Whole exome sequencing identifies causative mutations in the majority of consanguineous or familial cases with childhood-onset increased renal echogenicity

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Chronically increased echogenicity on renal ultrasound is a sensitive early finding of chronic kidney disease that can be detected before manifestation of other symptoms. Increased echogenicity, however, is not specific for a certain etiology of chronic kidney disease. Here, we performed whole exome sequencing in 79 consanguineous or familial cases of suspected nephronophthisis in order to determine the underlying molecular disease cause. In 50 cases, there was a causative mutation in a known monogenic disease gene. In 32 of these cases whole exome sequencing confirmed the diagnosis of a nephronophthisis-related ciliopathy. In 8 cases it revealed the diagnosis of a renal tubulopathy. The remaining 10 cases were identified as Alport syndrome (4), autosomal-recessive polycystic kidney disease (2), congenital anomalies of the kidney and urinary tract (3), and APECED syndrome (1). In 5 families, in whom mutations in known monogenic genes were excluded, we applied homozygosity mapping for variant filtering and identified 5 novel candidate genes (*RBM48, FAM186B, PIAS1, INCENP,* and *RCOR1*) for renal ciliopathies. Thus, whole exome sequencing allows the detection of the causative mutation in 2/3 of affected individuals, thereby presenting the etiologic diagnosis, and allows identification of novel candidate genes.

Kidney International advance online publication, 21 October 2015; doi:10.1038/ki.2015.317

KEYWORDS: chronic kidney disease; genetic kidney disease; pediatric nephrology

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Received 16 May 2015; revised 10 August 2015; accepted 20 August 2015

Renal ultrasound imaging (RUS) represents a simple and broadly accessible tool for the noninvasive early diagnosis of chronic kidney disease (CKD) in children and young adults. Often abnormal findings on RUS are detectable years before the kidney function deteriorates and before other symptoms develop. A typical abnormal finding in early stages of CKD is chronically increased echogenicity on RUS. It is frequently accompanied by loss of cortico-medullary differentiation and renal cysts. Increased echogenicity is easily detected as a

degree of echogenicity that is equal to or more pronounced than the echogenicity of the liver. Unfortunately, chronically increased echogenicity is not specific to certain types of kidney disease.[1–3] Particularly in early stages, in which other symptoms are not yet present, a correct diagnosis can be challenging. In these cases, whole-exome sequencing (WES) provides a novel means of establishing an etiologic diagnosis. By revealing the causative monogenic mutation, it provides affected individuals and their families with an unequivocal, early diagnosis.[1] As a result, a targeted therapeutic regimen can be initiated early if available.

Chronically increased echogenicity on RUS is often found in the early stages of nephronophthisis-related ciliopathies (NPHP-RCs). NPHP-RCs represent a group of cystic and fibrotic kidney diseases with an autosomal recessive mode of inheritance that typically progress to end-stage renal failure within the first three decades of life.[4,5] Nephronophthisis can present as isolated renal disease (MIM #613550) or together with extrarenal symptoms such as retinal degeneration (Senior Loken syndrome MIM #266900), cerebellar vermis hypoplasia (Joubert syndrome MIM #213300), and hepatic fibrosis. The renal manifestation ranges from severe, early onset cystic kidney disease[6] to slowly progressive, fibrotic remodeling of the kidney with CKD starting in adolescence.[7] Interestingly, the genotype–phenotype correlation in NPHP-RC is dependent on the gene and the specific mutation involved, which can both give rise to a broad phenotypic disease spectrum. NPHP-RCs are a very heterogeneous disease group, as by now mutations in more than 90 genes have been identified as causative for renal ciliopathies in humans.[4] Mutations in some of these genes are very rare, accounting for only two[8] or three[9] families worldwide. WES with direct inspection of the coding regions of these genes therefore represents the most rational and currently, most cost-effective approach for mutation analysis in these patients.[1,10–12] So far, no more than 13 ciliopathy genes have been systematically studied in a larger patient cohort.[13,14]

Here, we performed WES combined with homozygosity mapping in an international cohort of 79 families with pediatric onset of CKD and suspected nephronophthisis based on renal ultrasound presentation with chronically increased echogenicity, loss of cortico-medullary differentiation, and/ or $\geqslant 2$ cysts. All individuals were born of consanguineous union, or represented familial cases of CKD, and were therefore at high risk for recessive, monogenic diseases. In summary, we were able to identify a causative mutation in a known monogenic disease gene in 50 families (63.3%). In 32 of these families (64%), WES identified mutations in NPHP-RC genes as the molecular disease cause and confirmed the suspected clinical diagnosis. However, in 18 families (36%), we discovered a molecular diagnosis of a monogenic kidney disease that was not NPHP-RC, specifically renal tubulopathies ($n=8$, 16%), Alport syndrome ($n=4$, 8%), congenital anomalies of the kidney and urinary tract (CAKUT) ($n=3$, 6%), autosomal recessive polycystic kidney disease (ARPKD) ($n=2$, 4%), and autoimmune nephropathy (APECED (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy) syndrome) ($n=1$, 2%). In five consanguineous families, in whom we excluded mutations in known monogenic disease genes, we identified five novel candidate genes for NPHP-RC (*RBM48, FAM168B, PIAS1, INCENP,* and *RCOR1*).

RESULTS

WES identifies the molecular disease cause in 63% of cases

We performed WES in 79 families with suspected NPHP-RC based on renal ultrasound criteria (chronically increased echogenicity, loss of cortico-medullary differentiation, and/or $\geqslant 2$ renal cysts). In 50/79 families (63.3%), we identified a mutation in a gene that is known to cause monogenic renal disease when mutated (Figure 1a, Supplementary Tables S1 and S2 online).

NPHP-RCs account for 64% of molecularly diagnosed individuals

Thirty-two of the 50 families with established molecular diagnosis after WES (64.0%) harbored a mutation in a known NPHP-RC gene (Figure 1b). Of the 90 genes that are known to cause renal ciliopathies when mutated, which were systematically analyzed in this study, mutations in 18 genes contributed to this result. Mutations in the genes *NPHP3, NPHP4*, and *NPHP5* accounted for the majority of NPHP-RC cases (Table 1, Supplementary Tables S1 and S2 online). In addition to mutations in NPHP-RC genes, we detected causative mutations in monogenic genes of renal tubulopathies (8/50), Alport syndrome (4/50), CAKUT (3/50), and ARPKD (2/50). Furthermore, we established the molecular diagnosis of APECED in one previously undiagnosed individual (Figure 1b, Table 1, Supplementary Tables S1 and S2 online). In 10 individuals, the molecular diagnosis after WES was different from the previous clinical diagnosis. In particular, in one case of previously undiagnosed nephropathic cystinosis the molecular finding changed the therapeutic regimen (Supplementary Tables S1 and S2 online).

Mutations in more than one recessive disease gene can be present in consanguineous families

In one example of a highly inbred family (412 Mb of cumulative homozygosity), we identified homozygous mutations in seven known monogenic disease genes. Four of them are known to cause diseases with renal involvement (Supplementary Table S4 online). The affected child showed a complex phenotype suggestive of a renal ciliopathy, including Caroli's disease with massive cystic dilation of intrahepatic bile ducts, CKD that progressed to end-stage renal failure within the third year of life, post-axial polydactyly, nystagmus, and rod cone dystrophy. In addition to chronically increased echogenicity as the classical symptom of NPHP-RC, the child showed congenital hydronephrosis due to uretero-pelvic junction obstruction that required corrective surgery, as well as renal tubular acidosis type 4 (Supplementary Table S4 online). An older brother, from whom no DNA sample was available for mutation analysis, deceased at 15 months of age due to end-stage renal and liver disease. In addition, he