Simultaneous sequencing of 37 genes identified causative mutations in the majority of children with renal tubulopathies

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The clinical diagnosis of inherited renal tubulopathies can be challenging as they are rare and characterized by significant phenotypic variability. Advances in sequencing technologies facilitate the establishment of a molecular diagnosis. Therefore, we determined the diagnostic yield of a next generation sequencing panel assessing relevant disease genes in children followed through three national networks with a clinical diagnosis of a renal tubulopathy. DNA was amplified with a kit provided by the European Consortium for High-Throughput Research in Rare Kidney Diseases with nine multiplex PCR reactions. This kit produced 571 amplicons covering 37 genes associated with tubulopathies followed by massive parallel sequencing and bioinformatic interpretation. Identified mutations were confirmed by Sanger sequencing. Overall, 384 index patients and 16 siblings were assessed. Most common clinical diagnoses were 174 patients with Bartter/Gitelman syndrome and 76 with distal renal tubular acidosis. A total of 269 different variants were identified in 27 genes, of which 95 variants were considered likely, 136 definitely pathogenic and 100 had not been described at annotation. These mutations established a genetic diagnosis in 245 of the index patients. Genetic testing changed the clinical diagnosis in 16 cases and provided insights into the phenotypic spectrum of the respective disorders. Our results demonstrate a high diagnostic yield of genetic testing in children with a clinical diagnosis of a renal tubulopathy, consistent with a predominantly genetic etiology in known disease genes. Thus, genetic testing helped establish a definitive diagnosis in almost two-thirds of patients thereby informing prognosis, management and genetic counseling.

Kidney International (2018) ■, ■–■; https://doi.org/10.1016/j.kint.2017.10.016

KEYWORDS: Bartter syndrome; children; genetic testing; Gitelman syndrome; next generation sequencing; renal tubular acidosis; tubulopathy

The renal tubules reabsorb the vast majority of the glomerular filtrate, and in this way, preserve the "milieu interieur" and maintain homeostasis critical for normal physiology.[1] This task is performed by an array of specialized transporters and channels, the dysfunction of which can lead to a number of specific disorders, collectively referred to as tubulopathies. While tubulopathies can be inherited or acquired, identification of a genetic basis in inherited forms is desirable as it establishes a clear diagnosis, enabling specific work-up, genetic counseling, and cascade screening of at-risk relatives. Moreover, clinical observations in genetically stratified cohorts of patients not only improve understanding of the role of the causal gene, but also enable collection of long-term outcome data that inform prognosis and management of patients affected by the respective disorders.[2] Previously,

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Received 15 April 2017; revised 29 September 2017; accepted 5 October 2017

individual candidate genes based on clinical suspicion were sequenced; a process suitable if the clinical diagnosis is convincing and only a single gene needs to be screened. However, several tubulopathies can be caused by multiple genes or have phenotypic overlap with other disorders or be a combination of both, such as Bartter and Gitelman syndromes or distal renal tubular acidosis, making a single gene approach cumbersome and expensive. With the advent of next generation sequencing (NGS), simultaneous sequencing of multiple genes has become feasible and is increasingly performed. Driven by the rapidly decreasing costs of NGS, whole exome or even whole genome sequencing is increasingly utilized, but panel sequencing of selected genes provides the advantage of achieving high coverage of genes of interest at lower costs.[3] The working group for tubulopathies in the European Consortium for High-Throughput Research in Rare Kidney Diseases (EURenOmics)[4] designed a kit for targeted amplification of 37 known tubulopathy disease genes. Here we describe our experience with this kit in a cohort of 410 patients from 384 families, recruited predominantly from dedicated networks for renal tubulopathies centralized in London, Paris, and Brussels.

RESULTS

Gene amplification and sequencing

The depth and horizontal coverage of the panel were assessed after the first run (23 samples). All of the targeted regions were covered at >30X except for exon 1 of *OCRL* and *WNK1*, both of which have a high guanine-cytosine content. These exons were assessed by Sanger sequencing in those patients with a clinical diagnosis compatible with 1 of these genes and no other identified causative mutation.

Patients

A total of 384 index patients and 26 siblings were assessed. The most common clinical diagnosis was "Bartter/Gitelman syndrome" ($n = 174$) and distal renal tubular acidosis (dRTA; n = 76), followed by pseudohypoaldosteronism type 1 (PHA1; $n = 31$) and nephrogenic diabetes insipidus ($n = 23$), and a genetic diagnosis could be established in 74%, 58%, 42%, and 83% of cases, respectively. A list of the clinical diagnoses, the respective numbers of patients, and the diagnostic yields are provided in Table 1.

Genes

A total of 37 known tubulopathy disease genes were assessed. The genes that most commonly provided a genetic diagnosis were *SLC12A3* (63 patients), *CLCNKB* (29 patients), *SLC12A1* (22 patients), and *ATP6V0A4* (22 patients). A list of the 37 genes, the number of different mutations identified, and the number of patients in whom they provided a genetic diagnosis is provided in Table 2.

Variants

A total of 269 different variants were identified in 27 genes, of which 136 were deemed definitely (class 5) and 95 likely pathogenic (class 4), as well as 36 of unknown significance (class 3). One hundred variants had not been reported previously at time of annotation. The class 4 and 5 mutations provided a likely or definite genetic explanation of the clinical phenotype in 245 of the 384 tested index patients (64%) and in 270 (66%) of the overall cohort. Twenty-three index patients had affected siblings and the identified mutation(s) were subsequently also found in the siblings. A list of all patients and their identified mutations is provided in Supplementary Table S1, with reference sequences used for annotation provided in Supplementary Table S2. A list of all mutations identified with assigned variant class, arranged by gene and with novel mutations highlighted is provided in Supplementary Table S3. The previously known mutations (positive controls, see Supplementary Table S4) were all identified (100% sensitivity). All putative disease-causing variants identified by the panel were confirmed by Sanger sequencing.

Table 1 | Clinical and molecular diagnosis in the 384 index patients

Clinical diagnosis	Patients (*n*)	Genetic diagnosis [*n* (%)]
Bartter or Gitelman syndrome	174	128 (74)
EAST syndrome	3	2 (67)
PHA1	31	13 (42)
PHA2	4	3 (75)
dRTA	76	44 (58)
pRTA	1	1 (100)
NDI	23	19 (83)
FHH	12	5 (42)
ADH	1	1 (100)
Dent disease	15	6 (40)
Lowe syndrome	1	0 (0)
FHHNC	5	4 (80)
HOMG	7	4 (57)
Infantile hypercalcemia	14	6 (43)
Hypophosphatemic rickets	5	4 (80)
HC/NC	11	4 (36)
Hypokalemia	1	1 (100)
Total	384	245 (64)

ADH, autosomal dominant hypercalcemia; dRTA, distal renal tubular acidosis; EAST, epilepsy, ataxia, sensorineural deafness, tubulopathy; FHH, familial hypercalcemic hypocalciuria; FHHNC, familial hypomagnesemia with hypercalciuria and nephrocalcinosis; HC/NC, isolated hypercalciuria/nephrocalcinosis; HOMG, hypomagnesemia; NDI, nephrogenic diabetes insipidus; PHA1, pseudohypoaldosteronism type 1; PHA2, pseudohypoaldosteronism type 2; pRTA, proximal renal tubular acidosis.

Genetic revision of the clinical diagnosis

Genetic results lead to revision of the clinical diagnosis in 22 (16 index) patients (see Table 3). Ten (4 index) patients with a clinical diagnosis of idiopathic hypercalciuria or nephrocalcinosis or both were found to have either heterozygous ($n = 7$) or biallelic mutations ($n = 3$) in *SLC34A3*, establishing a diagnosis of hypophosphatemic rickets with hypercalciuria or its carrier status. In 9 patients, the revision was from Bartter to Gitelman syndrome ($n = 6$) or vice versa ($n = 3$, all with mutations in *CLCNKB*). One patient with a clinical diagnosis of "Dent disease/rickets" (P151) and one with "hypokalemia" (P103) were found to have dRTA by