Identifying causative variants in patients with monogenic stone disease
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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, GCHGR, 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, CLDN19, CYP24A1, KCNJ1, SLC34A1, and SLC4A1 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 Project (BdGF)
  • 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.

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 monogenic variants.
• Using these NGS results and careful 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
• Ronald Shah and Brenna Walton for previous work
• Rare Stones Genetics Review Board for phenotypic review
• Rachael Baker, Amy Wiitherman, and Brendan Lowery for modeling assistance
• NIDDK for funding
  • gnomAD (ES-0310145)

References

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.

SLC34A1 (sodium-dependent phosphate transporter 2A; NaPi-2a)
c.937C>T (p.Arg45Trp)
• A PH-negative patient presenting with echogenic kidneys was found to have a pathogenic variant in homozygosity.

HNF4A (hepaticocyte nuclear factor 4-alpha)
c.253C>T (p.Arg85Trp)
• Two unrelated DD-negative patients both presenting with Fanconi syndrome and nephrocalcinosis were found to have a missense HNF4A variant in homozygosity.

Figure 2a. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2b. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2c. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2d. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2e. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2f. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2g. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2h. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2i. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2j. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2k. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2l. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2m. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2n. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2o. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2p. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2q. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2r. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2s. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2t. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2u. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2v. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2w. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2x. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2y. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 2z. LRP1B (Low density lipoprotein receptor-related protein 1); B (arginine) found in homozygosity.

Figure 3a. (top left) and (top right) predicted alignment for the SLC4A1 variants in Fig. 2. Inset: SLC4A1 (sodium-dependent phosphate transporter 2A; NaPi-2a) variant in homozygosity.

Figure 3b. (middle) predicted alignment for the SLC4A1 variants in Fig. 2. Inset: SLC4A1 (sodium-dependent phosphate transporter 2A; NaPi-2a) variant in homozygosity.

Figure 3c. (bottom left) predicted alignment for the SLC4A1 variants in Fig. 2. Inset: SLC4A1 (sodium-dependent phosphate transporter 2A; NaPi-2a) variant in homozygosity.

Figure 3d. (bottom right) predicted alignment for the SLC4A1 variants in Fig. 2. Inset: SLC4A1 (sodium-dependent phosphate transporter 2A; NaPi-2a) variant in homozygosity.
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