

Evaluation of Potentially Pathogenic Mutations that Cause Nephrolithiasis and Nephrocalcinosis

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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

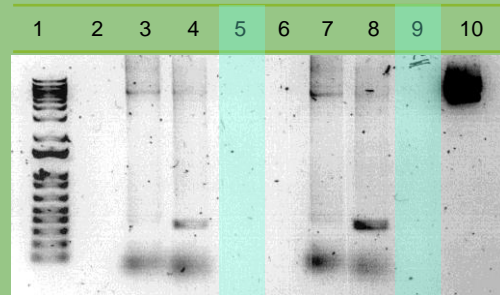


Fig1. Gel electrophoresis to evaluate primer set 15 designed to amplify targeted DNA sequence. Wells 5 & 9 were loaded with PCR product from primer set 15; no amplification was observed. Wells 3 & 7 were loaded with template DNA from primer set 1 and wells 4 & 8 were loaded with PCR product from primer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

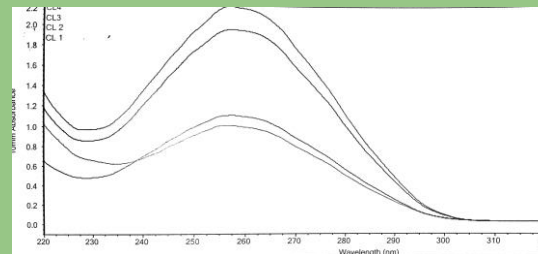


Fig2. Plasmid DNA concentration quantified using a nanodrop post transformation, growth and plasmid preparation.

Aims

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

Conclusions

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

Take-Aways

Working as a team is crucial for success in research

The interconnection between research and practice became readily apparent

Research is full of opportunities for failure, but one learns to persevere in the process

References

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