Metabolome- and Genome-wide Association To Discover Genetic Drivers of Calcium Oxalate Kidney Stone Disease in a Dog Model

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Introduction

- Miniature Schnauzer dogs are at high genetic risk of spontaneous calcium oxalate (CaOx) stone disease with **14x greater risk of CaOx stone development** relative to other dog breeds
- Multiple underlying metabolic abnormalities may contribute to stone formation, each with their own genetic drivers
- Metabolomics analysis allows us to precisely define metabolic phenotypes associated with stone development; genetic drivers of differential metabolites can then be defined through genome-wide association study (GWAS)
- **OBJECTIVE: Leverage the Miniature Schnauzer dog model to identify genetic drivers of metabolism that influence CaOx stone development**

Methods

Study Criteria

- Purebred Miniature Schnauzers (pets) were recruited through the University of Minnesota Canine Genetics Lab
- **Controls (N=20) –** >8 years old, confirmed stone-free with radiography and/or ultrasound
- **Case (N=23) –** any age with history of CaOx urolithiasis

Metabolomics

- Generated serum metabolomics profiles comprising 836 metabolites from 98 pathways (Metabolon Inc., Morrisville, NC)
- Differential analysis performed using MetaboAnalyst software v5.0 (Pang et al. 2021)

Genomics

- 305,846 single nucleotide polymorphism (SNP) genotypes were available for analysis after standard quality control for GWAS
  - Corrections for minor allele frequency >0.05, Hardy-Weinberg equilibrium, individual- and SNP-level missingness <0.1.
- Genome-wide association analysis was performed for top metabolites using a general linear model with corrections for age, sex, kinship, and case-control status; PLINK v2.0 (Chang et al. 2015)

Results

Metabolite	Pathway	Link to CaOx Stones	T-test P	FDR	GWAS Region (Chr:Position)	GWAS P	Gene
7-methylguanine	Purine Metabolism	**Downregulated in rodent models of CaOx**	2.9E-03	0.24	35:11088620-11741863	2.2E-06	*TMEM170B*, *ATRP*, *HIVEP1*, ***EDN1***
10-undecenoate	Medium Chain Fatty Acid	Unknown	1.3E-03	0.24	17:22944696-23641286, 22:50766021-50767582	5.95E-06, 6.09E-06	*CLIP4*, *ALK*; intergenic
Dihomo-linoleoylcarnitine	Fatty Acid Metabolism	Unknown	1.8E-03	0.24	27:35412959-35471771	1.6E-06	intergenic
N-delta-acetylornithine	Arginine and Proline Metabolism	Unknown	3.2E-03	0.24	16:27036665-27037871	7.8E-06	***FGFR1***
Hydroxyproline	Arginine and Proline Metabolism	**Major source of oxalate production**	2.6E-03	0.24	7:18021002	2.6E-06	*FAM129A*
(16 or 17)-methylstearate	Fatty Acid, Branched	Unknown	1.8E-02	0.36	3:11675370	3.1E-05	intergenic
12,13-DiHOME	Fatty Acid, Dihydroxy	Unknown	2.6E-03	0.24	29:37583170-38139511	2.8E-05	*TRIQK*
Pentadecanoate	Long Chain Saturated Fatty Acid	Unknown	1.6E-04	0.13	24:10531327-10549765	1.7E-05	intergenic
Malate	TCA Cycle	**Increases citraturia**	4.1E-03	0.24	13:23498223-25130364	4.0E-05	*LRATD2*

Table 1. GWAS results for top metabolites associated with CaOx stone status. The listed metabolites were consistently reported among top results in MetaboAnalyst analyses including T-test, partial least squared discriminant analysis (PLS-DA), and random forest classification. Top genetic loci for each metabolite are reported. Gene annotations were defined using the Broad Improved Canine Annotation for CanFam3.1 in the UCSC Genome Browser.

Discussion

- Top metabolomics features included metabolites previously implicated in CaOx stone risk—7-methylguanine, hydroxyproline, and malate
- 5/9 top metabolites are fatty acids, suggesting that fatty acid metabolism might be deranged in this model of CaOx stone disease
- Notable gene associations include *EDN1,* which has been associated with calcium homeostasis and hypercalciuria, and *FGFR1* which has a distinct role in phosphate and calcium transport in the kidney (Nicolaidou et al. 2003; Han et al. 2016)
- Validation cohorts are needed

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Key Citations

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