Mutations of *NPHP2* and *NPHP3* in infantile nephronophthisis

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Nephronophthisis is an autosomal recessive chronic tubulointerstitial disease that progresses to end-stage renal disease (ESRD) in about 10% of cases during infancy. Mutations in the *INVS* (*NPHP2*) gene were found in a few patients with infantile nephronophthisis. Mutations of *NPHP3*, known to be associated with adolescent nephronophthisis, were found in two patients with early-onset ESRD. Here we screened 43 families with infantile nephronophthisis (ESRD at less than 5 years of age) for *NPHP2* and *NPHP3* mutations and determined genotype–phenotype correlations. In this cohort there were 16 families with *NPHP2* mutations and 7 with *NPHP3* mutations. Three patients carried only one heterozygous mutation in *NPHP3*. ESRD arose during the first 2 years of life in 16 of 18 patients with mutations in *NPHP2* but in only two patients with mutations in *NPHP3*. Renal morphology, characterized by hyper-echogenic kidneys on ultrasound and tubular lesions with interstitial fibrosis on histology, was similar in the two patient groups. The kidney sizes were highly diverse and ultrasound-visualized cysts were present in a minority of cases. Extra-renal anomalies were found in 80% of the entire cohort, including hepatic involvement (50%), cardiac valve or septal defects (20%) and recurrent bronchial infections (18%). We show that *NPHP3* mutations in both infantile and adolescent nephronophthisis point to a common pathophysiological mechanism despite their different clinical presentations.

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Nephronophthisis (NPH) is an autosomal recessive chronic tubulointerstitial nephropathy, which in all cases progresses to end-stage renal disease (ESRD). Three clinical variants have been described according to the age at onset of ESRD: infantile, juvenile, and adolescent. Juvenile NPH is the most common, and it accounts for 5–10% of cases of ESRD in children. The kidneys in juvenile and adolescent NPH are of normal or even reduced size, and are characterized histologically by interstitial fibrosis and tubular atrophy with basement membrane splitting and thickening. Cysts develop late in the course of the disease, typically at the corticomedullary junction.[1,2]

A clinically distinct disorder was later recognized and characterized by autosomal recessive inheritance and rapidly progressive, chronic tubulointerstitial nephritis leading to ESRD before the age of 3 years.[3–5] This condition was considered to be the infantile form of NPH because of the presence of interstitial fibrosis. However, the enlargement of the kidneys with cortical cysts, the lack of basement membrane thickening, and the frequent association with hepatic fibrosis were more reminiscent of polycystic kidney disease than of NPH.

Mutations in seven genes, *NPHP1* and *NPHP3* to *NPHP8*, have been found in juvenile or adolescent NPH.[6–16] *NPHP2* (*INVS*) was the only gene implicated in the infantile form[17] until recently, when two patients with early-onset ESRD were found to carry *NPHP3* mutations.[18,19] Here we present a comprehensive study of a cohort of 43 families to assess the prevalence of mutations of *NPHP2* and *NPHP3* in cases of infantile NPH. We analyzed the genotype–phenotype correlation with respect to the clinical and pathological presentation.

RESULTS

NPHP2 mutations

Thirteen different *NPHP2* mutations were identified in 16 unrelated families (Table 1, Figure 1a). Five mutations were

Table 1 | Genetic and clinical characteristics of patients with *NPHP2* mutations

Ind	CS	Origin	Nucleotide seq (exon, segregation)	Alterations in coding seq	Diagnosis (months)	Symptoms at dg	ESRD (months)	Renal ultrasound	Renal histology	HT	Liver	Other extra-renal symptoms
A12-1[a]	+	Israel	c.2719C>T H (14)	p.R907X	5	ND	5	ND	Infantile type	+	—	—
F339-1	+	Israel	c.2719C>T H (14)	p.R907X	ND	Anemia, PU–PD, FT	32	ND	Infantile type	ND	—	—
F339-2			Not sequenced	ND	ND	ND	19	ND	ND	ND	ND	ND
1499	+	Israel	c.2719C>T H (14, P, M)	p.R907X	9	ND	16	SK, HE, cyst	End-stage K	+	—	Developmental delay
F331-1	+	Morocco	c.2011C>T H (13)	p.Q671X	11	Anemia, PU–PD, FT	11	NSK, HE, no cyst	Infantile type	+	—	Hypertriglyceridemia (6.6 mmol/l), bilateral hip dysplasia
A8-1[a]	+	Turkey	c.1807C>T H (13)	p.R603X	12	Anemia	18	EK, HE, no cyst	Infantile type	+	ET	Situs inversus, ventricular septal defect, recurrent bronchitis
1383	–	France	c.1453delC h (10, P) c.2695C>T h (14, M)	p.Q485fsX509 p.R899X	18	Anemia, PU–PD, FT	18	NSK (−1SD), HE, no cyst	ND	+	—	—
F411-1	–	France	c.1453delC h (10, P) c.2908delG h (15, M)	p.Q485fsX509 p.E970fsX971	36	Anemia, FT	36	SK (−2,5SD), HE, no cyst	Juvenile type	+	—	Cerebellar atrophy without vermis aplasia, mitral insufficiency
A6-1[a]	–	France	c.1453delC h (10, M) c.2695C>T h (14, P)	p.Q485fsX509 p.R899X	21	Anemia, FT	21	NSK (−1SD), HE, no cyst	Juvenile type	+	—	Recurrent bronchitis and upper respiratory infections, onycholysis
A7-1[a]	–	Portugal	c.1478T>C H (11)	p.L493S	5	Anemia, FT	18	EK (+2SD), HE, cysts	Infantile type	+	—	Perception deafness
F413-1	–	France	c.1453delC h (10, P) c.2695C>T h (14, M)	p.Q485fsX509 p.R899X	9	PU–PD, FT	9	NSK, HE, cysts	Infantile type	+	HF	—
A9-1[a]	–	France	c.1186C>T h (9, M) c.1445C>G h (10, P)	p.R396X p.P482R	11	Anemia, PU–PD, FT	20	EK, HE, cysts	Juvenile type	+	—	Mitral insufficiency
A9-2[a]			Not sequenced		5	Anemia, PU–PD	20	EK (+2SD), HE, cysts	ND	+	—	Supravalvular pulmonary stenosis, patent foramen ovale
F320-1	+	France	c.2719C>T H (14)	p.R907X	12	Anemia, FT	13	SK (−2,5SD), HE, no cyst	Infantile type	+	—	Developmental delay, recurrent bronchitis and otitis
F398-1	–	France	c.615+1G>A h (5, P) c.2011C>T h (13, M)	Abn transcript p.Q671X	5	FT	10	NSK, no cyst	Infantile type	ND	HF	—
F434-1	–	France	c.1194T>G h (9) c.2695C>T h (14)	p.C398W p.R899X	18	Anemia, FT	18	EK, HE, no cyst	Infantile type	+	HF	Situs inversus, aortic and pulmonary valve stenosis, recurrent bronchitis
F340-1	–	France	c.563G>A h (5, M) c. 2747insA h (14, P)	p.W188X p.K916fsX1002	5	ND	12	EK, HE, no cyst	Infantile type	+	—	—
A10-1[a]	–	France	c.2908delG h (15, M)	p.E970fsX971	12	Anemia, PU–PD	12	EK, HE, no cyst	ND	+	—	—

Abn, abnormal; CS, consanguineous; EK, enlarged kidney; ET, elevated serum transaminase level; FT, failure to thrive; h, heterozygous; H, homozygous; HE, hyperechogenic kidney; HF, hepatic fibrosis; HT, hypertension; Ind, individual; K, kidney; ND, no data available; NSK, normal-sized kidney; PU–PD, polyuria–polydipsia; SD, standard deviation; SK, small kidney.
[a]Families already published.[17]

novel, three of which were truncating mutations (p.Q671X, p.W188X, p.K916fsX1002), one affected an obligatory splice site (c.615 + 1G > A), and one was a missense mutation (p.C398W). The amino-acid substitution (p.C398W) resulting from the missense mutation was considered to be pathogenic because the affected cysteine, located in the ankyrin domain (Figure 1a), is conserved in vertebrates, and a tryptophan was not found in this position in any of 166 ethnically matched controls. This substitution was also predicted to be damaging by the PolyPhen program (PSIC score: 3.15). One patient was heterozygous for the variant p.A650P (F75-1, Supplementary Table 1): although this variant was previously considered to be a missense mutation,[19] we found it in 6/152 ethnically matched controls, and therefore we considered it to be a polymorphism.

Our study and earlier work by others[17,18] have thus revealed five *NPHP2* mutations that are recurrent in unrelated families (p.R899X, p.R907X, p.Q485fsX509, p.Q671X, and p.E970fsX971) (Table 1).

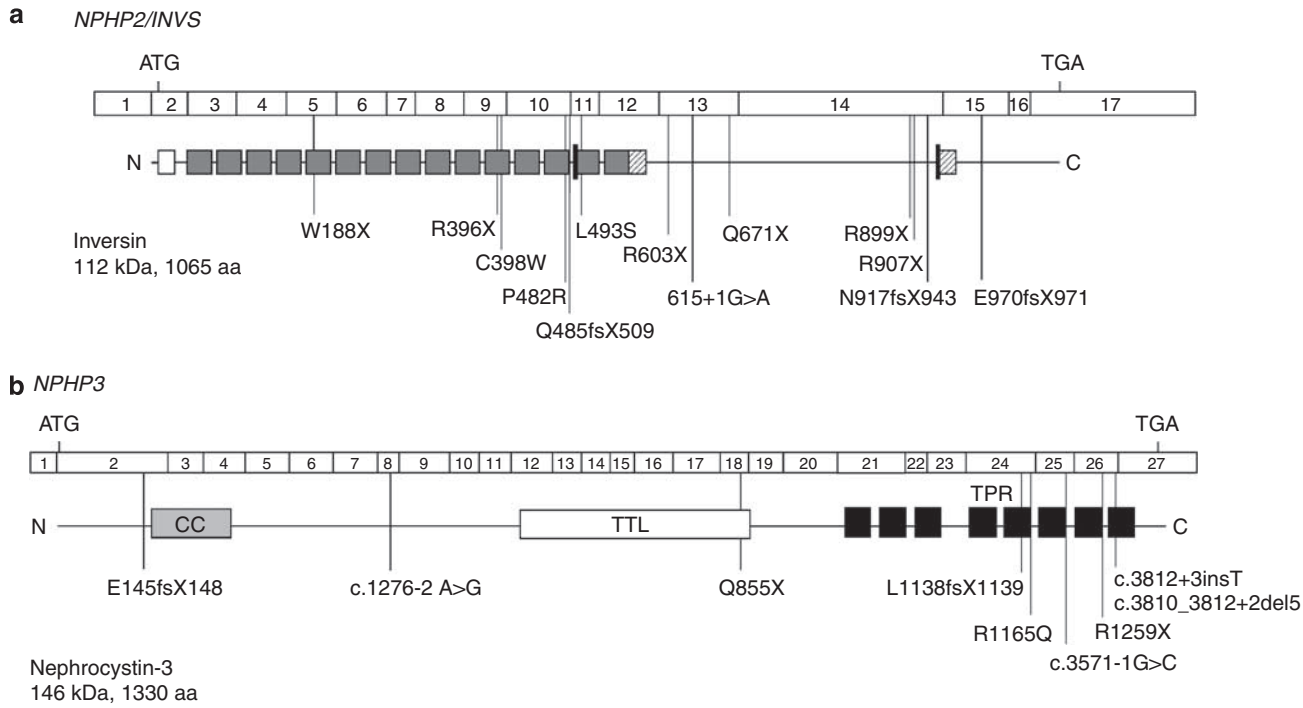

Figure 1 | Schematic diagram of the localization of the detected *NPHP2* and *NPHP3* mutations with respect to the exons and to the functional protein domains. (**a**) Inversin, the product of the *NPHP2/INVS* gene, contains 16 tandem ankyrin repeats (gray boxes), two IQ calmodulin-binding domains (hatched boxes), and two destruction box regions (black bars). (**b**) Nephrocystin-3, the product of the *NPHP3* gene, contains a coiled-coil domain (CC), a tubulin tyrosine ligase domain (TTL), and a tetratricopeptide-repeat domain (TPR). Most of the mutations map within the TPR domain.

In our cohort, seven families were homozygous for either a truncating ($n=6$) or a missense[1] mutation and eight families were compound heterozygous for two truncating mutations,[5] one truncating and one missense mutation,[2] or one truncating and one splice site mutation[1] (Table 1). In family A10, only one heterozygous truncating mutation was identified.

Phenotype of patients with *NPHP2* mutations

In the 16 families with *NPHP2* mutations, 18 children were affected (Table 1). The median age of the patients at diagnosis was 11 months (range: 5–36 months). Renal function deteriorated rapidly; ESRD was already present in half of the patients at the time of diagnosis and developed in all but two patients before the age of 2 years (median age: 18 months (range: 5–36 months), Table 1). Hypertension was a consistent finding. Ultrasound investigations revealed hyperechogenic kidneys in all but one case. In contrast, kidney size was variable: kidneys were enlarged in seven, normal-sized in five, and small in three cases. Cysts were detected in five of 15 patients.

Severe tubulointerstitial lesions were present in the 12 renal biopsies and in the five nephrectomy specimens studied. In nine biopsies (patients A12-1, F339-1, F331-1, A8-1, A7-1, F320-1, F398-1, F434-1, F340-1, Table 1), the histological pattern was typical of infantile NPH. In these cases, cortical tubules showed focal cystic dilatation and were lined by small cuboidal epithelial cells, or alternatively appeared collapsed and lined by a flat dedifferentiated epithelium (Figure 2a). Furthermore, diffuse moderate interstitial fibrosis was a constant feature in these patients, associated with mild focal cellular infiltration. Glomeruli were normal, sclerotic, or ischemic with a retracted capillary tuft surrounded by a thickened Bowman's capsule. Importantly, thickened tubular basement membranes were rare. In contrast, the lesions observed in two of the 12 biopsies (F411-1, A6-1) were similar to those in juvenile NPH with severe and nearly diffuse atrophy of tubules surrounded by a thick and irregularly laminated basement membrane (Figure 2b). In the remaining biopsy (1499), the lesions were advanced and too severe for precise classification. All five nephrectomy specimens (A8-1, A7-1, F413-1, A9-1, F320-1) displayed advanced chronic tubulointerstitial nephropathy with marked corticomedullary atrophy. A few medullary cysts were found in three of them (A7-1, F413-1, A9-1), and cortical microcysts in two (A8-1, F413-1). Marked medial hypertrophy of the arteries was present in all cases.

Extra-renal manifestations were found in 12 of 17 patients with available clinical information (Table 1). Heart valve or septal defects ($n=5$), hepatic involvement ($n=4$), and recurrent bronchial infections ($n=4$) were the most frequent findings. Developmental delay ($n=2$) and situs inversus ($n=2$) were also found. In the latter two cases, situs inversus was associated with heart valve or septal defects.

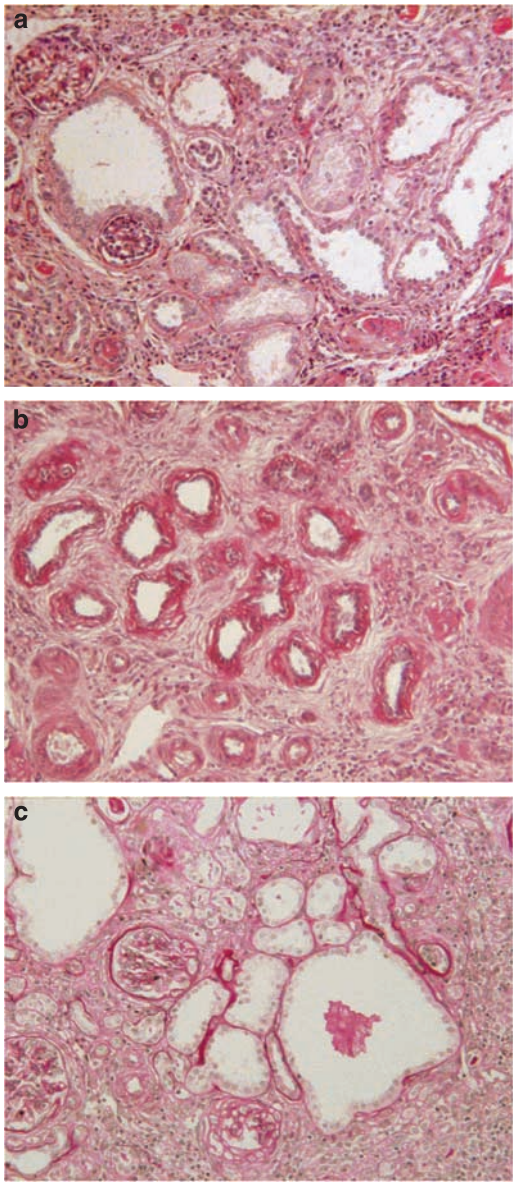

Figure 2 | Both 'juvenile' and 'infantile' type histological features were found in patients with infantile NPH. (**a**) Renal cortex characteristic of the infantile-type NPH. Irregular microcystic dilatation of cortical tubules. Absence of significant tubular basement membrane thickening. Interstitial fibro-edema. Normal glomeruli (light microscopy, periodic acid-Schiff (PAS) stain, × 250). (**b**) Renal cortex characteristic of the juvenile type. Most of the tubular basement membranes are thickened and laminated. Tubules are surrounded by a marked interstitial fibrosis with focal and moderate cell infiltration (light microscopy, PAS stain, × 280) (**c**). Microcystic dilatations of cortical tubules in a patient without *NPHP2*/*NPHP3* mutations (F299-1). Most adjacent tubules are dedifferentiated; few are surrounded by a thickened basement membrane (light microscopy, PAS stain, × 280).

NPHP3 mutations

We identified nine different *NPHP3* mutations in seven unrelated patients (Table 2, Figure 1b). Six mutations were novel: two were nonsense or frameshift mutations (p.Q855X, p.L1138fsX1139), three were splice site mutations (c.1276-2 A>G, c.3810_3812+2del5, c.3812+3insT), and one was a missense mutation (p.R1165Q). All three splice site mutations completely abolished the identification of the junction sequence by the NetGene2 program. Neither the consensus splice site mutation (c.3812+3insT) nor the other splice site mutation that affects the donor site of exon 26 (c.3810_3812+2del5) was found in 100 ethnically matched controls. The amino-acid substitution p.R1165Q was predicted to be benign by PolyPhen (PSIC score: 1.36). This mutation is likely to be pathogenic because the substituted arginine, located in the tetratricopeptide domain of nephrocystin-3 (Figure 1b), is conserved throughout vertebrate evolution and was not changed to glutamine in any of 107 ethnically matched control subjects.

Four of the seven patients were either compound heterozygous for a truncating and a consensus splice site mutation ($n=3$) or homozygous for a consensus splice site mutation ($n=1$) (Table 2). One of these patients (A11-1) carries a heterozygous *NPHP4* truncating mutation and was published earlier.[19] In three of the seven patients, we identified only one heterozygous *NPHP3* mutation (Table 2): one affected an obligatory splice site, one was truncating, and one was the missense mutation p.R1165Q.

Phenotype of patients with *NPHP3* mutations

The renal function of the seven unrelated patients with *NPHP3* mutations deteriorated less rapidly than did that of patients with *NPHP2* mutations: they were diagnosed with renal involvement at the median age of 30 months (range: 2–48) (Tables 2 and 3) and developed ESRD at the median age of 36 months (range: 6–52). Nevertheless, similar to patients with *NPHP2* mutations, all but one of the patients with *NPHP3* mutations were hypertensive. Renal ultrasound revealed hyperechogenic kidneys in all patients, and the kidneys were large in two patients, normal-sized in four, and small in one patient. Cysts were found by ultrasound in only two patients.

Diffuse tubulointerstitial lesions were present in all six renal biopsies studied. In two patients (1348, F271-1; Table 2), some cortical tubules showed cystic dilatation, and others were undifferentiated or atrophic, giving a histological picture consistent with that of infantile NPH (Figure 2a). Two other patients (F367-1, F369-1) showed thickening and splitting of the basement membrane, characteristic of juvenile NPH (Figure 2b). One patient showed severe lesions that precluded precise classification (patient 1364). Finally, the major abnormalities in one patient (F440-1) were glomerulocystic lesions that were also noted in the patient's nephrectomy specimen. The other available nephrectomy specimen (F271-1) showed severe corticomedullary atrophy without medullary cysts. Marked thickening of the arterial walls was observed in all cases.

All patients showed extra-renal manifestations, with hepatic involvement affecting all but one patient. Liver biopsies, when available, showed hepatic fibrosis (Table 2). The liver biopsy from patient 1348 showed massive portal

Table 2 | Genetic and clinical characteristics of patients with *NPHP3* mutations

Ind	CS	Origin	Nucleotide seq (exon, segregation)	Alterations in coding seq	Diagnosis (months)	Symptoms at dg	ESRD (months)	Renal ultrasound	Renal histology	HT	Liver	Other extra-renal symptoms
1348	+	Algeria	c.1276-2A>G H (8, P, M)	Abn transcript	2	Anemia, PU–PD, FT	6	NSK (+1SD), HE, no cyst	Infantile type	–	HF	Ventricular septal defect
F369-1	–	Norway	c.2563C>T h (18) c.3812+3insT h (26)	p.Q855X abn transcript	30	ND	36	NSK, no cyst	Juvenile type	ND	ET	Recurrent subdural collections
A11-1[a]	–	France	c.435_438del4 h (2, M) c.3571-1G>C h (25, P)	p.E145fsX148 abn transcript	36	PU–PD, FT	37	NSK, cyst	ND	+	HF	Recurrent pneumonias and bronchitis
F367-1	–	France	c.3412_3413delTT h (24) c.3775C>T h (26)	p.L1138fsX1139 p.R1259X	48	Anemia, PU–PD, FT	52	EK, no cyst	Juvenile type	+	—	Mitral insufficiency, keratitis
F271-1	–	France	c.3810_3812+2del5 h (26)	Abn transcript	20	FT	24	SK, HE, no cyst	Infantile type	+	HF	Cone-shaped epiphyses, retinitis pigmentosa
F440-1	–	France	c.3412_3413delTT h (24)	p.L1138fsX1139	5	PU–PD, FT	12	EK, HE, cortical cysts	Glomerulocystic	+	HF	Hypercholesterolemia
1364	–	Algeria	c.3494G>A h (24)	p.R1165Q	39	Anemia, PU–PD	39	NSK, HE, no cyst	End-stage K	+	ET EG	Cone-shaped epiphyses

Abn, abnormal; CS, consanguineous; EG, elevated serum GGT level; EK, enlarged kidney; ET, elevated serum transaminase level; FT, failure to thrive; h, heterozygous; H, homozygous; HE, hyperechogenic kidney; HF, hepatic fibrosis; HT, hypertension; Ind, individual; K, kidney; ND, no data available; NSK, normal-sized kidney; PU–PD, polyuria–polydipsia; SD, standard deviation; SK, small kidney.
[a]Family already published and known also to carry a heterozygous *NPHP4* mutation (c.3364-3367delACTG h).[19]

Table 3 | Summary of the phenotypes associated with *NPHP2* and *NPHP3* mutations

	NPHP2 mutations (*n*=18[a])	*NPHP3* mutations (*n*=7[a])
Age at onset of ESRD (median (range))	18 (5–36)	36 (6–52)
Hypertension	15/15	5/6
Size of the kidney (small/normal/large)	3/5/7	1/4/2
Hyperechogenic kidneys on ultrasound	14/15	7/7
Renal cyst on ultrasound	5/15	2/7
Histology		
Juvenile type[b]	3/14	2/6
Infantile type[b]	10/14	2/6
Glomerular cysts	0/14	1/6
Hepatic involvement	4/17	6/7

[a]The number of patients with known phenotype is indicated in each line.
[b]For the precise description of juvenile-type and infantile-type histological findings, see the text.

fibrosis with fibrous extensions bridging adjacent portal areas. No true bile duct proliferation was observed, but neo bile canaliculi were present within the fibrous areas.

Other recurrent extra-renal manifestations in patients with *NPHP3* mutations were cone-shaped epiphyses ($n=2$) and heart valve or septal defects ($n=2$). One patient suffered recurrent bronchial infections and retinitis pigmentosa.

Phenotype of patients without *NPHP2*/*NPHP3* mutations

No *NPHP2* or *NPHP3* mutations were found in 22 patients from 20 families. The ages at diagnosis of renal involvement (median: 27 months (range: 0–58)) and onset of ESRD (median: 47 months (range: 9–60)) were variable (Supplementary Table 1). Nevertheless, only two patients developed ESRD before 2 years of age. Most of the patients were hypertensive. On ultrasound, hyperechogenicity of the kidneys was a frequent finding, but cysts were found in only 5 of 14 patients. Similar to patients with identified mutations, kidney sizes were diverse: enlarged in 1 patient, normal-sized in 10, and small in 3 patients (Table S1).

Renal histology was studied in 11 patients by analysis of nine biopsies and five nephrectomy specimens. Juvenile- and infantile-type histological findings were found in four and three renal biopsies, respectively (Table S1, Figure 2c). In the two remaining biopsies, either focal nonspecific tubulointerstitial lesions (P1521) or arterial/arteriolar lesions (P1558) were prominent. Severe corticomedullary atrophy was present in the five end-stage kidneys. Cortical cysts were present in four of them (F348-1, F348-2, F350-1, F249-1) and associated with medullary cysts in one (F249-1); no cystic lesions were observed in patient F237-1.

Hepatic involvement ($n=10$), retinitis pigmentosa ($n=6$), cone-shaped epiphyses ($n=5$), and lower respiratory diseases ($n=5$) were the most frequent extra-renal involvements in patients with relevant clinical information (Table S1). Heart valve or septal defects were found in two patients and situs inversus in another patient. The other extra-renal anomalies were diverse. Some, including craniosynostosis and thoracic dystrophy, were not observed in any patient with *NPHP2* or *NPHP3* mutations.

DISCUSSION

Infantile NPH was described two decades ago. On the basis of the different macro- and microscopic renal morphology and the different genes mutated, infantile NPH and juvenile NPH

were considered to be distinct pathological entities. We found here that patients with infantile NPH may also present with a renal histology characteristic of juvenile NPH. Until recently, *NPHP2* was the only causative gene identified. We show in this study that *NPHP3* mutations are also associated with infantile NPH and that these two genes are mutated in half of the cases in a large cohort of patients with early-onset ESRD. The onset of ESRD occurs earlier in patients harboring *NPHP2* mutations than in those with *NPHP3* mutations, and *NPHP3* mutations are generally associated with liver abnormalities.

High *NPHP2* mutation rate in patients with ESRD before 2 years of age

The *NPHP2* mutation rate in our cohort of families with infantile NPH was high (37%, 16/43), and was 78% (14/18) in the subgroup of patients developing ESRD before the age of 2 years. This high prevalence of mutation was unexpected in view of the findings of Otto *et al.*,[18] who reported *NPHP2* mutations in only 2 of 25 NPH patients with early-onset ESRD (<5 years). However, we found few *NPHP2* mutations in patients with an onset of ESRD between 2 and 5 years of age; therefore, a different age distribution in the two cohorts may explain the difference in the observed prevalence of mutations.

The variant p.A650P was described earlier in the heterozygous state in a patient with juvenile NPH, who also carried an *NPHP3* heterozygous missense mutation,[19] and in a sibling pair with adolescent NPH and ulcerative colitis.[19,20] However, the alanine affected by this mutation is not conserved and we found a proline at this position in 4% of the controls. Thus, it is unlikely that this variant is pathogenic.

Thus, *NPHP2* can be considered to be the major causative gene of infantile NPH, especially in patients developing ESRD during the first 2 years of life. Its role in juvenile and adolescent NPH has not been determined.

Different types of *NPHP3* mutations in infantile and adolescent NPH

NPHP3 was initially localized by means of linkage analysis in a large Venezuelan kindred in which ESRD developed at a median age of 19 years.[2] The *NPHP3* gene was thus considered to be responsible for an adolescent form of NPH. Subsequently, *NPHP3* mutations were found in patients with an onset of ESRD between 7 and 37 years of age,[10] showing that they can also cause juvenile NPH. More recently, *NPHP3* mutations were found in two patients with an onset of ESRD at 3 and 4 years of age.[18,19] Given these new data, we sought to assess the involvement of *NPHP3* in patients with infantile NPH. Surprisingly, we found a high prevalence of *NPHP3* mutations (7/43 families, 16%). However, in three of the seven families we found only one heterozygous mutation, raising the possibility that mutations in other genes also contribute to the disease phenotype.[19,21] Alternatively, a second *NPHP3* mutation could have gone undetected if it had involved a deletion or was located within an intron or promoter region.

In total, the age at the onset of ESRD linked to mutations of *NPHP3* was highly variable, ranging from infancy to adolescence. There are two possible explanations for this variability.

The epistatic effect of a second locus could account for this variability, as one patient with infantile NPH and two *NPHP3* mutations (A11-1) is also known to carry a heterozygous *NPHP4* frameshift mutation.[19] Alternatively, the variability in the age at the onset of ESRD could be explained by the different types of *NPHP3* mutations found in patients with infantile and adolescent NPH. In the original description of adolescent NPH, eight of nine families carried a hypomorphic mutation on at least one allele;[10] in our study, six of seven families with infantile NPH carried truncating or consensus splice site mutations. Although we found only one heterozygous mutation in two of these families, the putative genotype–phenotype correlation is also supported by the recent finding that four families with a Meckel-like syndrome and cystic kidney dysplasia carry truncating or splice site *NPHP3* mutations on both alleles in all but one case.[22] A similar genotype–phenotype correlation was shown in mice with *Nphp3* mutations.[22] Thus, missense mutations in *NPHP3* seem to be associated with a late-onset, adolescent NPH, whereas truncating and splice site mutations are associated with a more severe phenotype, which is either multicystic, dysplastic kidneys in a Meckel-like syndrome[22] or, as in our study, infantile NPH. A similar genotype–phenotype correlation was found in patients with *NPHP8* mutations, as hypomorphic mutations were associated with late-onset NPH (and Joubert syndrome), and loss-of-function mutations led in most cases to cystic dysplastic kidneys and Meckel–Gruber syndrome.[15]

Moreover, most *NPHP3* mutations identified in our cohort map within the tetratricopeptide-repeat motifs. Interestingly, these motifs have been reported to be over-represented in ciliary proteins, such as intraflagellar transport proteins and the proteins mutated in Bardet–Biedl syndrome,[23] suggesting that these *NPHP3* mutations might affect ciliary function.

Renal phenotype of patients with infantile NPH

Patients with *NPHP2* and *NPHP3* mutations and also patients without identified mutations presented with a similar renal phenotype. The only notable difference was the faster deterioration of renal function in patients with *NPHP2* mutations compared with patients in the two other groups, considering that, in all patients with infantile NPH, the constant features of the renal phenotype were (1) arterial hypertension, leading to marked medial hypertrophy of the arterial wall; (2) hyperechogenicity of the kidneys on ultrasound; and (3) tubular lesions with interstitial fibrosis. Cysts, found on ultrasound in two of seven patients in the original description by Gagnadoux *et al.*[5] and in four of nine patients by Otto *et al.*,[17] were also found in a minority of the

cases (12/36) in this study. The size of the kidney, reported to be enlarged in four of seven patients by Gagnadoux *et al.*,[5] was highly variable in our cohort. The characteristic histological feature of juvenile NPH is the severe and nearly diffuse tubular atrophy, surrounded by a thick and irregularly laminated basement membrane.[1] Gagnadoux *et al.*[5] reported on the absence of such anomalies and concluded that infantile NPH should be considered a distinct entity. Although the histological abnormalities in most of our patients are similar to those described by Gagnadoux *et al.*,[5] we also found tubular basement membrane alterations characteristic of juvenile NPH in a large proportion of patients in all three patient groups. Thus, the infantile and juvenile forms of NPH cannot be clearly distinguished on the basis of localization of the cysts, the size of the kidney, or renal histology. Screening for mutations should therefore be directed by age at the onset of ESRD and not by macro- or microscopic renal morphology.

Extra-renal phenotype of patients with infantile NPH

Extra-renal symptoms are present in approximately 30% of the cases of juvenile NPH, with retinitis pigmentosa being the one most frequently reported.[12,18] We found extra-renal symptoms in 80% (36/45) of patients with early-onset ESRD. Hepatic fibrosis was almost a constant feature in patients with *NPHP3* mutations (6/7), but was relatively rare in patients with *NPHP2* mutations (4/16). Thus, *NPHP3* is an infantile NPH gene associated with hepatic fibrosis, but as liver disease was also present in half of the patients without an identified mutation (10/18), there is apparently further genetic heterogeneity within this patient group.

Structural cardiac anomalies and situs inversus have rarely been described in patients with NPH, despite their high frequency in *inv/inv* and *Nphp3*$^{ko/ko}$ mice, which show laterality problems in 90 and 70% and cardiac defects in 37 and 100%, respectively.[22,24] Recently, Bergmann *et al.*[22] found heart septal or valve defects in three of seven patients with *NPHP3* mutations and situs inversus in one further patient. In our cohort, heart valve or septal defects were found in nine patients, although four of them had only mitral insufficiency; this association may be incidental or the result of hypertension.[25] Nevertheless, severe cardiac defects were found in one patient with *NPHP3* mutations and in three patients with *NPHP2* mutations, two of whom (A8-1, F434-1) also had situs inversus. One of them (A8-1) was described earlier by Otto *et al.*[17] The association of cardiac developmental and laterality defects is well known and can be a consequence of the dysfunction of the motile cilia, as in the case of primary ciliary dyskinesia.[26]

Recurrent lower respiratory disease was found in 10 of 44 patients with infantile NPH. Four of these 10 patients carried *NPHP2* mutations and one carried *NPHP3* mutations. Irregular ciliary motility of the respiratory epithelial cells has already been described in patients with *NPHP1* deletion.[27] This irregularity was mild compared with primary ciliary dyskinesia, and correspondingly only four NPH patients have been reported in the literature with chronic sinusitis or bronchitis.[27,28] Our findings show that respiratory diseases are more frequent in patients with infantile NPH and implicate inversin and nephrocystin-3 in the function of motile cilia.

No causative gene has thus far been identified in patients with NPH and bone abnormalities. Interestingly, we found an *NPHP3* mutation in two patients with cone-shaped epiphyses. However, the causative role of these mutations is questionable because they were present in only one of the two alleles. The development of cone-shaped epiphyses in these two cases may have been the consequence of mutations elsewhere. Such oligogenic properties in NPH and associated disorders have already been described.[19,21]

Retinitis pigmentosa is the most frequent extra-renal symptom in juvenile NPH, but it was found in only one patient with an *NPHP3* mutation and not in association with *NPHP2* mutations in this study.

In summary, in our cohort, the characteristic features of the renal phenotype in infantile NPH were the presence of hypertension, hyperechogenicity of the kidneys on ultrasound, and tubular lesions with interstitial fibrosis. Typical tubular lesions characteristic of juvenile NPH were also observed in some patients with infantile NPH. We show that *NPHP3* mutations, known to be associated with adolescent NPH, can also be found in patients with early-onset ESRD. These various findings suggest that infantile and juvenile forms are two different manifestations of the same disease.

MATERIALS AND METHODS

Patients

A total of 43 unrelated families with infantile NPH were selected from a worldwide cohort of 380 NPH families, originating mainly from France and North Africa. Inclusion criteria were age at the onset of ESRD (5 years, the oldest age reported in a patient with infantile type NPH[17]) in association with any of the following clinical and morphological characteristics of NPH: (1) a history of polyuria–polydipsia, anemia, or failure to thrive with a progressive decline in renal function with no or mild proteinuria (<1 g/l); (2) histological lesions of chronic tubulointerstitial nephritis without any sign of dysplasia; (3) renal ultrasound findings excluding congenital malformations of the kidney and the urinary tract; and (4) family pedigree compatible with autosomal recessive inheritance. Of the 43 families included, 10 were consanguineous and 4 were multiplex involving two affected siblings (Tables 1 and 2, Table S1). Six families with *NPHP2* mutations and one family with *NPHP3* mutations were published earlier.[17,19]

Genomic DNA was isolated from peripheral blood by standard methods, after obtaining informed consent from the patients or their parents. All experiments were in accordance with the French ethical committee recommendations and with the Declaration of Helsinki Principles.

Linkage analysis

Linkage analyses were performed in consanguineous and multiplex families by genotyping the polymorphic markers

D9S272, D9S176, D9S1857, and D9S1690, flanking the *NPHP2* locus, and D3S1292, D3S1596, D3S1290, and D3S1238 flanking the *NPHP3* locus. Linkage to *NPHP2* and to *NPHP3* was excluded in three (F299, F317, F348) and two (F299, F317) families, respectively (Table S1).

Mutational analysis

Screening for mutations in *NPHP2* and *NPHP3* was performed in one affected child in each family, if linkage could not be excluded. *NPHP2* was sequenced first. *NPHP3* was sequenced subsequently in patients without *NPHP2* mutations on both alleles. Sequence analysis of *NPHP3* was extended to its in-frame transcript FLJ12592 in families with no or only one mutation detected in *NPHP3*.

Screening for mutations involved direct sequencing of the coding exons and the adjacent intronic junctions (primer sequences available on request). PCR products were treated with Exo-SAP IT (AP Biotech, Princeton, NJ, USA), sequenced using the dideoxy chain termination method on a 3130 XL DNA sequencer (Applied Biosystems, Foster City, CA, USA), and analyzed with the Sequencher 3.1 program (Genecodes). Amino-acid conservation at the sites of missense mutations and their possible damaging effects were assessed using SIFT[29] and PolyPhen software.[30] Donor and acceptor sites for splicing were predicted by NetGene2.[31] More than 100 control subjects, matched in each case with the patient's ethnicity, were tested for the presence of each non-silent nucleotide variant. Segregation of the identified mutations was investigated in families with available parental DNA.

Histology

Thirty-nine renal specimens from 31 patients were analyzed by the same pathologist (MCG). These specimens consisted of 27 renal biopsies obtained between 6 and 50 months of age and of 12 kidneys removed at the time of transplantation (after a few months to 6 years on dialysis). Renal biopsies and nephrectomy specimens were fixed in Dubosq-Brazil or formalin fixative, respectively, then embedded in paraffin, and serially sectioned and stained with hematoxylin and eosin, Trichrome light green, periodic acid-Schiff (PAS), and Jones silver methenamine. Liver biopsy was reviewed in one patient (patient 1348).

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Table S1. Clinical characteristics of patients without either *NPHP2* or *NPHP3* mutations.

Supplementary material is linked to the online version of the paper at http://www.nature.com/ki

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