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Hereditary tubulopathies are rare diseases with unknown prevalence in adults. Often diagnosed in childhood, hereditary tubulopathies can nevertheless be evoked in adults. Precise diagnosis can be difficult or delayed due to insidious development of symptoms, comorbidities and polypharmacy. Here we evaluated the diagnostic value of a specific panel of known genes implicated in tubulopathies in adult patients and compared to our data obtained in children. To do this we analyzed 1033 non-related adult patients of which 744 had a clinical diagnosis of tubulopathy and 289 had a diagnosis of familial hypercalcemia with hypocalciuria recruited by three European reference centers. Three-quarters of our tubulopathies cohort included individuals with clinical suspicion of Gitelman syndrome, kidney hypophosphatemia and kidney tubular acidosis. We detected pathogenic variants in 26 different genes confirming a genetic diagnosis of tubulopathy in 29% of cases. In 16 cases (2.1%) the genetic testing changed the clinical diagnosis. The diagnosis of familial hypercalcemia with hypocalciuria was confirmed in 12% of cases. Thus, our work demonstrates the genetic origin of tubulopathies in one out of three adult patients, half of the rate observed in children. Hence, establishing a precise diagnosis is crucial for patients, in order to guide care, to survey and prevent chronic complications, and for genetic counselling. At the same time, this work enhances our understanding of complex phenotypes and enriches the database with the causal variants described.

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Inherited renal tubulopathies are rare diseases often diagnosed in children, particularly those with autosomal recessive transmission. Although some tubulopathies are diagnosed in adulthood, including recessive diseases with potentially mild presentation (e.g., Gitelman syndrome), slowly progressive dominant diseases (e.g., autosomal dominant tubulointerstitial kidney disease), and diseases with variable severity (e.g., Dent disease), the prevalence of tubulopathies in adults remains mostly unknown.[1–3] In addition, the clinical presentation may be atypical or insidious, complicating and delaying the diagnosis of tubular dysfunction.[4]

Advances in understanding renal tubular solute transport systems has been achieved through the elucidation of

monogenic tubular disorders and follow-up studies using cellular and animal models.[5] In turn, these advances allowed us to improve molecular diagnosis and genetic counselling, as well as to devise therapeutic or preventive measures.[6] The introduction of high-throughput gene panel sequencing makes the analysis of several genes at the same time possible for genetically heterogeneous diseases and allows for accurate diagnosis in patients with atypical presentations.[7]

We recently published the experience of 3 European centers using a next-generation sequencing panel of 37 known genes to assess children with suspected tubulopathies and confirmed genetic disease in two-thirds of those tested.[8] In this article, we describe our results in a large adult population using the same panel as well as an improved second version containing 9 additional genes.

RESULTS

Patients

The clinical diagnoses of the adult cohort of 1033 patients comprise 20 different tubulopathies and familial hypercalcemia hypocalciuria (FHH) (Table 1). Four diagnoses correspond to 75% of the cohort: FHH ($N = 289$); Gitelman syndrome (GS) ($N = 272$); renal hypophosphatemia (RH) ($N = 124$), which includes nephrolithiasis/osteoporosis hypophosphatemic and hereditary hypophosphatemic rickets with hypercalciuria; and distal renal tubular acidosis (dRTA) ($N = 86$). Details of additional suspected diagnoses and their proportion in the cohort are summarized in Table 1.

Overall diagnostic performance: initially suspected and diagnostic revision

In this cohort, pathogenic or likely pathogenic variants were detected in 26% of cases (269 of 1033), allowing a genetic confirmation. In 24.5% of cases (253 of 1033), these variants were directly related to the diagnosis initially suspected. In contrast, in 1.5% of cases (16 of 1033), the genetic findings corresponded to a different diagnosis, symptoms of which may phenocopy the suspected initial pathology. If we consider only tubulopathies, the revision rate is 2.1% (16 of 744). The global performance is summarized in Supplementary Figure S1.

Genetic variants. A total of 275 variants were detected in 26 of the 46 genes included in version 2 of the panel; one-third of these variants were not previously described (Table 2). And 86% of the variants (236 of 275) were classified as class 4 or 5 and 25% (59 of 236) were not previously described. Table 3 summarizes novel variants classified as class 4 or 5 along with the criteria used for their classification. The variants already reported as well as class 3 variants are summarized by disease and by gene in Supplementary Table S1.

The analysis of 90 relatives from 56 different families allowed confirmation of (i) the genetic diagnosis in 29 affected relatives; (ii) compound heterozygosity in patients with recessive diseases after the analysis of parents or children in 22 families, (iii) confirmation of heterozygous carrier status in 2 cases of X-linked disease; and exclusion of familial disease in 15 relatives of patients with autosomal dominant disorders.

Table 1 | Clinical and genetic diagnoses in the cohort of 1033 adult subjects

Clinical diagnoses	Patients, *n* (%)	Genetic diagnoses, *n* (%)	Diagnosis revision, *n*	Global diagnostic rate, confirmed + revised, %
FHH	289 (28)	36 (12.5)	0	12.5
GS	272 (26)	117 (43)	8	46
RH	121 (12)	22 (18.2)	0	18.2
dRTA[a]	86 (8)	18 (20.9)	3	24.4
ADTKD	47 (4.5)	8 (17)	1	19.1
Dent	37 (3.6)	18 (48.6)	1	51.4
BS	30 (3)	6 (20)	1	23.3
ADH	34 (3)	3 (8.8)	0	8.8
PHA2	28 (2.7)	11 (39.3)	0	39.3
HOMG	24 (2.3)	2 (8)	1	12.5
IH	15 (1.5)	3 (20)	1	26.7
RG	15 (1.5)	7 (47)	0	47
NC or FNL	10 (1)	1 (10)	0	10
NDI	3 (0.3)	0 (0)	0	0
PHA1	1 (0.1)	0 (0)	0	0
Mixed RTA	1 (0.1)	1 (100)	0	100
Hypertension[b]	2 (0.2)	0	0	0
NSIAD	4 (0.4)	0	0	0
Fanconi	2 (0.2)	0	0	0
Hyperuricemia	2 (0.2)	0	0	0
Hypouricemia	2 (0.2)	0	0	0
Total	1033	253 (24.5)	16	26

ADH, autosomal dominant hypocalcemia; ADTKD, autosomal dominant tubulointerstitial kidney disease; BS, Bartter syndrome; Dent, Dent disease; dRTA, distal renal tubular acidosis; Fanconi, renal Fanconi syndrome; FHH, familial hypercalcemic hypocalciuria; FNL, familial nephrolithiasis; GS, Gitelman syndrome; HOMG, hypomagnesemia; IH, infantile hypercalcemia; mixed RTA, mixed renal tubular acidosis; NC, nephrocalcinosis; NDI, nephrogenic diabetes insipidus; NSIAD, nephrogenic syndrome of inappropriate anti-diuresis; PHA 1 (2), pseudohypoaldosteronism type 1 (2); RG, renal glycosuria; RH, renal hypophosphatemia (including hypophosphatemic rickets).
[a]Complete and incomplete.
[b]With hypokalemia or normokalemia.

Table 2 | Molecular diagnosis

Disease (#MIM)	Gene	Patients			Variants[a]	
		Diagnostic confirmed[b]	Not confirmed[c]	Total	Previously reported	Novel
FHH1 (145980)	*CASR*	29	2	31	14	16
FHH3 (600740)	*AP2S1*	5	1	6	2	1
FHH2 (145981)	*GNA11*	2	1	3	0	3
GS (263800)	*SLC12A3*	120	23	143	92	18
RH (612286)	*SLC34A1*	2	2	4	2	2
(241530)	*SLC34A3*[@]	16	3	19	13	10
(612287)	*SLC9A3R1*	7	1	8	2	1
dRTA (267300)	*ATP6V1B1*	2	0	2	2	0
(602722)	*ATP6V0A4*	1	2	3	3	1
(179800)	*SLC4A1*	16	0	16	6	2
ADTKD (162000)	*UMOD*	8	1	9	3	6
Dent1 (300009)	*CLCN5*	15	1	16	13	1
Dent2 (300555)	*OCRL*	3	0	3	3	0
BS1 (601678)	*SLC12A1*	3	2	5	1	7
BS2 (241200)	*KCNJ1*	1	1	2	1	0
BS3 (607364)	*CLCNKB*	12	6	18	9	7
ADH (601198)	*CASR*	3	0	3	2	1
PHA2 (614495)	*KLHL3*	9	0	9	1	7
(614492)	*WNK1*	1	1	2	0	2
(614491)	*WNK4*	1	0	1	1	0
FHHNC (248250)	*CLDN16*	1	0	1	1	0
HOMG1 (602014)	*TRPM6*	1	0	1	0	1
IH1 (143880)	*CYP24A1*	3	1	4	2	1
IH2 (616963)	*SLC34A1*	0	1	1	0	1
RG (233100)	*SLC5A2*	7	2	9	6	5
pRTA (604278)	*SLC4A4*	0	1	1	0	1
Mixed RTA (259730)	*CA2*	1	0	1	1	0
Hypouricemia (220150)	*SLC22A12*	0	1	1	0	1
Total	26 genes	269	53	322	180	95

ADH, autosomal dominant hypocalcemia; ADTKD, autosomal dominant tubulointerstitial kidney disease; BS1(2, 3), Bartter syndrome type 1 (2, 3); Dent 1 (2), Dent disease type 1 (2); dRTA distal renal tubular acidosis; FHH1 (2,3), familial hypercalcemic hypocalciuria type 1 (2, 3); FHHNC, familial hypomagnesemia with hypercalciuria and nephrocalcinosis; GS, Gitelman syndrome; HOMG1 hypomagnesemia type 1; IH1 (2), infantile hypercalcemia type 1 (2); MIM, Mendelian Inheritance in Man (http://omim.org); mixed RTA, mixed renal tubular acidosis; PHA2, pseudohypoaldosteronism type 2; pRTA, proximal renal tubular acidosis; RG, renal glycosuria; RH, renal hypophosphatemia (including hypophosphatemic rickets).
[a]Variants of class 3 are included.
[b]Patients with class 4 and 5 variants.
[c]Patients with class 3 variants or only 1 heterozygous variant in a recessive disease. One patient of this group also has a pathogenic variant in the *SLC9A3R1* gene.

Performance by disease

The detection rates of mutations by disease entity are shown in Figure 1a.

Familial hypercalcemia hypocalciuria. FHH constitutes the largest part of our cohort (28%; 289 of 1033 patients). Patients with this diagnosis were mainly included by the Paris center. Among patients with this clinical diagnosis, 12.5% of cases (36 of 289) were genetically confirmed. Mutations were identified in the *CASR* gene in 81% (29 of 36 FHH1), in the *AP2S1* gene in 14% (5 of 36 FHH3), and in the *GNA11* gene in 6% (2 of 36 FHH2). The available clinical and biochemical characteristics of these patients are summarized in Supplementary Table S2.

Tubulopathies. GS represents the most important clinical diagnosis of the tubulopathies in this cohort (26%; 272 of 1033). Among these, 43% of cases (117 of 272) were genetically confirmed—detection of 2 heterozygous class 4 or 5 variants in the *SLC12A3* gene.

RH corresponds to 12% of the cohort (124 of 1033) with a mutation detection rate of 18% (22 of 124). Thirteen patients had variants in the *SCL34A3* gene: 8 had 1 heterozygous variant, and 5 had 2 heterozygous variants (unfortunately DNA from relatives was unavailable for segregation studies). Seven patients had heterozygous pathogenic variants in the *SLC9A3R1* gene. Finally, 2 patients had heterozygous pathogenic variants in the *SLC34A1* gene. Interestingly 1 of patients with clinical and genetic diagnosis of RH with pathogenic heterozygous variant *SCL34A3* has also a class 5 variant in the *SLC9A3R1* gene, raising the possibility of digenism.

The third most common tubulopathy in the cohort is dRTA, representing 8% of the patients (86 of 1033). Out of the 86 patients with this diagnosis, 18 were genetically confirmed (21%). *SLC4A1* was implicated in 15 patients (83%), *ATP6V1B1* in 2 patients, and *ATP6V0A4* in 1 patient.

Autosomal dominant tubulointerstitial kidney disease related to *UMOD* represents 4.5% of the cohort (47 of 1033), and 17% of these patients (8 of 47) had genetic confirmation. Dent disease represents 3.6% of the cohort (37 of 1033), with a rate of genetic confirmation of 49% (18 of 37). Fifteen patients carried *CLCN5* variants (Dent 1), and 3 patients carried *OCRL* variants (Dent 2). Bartter syndrome (BS) and autosomal dominant hypocalcemia each represent 3% of the cohort (31 and 34 of 1033) with a rate of genetic confirmation of 20% (6 of 31) and 9% (3 of 34), respectively.

Table 3 | Novel class 4 and 5 variants detected in this study and their classification according to ACMG

Gene	Nomenclature cDNA	Nomenclature protein	ACMG Class	Criteria
CASR	c.226T>C	p.Phe76Leu	4	PM1, PM2, PP2, PP3
	c.293T>C	p.(Phe98Ser)	4	PM1, PM2, PP2, PP3
	c.503C>A	p.(Ala168Asp)	4	PM1, PM2, PP2, PP3
	c.811T>C	p.(Ser271Pro)	4	PM1, PM2, PM5, PP2, PP3
	c.1104_1105del	p.(Leu368Phefs*17)	5	PVS1, PM1, PM2
	c.1345T>C	p.(Cys449Arg)	4	PM1, PM2, PP2, PP3
	c.1823G>A	p.(Trp608*)	5	PVS1, PM1, PM2
	c.2011_2020del	p.(Glu671ThrfsTer24)	5	PVS1, PM1, PM2
	c.2048C>T	p.(Ala683Val)	4	PM1, PM2, PP2, PP3
	c.2087T>C	p.(Leu696Pro)	4	PM1, PM2, PP2, PP3
	c.2159_2160dup	p.(Leu721Glyfs*21)	5	PVS1, PM1, PM2
	c.2188C>T	p.(Leu730Phe)	4	PM1, PM2, PP1, PP2, PP3
	c.2336A>G	p.(Tyr779Cys)	4	PM1, PM2, PP2, PP3
	c.2572T>C	p.(Tyr858His)	4	PM1, PM2, PP2, PP3
	c.2730del	p.(Ser911Profs*28)	5	PVS1, PM1, PM2
CLCNKB	c.577-8T>G	p.(?)	4	PM2, PM3, PP1, PP3
	c.(866+1_867-1)_(2016+1_2017-1)del	p.(?)	5	PVS1, PM1, PM2
	c.1297+G>A	p.(?)	4	PVS1, PM2, PP3, PP5
	c.577-8T>G	p.(?)	4	PM2, PM3, PP1, PP3
CYP24A1	c.612C>G	p.(Tyr204*)	5	PVS1, PM1, PM2, PP3, PP4
GNA11	c.49_57del	p.(Glu17_Lys19del)	4	PM1, PM2, PM4
	c.-12_53del	p.(?)	5	PVS1, PM1, PM2
KLHL3	c.233T>C	p.(Met78Thr)	4	PM2, PM5, PP2, PP3, PP4
	c.234G>A	p.(Met78Ile)	4	PM1, PM2, PM5, PP2, PP3
	c.444T>A	p.(His148Gln)	4	PM1, PM2, PP2, PP4
	c.922G>A	p.(Gly308Ser)	4	PM2, PP1, PP2, PP3, PP4
	c.1205T>C	p.(Phe402Ser)	5	PM1, PM2, PP1, PP2, PP3, PP4
	c.1216A>G	p.(Thr406Pro)	4	PM1, PM2, PP1, PP2
	c.1300G>A	p.(Val434Met)	4	PS4, PM1, PM2, PP2
SLC12A1	c.1493C>T	p.(Ala498Val)	4	PM1, PM2, PM3, PP5
	c.1875G>T	p.(Trp625Cys)	4	PS4, PM1, PM2, PP3
	c.1878G>A	p.(Trp626*)	5	PVS1, PM1, PM2
	c.2035A>G	p.(Asn679Asp)	4	PM1, PM2, PM3, PP1
	c.2873+1del	p.(?)	5	PVS1, PM1, PM2, PP3
SLC34A1	c.1222G>A	p.(Val408Met)	4	PM1, PM2, PM5
SLC34A3	c.496G>A	p.(Gly166Ser)	4	PS4, PM2, PP3
	c.926-2A>C	p.(?)	5	PVS1, PM1, PM2, PP3
	c.1361A>G	p.(Asn454Ser)	4	PS4, PM1, PM2, PP3
	c.1717_1732del	p.(Asn573Argfs*63)	5	PVS1, PM2
SLC12A3	c.658_663del	p.(Gly220_Leu221del)	4	PM1, PM2, PM4
	c.1670-8_1670-7delinsCA	p.(?)	4	PM2, PP1, PP3, PP4
	c.1687C>T	p.(Gln563*)	5	PVS1, PM2, PM3, PM4, PP1
	c.1861T>G	p.(Tyr621Asp)	4	PM1, PM2, PP1, PP3
	c.2186G>A	p.(Gly729Asp)	4	PM1, PM2, PM3, PM5, PP3, PP5
	c.2368+1del	p.(?)	5	PVS1, PM2, PP3
	c.2711T>A	p.(Ile904Asn)	4	PM1, PM2, PM5
SLC4A1	c.2703_2713del	p.(Asp902Argfs*14)	5	PVS1, PM1, PM2
	c.2716G>T	p.(Glu906*)	5	PVS1, PM1, PM2
SLC4A4	c.1107dup	p.(Ile370Tyrfs*2)	4	PVS1, PM2
SLC5A2	c.394C>T	p.(Arg132Cys)	4	PM2, PP1, PP3, PP4, PP5
	c.1450-1G>A	p.?	5	PVS1, PM2, PP3, PP4
	c.1639_1640dup	p.(Thr548AlafsTer50)	5	PVS1, PM2, PP3, PP4

(*Continued on next page*)

The following diagnoses each represent less than 3% of the cohort and have a rate of genetic confirmation between 18% and 50% (Table 1): Pseudohypoaldosteronism type 2 (PHA2), hypomagnesemia (HOMG), infantile hypercalcemia (IH), and renal glycosuria. Finally, 1 patient with clinical diagnosis of mixed RTA had a genetic confirmation (homozygous class 4 variant in the *CA2* gene).

Other clinical diagnoses, for which no genetic confirmation was made included: PHA1, nephrocalcinosis (NC), hypertension, nephrogenic diabetes insipidus, nephrogenic syndrome of inappropriate antidiuresis, Fanconi syndrome, hypouricemia, and hyperuricemia (Table 1).

Patients with variants of unknown significance. Thirty variations detected in 16 different genes in 40 patients were classed as class 3 or variants of unknown significance. They are described by disease and by gene in Supplementary Table S1. In 10 patients with clinical BS or GS, the class 3 variant was associated with a class 4 or 5 variant in the same gene.

Table 3 | (Continued) **Novel class 4 and 5 variants detected in this study and their classification according to ACMG**

Gene	Nomenclature cDNA	Nomenclature protein	ACMG Class	Criteria
TRPM6	c.278A>G	p.(Asp93Gly)	4	PM2, PM3, PP1, PP4
UMOD	c.179G>C	p.(Gly60Ala)	4	PM1, PM2, PP3, PP4
	c.478G>C	p.(Asp160His)	4	PM1, PM2, PP3, PP4
	c.692T>C	p.(Leu231Pro)	4	PM1, PM2, PP3, PP4
	c.851T>C	p.(Leu284Pro)	4	PM1, PM2, PP3, PP4
	c.274T>C	p.(Cys92Arg)	4	PM1, PM2, PM (segregation)
WNK1	c.1888G>A	p.(Glu630Lys)	4	PM1, PM2, PP1, PP3, PP4

American College of Medical Genetics (ACMG) criteria description: PM, moderate evidence of pathogenicity; PM1, located in a mutational hot spot or critical and well-established functional domain without benign variation; PM2, absent from controls (or at extremely low frequency if recessive) in gnomAD database (https://gnomad.broadinstitute.org/); PM3, detected *in trans* with a pathogenic variant (the phase was determined); PM4, protein length changes due to in-frame deletions or insertions in a nonrepeat region or stop-loss variants; PM5, novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before; PP, supporting evidence of pathogenicity; PP1, cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease; PP2, missense variant in a gene that has a low rate of benign missense variation and in which missense variants; PP3, multiple lines of computational evidence support a deleterious effect on the gene or gene product; PP4, patient's phenotype or family history is highly specific for a disease with a single genetic etiology; PP5, reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation; PS, strong evidence of pathogenicity; PS1, same amino acid change as a previously established pathogenic variant regardless of nucleotide change; PS3, well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect on the gene or gene product; PS4, the prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls; PVS, very strong evidence of pathogenicity; PVS1, null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function is a known mechanism of disease.

Genetic revision of the clinical diagnosis. In 16 cases, the analysis of other genes present in the panel allowed a revision of the diagnosis. Table 4 summarizes these cases.

First, one-half of these cases correspond to the known overlap of phenotypes of GS and classic BS that turned out to be genetically either BS type 3 or GS.

Second, 1 patient with clinical diagnosis of autosomal dominant tubulointerstitial kidney disease had a genetic diagnosis of BS type 1 (patient B26). This patient had a diagnosis of chronic kidney disease at the age of 32 years, in absence of other manifestations except polyuria; she had hyperuricemia and normal level of potassium. Her oldest sister had gouty arthritis before the age of 30 years. The patient and her younger sister had nephrocalcinosis. DNA samples were not available to test family segregation.

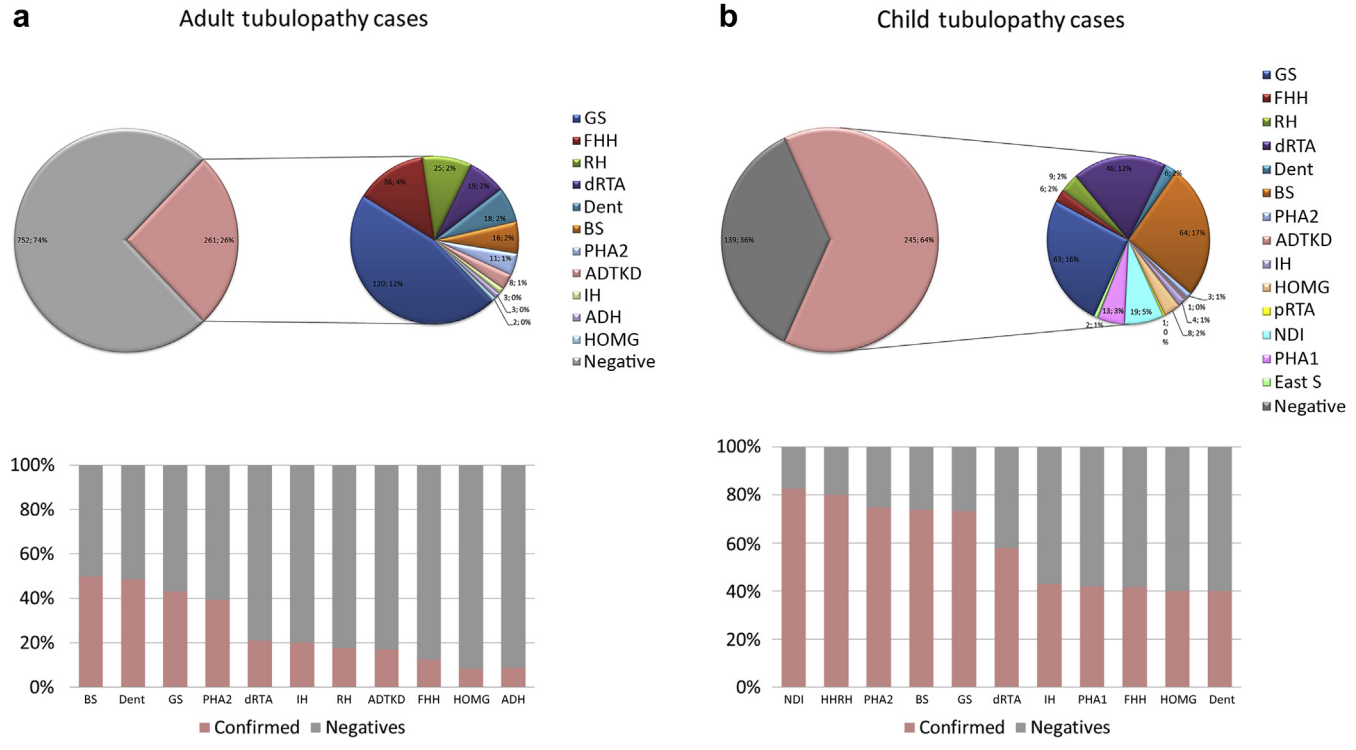

Figure 1 | Pie charts (*n;* %) and bar graphs comparing detection rates, distribution of genetically confirmed pathologies, and confirmation rate by disease in (a) adults and (b) children. ADH, autosomal dominant hypocalcemia; ADTKD, autosomal dominant tubulointerstitial kidney disease; BS, Bartter syndrome; Dent, Dent disease; dRTA, distal renal tubular acidosis; East S, epilepsy, ataxia, sensorineural deafness, and tubulopathy; FHH, familial hypercalcemia hypocalciuria; GS, Gitelman syndrome; HHRH, hereditary hypophosphatemic rickets with hypercalciuria; HOMG, hypomagnesemia; IH, infantile hypercalcemia; NDI, nephrogenic diabetes insipidus; PHA1 (2), pseudohypoaldosteronism type 1 (2); pRTA, proximal renal tubular acidosis; RH, renal hypophosphatemia.

Table 4 | Index cases with genetic revision of the clinical diagnosis

Patient	Clinical diagnosis	Genetic diagnosis
L81	Gitelman	Bartter 3
L117	Gitelman	Bartter 3
L123	Gitelman	Bartter 3
P56	Gitelman	Bartter 3
P104	Gitelman	Bartter 3
P116	Gitelman	Bartter 3
P608	Gitelman	Bartter 3
L85	Bartter 3	Gitelman
B26	ADTKD	Bartter 1
L84	Incomplete dRTA	Bartter 2
L150	Incomplete dRTA	Gitelman
P6	Incomplete dRTA	Renal hypophosphatemia
P113	Hypomagnesemia	Gitelman
P179	Dent	dRTA
B61	Familial nephrolithiasis	Renal hypophosphatemia
P582	Infantile hypercalcemia	Renal hypophosphatemia

ADTKD, autosomal dominant tubulointerstitial kidney disease; dRTA, distal renal tubular acidosis.

Third, 3 cases with clinical diagnosis of incomplete dRTA were genetically diagnosed as type 2 BS (patient L84), GS (patient L150), and RH (patient P6). Patient L84 had hypokalemia and nephrocalcinosis diagnosed age 35 years. She was referred to nephrology 3 years later and is on potassium supplements and amiloride. Her parents were first cousins and she had a strong family history of kidney stones. Plasma bicarbonate at presentation was 26 mmol/l, and the initial clinical diagnosis was of incomplete dRTA. Patient L150 is from Turkish Cyprus, who had severe acidosis (bicarbonate 12 mmol/l), hypokalemia (potassium 2.7–3 mmol/l), normal plasma magnesium and chloride, and a urinary pH of 8. He was on potassium supplements and spironolactone at the time of genotyping. Patient P6 had a history of kidney stones and hypocitraturia; an acid load test showed an inappropriate urinary pH (5.8) and an abnormal ammonia excretion establishing the diagnosis of incomplete dRTA, nevertheless a borderline phosphate concentration was also observed; in this patient 2 heterozygous variants in *SLC34A3* were detected.

Fourth, the patient (P113) with renal HOMG who had a final diagnosis of GS presented with severe HOMG (0.25 mmol/l) and borderline hypokalemia (3.4–3.9 mmol/l); the hypokalemia was initially considered secondary to HOMG.

Fifth, in 1 patient (P179) with clinical diagnosis of Dent disease, the genetic diagnosis was an autosomally dominant dRTA. This patient with a history of stone disease was given a diagnosis of medullary sponge kidney disease at 20 years old; an evaluation 3 years later showed proximal tubulopathy and chronic kidney disease: renal hypophosphatemia, hyperchloremic metabolic acidosis, hypocitraturia, proteinuria (1.41 g/d with moderate albuminuria 143 mg/d) and inulin clearance of 58 ml/min per 1.73 m^2. No variants were detected in genes responsible for Dent disease or renal Fanconi syndrome. Taking into account the presence of stone disease and hypocitraturia, we looked for variants in genes responsible of dRTA and detected a known class 5 variant in the *SLC4A1* gene.

Finally, 2 cases (B61 and P582) with clinical diagnosis of IH and nephrolithiasis (NL) had heterozygous variants in the *SLC34A3* gene.

DISCUSSION

Hereditary tubulopathies are less frequently diagnosed in adults. However, the establishment of a precise diagnosis is important to guide care, survey, and potentially prevent chronic complications as well as for genetic counselling. In this paper, we describe the analysis of a large multicenter cohort of adult patients with a panel of 46 genes involved in tubulopathies or in FHH, which yielded an overall genetic confirmation in 26% of cases after the detection of pathogenic variants in 26 different genes.

Twenty-eight percent of cases of this cohort have a clinical diagnosis of FHH, which is not primarily a tubulopathy. The genes responsible for this disease have been included due to the role of the corresponding proteins in tubular calcium reabsorption. In addition, patients with this diagnosis were mainly included by the Paris center. With the exclusion of this disease, the rate of genetic confirmation increases to 29% (216 of 744), which is approximately one-half of the rate we observed previously in children (64%).[8] This percentage remains unchanged when genes presented only in the second panel version are excluded (see Supplementary Table S3 for panel composition).

A recent study analyzed a panel of 30 genes that cause NC/NL in a cohort of children and adults showing that the detection rate of monogenic causes in adults was one-half that seen in children (11.4% vs. 20.8%).[9]

Several features may explain the lower rate observed in adults. First, autosomal recessive tubulopathies are mainly diagnosed in children. Accordingly, Halbritter *et al.*,[9] in their study of monogenic causes of NC/NL found that recessive causes were diagnosed more frequently in childhood, whereas dominant disease was diagnosed more commonly in adulthood. Nevertheless, in our cohort autosomal recessive diseases were confirmed in 153 of 269 patients (57%), primarily in patients with GS ($n = 120$) and BS ($n = 16$).

Second, the presence of comorbidities and polypharmacy in cohorts of adult patients can make interpretation of the phenotype more difficult (especially when the phenotype involves chronic kidney disease) or complicate the interpretation of urinary chemistry. Also acquired tubular dysfunction (e.g., autoimmune dRTA, renal Fanconi syndrome caused by drug or environmental toxicity) is much more common in adults, and genetic testing may be an important part of investigation for these patients.[10–12]

Third, a significant number of tests are performed as exclusion diagnosis. This is especially true for patients with clinical diagnosis of FHH, for which we have a low diagnosis rate (12.5%). FHH could have a very similar clinical presentation to primary hyperparathyroidism, and, despite proposed scores and algorithms, it remains difficult to have a

precise clinical diagnosis.[13] It is therefore recommended in these cases to exclude a diagnosis of FHH before committing to parathyroidectomy, which is inappropriate in FHH.[14] We were able to analyze the data of 86 patients for whom a genetic diagnosis of FHH was excluded and follow-up information was available. Thirty-five patients (41%) were parathyroidectomized and 31% of them (11 of 35) normalized their serum calcium level postoperatively while the remaining 24 patients had persistent hypercalcemia. This percentage is higher than the usual rate of unsuccessful parathyroidectomy in primary hyperparathyroidism (5%–10%). The diagnosis of FHH is not completely excluded in this population because there are probably other genes responsible for this disease.[15] This highlights the importance of pursuing further research genetic investigations to identify other genes responsible for these rare phenotypes. Concerning the clinical presentation of different types of FHH, although there are few cases of FHH2 and FHH3 in this cohort; analysis of clinical data (Supplementary Table S2) shows similar results to results described in the literature, that is, higher hypercalcemia in patients with FHH3.[16,17]

GS was the most common tubulopathy in adults with a high molecular confirmation rate (46%) (Figure 1). The main symptoms of GS are rather nonspecific (cramps, poorly defined discomfort, fatigue) and they evolve slowly, resulting in a late or incidental presentation. While the biochemical phenotype is typically well characterized, explaining the high level of genetic diagnosis, patient L150, presenting with acidosis, demonstrates that it can be surprisingly variable. Notably, in 55% of patients with available clinical information (42 of 76), the genetic test was performed to exclude the diagnosis (e.g., in patients with eating disorders or surreptitious diuretic abuse and questionable biological data) and as expected no variants were detected.[18]

In contrast with GS, BS, the most common tubulopathy in children, is rarely diagnosed in adulthood. BS was confirmed in 16 patients. As expected, most of them ($n = 12$) had BS type 3. Three patients (P103, B26, B46) had genetic diagnosis of BS type 1 and 1 patient (L84) had BS type 2. Patients B26 and L84 correspond to revised diagnosis. Patient P103 had a diagnosis of hypokalemia made when she was 30 years old on routine examination during her first pregnancy; later evaluation revealed alkalosis, high renin level, and hypercalciuria. She harbored 2 missense class 4 heterozygous variants in *SLC12A1* located *in trans* (her son is heterozygous for 1 of 2 variants). The presentation in the woman B46 was quite similar with reduced serum potassium concentration at age of 35 years with mild HOMG and hypercalciuria. The patient was heterozygous for a class 4 missense change and a complete deletion of exon 16. This rearrangement was expected to lead to a frameshifted transcript. Whereas BS type 1 is in general associated with polyhydramnios and severe neonatal manifestations, there is a description of late-onset manifestations related with partial loss of function of the mutant.[19] A similar situation could explain the late onset in these patients, which needs confirmation by *in vitro* studies.

Among patients with clinical BS and GS, 10 cases harbored 2 variants, but 1 of them was considered as class 3; for these variants there are no criteria for benignity and the confirmation that they are located *in trans* after familial segregation studies will allow us to reclassify them as class 4, increasing the confirmation rate.

NL/NC is a common clinical manifestation in adult patients. The main monogenic causes of NL/NC are Dent disease, RH, complete and incomplete dRTA, and IH, representing 17% of cases with genetic confirmation in our cohort. For patients with clinical diagnosis of Dent disease, we had a higher genetic confirmation rate in adults than in children (49% vs. 40%). This may be explained by the incomplete renal Fanconi syndrome that they have as infants, which is easy to miss clinically, and the fact that nephrocalcinosis and renal failure may only become evident during adult life. Causative genes were *CLCN5* in 83% of cases and *OCRL* in 17%, which is similar to that described in the literature.[20] The contribution of molecular diagnosis for these patients is crucial, given the progression to end-stage kidney disease (typically in the fourth or fifth decade of life), allowing genetic counselling and detection of heterozygous female carriers. For RH and dRTA, we observed a relatively lower detection rate that interestingly is similar to the rate described by Halbritter *et al.*[9] in adults. This lower detection rate may be explained by the multifactorial origin of NL/NC, but we cannot exclude variants not detected by our method (i.e., deep intronic variants), known genes not present in our panel (i.e., *SLC7A9*, *ADCY10*), or a supplementary genetic heterogeneity.[9,21] In 5 patients with NL, we detected 2 pathogenic heterozygous variants of the *SLC34A3* gene; unfortunately DNA from relatives was unavailable to confirm that they are biallelic; 2 of these 5 patients presented with osteopenia and 2 others with nephrocalcinosis. Finally, type 1 IH was confirmed in 3 patients. Previous reports and this data confirm that IH can also be an adult disease. Heterozygous patients can have hypercalciuria and NL as well as high calcitriol levels.[22,23] In 1 of our index cases (P580) only 1 heterozygous class 5 variant was detected. Although we cannot exclude the presence of a second variant in a nonanalyzed region of the gene; this observation rises also the possibility of clinical manifestation in heterozygous patients.

In PHA2, we found a high rate of confirmed diagnoses (39%), mainly explained by pathogenic variants in *KLHL3*. The biochemical phenotype of PHA2 (hypertension, hyperkalemia, and metabolic acidosis) is quite specific, which may explain this high rate. *KLHL3* molecular abnormalities explain a high proportion of patients with this disease in the cases described in the literature.[24]

For the patients with HOMG suspected of genetic origin, 2 patients had HOMG related to *TRPM6* mutations (HOMG1). This syndrome is usually diagnosed during the first year of life, with convulsions associated with severe hypocalcemia.[25] Patient P555 from a consanguineous family had a diagnosis of HOMG secondary to intestinal malabsorption at 32 years old; she had secondary hypoparathyroidism and no

neurological manifestations, again demonstrating the spectrum of clinical severity.

The rate of discrepancy between clinical and genetic diagnosis is relatively low (2.1%), which underlines the diagnostic accuracy of the referring clinicians. A total of 16 patients had their initial clinical diagnosis revised by the panel analysis. As in children, the revision was rarely substantial and mostly concerns salt-losing tubulopathies or diseases associated with NC/NL or a combination of these. Several tubulopathies present with either hypokalaemia or hypercalciuria or both and thus can constitute phenocopies of these findings (Figure 2).

The most common revision was from GS to BS type 3 or *vice versa* in 8 cases, which was expected taking into account the well-known phenotypic overlap between these 2 syndromes. Three cases with clinical diagnosis of incomplete dRTA were found to type 2 BS, GS, and RH (L84, L150, P6), respectively. Two cases (B61 and P582) with clinical diagnosis of IH and NL had heterozygous variants in the *SLC34A3* gene. This interesting observation shows that patients with absorptive hypercalciuria due to increased calcitriol as in IH or renal hypophosphatemia can have overlapping phenotypes and clinical and biological manifestations heterozygously.[22,23,26] For patient B26 with genetic diagnosis of BS type 1, the clinical diagnosis of autosomal dominant tubulointerstitial kidney disease could have been excluded due to the presence of nephrocalcinois.[2] Finally, in 1 patient (P179) with clinical diagnosis of Dent disease the genetic diagnosis was an autosomally dominant dRTA.

In conclusion, this work demonstrates that a genetic cause can be demonstrated in 1 of 3 adult patients presenting with a clinical suspicion of tubulopathy. This rate of detection is one-half of that observed in children. The establishment of a genetic diagnosis is crucial for the patient to guide care, survey, and prevent chronic complications, as well as for genetic counselling. These results enhance our understanding of complex phenotypes and enrich the database of causal variants associated with clinically defined tubulopathies.

METHODS

Patients

A total of 1033 adult index cases with a clinical diagnosis of tubular dysfunction made after the age of 18 years and for which the implicated gene(s) are present in the panel were included and analyzed from 2014 to 2016. The clinical diagnosis was performed by physicians belonging to the network of 3 expert centers. Of these, 139 samples were analyzed in Brussels, 171 in London, and 723 in Paris. The last center has a large recruitment of patients with hypercalcemia of parathyroid origin, explaining the inclusion of a large number of patients with suspected FHH, which is not strictly speaking a tubulopathy. In addition, 90 relatives belonging to 56 families in whom a pathogenic variant was detected in the proband were analyzed; these included 29 affected individuals. 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.

Gene amplification, sequencing, and bioinformatic analysis

This was performed as described previously.[8] Briefly, genomic DNA was isolated from white blood cells using standard procedures, followed by massive parallel sequencing using the versions 1 and 2 of a tubulopathies panel designed by the work package tubulopathies of the European consortium EURenOmics. The first version was previously described in our paediatric cohort.[7] The second version includes 9 additional genes. Supplementary Table S3 compares the composition of these 2 versions.

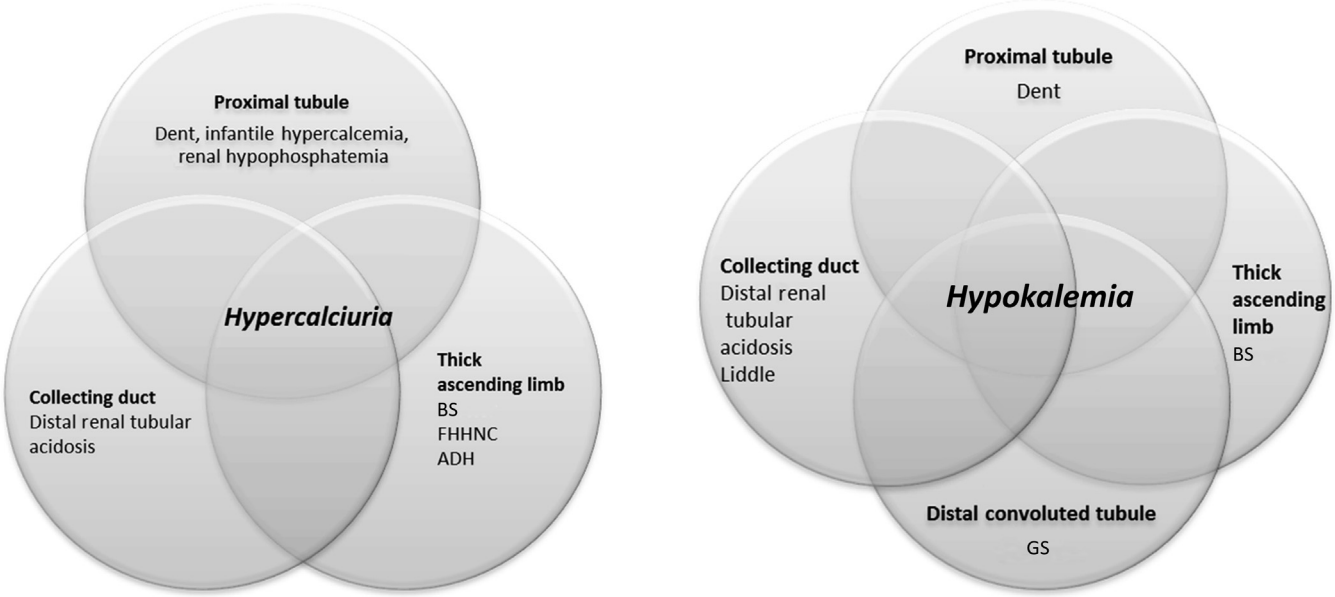

Figure 2| Tubulopathies phenocoping 2 frequent clinical presentations in the adult cohort. ADH, autosomal dominant hypocalcemia; BS, Bartter syndrome; Dent, Dent disease; FHHNC, familial hypomagnesemia with hypercalciuria and nephrocalcinosis; GS, Gitelman syndrome; Liddle, Liddle syndrome.

The depth for each region of interest was at least 30×, except for exon 1 of *OCRL* and *WNK1*, analysis of which was completed by Sanger sequencing in those with suspected disease related to these 2 genes and with no other causative mutation identified. Regions with coverage lower than 30X were sequenced by Sanger. Library preparation and bioinformatics analysis was performed according to the techniques and pipelines routinely used by each laboratory (Supplementary Methods). The variants were classified according to the guidelines published by the American College of Medical Genetics 2015.[27] Variants of interest were verified by Sanger sequencing.

The 3 centers are accredited laboratories proposing genetic diagnostic tests referenced in Orphanet (https://www.orpha.net/consor/cgi-bin/index.php?lng=EN or https://ukgtn.nhs.uk/find-a-test/search-by-laboratory/laboratory/london-north-east-rgc-gosh-43/).

DISCLOSURE

AR and JDF are employees of Multiplicom, provider of the kits used for amplification of the 46 genes. All the other authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Figure S1. Flow diagram of global performance.
Table S1. American College of Medical Genetics classification of all variants detected in this study summarized by disease and by gene.
Table S2. Phenotype-genotype correlation in patients with genetically confirmed FHH.
Table S3. Panel composition of versions 1 and 2 of the tubulopathies panel.
Supplementary Methods.
Supplementary material is linked to the online version of the paper at www.kidney-international.org.

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