Genetic Association Patterns are Shared Between Blood Ionized Calcium, Urinary Calcium, and Risk of Calcium Oxalate Urinary Stones in a Dog Model

Lauren A. Baker[1], Eva Furrow[2]

[1]School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI, USA

[2]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of Minnesota, Minneapolis, MN, USA

Introduction

- **Our group is utilizing a spontaneous dog model** to discover genetic drivers of calcium oxalate (CaOx) kidney stones
- Most dogs with CaOx stones are **hypercalciuric** and have **increased blood ionized calcium**
- Hypothesis: Genetic predisposition to CaOx stones may be driven by effects on calcium homeostasis
- CaOx stones are complex, with many risk factors—this analysis focuses on *calcium variables only* to understand just this aspect of the condition
- **OBJECTIVE: To identify patterns of genetic associations that are shared among three conditions: calcium measured in the blood, calcium measured in the urine, and CaOx stone formation in a dog model**

Why dogs? Artificial selection through breed creation and maintenance reduces genetic diversity and increases prevalence of genetic variants that cause disease. This dramatically reduces samples sizes needed for GWAS, making dogs ideally suited for genetic research. The Miniature Schnauzer has **20x** greater risk of developing CaOx stones relative to other dog breeds.

Methods

Dataset

- 373 Purebred Miniature Schnauzers
- Genotyped at 390,150 single nucleotide polymorphisms (SNPs)
- Measured traits:
  - Blood ionized Ca (iCa)
  - Urine calcium:creatinine (UCaCr)
  - Stone forming status (CaOx)

		CaOx Stone-forming Status		
Trait	**Total**	**Cases**	**Controls**	**Unknown**
CaOx	249	148	101	N/A
iCa	82	47	34	1
UCaCr	92	52	38	2

Table 1. Number of dogs in each GWAS analysis. Blood ionized calcium (iCa) and urine calcium to creatinine ratio (UCaCr) were not measured in all dogs. A small number of dogs with measured iCa or UCaCr had unknown stone-forming status.

1) Find genotypes associated with each variable

- Genome-wide association analysis (GWAS)
  - Linear mixed model
  - R package: 'gaston' (Perdry et al., 2018)
- Separate analysis for each trait

2) Identify shared patterns of genetic associations across the traits

- Multivariate Adaptive Shrinkage (MASH) algorithm (Urbut et al., 2019)

Results

GWAS results show shared pattern from 14.6-16.5 Mb on Chromosome 9

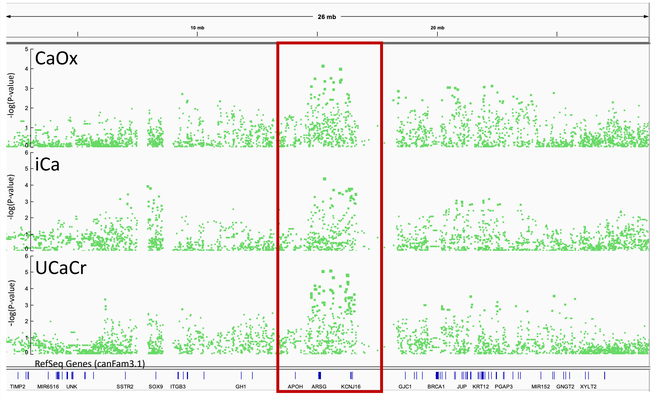

Figure 1. Manhattan plot of a 26Mb region on chromosome 9 shows a remarkably similar pattern across GWAS results spanning from approx. 14.6-16.5Mb, highlighted in red. Plot generated using Integrative Genomics Viewer (IGV) v.2.8.0 (available online from BROAD Institute. CaOx = calcium oxalate case/control GWAS; iCa = blood ionized calcium GWAS; UCaCr = urine calcium:creatinine GWAS.

Statistical analysis using MASH confirmed pattern's significance

Chr.	Location (Mb)	CaOx	iCa	UCaCr	Gene
9	14.91	8.05E-03	5.04E-03	3.72E-03	
9	14.91	4.34E-03	2.74E-03	1.99E-03	
9	15.08	1.05E-02	6.97E-03	5.33E-03	*ARSG*
9	15.25	1.83E-03	1.98E-03	7.38E-04	
9	15.33	8.35E-03	1.73E-03	3.21E-03	Unchar.
9	15.56	7.61E-03	4.70E-03	3.41E-03	*ABCA9*
9	15.59	2.08E-03	1.46E-03	6.45E-04	*ABCA9*
9	15.69	2.87E-03	1.18E-03	8.21E-04	
9	16.23	7.70E-03	1.38E-03	1.47E-03	
9	16.34	1.72E-02	2.23E-03	3.12E-03	
9	16.36	1.61E-02	2.18E-03	3.03E-03	
9	16.36	2.08E-02	3.20E-03	4.60E-03	
9	16.44	2.10E-02	4.08E-03	4.97E-03	*KCNJ16*
9	16.44	1.87E-02	2.29E-03	3.24E-03	*KCNJ16*
9	16.55	1.79E-02	3.72E-03	4.01E-03	

Table 2. Local false sign rate (LFSR) and genes from top 15 most-significant SNPs from MASH analysis. LFSR < 0.05 is considered significant. LFSR is analogous to a false discovery rate (FDR), but more stringent because it requires true discoveries to be not only nonzero, but also correctly signed (Urbut et al. 2018).

The pattern overlaps the boundaries of multiple genes, including ***KCNJ16***

In total, 66 SNPs from this region were significant in all 3 conditions

Discussion

- The identified region contained 6 genes: *GNA13, SLC16A6, ARSG, ABCA9, MAP2K6,* and *KCNJ16*
- Of these, ***KCNJ16*** is the most promising candidate
- *KCNJ16* encodes a subunit of a potassium channel highly expressed in the basolateral membrane of the **distal renal tubule**
- *KCNJ16* regulates pH and electrolyte balance, and knockout mice develop a metabolic acidosis and **hypercalciuria** (Paulais et al., 2011)
- We plan to use whole genome sequencing data to interrogate significant regions, including *KCNJ16*, for variants within or near candidate genes
- The approach used for this analysis will be extended to other non-calcium variables that are relevant to CaOx stones