorphism and dogs with the wild-type sequence. The median baseline ACE activity was significantly lower for the ACE polymorphism (27.0 U/l) than the wild-type sequence dogs (31.0 U/l) ($P = 0.02$). Dogs with more severe disease and the ACE polymorphism had significantly lower levels of ACE activity than dogs with the wild-type sequence ($P = 0.03$).**

Conclusion **The CKCS appears to have a high prevalence of the ACE variant. Dogs with the ACE variant had lower levels of ACE activity even in more advanced mitral valve disease than dogs without the variant. The clinical significance of this finding and its impact on the need for ACE-I in dogs with the polymorphism and heart disease deserves further study. *Pharmacogenetics and Genomics* 28:37–40**

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Introduction

Myxomatous mitral valve disease (MMVD) is the most common cause of heart disease in the dog [1]. It is particularly common in the Cavalier King Charles Spaniel (CKCS) [1]. The disease course is typically characterized by an extended preclinical period, progressive cardiac remodeling, and in some cases, the development of congestive heart failure [1]. Angiotensin-converting enzyme inhibitors (ACE-I) are often prescribed for the management of MMVD during both the cardiac remodeling and clinical heart failure stages [2–5].

We have previously identified a single base pair polymorphism in the angiotensin-converting enzyme (*ACE*) gene in several breeds of dogs including the CKCS [6]. The *ACE* gene encodes for the production of angiotensin-converting enzyme, a metalloenzyme central to the activity of the renin–angiotensin–aldosterone system (RAAS). Within this system, ACE primarily cleaves angiotensin I to angiotensin II, the peptide responsible for activating angiotensin II type I receptors [7,8]. ACE activity is particularly important in heart failure, where its role in the production of angiotensin II helps maintain blood pressure in a failing heart by causing peripheral vasoconstriction and aldosterone secretion [8]. Although this activity is initially a hemodynamically beneficial response, prolonged pathological activation of the system is counterproductive, producing increases in preload, afterload, and myocardial fibrosis that eventually results in worsening heart failure [8]. ACE inhibition has become an important pharmacologic tool in suppressing

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the RAAS system as it becomes activated in cardiac disease and heart failure in an effort to try to control the detrimental effects of angiotensin II and aldosterone in both human beings and dogs [2].

The ACE polymorphism has been associated with a decrease in ACE activity in the dog [9]. As the CKCS is one of the most commonly affected breeds of dogs with mitral valve disease, the aim of this study was to evaluate for the prevalence of the ACE polymorphism in CKCS with mitral valve disease and to determine whether the presence of the polymorphism is associated with alterations in ACE activity at different stages of cardiac disease. We hypothesized that: (a) the ACE polymorphism would be prevalent in CKCS; (b) CKCS with the polymorphism would have reduced *ACE* activity compared with CKCS with the wild-type *ACE* gene; and (c) the difference in *ACE* activity between dogs with the ACE polymorphism and dogs with the normal (wild type) sequence would be more pronounced in dogs with more severe mital valve disease.

Methods

This study was carried out with participating dogs from the University of Copenhagen and the North Carolina State University's College of Veterinary Medicine. It was approved by the Danish Animal Experiments Inspectorate (license no. 2012-15-2934-00700) and was carried out in accordance with the guidelines of the North Carolina State University Institutional Animal Care and Use Committee (IACUC 13-103-0). Written consent was obtained from all owners.

CKCS with a diagnosis of preclinical (asymptomatic) mitral valve disease were recruited for participation in a study to evaluate different aspects of mitral valve endocardiosis in this breed. Some of the dogs were included in a previous study [10]. All dogs were at least 4 years of age and none of the dogs were receiving any cardiac medication. A small number of dogs were receiving additional medications for minor health issues and this information was recorded. All dogs underwent a standardized examination including a history, physical examination, and echocardiogram. Approximately 3–4 ml of blood was drawn for ACE polymorphism genotyping as described previously [6].

Briefly, PCR amplification primers for the previously reported SNP at canine chromosome 9:11507816 (dbSNP rs#: 850683722) were designed using Primer 3 software (*http://frodo.wi.mit.edu/*) and the canine nucleotide sequences from the Ensemble genomic database (*http://www.ensembl.org/index.html*). The forward primer was 5′ TCAGCTCCATGCAATCCATA 3′ and the reverse primer was 5′ CCCCTTGCCCTATCTGTAAA 3′.

Products were sequenced with both forward and reverse primers. PCR was carried out using a cocktail of water, 10× KCL Taq buffer, 1 mmol/l $MgCl_2$, 0.2 U/μlof reaction volume Taq DNA polymerase, 0.5 U/μl 0.4 mmol/l dNTPs, 0.4 μmol/l PCR amplification primers, and 100–200 μg DNA. The PCR protocol included 5 min at 95°C, 40 cycles of 94°C for 30 s, 57°C for 30 s, 72°C for 30 s, and 72°C for 7 min. Products were sequenced with both forward and reverse primers and analyzed on an ABI Prism 377 Sequencer (Foster City, California, USA). Nucleotide sequences were evaluated visually for sequence quality and aligned using SeqMan Pro (DNAStar, Madison, Wisconsin, USA) software to evaluate for DNA variants between the individual animals.

After the initial examination, 2–4 ml of blood was immediately placed in a red top tube and serum was separated by centrifugation and frozen at −80° for subsequent analysis of ACE activity using a radioenzymatic assay for the direct determination of ACE activity (Mayo Clinic, Rochester, Minnesota, USA). The severity of mitral valve disease was estimated by the degree of mitral valve regurgitation as described previously [11,12]. Briefly, the severity of mitral valve regurgitation was assessed in a blinded manner on the basis of a left apical four-chamber view of the systolic mitral regurgitation (MR) jet area using two-dimensional color flow Doppler flow mapping. The dogs were grouped as follows: group 1, mild (<20%), group 2, moderate (20–50%), and group 3, severe (>50%) [10,11].

Statistical analysis

ACE activity was compared between dogs that were homozygous for the wild type (normal canine reference sequence) or homozygous for the ACE polymorphism. Data were visually and statistically tested for normality using the D'Agostino Pearson omnibus normality test. Normally distributed data are reported as mean ± SD, whereas non-normally distributed data are reported as median with interquartile range.

An unpaired *t*-test was used to compare normally distributed data and a Mann–Whitney test was used to compare non-normally distributed data between the two genotype groups and between the two genotype groups at different stages of MR.

Results

The study included 73 CKCS (29 females, 15 spayed females, 21 male, eight castrated males). The mean age of the dogs was 8 ± 2 years.

Forty-three (59%) dogs were homozygous for the ACE polymorphism, five (7%) were heterozygous for the ACE polymorphism, and 25 (34%) were homozygous wild type. The CKCS population frequency of the ACE polymorphism allele was 0.62 and that of the wild-type allele was 0.38. On the basis of the allele frequencies within the population, the genotype frequencies were significantly different from that expected under Hardy–Weinberg equilibrium ($P < 0.00001$).

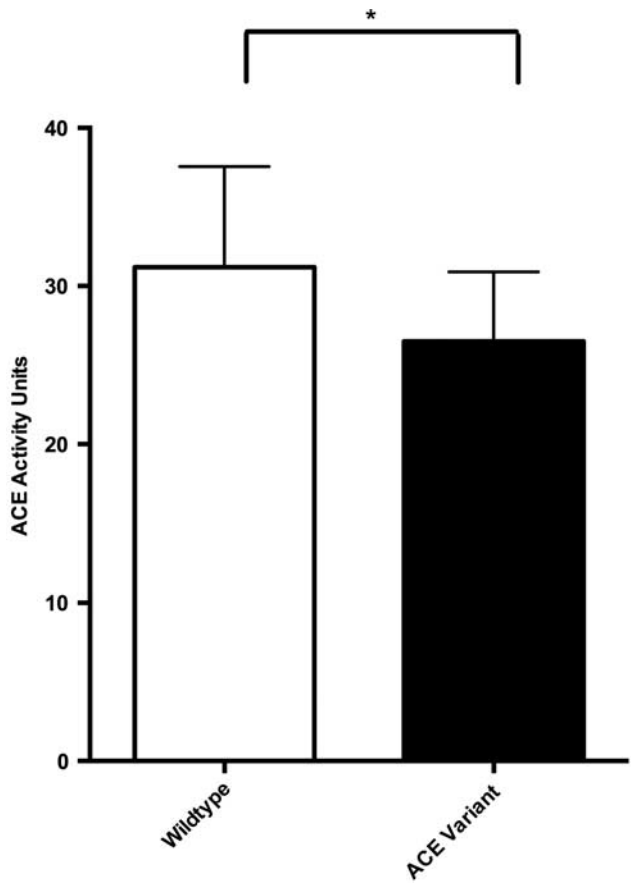
A small number of dogs were receiving medications for noncardiac-related issues including keratoconjunctivitis sicca and pruritis.

The mean age of ACE polymorphism dogs (7 ± 2 years) was not different from the wild-type group (8 ± 2 years; $P = 0.15$).

The median grade of heart murmur was two for both groups.

The median severity of MR for the ACE variant and the wild-type group was not different (2, 25–75% percentile of 1–3; 2, 1–3, respectively, $P = 0.20$).

Fig. 1



Median baseline ACE activity was significantly lower for dogs ($n = 43$) homozygous for the ACE variant (27.0 U/l) than for dogs ($n = 25$) with the wild-type sequence (32.0 U/l) ($P = 0.02$). *Statistical significance. The error whiskers indicate the interquartile range of the median. ACE, angiotensin-converting enzyme.

The median baseline ACE activity was significantly lower for dogs homozygous for the ACE polymorphism (27.0 U/l, 21–31) than for dogs with the wild-type sequence (31.0 U/l, 26–38, $P = 0.02$) (Fig. 1).

The severity of MR in group 1 included 14 dogs that were homozygous for the ACE variant and three dogs that were homozygous wild type. Group 2 included 13 dogs that were homozygous for the ACE variant and 13 dogs that were homozygous for the wild type. Group 3 included 16 dogs that were homozygous for the ACE variant and nine dogs homozygous for the wild type (Table 1). Dogs in MR severity groups 2 and 3 with the ACE polymorphism had significantly lower levels of ACE activity than dogs in these groups with the wild-type sequence [group 2 ($P = 0.02$) and group 3 ($P = 0.03$)] (Fig. 2 and Table 1).

Discussion

The results of this study support our first hypothesis that the *ACE* gene polymorphism is frequently observed in the CKCS breed. A previous study identified the ACE polymorphism in Boxers, Doberman pinschers, and CKCS, but the number of dogs evaluated was small and breed comparisons were not performed [6]. In this study, the majority (66%) of CKCS were homozygous positive for the ACE polymorphism. This study also confirmed our second hypothesis that CKCS with the ACE polymorphism had lower baseline levels of ACE activity than wild-type dogs.

In terms of our third hypothesis involving the relationship of ACE activity with mitral valve regurgitation severity, dogs with the ACE polymorphism appeared to experience only a small increase in ACE activity with worsening MR severity. This is surprising as it has been shown previously that dogs with mitral valve disease have greater ACE activity than healthy dogs, and that ACE activity in dogs with MR increases with disease severity as assessed by left ventricular diastolic wall stress [13,14]. In the study presented here, the relationship between ACE activity and disease severity differed on the basis of the ACE genotype.

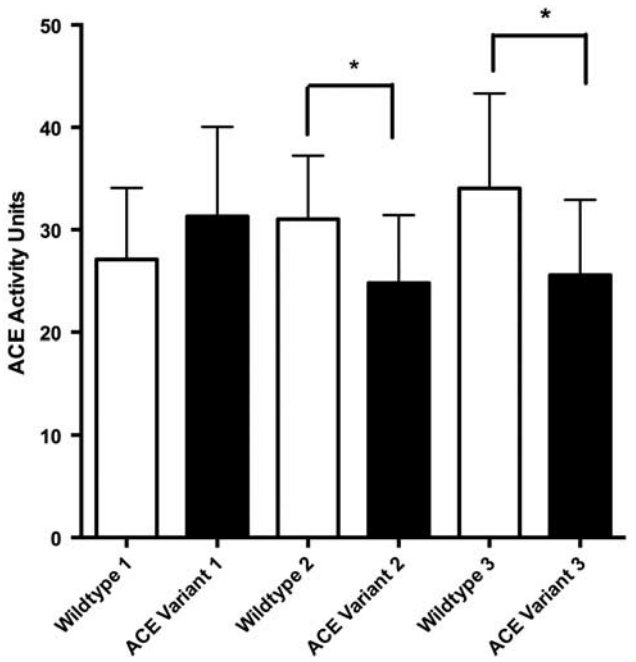
This study has several limitations. We only statistically compared the ACE activity for the two genotypes (homozygous variant, homozygous wild type), although there are three possible genotypes in the canine population (homozygous wild type, heterozygous, homozygous

Table 1 Angiotensin-converting enzyme activity and mitral regurgitation severity

Genotypes	MR 1 wild type 1	MR 2 wild type 2	MR 3 wild type 3	MR 1 variant 1	MR 2 variant 2	MR 3 variant 3
Number of dogs	3	13	9	14	13	16
Mean ACE activity	27.10	31.0	34.0	31.31	24.78	25.57
Range ACE activity	19.6–33.4	19.6–38.9	20.6–47.3	21.4–48.5	18.6–42.7	11.5–37.7

The number of dogs with each genotype and mitral regurgitation severity is indicated.
Mean and range of ACE activity for each group are indicated.
ACE, angiotensin-converting enzyme; MR, mitral regurgitation.

Fig. 2



Dogs with the ACE variant had a significantly lower level of ACE activity than dogs with the wild type in mitral regurgitation severity groups 2 ($P=0.02$) and 3 ($P=0.03$). The number of dogs in each group is as follows: group 1 wild type, three dogs; group 1 ACE variant, 14 dogs; group 2 wild type, 13 dogs; group 2 ACE variant, 13 dogs; group 3 wild type, nine dogs; group 3 ACE variant, 16 dogs.*Statistical significance. The error whiskers indicate the SD of the mean. ACE, angiotensin-converting enzyme.

ACE polymorphism) at this genetic location. The number of dogs that were heterozygous for the variant was small (7% of the population studied, five dogs) and was deemed too small for statistical analysis. Although we studied a single breed and found the variant in 66% of the cohort studied, the frequency of the variant is likely to vary between families and breeding lines potentially influencing the overall prevalence of the variant in this breed. Finally, although we did identify a significant difference in ACE activity in dogs with the polymorphism, we did not assess the functional implications of this finding.

Conclusion

The CKCS breed appears to have a high prevalence of the ACE variant in the population. The clinical significance of the findings of lower overall ACE activity in dogs with the ACE polymorphism and a failure to increase with worsening heart disease as characterized by mitral valve regurgitation is not yet known. It is possible that a lower level of ACE activity could actually have a beneficial effect in dogs with heart disease, similar to medical management with an ACE inhibitor. The clinical significance of this finding and its impact on the need for ACE-I in dogs with the polymorphism and heart disease deserves further study.

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Conflicts of interest

There are no conflicts of interest.

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