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

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Running title: Genetic testing in children with tubulopathies

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word count: main text: 2867 (excluding concise methods), abstract: 249

Abstract

The clinical diagnosis of inherited renal tubulopathies can be challenging, as they are rare characterized by significant phenotypic variability. Advances in sequencing technologies facilitate the establishment of a molecular diagnosis. We aimed to investigate the diagnostic yield of a next generation sequencing panel assessing relevant disease genes in children with a clinical diagnosis of a renal tubulopathy followed through three national networks. DNA was amplified with a kit provided by the European Consortium for High-Throughput Research in Rare Kidney Diseases with 9 multiplex PCR reactions producing 571 amplicons to cover 37 genes associated with tubulopathies, followed by massively parallel sequencing and bioinformatic interpretation. Identified mutations were confirmed by Sanger sequencing. Overall, we assessed 384 index patients and 16 siblings. Most common clinical diagnoses were Bartter/Gitelman syndrome (N=174) and distal renal tubular acidosis (N=76). A total of 269 different variants were identified in 27 genes, of which 95 were considered likely and 136 definitely pathogenic and 100 had not been described at annotation. These mutations established a genetic diagnosis in 245 (64%) of the index patients. . Genetic testing changed the clinical diagnosis in 16 (4%) of 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. Genetic testing helped establish a definitive diagnosis in almost two thirds of patients and thereby informed prognosis, management and genetic counselling.

Key words: Genetic testing, next generation sequencing, tubulopathy, children, Bartter syndrome, Gitelman syndrome, renal tubular acidosis

Introduction

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 specialised transporters and channels, the dysfunction of which can lead to 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 counselling and cascade screening of at-risk relatives. Moreover, clinical observations in genetically stratified cohorts of patients not only help 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 disorder.[2] Previously, 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 and/or have phenotypic overlap with other disorders, 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 cost.[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 one of *OCRL* and *WNK1*, both of which have a high GC content. These exons were assessed by Sanger sequencing in those patients with a clinical diagnosis compatible with one 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 dRTA (N=76), followed by Pseudohypoaldosteronism type 1 (PHA1, N=31) and Nephrogenic Diabetes Insipidus (NDI, N=23) and a genetic diagnosis could be established in 74, 58, 42 and 83%, respectively. A list of the clinical diagnoses, the respective number of patients and the diagnostic yield is 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* and *ATP6V0A4* (22 patients each). 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 (64%) of the 384 tested index patients and in 270 (66%) of the overall cohort. 23 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 supplemental table 1, with reference sequences used for annotation provided in supplemental table 2. A list of all mutations identified with assigned variant class, arranged by gene and with novel mutations highlighted is provided in supplemental table 3. The previously known mutations (positive controls, see supplemental table 4) were all identified (100% sensitivity). All putative disease causing variants identified by the panel were confirmed by Sanger sequencing.

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/nephrocalcinosis were found to have either heterozygous (N=7) or bi-allelic mutations (N=3) in *SLC34A3*, establishing a diagnosis of hypophosphatemic rickets with hypercalciuria (HHRH) 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 distal renal tubular acidosis (dRTA) by genetic testing. Patient L70 had a clinical diagnosis of Gitelman syndrome, yet was found to have autosomal dominant tubulointerstitial kidney disease (ADTKD), based on a heterozygous deletion of *HNF1B*. A renal ultrasound at the age of 14

years was initially reported as normal, but on review showed increased echogenicity with reduced corticomedullary differentiation.

Discussion

We report the use of targeted amplification and NGS of 37 "tubulopathy genes" in a large multinational cohort of patients with childhood-onset renal tubulopathies. Our most striking finding is the high diagnostic yield, which far surpasses the experience in other disease areas. In steroid-resistant nephrotic syndrome, the reported diagnostic yield varies between 23- 30%, although it is far lower for patients presenting after the first year of life.[5, 6] In nephronophthisis-related ciliopathies the reported yield is around 20-25%,[7, 8] while it is even lower, around 5-15%, in CAKUT.[9, 10] Our high diagnostic yield is comparable to previously reported cohorts of patients with autosomal recessive dRTA and Gitelman syndrome.[11, 12] This is consistent with the concept that the vast majority of childhood-onset tubulopathies have a genetic basis and that the majority of causative genes have been identified. Inclusion of more genes into the panel may have increased the yield further. For example, there were 8 index patients with a clinical diagnosis of infantile hypercalcemia, but no genetic diagnosis and one of the known causative genes, *CYP24A1*, was not included in the panel. Similarly, neither PHEX nor FGF23 were included to assess the patient with a clinical diagnosis of hypophosphatemic rickets, who had no underlying mutation identified. More genes associated with hypomagnesemia have been identified since the development of the panel, inclusion of which might have established a genetic diagnosis in the patients with a clinical diagnosis of hypomagnesemia and thus increased the diagnostic yield. The absence of these genes demonstrates the limitations of such a panel and the need for regular updates, whilst striking a balance between the expected diagnostic yield and cost efficiency.

Interestingly, we noted a high conformity of clinical and genetic diagnosis suggesting that an accurate clinical diagnosis can be established in most cases. Renal tubulopathies are characterized by typical constellations of clinical and biochemical findings, which can pinpoint a diagnosis.[13-15] Although there was discrepancy between the clinical and genetic diagnosis in 22 cases, the revision was rarely substantial. In most cases the diagnosis changed from Bartter to Gitelman or vice versa. Phenotypic overlap between Bartter type 3 and Gitelman syndromes is well recognized.[16, 17] Of interest is the

change in diagnosis in patient L70 from Gitelman syndrome to ADTKD. While electrolyte abnormalities, especially hypomagnesemia, but also hypokalemia have been previously associated with HNF1B mutations, this has been in patients with concurrent renal malformations, such as cystic dysplasia, which was not reported as present in the ultrasound from this patient.[18, 19]

In addition, genetic testing established a specific diagnosis in several patients with a clinical diagnosis of idiopathic hypercalciuria/nephrocalcinosis, mostly on the basis of mutations in *SLC34A3*. Recessive mutations in this gene cause HHRH and carriers have been described to have hypercalciuria.[20] The establishment of a diagnosis of dRTA in the 2 patients with a clinical diagnosis of "hypokalemia" and "Dent disease/rickets" likely reflects the occasional difficulties in establishing a diagnosis of dRTA early in life, as well as an associated proximal tubulopathy, which we reported in two-thirds of patients with dRTA at initial presentation and appears to be linked to the acidosis.[21] Indeed, the features of the proximal tubulopathy disappeared with correction of the acidosis also in this patient. Overall, the high diagnostic yield and the lack of substantial revision of the diagnosis in the vast majority of cases suggests a high level of clinical expertise present in the respective tubulopathy networks.

Yet, even in cases where the clinical diagnosis was confirmed, there was occasional diagnostic uncertainty, where genetic testing helped clarify the situation: for instance, there were 2 patients (patients L103 and L104), who initially presented to their local hospital in the first month of life with failure to thrive and were found to have hyponatremia, hyperkalemia and acidosis with elevated aldosterone levels, suggesting a clinical diagnosis of PHA1. However, when the children were assessed in the tubulopathy clinic at 3 and 5 months of life, respectively, no abnormalities were found: the children were thriving with no apparent electrolyte abnormalities, normal distal potassium secretion (transtubular potassium gradient, TTKG > 8) and remained well also after weaning off the salt supplementation. Consequently, there was uncertainty about the etiology of the initial presentation. There was no family history of PHA1 and an acquired form of PHA1 was considered, for instance in the context of a urinary tract infection, that had been unrecognised at the time of presentation (both had

initially received antibiotics due to their poor clinical state).[22] Yet, in both cases we identified heterozygous mutations in *NR3C2*, the gene encoding the mineralocorticoid receptor, establishing a diagnosis of autosomal dominant PHA1. Spontaneous resolution of symptoms during childhood has been reported in this disorder, as well a high proportion of asymptomatic adult *NR3C2* mutation carriers.[23, 24] Yet, our experience here suggests that symptoms can disappear even as early as during the first few months of life. Carriers of such mutations nevertheless are reported to have lifelong increased plasma renin and aldosterone levels.[25] Consistent with this observation, aldosterone levels in our patients subsequently returned elevated (L103: 5100, L104: 2200, normal <700 pmol/l). Apart from providing an explanation for the initial severe presentation, in one of these cases, the genetic diagnosis had further direct clinical implications, as it allowed early diagnosis in a subsequently born sibling.

Other cases of diagnostic uncertainty were those of patients with complex phenotypes, i.e. with symptoms beyond the tubulopathy. For instance, the pregnancy with patient L19 was complicated by antenatally noted cardiac calcifications, as well as polyhydramnios. Postnatally, an annular calcification at the root of the aorta was seen, as well as nephrocalcinosis and she was noted to have hyperkalemia and hyponatremia, which over the first two weeks of life changed to the typical electrolyte constellation of Bartter syndrome with hypokalemic alkalosis. This evolution of electrolyte abnormalities was consistent with a diagnosis of Bartter syndrome type 2, but it was unclear whether the patient suffered from an unknown complex syndrome that included a Bartter-like tubulopathy, or whether there were two separate diagnoses: Bartter syndrome plus aortic calcification. Genetic testing identified a homozygous mutation in *KCNJ1*, thus suggesting the co-existence of two separate diseases in this offspring of a consanguineous marriage.

An intriguing finding are the heterozygous mutations in *ATP6V1B1* c.1181G>A, p.(Arg394Gln) in patients L80 and L82 and c.1180C>G, p.(Arg394Gly) in patient P73, affecting the same amino acid. The clinical features of patient L80 have been described recently and like those of L82 and P73 clearly fit

the phenotype of dRTA,[21] but with no evidence of hearing loss, a feature typical for patients with recessive *ATP6V1B1* mutations. Given that *ATP6V1B1* is a recessive disease gene, the dRTA in these 3 patients was defined as "genetically unsolved". Yet, interestingly, this mutation has been described previously in only heterozygous state in patients with dRTA with (mild) or without hearing loss.[12, 26] None of these patients have a recognized family history of dRTA, arguing against a dominant negative effect, unless the mutation occurred *de novo* in all. We were only able to test the parents of patient L80, and both did not carry this variant, arguing for a *de novo* mutation. The variants are not reported in the ExAC browser and thus likely rare and present in these patients not just by coincidence. Further studies are needed to better understand a potential causality of this variant.

It is important to note that in this study only variants classified as "probably" or "definitely" pathogenic have been accepted as causative and used for the analysis. Yet, there were additional patients carrying variants of unknown significance (VUS), where a causative role is possible. For instance, patient L40 has a clinical diagnosis of Bartter syndrome type 4: She was born at 27 weeks gestation after a pregnancy complicated by polyhydramnios, noted to have hypochloremic, hypokalemic metabolic alkalosis and sensorineural deafness, treated with cochlear implants, all consistent with Bartter syndrome type 4. Yet, her subsequent clinical course was unusually mild for this diagnosis. She has grown and developed normally (at the age of 16 years now on the 25th to 50th percentile for height and weight), attending mainstream schools and with only borderline abnormal plasma electrolytes. Genetic testing in her revealed a recognized pathogenic mutation in Barttin on one allele (p.(Gly47Arg)) and a VUS on the other (p.(Ser42Asn)). This VUS is not in the EXaC database and thus unlikely a common polymorphism, but it scores low for pathogenicity in prediction algorithms (see supplemental table 1). It is tempting to speculate that the Ser42Asn variant retains some Barttin functionality and, in trans with the Gly47Arg variant, which itself has been associated with a less severe phenotype, can explain the unusually mild clinical course.[27] Overall, there were 25 patients, in which we identified VUS in genes consistent with the clinical diagnosis and where pathogenicity of these VUS would provide a genetic explanation. Thus, it is likely, that the true genetic

identification rate is even higher than we currently state. Identification of further patients carrying these variants, familial segregation analysis and/or functional studies may change the classification of these variants in the future. Moreover, a potential disadvantage of NGS is the limited ability to call copy number variations and it is possible that we may have missed some in our cohort. Similarly, the genetic testing in this cohort was performed by three independent clinical genetic laboratories using their own respective NGS analysis pipelines. Different platforms can yield different results and while there was no evidence for false positive findings, as all identified variants could be confirmed by Sanger sequencing, it is possible that individual variants may have been missed.[28] Again, this suggests that the true number of patients with a genetic cause in a recognized disease gene is even higher than we state.

An interesting finding is the large number of patients with a genetic diagnosis of Gitelman syndrome (N=73), accounting for almost 20% of the entire patient cohort and similar in number to patients with Bartter syndrome (N=72, all 4 tested subtypes combined) and substantially more than patients with dRTA (N=50, all 3 tested subtypes combined). Gitelman syndrome is sometimes considered a disorder mostly presenting in adulthood, but our findings clearly argue for its relevance in children, as well.[16] Our findings are consistent with the notion of Gitelman syndrome being the most common tubulopathy, even in children.[29]

In contrast, the almost complete absence of patients with a clinical or genetic diagnosis of ADTKD is surprising and may represent a bias in our cohort. Whilst mutations in *MUC1* were not tested for, due to the technical difficulties of identifying the recognized pathogenic variant by NGS, this mutation is very rare.[30] Patients with mutations in *UMOD* may predominantly present in adulthood, but mutations in *HNF1B* have been recurrently reported in children with clinical signs of a tubulopathy.[18, 31] Presumably, the usually concurrent renal malformation combined with the relatively easy availability

of genetic testing for *HNF1B* in the three involved countries has led to a lack of referring of such patients to the laboratories, as most already had a genetic diagnosis.

Summary

In conclusion, we find that comprehensive genetic testing of childhood-onset renal tubulopathies establishes a likely genetic diagnosis in the majority[32] of patients referenced through a tubulopathy network. While clinical assessment in experienced hands can accurately identify the general diagnosis in most cases, identification of the molecular basis is still needed for precise genetic counselling and early diagnosis of subsequent siblings. Genetic testing enables dissection of complex phenotypes and patient stratification by genetic sub-diagnosis, such as the different types of Bartter syndrome and Gitelman syndrome, to improve our understanding of the associated phenotype, which in turn informs patient management. The early resolution of clinical symptoms in autosomal dominant PHA1 and the Gitelman-like tubulopathy without obvious renal malformation associated with an *HNF1B* mutation are examples for such insights gained. Moreover, renal tubulopathies are rare kidney diseases, many of which nephrologists practicing outside a specialized center may never encounter in their lifetime, leading to challenges in diagnosis and treatment.[4] Our study suggests that genetic testing is highly likely to help establish a specific diagnosis in children with symptoms suggestive of a tubulopathy, allowing targeted management.

Methods

A detailed description of the methods is provided in the supplemental appendix.

Gene selection

In 2012, the tubulopathy working group of the European Consortium for High-Throughput Research in Rare Kidney Diseases (EURenOmics) met to decide on gene inclusion for a comprehensive genetic

testing panel. At the time, the group identified a total of 37 genes, mutations in which were recognized causes of tubulopathies. Only these and no candidate genes were included in the panel (table 2).

Patients

DNA samples from a total of 410 patients from 384 families were assessed. Included were patients with a clinical diagnosis of a tubulopathy that was made before the age of 18 years and was consistent with the 37 genes tested. Of these, 139 samples were analysed in London, 195 in Paris and 76 in Brussels. Informed consent for genetic testing was obtained by the respective treating physician after approval by the respective institutional review boards, in accordance with the Declaration of Helsinki. An overview of the clinical diagnoses is provided in table 1. A list of all patients with their respective clinical and molecular diagnosis is provided in supplemental table 1. Detailed clinical features of some patients with dRTA and Bartter syndrome have been reported previously.[21, 32-35]

Controls

Samples were tested from 22 patients with known mutations in 17 different genes in the panel to determine the sensitivity of the assay. These "positive control" mutations included a spectrum of different types including missense, nonsense, splice site and whole exon deletions present in either homozygous, heterozygous and hemizygous state and are listed in supplemental table 4.

Laboratory

Genetic testing was performed in three clinical genetic laboratories:

1) The North East Thames Regional Genetics Services, located at GOSH, which is accredited to ISO 15189 standard by the relevant UK authority (UKAS reference 7883), allowing us to perform genetic testing on a clinical basis with provision of formal reports.
2) The laboratory of molecular genetics of the HEGP is accredited to ISO 15189 standard by the relevant French authority (COFRAC reference N°8-3147).
3) The laboratory of molecular and cellular biology at the IPG institute, which is accredited to ISO 15189 standard by the relevant Belgian authority (BELAC reference 381-MED).

Gene amplification and next generation sequencing

The 37 target genes were amplified with a total of 9 multiplex PCR reactions generating 571 amplicons. MASTR kits for multiplex PCR were developed and provided by Multiplicom (www.multiplicom.com), an industry partner in the EURenOmics consortium. A detailed description of the NGS library generation is provided in the supplementary data.

Bioinformatic analysis

Bioinformatic analysis was performed by various in house pipelines in the three laboratories involved, detailed in the supplemental methods. In general, variants had to be present in 20% of at least 30 reads to be called. Further filtering excluded variants present at 2% or greater in exome variant server (EVS) or 1000 genomes datasets or in greater than four patients on a run of 23 (unless seen in patients with same clinical diagnosis). Samples were checked for copy number variations (CNV) using a combination of read depth (for homozygous deletions) and Exome Depth (http://cran.r-project.org/web/packages/ExomeDepth/index.html). Alamut®Visual software (www.interactive-biosoftware.com) was used to assist in determining variant pathogenicity. The respective reference sequences used for annotation are provided in supplemental table 2.

Identified variants were checked against relevant databases, such as Clinvar (https://www.ncbi.nlm.nih.gov/clinvar/); HGMD (http://www.hgmd.cf.ac.uk/ac/index.php), Varsome (https://varsome.com/) and local databases to assess for previous publication.

Variant annotation

Identified variants were annotated by the three involved clinical genetic laboratories according to published guidelines.[36] Variants were considered disease-causing, if they were consistent with the inheritance pattern, if the gene in which they were identified was compatible with the clinical findings and if there was evidence, based on previous reports or prediction algorithms (SIFT, Align GVD, mutation taster and Polyphen2 for pathogenicity, so that variants were either classified as "likely pathogenic"(class 4) or "definitely pathogenic" (class 5). If available, segregation was assessed within the family to confirm pathogenicity. "Variants of uncertain significance" (class 3) were recorded but were not considered disease-causing for the purpose of this study.

Each variant identified and annotated in one laboratory was independently annotated by at least one of the other laboratories. For those variants with discordant annotation, final classification was reached by consensus of the three laboratories using the relevant guidelines.[36]

Mutation confirmation

All identified mutations were confirmed by Sanger sequencing. Primers for mutation confirmation were designed using Primer 3 software (http://bioinfo.ut.ee/primer3). Sequencing was performed according to standard methods for point mutations.

Disclosures

AR and JDF are employees of Multiplicom, provider of the kits used for amplification of the 37 genes.

The other authors have no interests to disclose.

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Acknowledgements

Funding for this study was kindly provided by the European Union, FP7 (grant agreement 2012-305608 "European Consortium for High-Throughput Research in Rare Kidney Diseases (EURenOmics)"; by the British Kidney Patient Association (BKPA); and by the clinical research priority program (KFSP) radiz (Rare Disease Initiative Zurich) of the University of Zurich.

Table legends

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

Shown are the clinical diagnoses, respective number of patients, as well as the number (proportion) of patients with a genetic diagnosis.

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

Table 2: The 37 genes included in the panel with associated diagnosis, number of index patients with causative mutations and total number of different variants.

Shown is a list of the 37 genes included in the panel and the number of index patients with mutations in the respective genes. Variants were detected in 26 of the 37 genes tested. Note that the total number of variants includes those of uncertain significance and thus number of variants may exceed the number of causative mutations.

ad: autosomal dominant; ar: autosomal recessive; Xl: X-linked; dRTA: distal renal tubular acidosis; pRTA: proximal renal tubular acidosis; PHA1: pseudohypoaldosteronism type 1; PHA2:

pseudohypoaldosteronism type 2; FHHNC: familial hypomagnesemia with hypercalciuria and nephrocalcinosis; HOMG: hypomagnesemia; NDI: nephrogenic diabetes insipidus; pRTA: proximal renal tubular acidosis; PHA2: pseudohypoaldosteronism type 2, ADTKD: autosomal dominant tubulointerstitial kidney disease; HHRH: hereditary hypophosphatemic rickets with hypercalciuria

3: Index patients with discrepant clinical and genetic diagnosis

Listed are the 16 index patients in whom genetic testing changed the clinical diagnosis, as well as their initial clinical and subsequent genetic diagnosis.

ADTKD: autosomal dominant tubulointerstitial kidney disease; dRTA: distal renal tubular acidosis; HHRH: hereditary hypophosphatemic rickets with hypercalciuria

Table 1: Clinical and genetic diagnosis in the 384 index patients

Clinical diagnosis	Patients (N)	Genetic diagnosis N (%)
Bartter/ 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	385	246 (70)

Table 2: The 37 genes included in the panel with associated diagnosis, number of index patients with causative mutations and total number of different variants.

Gene	Associated Diagnosis	MIM	Patients	Different variants
SLC12A1	Bartter syndrome type 1	601678	22	29
KCNJ1	Bartter syndrome type 2	241200	9	9
CLCNKB	Bartter syndrome type 3	607364	29	21
BSND	Bartter syndrome type 4	602522	4	5
SLC12A3	Gitelman syndrome	263800	63	70
KCNJ10	EAST syndrome	612782	2	2
NR3C2	PHA1, ad	177735	8	6
SCNN1A	PHA1, ar	264350	4	7
SCNN1B			0	5
SCNN1G			1	1
WNK1	PHA2	614492	0	0
WNK4		614491	0	0
CUL3		614496	0	0
KLHL3		614495	3	3
SLC4A1	dRTA, ad and ar	179800	6	8
ATP6V0A4	dRTA, ar	602722	22	24
ATP6V1B1		267300	18	14
SLC4A4	pRTA with eye findings	604278	1	2
AQP2	NDI, ar	125800	2	4
AVPR2	NDI, Xl	304800	17	13
CaSR	FHH	145980	5	6
GNA11		145981	0	0
AP2S1		600740	1	1
CLCN5	Dent disease 1	300009	3	3
OCRL	Dent disease 2/Lowe syndrome	300555	3	5
CLDN16	FHHNC	248250	4	5
CLDN19		248190	0	2
TRPM6	HOMG	602014	4	5
EGF		611718	0	0
FXYD2		145020	0	0
KCNA1		176260	0	0
HNF1B	ADTKD	137920	1	1
REN		613092	0	0
UMOD		162000	0	0
SLC34A1	Infantile Hypercalcemia	616963	4	7
SLC34A3	HHRH	241530	9	11
SLC9A3R1	Nephrolithiasias,	612287	0	0

Table 3: Index patients with discrepant clinical and genetic diagnosis

Patient	Clinical diagnosis	Genetic diagnosis
B39	Gitelman syndrome	Bartter syndrome type 3
L28	Gitelman syndrome	Bartter syndrome type 3
L38	Gitelman syndrome	Bartter syndrome type 3
B38	Bartter syndrome	Gitelman syndrome
L54	Bartter syndrome	Gitelman syndrome
P19	Bartter syndrome	Gitelman syndrome
P20	Bartter syndrome	Gitelman syndrome
P22	Bartter syndrome	Gitelman syndrome
L44	Bartter syndrome	Gitelman syndrome
L70	Gitelman syndrome	ADTKD HNF1B
P151	Dent disease/rickets	dRTA
P103	Hypokalemia	dRTA
P146	Nephrocalcinosis	HHRH carrier
P147	Nephrocalcinosis	HHRH carrier
P149	Nephrocalcinosis	HHRH carrier
P150	Nephrocalcinosis	HHRH