Identifying causative variants in patients with monogenic stone disease

Kalina J. Reese[1,2], Andrea G. Cogal[3], John C. Lieske, M.D.[3], Peter C. Harris, Ph.D.[2,3],
[1]Calvin University, Grand Rapids, MI [2]Department of Biochemistry and Molecular Biology
[3]Division of Nephrology and Hypertension
Mayo Clinic, Rochester, MN, USA

Introduction

- Primary hyperoxaluria (PH) and Dent disease (DD) are two monogenic causes of kidney stones, nephrocalcinosis, and chronic kidney disease.
  - Known genetic causes of PH: *AGXT*, *GRHPR*, *HOGA1*
  - Known genetic causes of DD (X-linked): *CLCN5, OCRL*
- Over 50% of patients clinically suspected of having PH or DD were negative from Sanger screening of these genes.
- Therefore, we employed a next generation sequencing (NGS) panel of 102 known or candidate stone disease genes to screen the 297 unresolved cases. This analysis identified biallelic mutations in other genes including *APRT*, *CLDN16*, *CLDN19*, *CYP24A1*, *KCNJ1*, *SLC34A1*, and *SLC34A3* in 29 (9.8%) cases.
- Here, we employed an improved bioinformatics pipeline to identify likely causative mutations in residual unresolved cases.

Approach

- Cases with heterozygous variants in possible dominant genes or biallelic variants in recessive genes were identified.
- NGS data from the 102 candidate gene panel was available for 268 unresolved presumed PH/DD patients.
- Potentially pathogenic variants were analyzed with *in silico* and population tools.
  - HGMD and PubMed literature search
  - OMIM phenotype identification
  - gnomAD population data
  - Multi-sequence protein alignments
  - Prediction Programs
    - SIFT, PolyPhen-2 HVAR, MutationTaster, Mutation Assessor, FATHMM, FATHMM MKL, Human Splice Finder (HSF), and Berkley Drosophila Gene Project (BDGP)
  - Tertiary/Quaternary Structural Modeling (SWISSMODEL/Protein Data Bank (PDB)/PyMol)
  - UniProt protein data
  - ClinVar pathogenicity designation
- Possible pathogenic variants were carefully compared with the clinical phenotype to determine if they were consistent.

SLC4A1 (anion exchanger 1; AE1)

c.1765C>T (p.Arg589Cys)

- A PH-negative patient presenting with nephrocalcinosis was found to have a missense *SLC4A1* variant in heterozygosity.

Gene Information
Renal Function: Chloride-bicarbonate co-transporter; regulates urinary pH
Renal Phenotype: Renal Tubular Acidosis
Inheritance: AD/AR

Literature	ClinVar	Predicted Damage	gnomAD
All variants (including Cys, Ser, and His) at this residue are pathogenic in heterozygous presentation.	3 X Pathogenic	6/6	Not Present

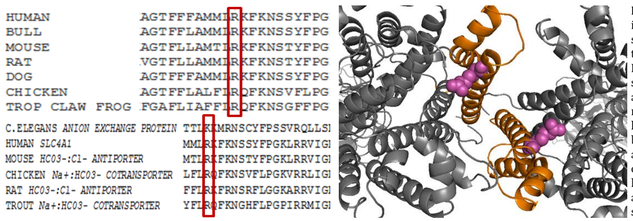


Figure 1B. SLC4A1 is critical to structure of anionic transporter region. Residue 589 (pink spheres) is part of anionic transporter region (orange), likely impairing both the structure and function of the heteromeric active site.

Figure 1A. *(Above left - top)* Multi-sequence alignment for *SLC4A1* variant p.Arg589Cys. Arginine 589 is conserved in 7/7 orthologous protein sequences of *SLC4A1* with relatively high conservation surrounding the position. *(Above left - bottom)* Conserved Domains for *SLC4A1* variant p.Arg589Cys. Variant is highly conserved in anion exchanger (antiport/cotransport of anions) family domains.

HNF4A (hepatocyte nuclear factor-4-alpha)

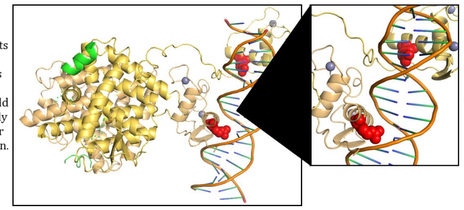
c.253C>T (p.Arg45Trp)

- Two unrelated DD-negative patients both presenting with Fanconi syndrome and nephrocalcinosis were found to have a missense *HNF4A* variant in heterozygosity.

Gene Information
Renal Function: Hepatocyte transcription factor; contributes to kidney development
Renal Phenotype: Fanconi Syndrome
Inheritance: AD

Literature	ClinVar	Predicted Damage	gnomAD
Variant is reported as pathogenic in heterozygous presentation.	5 X Pathogenic	6/6	Not Present

Figure 2A. Arg45 is critical to function of HNF4A. *(Left)* Dimeric HNF4A (yellow/tan) acts to initiate DNA transcription. *(Right)* Arg45 (red spheres) acts directly on the DNA helix, implying that its alteration would significantly impair function.



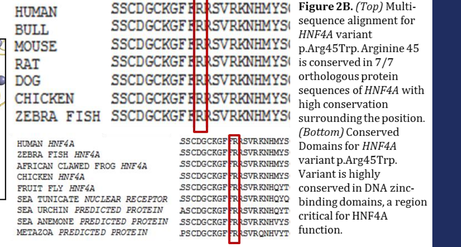


Figure 2B. *(Top)* Multi-sequence alignment for *HNF4A* variant p.Arg45Trp. Arginine 45 is conserved in 7/7 orthologous protein sequences of *HNF4A* with high conservation surrounding the position. *(Bottom)* Conserved Domains for *HNF4A* variant p.Arg45Trp. Variant is highly conserved in DNA zinc-binding domains, a region critical for HNF4A function.

SLC12A1 (Na-K-2Cl cotransporter; NKCC2)

c.769G>A (p.Gly257Ser)/c.1424G>A (p.Cys475Tyr)

- A DD-negative patient presenting with polyuria and hypercalcemia was found to have two missense *SLC12A1* variants in compound heterozygosity.

Gene Information
Renal Function: Sodium/Potassium-chloride symporter; regulation of ion balance and cell volume
Renal Phenotype: Bartter's syndrome
Inheritance: AR

Literature	ClinVar	Predicted Damage	gnomAD
Exact genotype is reported with symptoms of nephrolithiasis, polyuria, and hypercalcemia.	p.Gly257Ser: 1 X Pathogenic p.Cys475Tyr: 1 X Pathogenic	p.Gly257Ser: 5/6 p.Cys475Tyr: 5/6	p.Gly257Ser: 1/31402 p.Cys475Tyr: Not Present

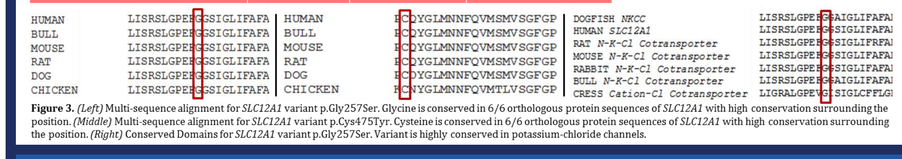


Figure 3. *(Left)* Multi-sequence alignment for *SLC12A1* variant p.Gly257Ser. Glycine is conserved in 6/6 orthologous protein sequences of *SLC12A1* with high conservation surrounding the position. *(Middle)* Multi-sequence alignment for *SLC12A1* variant p.Cys475Tyr. Cysteine is conserved in 6/6 orthologous protein sequences of *SLC12A1* with high conservation surrounding the position. *(Right)* Conserved Domains for *SLC12A1* variant p.Gly257Ser. Variant is highly conserved in potassium-chloride channels.

SLC34A1 (sodium-dependent phosphate transporter 2A; NaPi-2a)

c.937-8T>A (p.Ala313_insIleSTOP)

- A PH-negative patient presenting with echogenic kidneys was found to have an atypical splicing missense *SLC434A1* variant in homozygosity.
- Prediction programs designated a strong new splice site 8 base pairs upstream that inserted two coding codons at the beginning of exon 9. The first amino acid codes for Ile and the other for STOP, resulting in a truncation of the protein.

Gene Information
Renal Function: Sodium-phosphate co-transporter; makes up most of apical pyramid flux
Renal Phenotype: Fanconi Syndrome, Hypercalcemia, Nephrolithiasis
Inheritance: AD/AR

Literature	ClinVar	Predicted Damage	gnomAD
No Data	No Data	HSF: 60.22 to 89.17; BDGP: 0.67 to old site lost – new site: 0.81	41/282788

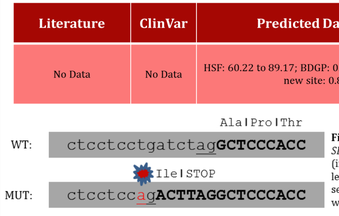


Figure 4A. Intronic substitution results in early termination of *SLC34A1* gene product. Top panel shows WT DNA sequence (intron in lowercase, exon in uppercase bold; reading frame is left to right) and typical ag splice site (underlined) with protein sequence above. Bottom panel shows base pair change (red) with novel splice site (underlined) and resulting codons.

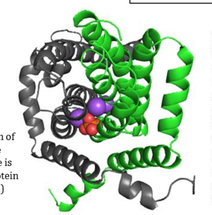


Figure 4B. SLC34A1 truncation results in estimated complete loss of channel function. Sodium (purple) and phosphate (yellow/red) are unable to be transported when p.Ala313_insIleSTOP creates a truncated protein product (loss indicated in grey).

Conclusions and Further Directions

- 5/268 (1.9%) cases were fully resolved from the analysis performed here; 34/297 (11.4%) in total from the NGS.
- 35 further families were found to have complex inheritance or at least likely contributing monoallelic variants.
- Using these NGS results and careful *in silico* analysis, we can identify additional genes that are likely contributing to the clinical phenotype, thus improving patient diagnosis and treatments.
- Future steps include screening of unresolved families with exome and whole genome sequencing.

Acknowledgements

- Peter Harris and John Lieske for mentorship
- Andrea Cogal and Zejfa Haskic for project support
- Ronak Shah and Brenna Walton for previous work
- Rare Stones Genetics Review Board for phenotypic review
- Rachael Baker, Amy Wilstermann, and Brendan Looyenga for modeling assistance
- NIDDK for funding
  - nuSURF (R25-DK101405)

References: Human Splice Finder (Desmet et. al. 2009) ; Berkley Drosophila Gene Project (Reese et. al. 1997); Multi-Sequence Alignments/Conserved Domains, NCBI database; Variant Pathogenicity, *HNF4A* (Hamilton et. al. 2014), *SLC4A1* (Bruce et. al. 1997, Karet et. al. 1998), *SLC12A1* (Daga et. al. 2018); *SLC34A1* model (Fenollar-Ferrer et. al. 2014); *In silico* modeling for pathogenic variants (Baker et. al. 2018)

MAYO CLINIC

Title Safe Area: Title text should appear within this area

Author/Affiliation Area: Authors, affiliations and subbrand names should snap to the top of this area and flow downward.

Brand Safe Area: The upper title banner section of the poster provides a brand safe area for the logo, title and author/affiliation text. No photos, illustrations, patterns, high-contrast backgrounds, or graphics are allowed within this area. A logo representing another non-Mayo listed contributing affiliation may be placed in upper right corner within green guideline space.

Poster Body Area: Research text, figures, tables and graphs should appear within this area. No photos, illustrations, patterns, high-contrast backgrounds, or graphics are allowed in the margins.

Use the text boxes in the template when possible.

Copyright Line: Copyright graphic should appear at bottom right under last text/figure box. Recommend graphic be placed no more than 1.5" from bottom of poster.