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The association between single nucleotide polymorphism in vitamin D receptor and calcium oxalate urolithiasis in dogs

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Abstract

Background: Polymorphisms of the vitamin D receptor (VDR) are associated with calcium oxalate (CaOx) nephrolithiasis in humans.

Objectives: To investigate the association between *VDR* polymorphisms and susceptibility to CaOx urolithiasis in dogs.

Animals: Thirty-five dogs with CaOx urolithiasis were compared with 40 stone-free dogs.

Methods: This was a case-control study. Two *VDR* gene polymorphisms (rs851998024 and rs852900542) were detected by specific TaqMan real-time polymerase chain reaction assay, and their relationship with serum 1,25-dihydroxyvitamin D, serum and urinary electrolyte concentrations was evaluated.

Results: The distribution of the rs852900542 polymorphism was significantly different between the case and the control dogs ($x^2 = 6.369$, $P = .04$). Dogs with a CC or CT genotype had an increased risk of CaOx stones than those with the TT genotype (odds ratio = 3.82, 95% confidence interval 1.04-13.98). The CaOx dogs with the TT genotype had a significantly lower urinary calcium-to-creatinine ratio than the CT +CC genotypes. 1,25-(OH)2D concentrations did not differ between the cases and the controls (308.7 ± 217.4 vs 286.7 ± 185.1 pg/mL, $P = .45$).

Conclusions and Clinical Importance: This finding suggests that vitamin D metabolism might play a role in CaOx stone formation in dogs.

KEYWORDS
genetic variation, stone, VDR

1 | INTRODUCTION

Calcium oxalate (CaOx) is 1 of the most common uroliths in dogs, and the prevalence of CaOx urolithiasis in dogs has increased in most countries since the early 2000s.[1] The pathogenesis of CaOx urolithiasis is poorly understood and might be a result of both genetic and environmental

Abbreviations: 1,25(OH)$_2$D, 1,25-dihydroxyvitamin D; CaOx, calcium oxalate; PTH, parathyroid hormone; SNP, single nucleotide polymorphism; UCa/Cr, urinary calcium-to-creatinine ratio; UMg/Cr, urinary magnesium-to-creatinine ratio; UP/Cr, urinary phosphate-to-creatinine ratio; VDR, vitamin D receptor.

factors. Despite multiple underlying causes, hypercalciuria seems to be a more important risk factor than hyperoxaluria and hypocitraturia.[2-4] High urinary calcium excretion results from various pathogenic mechanisms, including increased gut calcium absorption, decreased renal calcium resorption and increased bone resorption.[5,6] Interestingly, most dogs with CaOx urolithiasis have high urinary calcium excretion with normal to high-normal blood calcium concentration.[4]

Vitamin D is 1 of the regulating factors that maintains calcium homeostasis. Concentration of parathyroid hormone (PTH) or 1,25-dihydroxyvitamin D (1,25-(OH)2D) in serum is similar between CaOx dogs and healthy dogs.[2] Human patients with idiopathic hypercalciuria have an increase in vitamin D receptor (VDR) levels in the peripheral blood monocytes, although serum vitamin D concentrations were within the reference range. An increased effect of vitamin D in target tissues could result from an increased level of VDR.[7] Furthermore, the overexpression (or overactivity) of VDR is involved in stone formation in genetic hypercalciuric stone-forming (GHS) rats.[8] Given the crucial role of vitamin D in calcium homeostasis, the genetic alterations in the *VDR* gene could affect vitamin D action both during intestinal absorption and renal calcium handling, leading to hypercalciuria and stone formation. There is epidemiological evidence of allelic variation in the *VDR* gene that might be involved in calcium stone etiology in definite human populations.[9-13]

Multiple *VDR* gene polymorphisms occur in dogs (www.ensembl.org). However, the effect of *VDR* polymorphisms on calcium metabolism and risk for CaOx urolithiasis in dogs is unknown. To investigate the association of *VDR* gene polymorphisms with CaOx urolithiasis in dogs, rs851998024, a missense variant and the only nonsynonymous variant in canine VDR, and rs852900542, 1 of the 4 single nucleotide polymorphisms (SNPs) and located in the intron region of the *VDR* gene were selected.[14]

2 | MATERIALS AND METHODS

2.1 | Study samples

All procedures were approved by the Chulalongkorn University Animal Care and Use Committee (CU-ACUC), Faculty of Veterinary Science, Chulalongkorn University (Protocol number 1831101). Seventy-five dogs at the Small Animal Hospital, Chulalongkorn University, were included in this study. Informed consent was obtained from the owners between February 2019 and January 2020. All dogs in this study were client-owned dogs that lived in the city of Bangkok. The dogs in the control group (n = 40) consumed a regular adult diet that was commercially available, while dogs included in the stone-forming cases (n = 35) consumed a prescription diet for controlling urolithiasis (Canine urinary SO, Royal Canin Veterinary Diet, Waltham Centre for Pet Nutrition, USA or c/d Canine Prescription Diet, Hill's Pet Nutrition Inc, USA).

Specific cases (n = 35) that were diagnosed with CaOx stone disease during any episode were treated at the Small Animal Hospital, Faculty of Veterinary Science, Chulalongkorn University. Dogs were excluded from the study if they had remnant stones detected by either abdominal radiographs or ultrasonography, or had active urinary tract infection. The stone composition was analyzed by standard stone analysis (polarizing light microscopy and infrared spectroscopy) at the Minnesota Urolith Center, and the major composition of uroliths was CaOx (≥70%). Blood and urine samples were collected at least 14 days after stone removal. The control samples (n = 40) were obtained from the dogs that presented for health checkups or neuter appointments with no history of uroliths or lower urinary tract disease confirmed by radiography or ultrasonography. Dogs that received any kind of drugs that alter urinary calcium excretion (such as glucocorticoid, furosemide, thiazide diuretic, potassium citrate, calcium, and vitamin D supplement) or were affected by any disease that changed calcium excretion, were also excluded.

2.2 | Experimental protocol

Feed was withheld from all dogs with free access water for at least 8 hours before the experimental study. On the experimental day, approximately 5 mL of blood samples were collected into tubes containing ethylenediaminetetraacetic acid (EDTA) (3 mL) for CBC and DNA extraction. Another portion of the blood (3 mL) was collected in a heparin tube. The plasma was separated and stored at −20°C for analysis of electrolyte concentrations (Ca, P, and Mg), and 1,25-(OH)2D concentrations.

Approximately 10 mL of urine samples were collected via voiding or catheterization. The urine samples were stored at −80°C for further analysis of electrolytes (Ca, P, and Mg), and creatinine concentrations.

2.3 | Analytical procedures

2.3.1 | DNA collection and extraction

Peripheral blood mononuclear cells (PBMCs) were separated from the blood EDTA-samples using the Ficoll-Paque method.[15] Genomic DNA was extracted from PBMCs using the phenol-chloroform-isoamyl alcohol method as described previously[16] and kept at −20°C until further analysis. The DNA concentration and purification were analyzed using a DenoVix DS-11 spectrophotometer (DeNovix Inc, Wilmington, Delaware).

2.4 | Genotyping of *VDR* polymorphisms

Genotypic analysis of *VDR* gene polymorphisms (rs852900542 and rs851998024) was performed using TaqMan real-time polymerase chain reaction assay (Thermo Fisher Scientific, Waltham, Massachusetts). The forward primer for rs852900542 was 5′-CTCTTCCTCCTGCTCGGATC-3′, and the reverse primer was 5′-CGGGTAGGGACCACTGGCAA-3′. The forward primer for rs851998024 was 5′-GTCAGTGATGTGGCCAAAGGTA-3′, and the reverse was primer 5′-TGTGCCTCATCAGGGTCTATG-3′. Positive and negative controls for each SNP were also included in each experiment to validate the assay accuracy. Thermal cycling conditions included an initial step at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 30 minutes, primer annealing

TABLE 1 Characteristics and biochemical data of study population

Characteristics	Controls (n = 40)	Cases (n = 35)	*P* value
Sex			.14
Males [M/Mc]	22 [11/11] (55%)	26 [9/17] (74.3%)	
Females [F/Fs]	18 [10/8] (45%)	9 [4/5] (25.7%)	
Age (years)	7.3 ± 3.0	9.0 ± 3.0	**.01**
Breed			
Pomeranian	19 (47.5%)	12 (34.3%)	
Shih Tzu	11 (27.5%)	10 (28.6%)	
Chihuahua	6 (15%)	6 (17.1%)	
Others (Schnauzer, Yorkshire Terrier, Maltese)	4 (10%)	7 (20%)	
Episode			
First	–	24 (68.6%)	
Second	–	11 (31.4%)	
Location			
Upper urinary tract (kidney, ureter)	–	3 (8.6%)	
Lower urinary tract (bladder, urethra)	–	32 (91.4%)	
Serum electrolytes and creatinine (mg/dL)			
Calcium	8.18 ± 0.87	9.92 ± 1.30	**<.001**
Phosphorus	4.31 ± 0.98	3.96 ± 2.00	**.04**
Magnesium	2.35 ± 0.53	2.38 ± 0.91	.92
Creatinine	0.90 ± 0.20	0.80 ± 0.20	.07
$1,25(OH)_2D$ (pg/mL)	286.7 ± 185.1	308.7 ± 217.4	.45

Notes: Results presented as mean ± SD. *P* values in bold denote significance (<.05).
Abbreviations: $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; F, intact female; Fs, spayed female; M, intact male; Mc, castrated male.

at 60°C (rs852900542) or 62°C (rs851998024) for 30 seconds, and extension at 72°C for 30 seconds, followed by a final extension step at 72°C for 5 minutes and fluorescent signals from VIC and FAM were obtained at the end of each cycle. The allelic discrimination plot was analyzed using StepOne software (version 2.2; Thermo Fisher Scientific).

2.5 | Analysis of plasma and urine electrolytes

Plasma and urinary concentrations of Ca and P were measured using automate analyzer (The IL ILab 650 Chemistry Analyzer, Diamond Diagnostic, Massachusetts), while Mg was determined by inductively coupled plasma optical emission spectrometry (ICP-OES) Optima 5400 (Perkin Elmer Optima, Waltham, Massachusetts). Plasma and urinary creatinine concentrations were measured using automated analyzer (enzymatic method). The urinary excretion rate of each mineral is presented as per creatinine ratio.

2.6 | Determination of vitamin D concentration

Serum 1,25-(OH)2D was assessed using a competitive direct enzyme-linked immunosorbent assay (MBS734600, MyBioSource, San Diego, California) according to the manufacturer's instructions.

2.7 | Statistical analysis

Data are presented as mean ± SD. The genotype and allele distribution between the case and control groups were determined using the Chi-square or Fisher's exact test. Breed was used as a covariate in the regression for breeds with >3 dogs per group. The breeds with 3 or fewer dogs per group were combined as "other" in the breed category. Possible determinants for calculi risk factors were assessed through generating multiple logistic regression using the dichotomy data of stone status as outcomes and genotype, breed, sex, and age as variables. The results were expressed in the form of adjusted odds ratios (ORs) with 95% confidence intervals (CIs). When performing multiple linear regression analysis, the urinary calcium-to-creatinine ratio (UCa/Cr) value was log-transformed and was a dependent variable. The independent variables were stone status and genotype. The relations between variables were assessed using *R* values. Student's *t* test or Mann-Whitney *U* test was used to compare the mean of variables between groups. All analyses were performed using the commercial SPSS Statistics 22 software (IBM Corp, Armonk, New York). Statistical value <.05 was set as statistically significance.

3 | RESULTS

Seventy-five dogs were enrolled in this study (Table 1) being 35 cases and 40 control dogs. The sex distributions between the cases and

controls were not significantly different ($P = .14$). The mean age of the dog in cases was significantly higher than that of the controls ($P = .01$). There were 3 breeds with >3 dogs per groups, the Pomeranian, Shih Tzu, and Chihuahua. The compositions of the stone body in all cases were constituted by 100% CaOx (dihydrate + monohydrate). Nine Pomeranians, 9 Shih Tzus, and 5 Chihuahuas had a stone body composition of CaOx monohydrate (≥70%). The stone type of the other breeds (n = 7) was composed of 100% CaOx monohydrate. Eleven of the 35 cases (31.4%) were recurrent CaOx stone formers. Most CaOx stones occurred with greater frequency in the lower urinary tract (91.4%).

3.1 | Serum and urinary electrolyte and vitamin D concentrations

All dogs had serum calcium concentrations within the reference interval. Serum calcium concentrations were significantly higher in cases than in controls ($P < .001$; Table 1). There was a significant decrease in serum phosphorus in the cases compared with controls ($P = .04$). No significant differences in serum magnesium concentrations were found between the cases and the controls. There was no significant difference in serum creatinine concentration and 1,25-(OH)2D concentration between the cases and controls. Regarding urinary excretion rate, dogs in the case group had significantly higher UCa/Cr (control = 0.056 ± 0.043 mg/mg, case = 0.083 ± 0.053 mg/mg, $P = .03$), and urinary magnesium-to-creatinine ratio (UMg/Cr) Cr (control = 0.063 ± 0.036 mg/mg, case = 0.104 ± 0.067 mg/mg, $P = .02$) than those in controls (Figure 1).

3.2 | *VDR* SNPs distribution in controls and urolithiasis dogs

The SNP distribution and allele frequency of rs852900542 and rs851998024 in the *VDR* gene were studied in 40 controls and 35 dogs with urolithiasis (Table 2). Single nucleotide polymorphism genotyping assays were successful in all the samples. The genotype frequencies of each SNP in the study samples did not deviate from the Hardy-Weinberg equilibrium ($P < .05$).

3.3 | rs852900542 genotype

Three genotypes of rs852900542 were identified including TT (homozygous wild-type), CT (heterozygous), and CC (homozygous mutant) with 50, 18, and 7 genotypes, respectively. There was a significant difference in the proportion of dogs homozygous for the reference allele (TT genotype) compared to those homozygous or heterozygous for the variant (CC or CT genotypes, respectively) between stone and control dogs (Table 2). The number of genotype and allele frequencies per breed were presented in supplemental table 1.

The frequency of T alleles in CaOx stone dogs was 0.69 vs 0.88 in control dogs ($P = .01$; Table 2). Dogs with a CC or CT genotype had an increased risk for CaOx stones than did those with the TT genotype (OR = 3.82, 95% CI 1.04-13.98; Table 3).

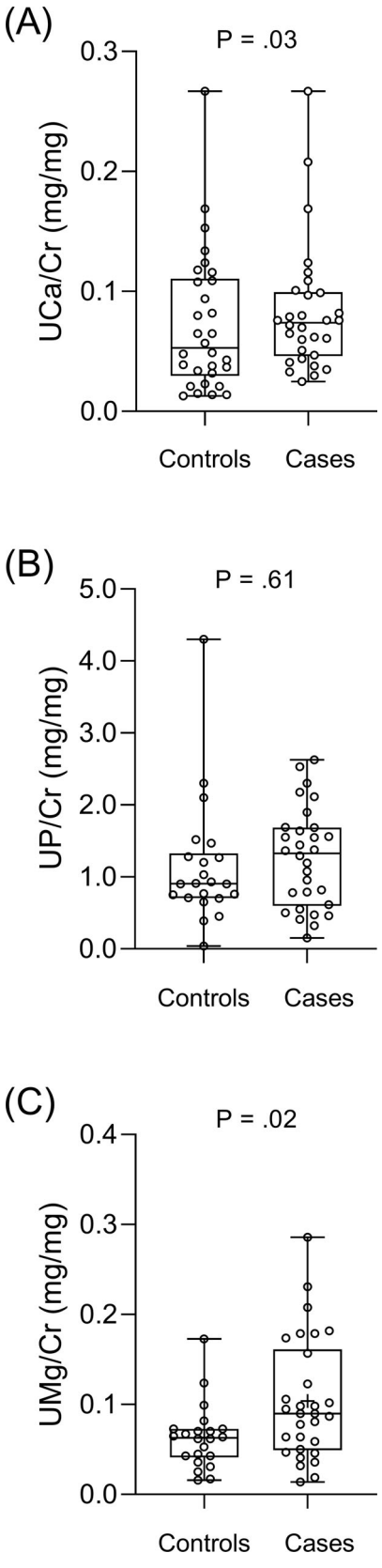

FIGURE 1 Box and whisker plots of spot urinary calcium-to-creatinine ratio (A) (UCa/Cr), urinary phosphate-to-creatinine ratio (B) (UP/Cr), and urinary magnesium-to-creatinine ratio (C) (UMg/Cr) in dogs with CaOx stone formation (cases) and breed-matched dogs (controls). Each open circles represent individual dog measurement

TABLE 2 Genotypic distribution and allele frequency of rs852900542 in studied subjects

	Controls (n = 40)	Cases (n = 35)	*P* value
Genotype distribution (%)			
rs852900542			**.04**[a]
TT	31 (77.5)	19 (54.3)	
CT	8 (20.0)	10 (28.6)	
CC	1 (2.5)	6 (17.1)	
CT+CC	9 (22.5)	16 (45.7)	
rs851998024			
TT	40 (100)	35 (100)	NA
Allele frequency (%)			
rs852900542			**.01**
T	70 (87.5)	48 (68.6)	
C	10 (12.5)	22 (31.4)	

Note: *P* values in bold denote significance (<.05).
Abbreviations: CC, homozygous mutant; CT, heterozygous; TT, homozygous wild-type.
[a]Using Fisher's exact test when compared between TT and CT+CC.

TABLE 3 Multiple logistic regression of stone status as outcome and various factors including genotypes (rs852900542), breed, sex, and age as predictors

	Adjusted OR	95% CI	*P* value
Genotype of rs852900542 (CT or CC)	3.82	1.04-13.98	**.04**
Breed[a]	0.78	0.38-1.64	.52
Sex	0.48	0.15-1.50	.20
Age	1.25	1.04-1.50	**.02**

Notes: *P* values in bold denote significance (<.05). Pearson Chi-square statistic: 64.14; $P = .27$. Likelihood ratio test statistic: 11.73; $P = .02$.
Abbreviations: CI, confidence interval; OR, odds ratio.
[a]Including the three main breeds (Pomeranian, Shih Tzu, and Chihuahua).

TABLE 4 Serum electrolytes and $1,25(OH)_2D$ level of CaOx stone-forming dogs according to rs852900542 genotype

Variables	TT (n = 19)	CT+CC (n = 11)	*P* value
Serum electrolytes and creatinine (mg/dL)			
Calcium	10.17 ± 1.34	9.48 ± 1.17	.17
Phosphorus	3.68 ± 0.86	4.45 ± 3.14	.86
Magnesium	2.49 ± 1.11	2.19 ± 0.35	.40
Creatinine	0.83 ± 0.21	0.73 ± 0.17	.16
$1,25(OH)_2D$ (pg/mL)	316.9 ± 263.1	294.7 ± 110.0	.67

Note: Results presented as mean ± SD.
Abbreviations: $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; CC, homozygous mutant; CT, heterozygous; TT, homozygous wild-type.

No significant differences in serum calcium, phosphorus, magnesium, creatinine, and 1,25-(OH)2D concentrations were observed between dogs that were homozygous (TT) vs homozygous or heterozygous for variant allele (CC and CT, respectively; Table 4).

Calcium oxalate dogs with the TT genotype had significantly lower UCa/Cr (TT = 0.072 ± 0.039 mg/mg, CT+CC = 0.110 ± 0.073 mg/mg, $P = .04$), and UMg/Cr concentrations than those with CT+CC genotypes (TT = 0.084 ± 0.054 mg/mg, CT+CC = 0.138 ± 0.076 mg/mg, $P = .03$; Figure 2). In controls, there was no difference in UCa/Cr between TT genotype and CT+CC genotype dogs (0.051 ± 0.043 mg/mg, 0.067 ± 0.046 mg/mg, $P = .60$). Urinary magnesium-to-creatinine ratio (0.063 ± 0.036 mg/mg, 0.063 ± 0.038 mg/mg, $P = .88$) and urinary phosphate-to-creatinine ratio (UP/Cr) (1.04 ± 0.94 mg/mg, 1.45 ± 0.65 mg/mg, $P = .10$). Calcium oxalate stone-forming dogs were associated with higher urinary calcium excretion compared with controls ($R = 0.42$, $P = .01$; Table 5). However, genotype had no association with urinary excretion of calcium when considering in all dogs ($P = .12$) although there were differences of urinary excretion of calcium between CT+CC and TT genotypes were found in case dogs.

3.4 | rs851998024 genotype

Among the 75 dogs that participated in the present study, only the TT genotype of rs851998024 was identified and was not associated with CaOx stone risk.

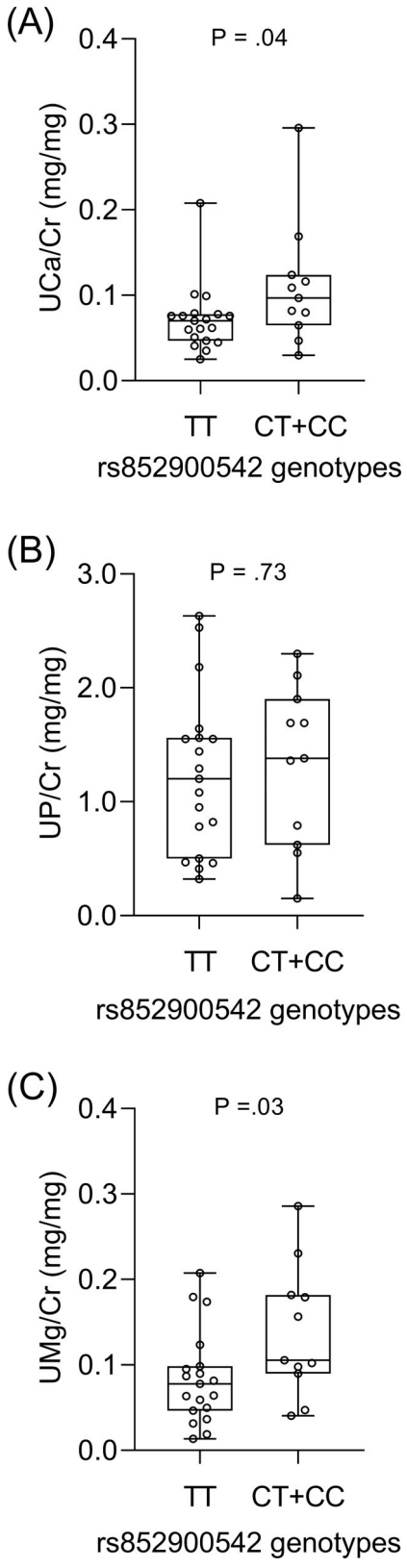

FIGURE 2 Box and whisker plots of spot urinary calcium to creatinine ratio (A) (UCa/Cr), urinary phosphate to creatinine ratio (B) (UP/Cr), and urinary magnesium to creatinine ratio (C) (UMg/Cr) in dogs with CaOx carrying TT genotype and CT+CC genotype of rs852900542. CC, homozygous mutant; CT, heterozygous; TT, homozygous wild type

TABLE 5 Multiple linear regression model for the effects of stone status and rs852900542 genotype on urinary calcium excretion[a]

	B	SE	*t*	*P* value
Constant	−0.99	0.13	−7.78	**<.001**
Group	−0.21	0.08	−2.64	**.01**
Genotype (CT or CC)	0.13	0.08	1.57	.12

Notes: Log UCa/Cr = −0.986 − (0.212 × group) + (0.133 × genotype).
$R = 0.42$; *F*-statistic: 5.16; *P* value = .01.
P values in bold denote significance (<.05).
Abbreviations: B, coefficient; SE, standard error.
[a]The regression was performed using log UCa/Cr as dependent variable.

4 | DISCUSSION

The present study revealed the possible genetic factors affecting CaOx susceptibility in dogs. We found that the rs852900542 SNP of the *VDR* gene was significantly different between case and control dogs. Dogs with the C allele had and increased risk of CaOx urolithiasis. The urinary calcium excretion concentrations in case dogs carrying the CT or CC genotypes were also significantly higher than those with TT. However, when considering all dogs in both control and case, there was no association between rs852900542 genotype and urinary calcium excretion. Thus, CaOx stone formation in dogs might be associated with multifactorial effects such as C allele of the *VDR* gene, urinary calcium excretion, or the presence of stone inhibitory agents.

The observed allele frequencies of T (0.88) and C (0.12) in control dogs from our study were similar to the control dogs in another study which accounted for 0.9 and 0.1 in T and C allele, respectively.[14] However, the allele frequencies of T and C in CaOx dogs were 0.69 and 0.31, respectively. This suggests that the allele frequency of the rs852900542 SNP varies according to different diseases and study samples. Thus, further studies with larger sample sizes are needed to confirm this result.

Although there were no differences in serum calcium, phosphate, and magnesium concentrations in CaOx dogs between the TT genotype and the CT or CC genotype, the UCa/Cr ratio was significantly higher in the dogs with CT or CC genotype. However, the serum vitamin D concentration and UP/Cr ratio were not different. Previous studies in both hypercalciuric humans and GHS rats that failed to reduce urinary calcium excretion under restriction of dietary calcium intake suggested a defect in renal calcium reabsorption.[17,18]

The mechanism of this *VDR* gene polymorphism in the pathogenesis of CaOx stone formation is still unclear, but several human studies suggest that *VDR* gene polymorphisms could influence calcium absorption and excretion.[13] For example, the T allele of the *TaqI VDR* polymorphism was associated with urinary calcium excretion and the severity of stone formation in the Japanese population.[10] The B allele of the *BsmI VDR* polymorphism was greater in hypercalciuria infants with urolithiasis than in normocalciuric urolithiasis human patients.[12] There is an association between the CT *Fok1* genotype and stone formation in human patients.[11] Many other VDR polymorphisms are

reported in dogs. Although the associated SNP in this study is located at the intronic region that might not alter the protein level, the intronic polymorphisms located within 200 base pairs from the nearest splicing site alter the transcription activity or splicing efficiency.[19] Hence, the intronic SNP might affect the VDR-binding affinity or mRNA stability, which could influence vitamin D efficiency.[20,21] Alternatively, the intronic variant might be tagging an associated haplotype, that is, in linkage disequilibrium with the true causal variant. Further studies are needed to test the association of additional VDR SNPs with CaOx risk with follow-up functional assays to determine the possible impact on gene regulation.

The present study showed no significant difference in 1,25-(OH) 2D concentrations between the case and the control groups. This was similar to other studies in CaOx stone-forming dogs.[2,22] Although the liquid chromatography-mass spectrometry provides more accurate information in which it measures only a single compound, the assay that was used in this study is verified to have less cross-reactivity or interference between 1,25-(OH)2D and its analogues. Our study did not measure 25-(OH)D/24,25-(OH)2D to definitely assess the vitamin D status, whereas difference in the 25-(OH)D/24,25-(OH)2D between cases and controls are reported.[22] In human studies, CaOx nephrolithiasis patients with hypercalciuric condition have either higher or lower in serum vitamin D,[23,24] and 25-(OH)D/24,25-(OH)2D concentration is associated with stones in humans.[25]

The proportion of male dogs is higher than female dogs in stone-forming cases, similar to reports that the prevalence of CaOx stone are the highest in intact male dogs compared with others (neutered male, intact female, and neutered female).[1,26,27] There were 6 types of small-breed dogs that are predisposed to CaOx uroliths. Similar to other studies that indicate that small-breed dogs are at high risk for CaOx stone formation.[1,28] Small-breed dogs have higher urinary calcium excretion, which contributed to CaOx stone formation.[29] However, the underlying mechanism is poorly understood.

Blood and urine samples were collected after 8 hours of withholding of food wherein the animals had free access to water to avoid the effect of calcium absorption during diet consumption. However, no postpandrial effect of UCa/Cr was observed within and between healthy and CaOx dogs.[30] Urinary calcium-to-creatinine ratio in CaOx dogs is significantly higher than that to healthy dogs during fasting and diet consumption.[2,4] From our results, the serum calcium concentration was significantly higher, but within the normal reference range in CaOx dogs compared with control dogs. Likewise, the UCa/Cr ratio was higher in CaOx dogs than that in breed-matched controls. These results suggest that hypercalciuria is 1 of the important metabolic factors contributing to CaOx formation in dogs, which has been reported.[2,4,31] The increase in calcium excretion in CaOx dogs could be related to the elevated filtered load of calcium, wherein the calcium flux might be because of the increase in gastrointestinal absorption of calcium. On the other hand, the serum phosphate concentration was significantly reduced in CaOx dogs, while phosphate excretion was slightly increased. These findings might explain the effect of PTH on renal tubules, which can cause decreased renal reabsorption of phosphate or the high P-containing diet in control. The UMg/Cr ratio was significantly increased in CaOx dogs, although there was no difference in serum concentrations. Magnesium is known to inhibit CaOx stone formation by reducing calcium and oxalate aggregation, which is effective in acidic urine conditions.[32] However, the exact mechanism of its effect is uncertain.

This study has limitations. A small number of dogs were included in this study. Moreover, it was difficult to match between the control and CaOx urolithiasis dogs. There is still potential for sample stratification, even when breeds are matched. We could not obtain 24-hour urine samples to analyze the correlation between urine calcium excretion and *VDR* polymorphism because of technical difficulties and inconvenience. Dogs in CaOx groups were fed prescription diets which contains ingredient such as potassium citrate that might affect some serum and urinary variables. Each of the control dogs was fed a varied diet, which might have affected the excretion of some electrolytes, albeit dogs were subjected to fasting before sample collection to minimize this effect. Some biochemical measurements were not available for all dogs because of inadequate urine and serum samples. Other factors and hormones that could be involved in CaOx stone formation, such as oxalate, citrate, and PTH, were not assessed in the present study.

In conclusion, this study elucidates the association between rs852900542 *VDR* polymorphism and CaOx susceptibility in dogs, which is related to urinary calcium excretion. This finding suggests that vitamin D metabolism might play a role in CaOX stone risk in dogs. Further investigations are needed to identify the role of vitamin D and *VDR* polymorphism in preventing the incidence and recurrence of CaOx urolithiasis in dogs.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Chulalongkorn University Animal Care and Use Committee (CU-ACUC): Animal Use protocol No. 1831101.

HUMAN ETHICS APPROVAL DECLARATION

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

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REFERENCES

1. Hunprasit V, Osborne CA, Schreiner PJ, Bender JB, Lulich JP. Epidemiologic evaluation of canine urolithiasis in Thailand from 2009 to 2015. *Res Vet Sci*. 2017;115:366-370.
2. Lulich JP, Osborne CA, Nagode LA, et al. Evaluation of urine and serum metabolites in Miniature Schnauzers with calcium oxalate urolithiasis. *Am J Vet Res*. 1991;10:1583-1589.
3. Stevenson AE, Robertson WG, Markwell P. Risk factor analysis and relative supersaturation as tools for identifying calcium oxalate stone-forming dogs. *J Small Anim Pract*. 2003;11:491-496.
4. Furrow E, Patterson EE, Armstrong PJ, et al. Fasting urinary calcium-to-creatinine and oxalate-to-creatinine ratios in dogs with calcium oxalate urolithiasis and breed-matched controls. *J Vet Intern Med*. 2015;1:113-119.
5. Coe FL, Worcester EM, Evan AP. Idiopathic hypercalciuria and formation of calcium renal stones. *Nat Rev Nephrol*. 2016;9:519-533.
6. Park S, Pearle MS. Pathophysiology and management of calcium stones. *Urol Clin North Am*. 2007;3:323-334.
7. Favus MJ, Karnauskas AJ, Parks JH, et al. Peripheral blood monocyte vitamin D receptor levels are elevated in patients with idiopathic hypercalciuria. *J Clin Endocrinol Metab*. 2004;10:4937-4943.
8. Li XQ, Tembe V, Horwitz GM, et al. Increased intestinal vitamin D receptor in genetic hypercalciuric rats. A cause of intestinal calcium hyperabsorption. *J Clin Invest*. 1993;2:661-667.
9. Yang SK, Song N, Wang YY, et al. Association of vitamin D receptor gene polymorphism with the risk of nephrolithiasis. *Ther Apher Dial*. 2019;5:425-436.
10. Nishijima S, Sugaya K, Naito A, et al. Association of vitamin D receptor gene polymorphism with urolithiasis. *J Urol*. 2002;5:2188-2191.
11. Bid HK, Kumar A, Kapoor R, et al. Association of vitamin D receptor-gene (FokI) polymorphism with calcium oxalate nephrolithiasis. *J Endourol*. 2005;1:111-115.
12. Goknar N, Oktem F, Torun E, et al. The role of vitamin D receptor gene polymorphisms in Turkish infants with urolithiasis. *Ren Fail*. 2016;4:545-551.
13. Gonzalez-Castro TB, Blachman-Braun R, Hernandez-Diaz Y, et al. Association of vitamin D receptor polymorphisms and nephrolithiasis: a meta-analysis. *Gene*. 2019;711:143936.
14. Rodriguez-Cortes A, Martori C, Martinez-Florez A, et al. Canine leishmaniasis progression is associated with vitamin D deficiency. *Sci Rep*. 2017;1:33-46.
15. Fuss IJ, Kanof ME, Smith PD, et al. Isolation of whole mononuclear cells from peripheral blood and cord blood. *Curr Protoc Immunol*. 2009;1:1-8.
16. Fan H, Gulley ML. DNA extraction from fresh or frozen tissues. *Methods Mol Med*. 2001;49:5-10.
17. Coe FL, Favus MJ, Crockett T, et al. Effects of low-calcium diet on urine calcium excretion, parathyroid function and serum 1,25(OH) 2D3 levels in patients with idiopathic hypercalciuria and in normal subjects. *Am J Med*. 1982;1:25-32.
18. Frick KK, Asplin JR, Krieger NS, et al. 1,25(OH)(2)D(3)-enhanced hypercalciuria in genetic hypercalciuric stone-forming rats fed a low-calcium diet. *Am J Physiol Renal Physiol*. 2013;8:1132-1138.
19. Cooper DN. Functional intronic polymorphisms: buried treasure awaiting discovery within our genes. *Hum Genomics*. 2010;5:284-288.
20. Morrison NA, Qi JC, Tokita A, et al. Prediction of bone density from vitamin D receptor alleles. *Nature*. 1994;367:284-287.
21. Jurutka PW, Whitfield GK, Hsieh JC, Thompson PD, Haussler CA, Haussler MR. Molecular nature of the vitamin D receptor and its role in regulation of gene expression. *Rev Endocr Metab Disord*. 2001;2:203-216.
22. Groth EM, Lulich JP, Chew DJ, et al. Vitamin D metabolism in dogs with and without hypercalciuric calcium oxalate urolithiasis. *J Vet Intern Med*. 2019;33:758-763.
23. Ticinesi A, Nouvenne A, Ferraro PM, et al. Idiopathic calcium nephrolithiasis and hypovitaminosis D: a case-control study. *Urology*. 2016;40:40-45.
24. Kim WT, Kim YJ, Yun SJ, et al. Role of 1,25-dihydroxy vitamin D3 and parathyroid hormone in urinary calcium excretion in calcium stone formers. *Yonsei Med J*. 2014;5:1326-1332.
25. Ketha H, Singh RJ, Grebe SK, et al. Altered calcium and vitamin D homeostasis in first-time calcium kidney stone-formers. *PLoS One*. 2015;10:1-11.
26. Picavet P, Detilleux J, Verschuren S, et al. Analysis of 4495 canine and feline uroliths in the Benelux. A retrospective study: 1994–2004. *J Anim Physiol Anim Nutr*. 2007;91:247-251.
27. Okafor CC, Lefebvre SL, Pearl DL, et al. Risk factors associated with calcium oxalate urolithiasis in dogs evaluated at general care veterinary hospitals in the United States. *Prev Vet Med*. 2014;115:217-228.
28. Wisener LV, Pearl DL, Houston DM, Reid-Smith RJ, Moore AE. Risk factors for the incidence of calcium oxalate uroliths or magnesium ammonium phosphate uroliths for dogs in Ontario, Canada, from 1998 to 2006. *Am J Vet Res*. 2010;71:1045-1054.
29. Stevenson A, Blackburn J, Markwell P, et al. Nutrient intake and urine composition in calcium oxalate stone-forming dogs: comparison with healthy dogs and impact of dietary modification. *Vet Ther*. 2003;5:218-231.
30. Carr SV, Grant DC, DeMonaco SM, et al. Measurement of prepandial and postpandial urine calcium to creatinine ratios in male Miniature Schnauzers with and without urolithiasis. *J Vet Intern Med*. 2020;34:754-760.
31. Furrow E, McCue ME, Lulich JP. Urinary metals in a spontaneous canine model of calcium oxalate urolithiasis. *PLoS One*. 2017;5:1-13.
32. Riley JM, Kim H, Averch TD, et al. Effect of magnesium on calcium and oxalate ion binding. *J Endourol*. 2013;12:1487-1492.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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