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 Metabo Analyst 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

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Pathway</th>
<th>Link to CaOx Stones</th>
<th>T-test P</th>
<th>FDR</th>
<th>GWAS Region (Chr:Position)</th>
<th>GWAS P</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>7-methylguanidine</td>
<td>Purine Metabolism</td>
<td>Downregulated in rodent models of CaOx</td>
<td>2.9E-04</td>
<td>0.24</td>
<td>35:11088620-11741863</td>
<td>2.2E-06</td>
<td>TMEM170B, ATRP, HIVEP1, EDN1</td>
</tr>
<tr>
<td>10-undecenoate</td>
<td>Medium Chain Fatty Acid</td>
<td>Unknown</td>
<td>1.5E-03</td>
<td>0.24</td>
<td>17:2294496-23641286, 22:50766021-50767582</td>
<td>5.95E-06, 6.09E-06</td>
<td>CLP4, ALK, intergenic</td>
</tr>
<tr>
<td>Dihomoolinoeoylcarnitine</td>
<td>Fatty Acid Metabolism</td>
<td>Unknown</td>
<td>3.3E-03</td>
<td>0.24</td>
<td>27:35412953-35471771</td>
<td>1.6E-06</td>
<td>intergenic</td>
</tr>
<tr>
<td>N-delta-acetylornithine</td>
<td>Arginine and Proline Metabolism</td>
<td>Unknown</td>
<td>1.6E-03</td>
<td>0.24</td>
<td>16:27036665-27037871</td>
<td>7.8E-06</td>
<td>FGFR1</td>
</tr>
<tr>
<td>Hydroxyproline</td>
<td>Arginine and Proline Metabolism</td>
<td>Major source of oxalate production</td>
<td>2.6E-03</td>
<td>0.24</td>
<td>7:18021002</td>
<td>2.6E-06</td>
<td>FabM29A</td>
</tr>
<tr>
<td>(16 or 17)-methylstearate</td>
<td>Fatty Acid, Branched</td>
<td>Unknown</td>
<td>1.8E-02</td>
<td>0.36</td>
<td>8:11673570</td>
<td>3.1E-05</td>
<td>intergenic</td>
</tr>
<tr>
<td>12,5-DHOME</td>
<td>Fatty Acid, Dihydroxy</td>
<td>Unknown</td>
<td>2.0E-03</td>
<td>0.24</td>
<td>29:37583170-3831951</td>
<td>2.85E-06</td>
<td>TRGK</td>
</tr>
<tr>
<td>Pentadecanoic acid</td>
<td>Long Chain Saturated Fatty Acid</td>
<td>Unknown</td>
<td>1.6E-04</td>
<td>0.13</td>
<td>24:10513137-10549765</td>
<td>1.75E-05</td>
<td>intergenic</td>
</tr>
<tr>
<td>Malate</td>
<td>TCA Cycle</td>
<td>Increases citrullina</td>
<td>4.3E-03</td>
<td>0.24</td>
<td>13:23498223-25:383684</td>
<td>4.0E-05</td>
<td>LRATD2</td>
</tr>
</tbody>
</table>

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-methylguaninate, 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 hypercalcuria, 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

Funding Declaration

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Key Citations


Figure 1. Schematic of the process used in this study to identify genetic drivers of CaOx stone disease in dogs.