Whole exome sequencing frequently detects a monogenic cause in early onset nephrolithiasis and nephrocalcinosis

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The incidence of nephrolithiasis continues to rise. Previously, we showed that a monogenic cause could be detected in 11.4% of individuals with adult-onset nephrolithiasis or nephrocalcinosis and in 16.7-20.8% of individuals with onset before 18 years of age, using gene panel sequencing of 30 genes known to cause nephrolithiasis/nephrocalcinosis. To overcome the limitations of panel sequencing, we utilized whole exome sequencing in 51 families, who presented before age 25 years with at least one renal stone or with a renal ultrasound finding of nephrocalcinosis to identify the underlying molecular genetic cause of disease. In 15 of 51 families, we detected a monogenic causative mutation by whole exome sequencing. A mutation in seven recessive genes (*AGXT, ATP6V1B1, CLDN16, CLDN19, GRHPR, SLC3A1, SLC12A1*), in one dominant gene (*SLC9A3R1*), and in one gene (*SLC34A1*) with both recessive and dominant inheritance was detected. Seven of the 19 different mutations were not previously described as disease-causing. In one family, a causative mutation in one of 117 genes that may represent phenocopies of nephrolithiasis-causing genes was detected. In nine of 15 families, the genetic diagnosis may have specific implications for stone management and prevention. Several factors that correlated with the higher detection rate in our cohort were younger age at onset of nephrolithiasis/nephrocalcinosis, presence of multiple affected members in a family, and presence of consanguinity. Thus, we established whole exome sequencing as an efficient approach toward a molecular genetic diagnosis in individuals with nephrolithiasis/nephrocalcinosis who manifest before age 25 years.

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Nephrolithiasis (NL) is a highly prevalent condition affecting up to 10% of individuals worldwide.[1] It is associated with high morbidity, high recurrence rate, as well as high economic cost.[2] Although NL is less common among children than adults, the incidence of NL and nephrocalcinosis (NC) in the pediatric age group has been rising over the past 10 years.[3] The causes of NL are not well understood. Formerly monogenic causes of NL were thought to be restricted to rare tubulopathies and genetic syndromes. However, we recently revealed that a causative monogenic mutation can be detected in 1 of 30 known NL-causing genes in 20.8% of patients with onset of NL before age of 18 years.[4] We subsequently confirmed the high rate of a molecular diagnosis in 16.7% of early-onset NL in a 3-center cohort and found that important therapeutic and preventative measures may result from mutation detection.[5] These previous studies employed exon sequencing in gene panels, rather than whole exome sequencing (WES). Because WES offers the opportunity to detect dozens of additional genes at a rather low cost and has not yet been applied to patients with NL, we here employed WES in 51 families (65 individuals) with ≥1 episode of renal stone or evidence of NC on renal ultrasound before the age of 25 years, in order to identify monogenic causes in the 30 NL/NC known genes (Supplementary Table S1). We also evaluated WES data for 117 additional phenocopy genes (Supplementary Table S2; 30 known renal tubulopathy genes, 87 renal ciliopathy genes) and 16 hypothesized candidate genes. We confirm the high rate of detection (29.4%) of causative mutations in 1 of the 30 known NL/NC genes. We study genotype-phenotype correlations, determine factors such as early onset and familial disease correlating with high mutation detection rate, and determine that a molecular genetic diagnosis allows for finely tailored treatment plans that may prevent recurrent disease or delay progression to end-stage renal disease (ESRD).

RESULTS

We performed WES in 65 individuals from 51 families with NL or a finding of NC on renal ultrasound or both, who manifested before the age of 25 years. Of the 65 individuals, 32 had isolated NL, 22 had isolated NC, and 11 had both NL and NC. No affected individual had hypercalciuria in the absence of NL or NC (Supplementary Figure S1). When evaluating WES data for 30 genes known to cause NL or NC when mutated (Supplementary Table S1), we identified a mutation in 15 of 51 families (29.4%) (Figure 1). Recessive or dominant causative mutations were detected in 9 of the 30 genes (Tables 1 and 2).[6–16] Pathogenic mutations were detected in 8 recessive genes in 17 individuals from 12 families: *AGXT* (4 individuals, 3 families), *ATP6V1B1* (1 individual, 1 family), *CLDN16* (1 individual, 1 family), *CLDN19* (3 individuals, 1 family), *GRHPR* (2 individuals, 1 family), *SLC3A1* (1 individual, 1 family), *SLC12A1* (3 individuals, 2 families), and *SLC34A1* (2 individuals, 2 families) (Table 1).[6–14] Pathogenic mutations were detected in 2 dominant genes in 5 individuals from 3 families: *SLC9A3R1* (1 individual, 1 family)

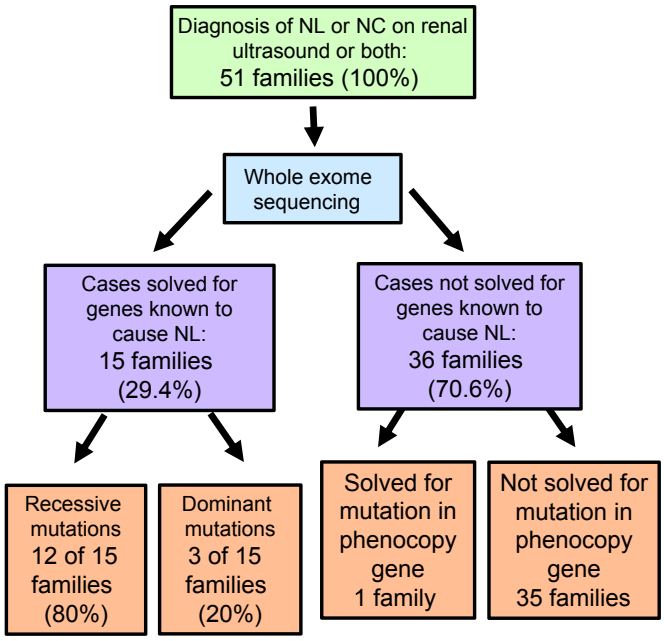

Figure 1 | Flow diagram on detection by whole exome sequencing of causative monogenic mutations in 30 nephrolithiasis or nephrocalcinosis or both (NL/NC) genes in 51 families with NL/NC. Of 100 available families with NL/NC, 51 were tested by whole exome sequencing for detection of monogenic causation of stone disease. A causative mutation was detected in a known NL/NC gene in 15 of 51 families (29.4%). A causative mutation was detected in a phenocopy gene (*CTNS*) in 1 patient with NC; 35 families remain unsolved.

and *SLC34A1* (4 individuals, 2 families) (Table 2).[15,16] *SLC34A1* gene mutations may follow an autosomal recessive mode of inheritance-causing infantile hypercalcemia[6] or an autosomal dominant mode of inheritance-causing NL/NC.[5,17] The family history, status of consanguinity, and detailed phenotype of individuals is shown (Tables 1 and 2).[6–16] The pedigrees of all 15 families show family history and consanguinity (Supplementary Figure S2).

Of the 22 individuals, in whom we detected causative mutations in this study, 9 presented with NL and 13 presented with NC on a renal ultrasound (Tables 1 and 2).[6–16] The radiographic evidence of NC in 6 of 13 individuals affected with NC is shown (Figure 2). The clinical characteristics of individuals with an identified monogenic cause for NL/NC are described (Supplementary Table S3). Seven of 19 detected mutations (36.8%) were novel pathogenic variants that have not been previously reported in databases of human disease-causing mutations.

Detection rate of causative mutations was not different between sexes (14 of 32 in male subjects and 8 of 33 in female subjects, $P = 0.27$). Median age of onset was significantly lower (Z score: 2.6, $P = 0.009$) in patients with a monogenic cause (3 years) versus those without detection of monogenic cause (7 years) (Supplementary Figure S3). We evaluated our cohort for differences regarding disease (NL/NC) at presentation, age of onset of disease, and causative mutation detection (Figure 3). Individuals with NC presented before