Evaluation of Potentially Pathogenic Mutations that Cause Nephrolithiasis and Nephrocalcinosis
Julia Licari1,2, Jen Arroyo, PhD2, Mohammed Arnous, MD2, John Lieske, MD2 Peter Harris, PhD2
1 Center for Learning Innovation, University of Minnesota Rochester
2 Division of Nephrology and Hypertension, Mayo Clinic Rochester, MN

Background
Urinary stone lifetime prevalence is approximately 7.2%.
Genetic factors explain 20-36% of incidents.
Underlying etiology are multifactorial and remain elusive.
Mono- and biallelic Napi2a and Napi2c mutations (encoded by SLC34A1 and SLC34A3) have been associated with both nephrolithiasis and nephrocalcinosis.
These sodium dependent phosphate transporters are important for proximal tubular phosphate reabsorption, and their dysfunction leads to hypercalciuria.
Hypercalciuria is an important risk factor for common calcium oxalate urinary stones (up to 80% of all stones!)

Methods
Use primers carrying patient variants and PCR to generate mutant plasmids
Visualization of plasmid after PCR construct using gel electrophoresis
Bacterial expression of plasmid and selection of single clones using antibiotic resistance contained in plasmid
Sequence verify plasmid, amplify, and store for future functional testing in cell models

Results

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
</table>

Fig1. Gel electrophoresis to evaluate primer set 15 designed to amplify targeted DNA sequence. Wells 5 & 9 were loaded with PCR product from primer set 15; no amplification was observed. Wells 3 & 7 were loaded with template DNA from primer set 1 and wells 4 & 8 were loaded with PCR product from primer set 14.

Fig2. Plasmid DNA concentration quantified using a nanodrop post transformation, growth and plasmid preparation.

Aims
In-vitro characterization of wildtype and patient-specific SLC34A1 and SLC34A3 variants.

Conclusions
Plasmids containing patient variants are awaiting functional characterization using Xenopus laeves oocytes and human immortalized kidney cells.

Take-Aways
Working as a team is crucial for success in research
Research is full of opportunities for failure, but one learns to persevere in the process
The interconnection between research and practice became readily apparent

References