

Variable Expressivity of HNF1B Nephropathy, From Renal Cysts and Diabetes to Medullary Sponge Kidney Through Tubulo-interstitial Kidney Disease



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Introduction: In humans, heterozygous mutations of hepatocyte nuclear factor 1beta (HNF1B) are responsible for a dominant inherited disease with both renal and extrarenal phenotypes. HNF1B nephropathy is the umbrella term that includes the various kidney phenotypes of the disease, ranging from congenital anomalies of the kidney and urinary tract (CAKUT), to tubular transport abnormalities, to chronic tubulointerstitial and cystic renal disease.

Methods: We describe 7 families containing 13 patients with ascertained HNF1B nephropathy. All patients underwent genetic testing and clinical, laboratory, and instrumental assessment, including renal imaging and evaluation of extrarenal HNF1B manifestations.

Results: Significant inter- and intrafamilial variability of HNF1B nephropathy has been observed. In our cohort, HNF1B pathogenic variants presented with renal cysts and diabetes syndrome (RCAD); renal cystic phenotype mimicking autosomal dominant polycystic kidney disease (ADPKD); autosomal dominant tubulointerstitial kidney disease (ADTKD) with or without hyperuricemia and gout; CAKUT; and nephrogenic diabetes insipidus (NDI). Of note, for the first time, we describe the occurrence of medullary sponge kidney (MSK) in a family harboring the HNF1B whole-gene deletion at chromosome 17q12. Genotype characterization led to the identification of an additional 6 novel *HNF1B* pathogenic variants, 3 frameshift, 2 missense, and 1 nonsense.

Conclusion: HNF1B nephropathy may present with a highly variable renal phenotype in adult patients. We expand the HNF1B renal clinical picture to include MSK as a potential new finding. Finally, we expand the allelic repertoire of the disease by adding novel HNF1B pathogenic variants.

Kidney Int Rep (2020) 5, 2341–2350; <https://doi.org/10.1016/j.ekir.2020.09.042>

KEYWORDS: ADPKD; ADTKD; CAKUT; cystic kidney disease; HNF1B; medullary sponge kidney; nephrogenic diabetes; RCAD; tubulointerstitial nephritis

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H*NF1B* gene, also known as transcription factor-2 (TCF2), is a developmental gene located on chromosome 17q12, coding for protein hepatocyte nuclear factor 1 homeobox B, with a crucial role in early

embryonic development. The TFC2 protein is required for tissue-specific gene expression in the epithelial cells of many organs, including the kidney, pancreas, liver, and genitourinary tract.^{1,2} *HNF1B* is also transiently expressed in the neural tube, epididymis, seminal vesicles, prostate, and uterus.^{1,2} This expression pattern explains the frequently highly variable presentation of *HNF1B*-related disorder.

In 1997, Horikawa *et al.*³ first reported, in a Japanese family, the association of pathogenic variants of *HNF1B* gene with maturity onset diabetes of the young 5 (MODY-5), a monogenic form of diabetes mellitus

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Received 27 June 2020; revised 24 August 2020; accepted 22 September 2020; published online 7 October 2020

characterized by onset before 25 years of age and autosomal dominant inheritance.³ Renal disease was also reported in some affected members of the family.³

Since then, renal involvement has emerged as the earliest and most prevalent finding described in almost all patients with *HNF1B* variants, which are now considered to be the most frequent monogenic cause of developmental renal disease.^{4–17}

In the last decade, various kidney phenotypes have been described among patients with *HNF1B* pathogenic variants, encompassing CAKUT and tubular transport disorders.^{4–17} To date, *HNF1B*-nephropathy phenotype includes the following: pre-natal hyper-echogenic kidney; chronic tubulointerstitial kidney disease; renal cystic disease; renal hypo-dysplasia; glomerulocystic kidney disease; single, horseshoe, and duplex kidneys; bilateral hydronephrosis; nephrocalcinosis; nephrogenic diabetes insipidus; and hypomagnesemia due to renal magnesium wasting.^{4–17}

Although the association of MODY with a heterogeneous clinical kidney disease is the core phenotype of *HNF1B*-associated disease, an ever-expanding spectrum of extrarenal manifestations have been reported, such as exocrine pancreatic failure, pancreatic hypoplasia, fluctuating liver tests abnormality, early-onset gout, and genital tract malformations.^{4–17} Therefore, *HNF1B* disease must be considered as an autosomal, dominant multi-system disorder (MIM 137920).

In approximately 40% to 50% of patients, the molecular defect is a whole *HNF1B* gene deletion, occurring in the context of the 17q12 recurrent deletion syndrome that includes several other genes (OMIM 614527); the 17q12 recurrent deletion syndrome can be associated with mild dysmorphic features and neurodevelopmental/neuropsychiatric disorders, attributable to deletion of the genes other than *HNF1B* located in the 17q12 region.¹⁶ In the remaining cases, heterozygous *HNF1B* variants are detected: about 52% are missense, 29% are frameshift, and 15% are nonsense (LOVD database; <https://databases.lovd.nl/shared/genes/HNF1B/graphs>).

De novo *HNF1B* variants are encountered in approximately 30% to 50% of cases.^{4–17} A close genotype–phenotype correlation is lacking. Some authors have suggested that patients with *HNF1B* sequence pathogenic variant may have a poorer renal prognosis than those with whole-gene deletion. This observation is based mainly on the evidence that in a large cohort of adult *HNF1B*-disease patients, those with sequence variants showed a higher frequency of chronic kidney disease stage 3 to 4 or end-stage renal disease and a lower median estimated glomerular filtration rate (eGFR) at follow-up compared with patients with whole-gene deletion.^{15,17} The mechanism underlying this possible genotype–phenotype

correlation remains unexplained; it can be hypothesized that some *HNF1B* variants may have a dominant negative effect worsening the phenotype.¹⁷

Here we describe 13 patients from 7 families of Italian ancestry with *HNF1B* nephropathy, showing a great inter- and intrafamilial variability in the renal disease, including the following: RCAD; renal cystic phenotype mimicking ADPKD; ADTKD with hyperuricemia and gout; CAKUT; and prenatal hyperechogenic kidney in association with NDI. Interestingly, for the first time, we describe, in a family with 17q12 recurrent deletion syndrome, a renal phenotype consistent with MSK, a rare developmental disorder for which the underlying defect is not fully understood.

Despite the single-genetic etiology, *HNF1B* nephropathy has a protean clinical presentation. Diagnosis of *HNF1B* nephropathy is often challenging, and the differential diagnosis is complex, as the disease may mimic a variety of renal disorders. For this reason, *HNF1B* nephropathy can be considered as one of the “great masqueraders” in nephrology.

METHODS

Since 2014, a total of 120 patients with clinical diagnosis of tubulo-interstitial kidney disease have been evaluated at the Division of Nephrology, University of Brescia (Brescia, Italy); 92 patients have undergone genetic testing. Diagnosis of tubulo-interstitial kidney disease was established on the basis of renal phenotype characterized by chronic tubulo-interstitial nephritis with nonsignificant urinalysis and slowly progressive renal failure, with or without hyperuricemia and gout. Genetic testing for ADTKD genes was offered to patients with a positive family history for tubulo-interstitial kidney disease or sporadic cases with renal disease onset at less than 50 years of age.

The genetic testing protocol included targeted gene panel NGS analysis to detect sequence variants in the following genes related to ADTKD or cystic kidney diseases: *PKD2*, *UMOD*, *HNF1B*, *REN*, *GANAB*, *PARN*, *ALG8*, and *DNAJB11*. To detect *HNF1B* gene deletion, multiple ligation probe amplification (MPLA) analysis was performed in all patients ([Supplementary Materials and Methods](#)).

In our series, 7 of 92 probands had pathogenic variants in *HNF1B* (7.6%). All family members underwent genetic testing and clinical, laboratory, and instrumental assessments, including renal function tests and renal imaging. At-risk family members were offered presymptomatic genetic testing. Extension of the clinical and molecular study to relatives allowed us to find an additional 6 affected patients, for a total of 7 families with 13 affected members ([Figure 1](#)).

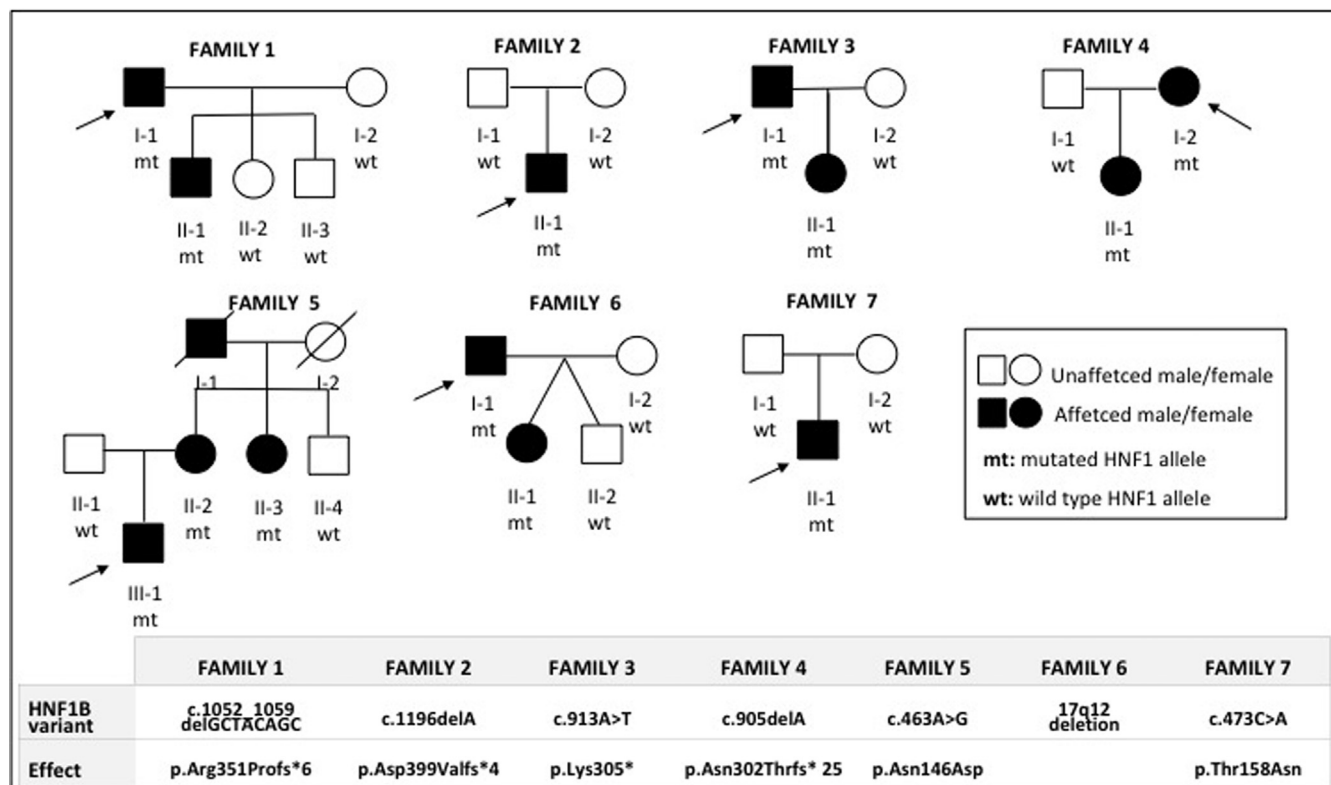


Figure 1. Pedigrees of families showing affected and unaffected individuals and HNF1B testing results. In the lower part of the figure, the HNF1B gene variants for each family are listed.

Medical histories were obtained as part of all patients' clinical workup. Clinical and genetic data were collected according to national laws ([Supplementary Materials and Methods](#)).

RESULTS

Clinical and molecular data of the patients are summarized in [Figure 1](#) and [Tables 1–3](#).⁸

Family 1

The index case (I-1, [Figure 1](#)), a 73-year-old man, was referred to the nephrologist because of large bilateral renal cysts. His medical history revealed severe aortic insufficiency and aortic bulb dilatation requiring cardiac surgery at age 55 years; serum creatinine (sCr) was 0.8 mg/dl (eGFR 99 ml/min per 1.73 m², calculated with the Chronic Kidney Disease Epidemiology Collaboration [CKD-EPI] formula). At referral, sCr was 1.14 mg/dl (eGFR 63 ml/min per 1.73 m²) and uric acid 8.4 mg/dl ([Table 1](#)). Urinalysis findings were nonsignificant, and hemoglobin A1c was in the normal range. A computed tomography (CT) scan revealed 2 enlarged kidneys with numerous bilateral cysts, no liver cysts, and mild pancreatic hypoplasia without alteration of glycemic status ([Tables 2 and 3](#), [Figure 2a](#)). For the cystic renal involvement and the vascular phenotype, sequence,

and MLPA analysis of PKD1 and PKD2 genes, results were negative.

One year later, we evaluated the 35-year-old son (II-1, [Figure 1](#)) with chronic kidney disease stage IIIB (sCr 2.17 mg/dl; eGFR 38 ml/min per 1.73 m²). His past history revealed onset of polyuria and polydipsia at age 2. Nephronophthisis was hypothesized by renal biopsy, which revealed a picture of chronic tubulointerstitial nephritis ([Table 1](#)). At referral, diabetes, hypomagnesemia, and hypokalemia were documented ([Tables 1 and 2](#)). A CT scan showed bilateral cortical cysts with normal kidney volume and pancreatic hypoplasia ([Table 3](#), [Figure 2b](#)). A mild aortic bulb dilatation was also recognized. Multigene NGS panel analysis for ADKTD revealed a novel heterozygous disease-causing frameshift variant in HNF1B: c.1052_1059delGCTACAGC; p. Arg351Profs*6. Segregation analysis revealed that the father also harbored the familial pathogenic HNF1B variant.

Family 2

The only affected family member of this family was a 54-year-old man (II-1, [Figure 1](#)) who was diagnosed with chronic renal disease stage III at age 27 (sCr 2.27 mg/dl, eGFR 38 ml/min per 1.73 m²). Urinalysis was not significant. Renal biopsy was performed at age 28 and showed diffuse tubulo-interstitial fibrosis ([Table 1](#)). A

Table 1. Renal manifestations in reported HNF1B patients

	Family 1		Family 2		Family 3		Family 4		Family 5 ^a			Family 6		Family 7
Pedigree ID (sex)	I-1 (M)	II-1 (M)	II-1 (M)	I-1 (M)	II-1 (F)	I-2 (F)	II-1 (F)	III-1 ^a (M)	II-2 ^a (F)	II-3 ^a (F)	I-1 (M)	II-1 (F)	II-1 (M)	
First evaluation (age)	56 yr	2 yr	28 yr	26 yr		28 yr		16 yr	47 yr	47 yr	23 yr		18 yr	
Serum creatinine (mg/dl)	0.80	0.8	2.27	1.78	N/A	1.32	N/A	1.4	1.2	1.04	1.2	N/A	1.5	
eGFR ^b (ml/min per 1.73 m ²)	99	46 ^c	38	51.5		54		73.9	53.8	63.9	84.7		67	
Suspected diagnosis at presentation	ADPKD	NPH	NPH	ADTKD	N/A	ADTKD	N/A	ARPKD	MCKD	MCKD	CAKUT/ MSK	NC	NPH	
Last follow-up (age)	77 yr	35 yr	54 yr	48 yr	19 yr	38 yr	9 yr	25 yr	58 yr	57 yr	51 yr	16 yr	38 yr	
Serum creatinine (mg/dl)	1.5	2.17	4.3	5.5	0.96	1.49	0.72	1.56	1.41	1.20	1.2	1.0	2.36	
eGFR ^b (ml/min per 1.73 m ²)	44.3	38	14.4	12	114.4	44	>75 ^c	60.8	41	50.3	69.6	110	33.7	
Hyperuricemia/gout	Yes/no	No/no	Yes/yes	Yes/no	Yes/no	Yes/no	No/no	No/no	No/no	No/no	No/no	No/no	No/no	
Magnesemia (n.v. 0.66–1.07 mEq/l)	0.70	0.60	0.31	0.56	0.61	0.60	0.66	0.78	0.75	0.90		0.66	0.90	
Kalemia/serum calcium (K ⁺ n.v. 3.5–5.3 mEq/l)	3.4/ 9.3	3.6/ 9.5	3.1/ 6.79	3.3/ 9.6	3.3/ 9.9	3.6/ 9.36	3.6/ 10.6	5.1/ 10	4.9/ 8.4	4.9/ 9	3.8 / 8.4	4.8/ 9.7	4.9/ 10.1	
(Ca ⁺⁺ n.v. 9.3–10.6 mg/dl)														
Renal biopsy (age)	No	Yes ^d (2 yr)	Yes ^d (28 yr)	No	No	No	No	No	No	No	No	No	Yes ^d (31 yr)	
Other renal features	No	POL	No	No	No	No	No	¹¹ POL ¹² NDI	No	No	No	No	No	
Type of HNF1B nephropathy	ADTKD	Early-onset ADTKD	ADTKD	ADTKD	ADTKD	ADTKD	ADTKD	Early-onset ADTKD	RCAD (ADTKD)	RCAD (ADTKD)	CAKUT/ MSK	NC/ MSK	ADTKD	

ADPKD, autosomal dominant polycystic kidney disease; ADTKD, autosomal dominant tubulo-interstitial kidney disease; ARPKD, autosomal recessive polycystic kidney disease; CAKUT, congenital anomalies of kidney and urinary tract; DI, nephrogenic diabetes insipidus; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; F, female; M, male; MCKD, medullary cystic kidney disease; MSK, medullary sponge kidney; N/A, not available; NC, nephrocalcinosis; No, absent; NPH, nephronophthisis; n.v., normal value; POL, polyuria in infancy; RCAD, renal cysts and diabetes; Yes, present.

^aPatients reported in Izzi et al.⁸

^beGFR calculated with Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.

^ceGFR calculated with Schwartz formula.

^dRenal biopsy findings: interstitial fibrosis and tubular atrophy.

diagnosis of nephronophthisis was made. At referral, renal function deterioration was documented (sCr 4.3 mg/dl, eGFR 14.4 ml/min per 1.73 m²) (Table 1). A CT scan revealed 2 small kidneys with few small cortical cysts (Figure 2c, Table 3). His medical history revealed hyperuricemia and recurrent gouty attacks, unexplained elevated liver enzymes, and normal serum magnesium levels (Table 1). Multigene NGS panel analysis for ADKTD disclosed a novel pathogenic heterozygous frameshift mutation in *HNF1B*: c.1196delA; p. Asp399Valfs*4 (Table 2).

Family 3

The index case, a 52-year-old man (I-1, Figure 1), was diagnosed with chronic renal disease stage III at age 26

(sCr 1.78 mg/dl, eGFR 51.5 ml/min per 1.73 m²). Abdominal ultrasound (US) revealed normal kidney volume and left renal cysts, and urinalysis was nonsignificant (Table 3). Hypomagnesemia, uncomplicated hyperuricemia, and diabetes were also found (Table 1). At age 48, sCr 5.5 mg/dl was documented (eGFR 12 ml/min per 1.73 m²), and at age 49, a living-donor kidney transplantation was performed (Table 1). A CT scan revealed 2 small kidneys with few cysts and pancreas hypoplasia (Figure 2d, Table 3). A multigene NGS panel analysis for ADKTD revealed a heterozygous nonsense mutation in *HNF1B* gene c.913A>T; p. Lys305* (Table 2). The 19-year-old daughter (II-1, Figure 1) inherited the familial pathogenic variant of *HNF1B*. A tailored clinical evaluation was performed,

Table 2. Extrarenal manifestations in reported HNF1B patients

	Family 1		Family 3	Family 5 ^a		Family 6
Pedigree ID (sex)	I-1 (M)	II-1 (M)	I-1 (M)	II-2 ^a (F)	II-3 ^a (F)	I-1 (M)
Pancreas abnormalities	Hypoplasia	Hypoplasia	Hypoplasia	—	—	Hypoplasia
Genital tract abnormalities	—	—	—	Septate uterus	—	EDO
Glycemic status: diabetes	—	Yes	Yes	Yes	GD	Yes
Neurologic impairment	—	—	—	—	—	Epilepsy, mild cognitive impairment
Cardiovascular disease	Severe aortic insufficiency	Mild aortic bulb ectasia, MVP	—	—	—	MVP

—, not present; EDO, ejaculatory duct obstruction; F, female; GD, gestational diabetes; M, male; MVP, mitral valve prolapse; No, absent; Yes, present.

^aPatients reported in Izzi et al.⁸

Table 3. Renal imaging (US, CT, or MRI scan) in reported HNF1B patients

Family	Pedigree ID	Presentation	Last follow-up
Family 1	I-1	Multiple bilateral cysts, increased TKV, renal stone (56 yr)	Multiple bilateral cysts, increased TKV (73 yr)
	II-1	Normal kidneys (2 yr)	Multiple bilateral cysts, normal kidneys (36 yr)
Family 2	II-1	Few bilateral cysts, normal kidneys (28 yr)	Bilateral small cysts, small kidneys, renal stones (54 yr)
Family 3	I-1	Few cysts, normal kidneys (35 yr)	Few cysts, small kidneys (49 yr)
	II-1	Normal kidneys (19 yr)	NA
Family 4	I-2	Normal kidneys (28 yr)	Few cortical cysts, small kidneys (38 yr)
	II-1	Few bilateral cysts (9 yr)	NA
Family 5	III-1	Antenatal: HE, enlarged kidneys (22 wk); bilateral cysts, normal kidneys (3 yr)	Multiple bilateral cysts (25 yr)
	II-2	Few bilateral cysts	Few small bilateral cysts (45 yr)
	II-3	NA	Bilateral cysts (40 yr)
Family 6	I-1	MSK; few left renal cysts, right hypoplastic kidney (23 yr)	MSK, bilateral cysts, right hypoplastic kidney (45 yr)
	II-1	Echogenic foci in renal medulla suggestive for calcifications (8 yr)	MSK, few small cysts (16 yr)
Family 7	II-1	NA	Bilateral cysts, small kidneys (33 yr)

Age in parentheses is age at evaluation. CT, computed tomography; HE, hyperechogenic kidneys; MRI, magnetic resonance imaging; MSK, medullary sponge kidney; NA, not available; TKV, total kidney volume; US, ultrasound; wk, week of gestation.

which showed normal renal function, hypomagnesemia, and hyperuricemia (Table 1). Abdominal US findings were normal (Table 3).

Family 4

The 38-year-old proband (I-2, Figure 1) was incidentally found to have sCr 1.32 mg/dl (eGFR 54 ml/min per 1.73 m²) at age 28; urinalysis was negative except for the low specific gravity (Table 1). At last follow-up, at age 38, sCr was 1.49 mg/dl and eGFR 44 ml/min per 1.73 m² (Table 1). Renal US showed moderately reduced kidneys and few cortical cysts (Table 3). Multigene

panel for ADTKD was scheduled and revealed a novel heterozygous frameshift mutation in *HNF1B*: c.905delA; p.Asn302Thrfs* 25 (Table 2). In the 10-year old daughter (II-1, Figure 1), US showed few bilateral cortical cysts (Table 3). Renal function was in the normal range for age, and magnesium level was in the lower range (Table 1). The maternal *HNF1B* variant was identified (Figure 1).

Family 5

This family has already been described in a previous report.⁸ Briefly, in the index case (III-1, Figure 1), renal

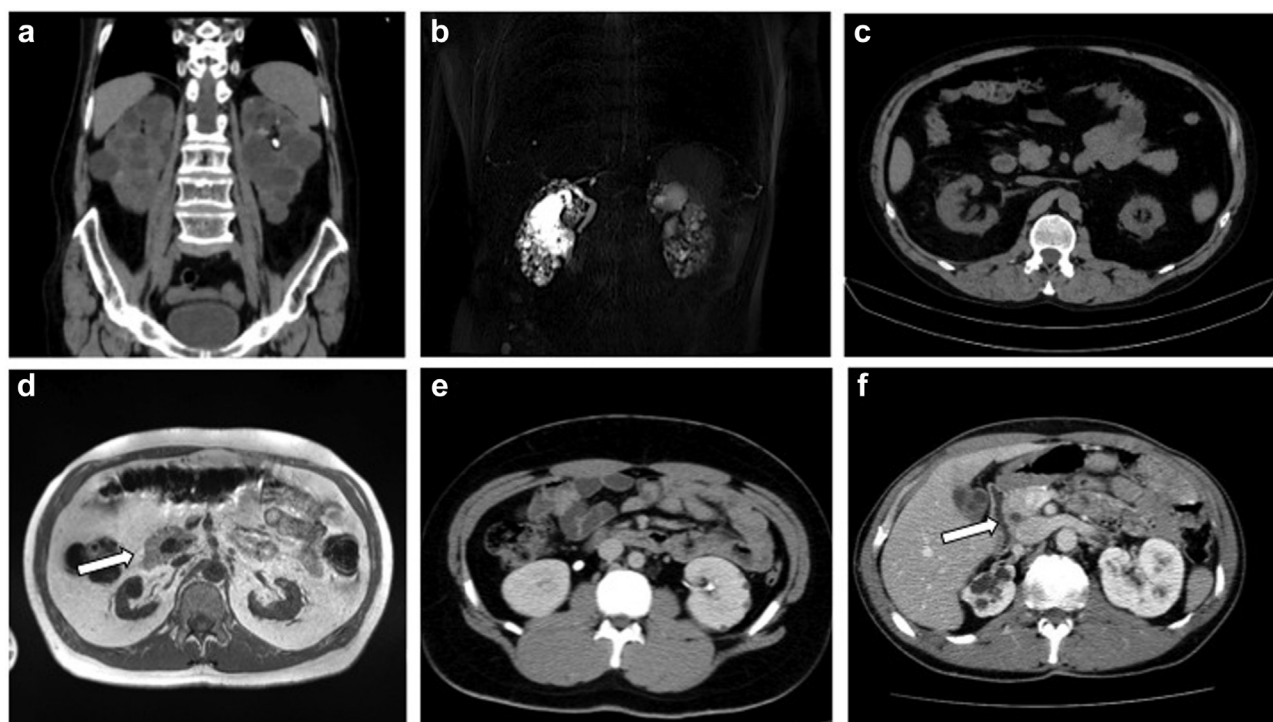


Figure 2. (a) I-1, family 1: computed tomography (CT) scan showing enlarged bilateral cystic kidneys, left renal stone. (b) II-1, family 1: magnetic resonance imaging (MRI) scan showing multiple small bilateral kidney cysts in normal sized kidneys. (c) II-1, family 2: CT scan showing bilateral small kidneys and few cysts. (d) I-1, family 3: MRI scan showing bilateral small kidneys and annular pancreas (white arrow). (e) III-1, family 5: CT scan showing bilateral normal sized kidneys and several cysts. (f) I-1, family 6: CT scan showing right kidney hypoplasia with cysts, including multiple cysts in the left kidney and annular pancreas (white arrow).

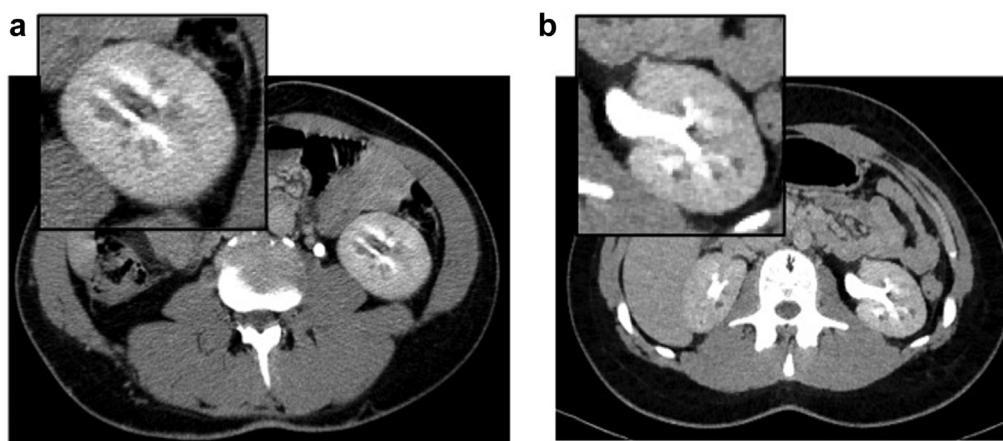


Figure 3. Computed tomography scan of (a) I-1 and (b) I-2 of family 6: insets show magnification of left kidney with nephrocalcinosis and cystic dilatation of the renal medullary collecting ducts.

disease was diagnosed prenatally, when fetal US disclosed enlarged hyperechogenic kidneys and polyhydramnios (Table 3). After birth, the clinical course was characterized by normal-sized kidneys showing numerous bilateral renal cysts; severe polyuria and polydipsia, consistent with a diagnosis of partial NDI, was present; slowly progressive chronic kidney disease was also evident (Tables 1 and 3). At last follow-up, at age 25, sCr was 1.56 mg/dl and eGFR 60 ml/min per 1.73 m² (Table 1). Diagnosis of early-onset *HNF1B* nephropathy was made following the detection of a novel heterozygous missense variant in *HNF1B* c.463A>G; p.Asn146Asp (Table 2). Family history and segregation analysis revealed an affected 58-year-old mother (II-2, Figure 1) (eGFR 41 ml/min per 1.73 m², bilateral renal cysts by US, diabetes mellitus, and septate uterus) and an affected 57-year-old maternal aunt (II-3, Figure 1) (eGFR 50.3 ml/min per 1.73 m², bilateral renal cysts, and gestational diabetes) (Tables 1–3).

Family 6

In the index case (I-1, Figure 1), hypoplastic right kidney and MSK were diagnosed at age 23 by urography; sCr was 1.2 mg/dl and eGFR 84.7 ml/min per 1.73 m² (Table 1). At last follow-up, at age 51, sCr was 1.2 mg/dl and eGFR 69.6 ml/min per 1.73 m² (Table 1). Clinical history was also remarkable for multifaceted extrarenal manifestations, including mild cognitive impairment, juvenile diabetes mellitus, generalized epilepsy, ejaculatory duct obstruction (recognized during ascertainment for infertility) (Table 2). A CT scan showed nephrocalcinosis, cystic dilatation of the renal medullary collecting ducts, and pancreas hypoplasia (Figure 3a, Tables 2 and 3). Because of the diagnosis of MSK, a nephropathy of unknown cause, the patient was enrolled 5 years ago for whole-exome

sequencing in a research project dedicated to families with genetic nephropathy of unknown origin. The analysis did not identify pathogenic sequence variants. Recently, on suspicion of genomic syndrome because of neurologic extrarenal manifestations in the proband, we searched for *HNF1B* whole-gene deletion with MLPA analysis. Because MPLA analysis revealed a whole-gene *HNF1B* deletion, CGH-array analysis was performed to define the extension of the chromosomal deletion, revealing a 17q12 deletion of 1.77 to 2.08 Mb, including the *HNF1B* gene (arr[GRCh37]17q12(34438350_36207539) x1), enabling the diagnosis of the recurrent 17q12 microdeletion syndrome.

Molecular study documented that the daughter (I-2, Figure 1) inherited the 17q12 deletion from the father (Table 2). Her clinical history was unremarkable except for echogenic foci in the renal medulla suggestive for calcifications, revealed on abdominal US at 8 years of age (Table 3). At age 16, renal function was normal and urinalysis was negative (Figure 3b, Tables 1 and 3). A CT scan of the abdomen showed cystic dilatation of the renal medullary collecting ducts, consistent with a diagnosis of MSK; few cortical cysts were also recognized (Table 3). Neurologic involvement was not present (Table 2).

Family 7

The 33-year-old index case (II-1, Figure 1) was noted to have renal impairment at age of 18, when sCr was 1.4 mg/dl (eGFR 67 ml/min per 1.73 m²) (Table 1). Renal biopsy indicated a picture of tubulo-interstitial nephritis, and a diagnosis of nephronophthisis was made (Table 1). At referral, at age 33, sCr was 2.2 mg/dl and eGFR 38 ml/min per 1.73 m² (Table 1). Renal imaging revealed reduced kidney volume and bilateral cysts (Table 3); urinalysis was normal except for a

decreased urinary concentration ability. Multigene NGS analysis for ADTKD disclosed a *de novo* heterozygous missense mutation in *HNF1B* c.473C>A; p.Thr158Asn (Figure 1). Extrarenal manifestations were not recognized (Table 2).

Classification of Sequence Variants in *HNF1B* Gene

All *HNF1B* variants detected in families 1, 2, 3, 4, 5, and 7 are novel, not listed in any public repository of population-based exome sequencing project (ExAC, gnomAD) and not found in 169 ethnically matched controls. Phenotype is consistent with the disease associated with *HNF1B*, and the variants co-segregated with the disease in family affected members. These data provide strong evidence of pathogenicity for the nonsense variants p.Arg351Profs*6, p.Asp399Valfs*4, p.Lys305*, and p.Asn302Thrfs*25, according to the proposed classification of pathogenicity by the American College of Medical Genetics (ACMG).¹⁸

The p. Asn146Asp variant co-segregated with the disease in family affected members and has been excluded in nonaffected members. Segregation analysis in the unaffected parents in family 7 revealed that the p. Thr158Asn variant is *de novo*, supporting evidence of probable pathogenicity, according to the ACMG classification.¹⁹ The most common *in silico* prediction tools (Align-GVGD, SIFT, PolyPhen-2, MutationTaster2) based on sequence homology, protein structure, and evolutionary conservation classified the 2 missense variants as deleterious.

DISCUSSION

HNF1B nephropathy is a condition characterized by an autosomal dominant inheritance and a highly variable renal phenotype, including cystic abnormalities, CAKUT, tubular transport disorders, and slowly progressive renal decline.^{1–6} Renal presentation varies widely according to age at recognition. In the fetus, hyperechogenic kidneys with normal or slightly enhanced size and normal amniotic fluid or polyhydramnios is the more frequent renal phenotype.^{20,21} In childhood, HNF1B nephropathy is typically characterized by renal cystic hypo-dysplasia and CAKUT.^{19,22} In adults, renal phenotype is less frequently described, and is usually characterized by slowly progressive chronic renal failure with a tubulointerstitial pattern.^{12–15,17,23}

In our study, we focused on HNF1B nephropathy in 11 affected adults and 2 young patients belonging to 7 families, providing some new clinical and imaging findings useful for the recognition of HNF1B nephropathy. With respect to the renal phenotype, the key findings were 3-fold.

First, the most common clinical renal presentation was that of chronic tubulo-interstitial nephritis, characterized by nonsignificant urinalysis and slowly progressive renal failure; when performed, renal biopsy confirmed a picture of interstitial fibrosis and tubular atrophy. In some patients, this picture was associated with hyperuricemia and gout. Varying degrees of renal impairment were found, from mild to moderate renal involvement to end-stage renal disease. A similar phenotype is usually found in patients with heterozygous variants of *UMOD*, *MUC1*, and *REN* genes, causing an autosomal dominant form of tubulo-interstitial nephritis.^{24–27} For this, HNF1B nephropathy is now categorized as part of the group of disorders named ADTKD, together with ADTKD-*UMOD*, ADTKD-*MUC1*, ADTKD-*REN*, and ADTKD-*SEC61A1*.^{24–27} In our series, 9 of 13 patients had a renal presentation of chronic tubulo-interstitial nephritis; only 2 of these patients had diabetes, making the phenotype indistinguishable from that of other ADTKD subtypes. The renal phenotypes associated with *HNF1B* variants, however, only partly overlap with mutations in *REN*, *UMOD*, and *MUC1*. Indeed, although the clinical manifestations of diseases caused by variants in *UMOD*, *MUC1*, and *REN* are usually confined to the kidney, *HNF1B* variants result in variable renal and extrarenal manifestations.^{12–15,24–27} For this, it appears reasonable to confine the term “ADTKD” to those *HNF1B*-related cases in which tubulo-interstitial fibrosis is the leading renal manifestation. The mechanism whereby mutated *HNF1b* leads to renal fibrosis is not fully understood. In ADTKD-*UMOD*, impaired intracellular trafficking and retention of mutant uromodulin may initiate an inflammatory process, resulting in progressive interstitial fibrosis, suggesting a gain-of-function effect of *UMOD* variants.²⁷ Although HNF1b regulates the transcription of *UMOD*, it is unlikely that a mechanism similar to the gain-of-function effect of *UMOD* mutations in ADTKD-*UMOD* is responsible for the phenotype of ADTKD-*HNF1B*, as patients with HNF1B-related ADTKD have normal *UMOD* expression.^{26,27} On the other hand, *HNF1b* has been found to be implicated in epithelial–mesenchymal transition, a process by which epithelial cells acquire mesenchymal characteristics. Epithelial–mesenchymal transition is essential for tissue regeneration and, when sustained, is associated with fibrogenesis.²⁸ Recently, Chan *et al.* found that loss of *HNF1b* may induce epithelial–mesenchymal transition both *in vitro* and *in vivo*, leading to up-regulation of expression of transforming growth factor- β ligands in renal epithelial cells, which can ultimately cause renal fibrosis.²⁹ These findings reveal a potential novel mechanism of renal fibrosis in HNF1B nephropathy.^{2,29}

The second important finding of our study was that cystic disease was the predominant renal imaging phenotype, being found in 92% of patients (Table 3). This is consistent with previous reports describing cystic phenotype in 62% to 81% of adult patients and in 73% of a large case series including both pediatric and adult patients.^{5,6,15,17,22} The finding of cystic kidneys is not unexpected in patients with *HNF1B* mutation. In an animal model, HNF1b regulates the transcription of numerous cystic disease genes, including *PKHD1*, *PKD2*, *UMOD*, *GLIS2*, and *KIF12*, a polycystic kidney disease (PKD) modifier gene.^{2,30} Moreover, it is of note that humans carrying heterozygous mutations of both *PKD1* and *HNF1B* have a more severe cystic phenotype, suggesting that HNF1B may virtually function as a modifier gene in ADPKD.^{31,32} Taken together, these data indicate that HNF1b produces kidney cysts by downregulating the expression of multiple cystic disease genes.^{2,30} In keeping with previous reports, renal cysts were usually small.^{7,12} However, in the index case of family 1, massively enlarged cystic kidneys mimicking those of ADPKD were found (Tables 1 and 3). This uncommon renal presentation, already described by Faguer *et al.* in two 20-year-old monozygous twins,³³ suggests that phenocopies of the ADPKD phenotype can be due to *HNF1B* variants. In our case, the identification of the affected son with a picture of chronic tubulointerstitial disease since infancy and diabetes paved the way for accurate diagnosis and confirmed that, in HNF1B nephropathy, phenotypic variability is often extensive, even within the same family (Tables 1 and 3). A more severe cystic phenotype was also found in the index case of family 5; in this patient, the kidneys, although normal in size, showed numerous bilateral cysts, which were probably responsible for a secondary form of polycythemia with inappropriately high serum erythropoietin produced by renal cysts (Tables 1 and 3).

The third major finding was the occurrence of 2 unusual renal phenotypes in the context of HNF1B nephropathy, such as severe polyuria attributable to NDI and MSK. In the index case of family 5, already reported by our group as a case report,⁸ the course of the renal disease since infancy was characterized by severe polyuria, increase in size and number of renal cysts, and slow progression of chronic kidney disease (Table 1). In the absence of mutations in *AVPR2* and *AQP2* genes, for the first time a diagnosis of partial NDI in the context of HNF1B nephropathy was made.⁸ Polyuria has already been observed in humans with mutations of *HNF1B*^{2,4}; however, it is not a universal finding, particularly in adult patients.^{12–15,24,26} Usually, the causes of polyuria include glycosuria due to early-onset diabetes, and renal sodium and potassium

wasting. Recently, however, a murine model with HNF1B deletion in the renal collecting ducts exhibited persistent polyuria due to partial nephrogenic diabetes insipidus through multiple mechanisms, including impaired intracellular trafficking of AQP2.³⁴ These experimental findings disclose a role of HNF1b in osmoregulation, expanding the spectrum of renal disorders caused by HNF1B mutations.³⁴

Possibly the most intriguing finding of our study was the presence, in the index case of family 6, of a clinical and imaging picture of MSK (Tables 1–3). MSK is a rare developmental disorder, characterized by ectasia and cystic formation in the medullary collecting ducts, which usually manifest with nephro-calcinosis and recurrent kidney stones.³⁵ Both the proband and the daughter of family 6 carrying the recurrent 17q12 deletion showed the MSK phenotype (Tables 1 and 3). This deletion comprises several genes, and none of these are known to be involved in renal development, apart from *HNF1B*.⁶ To our knowledge, MSK has never been reported in association with *HNF1B* variants or deletion; only 2 cases of nephro-calcinosis, apparently in the absence of MSK, have been reported by Faguer *et al.*⁶ The underlying defect of MSK is not fully understood. Interestingly, MSK may occur in association with a number of renal and urinary tract abnormalities and with hemihypertrophy and in Beckwith–Wiedemann syndrome,³⁵ reinforcing the hypothesis that MSK is a developmental abnormality resulting from alterations in critical developmental genes. Familial clustering of MSK, with an apparent autosomal dominant inheritance, further suggests a genetic component in the pathogenesis of the disease.³⁶ In 2010, Torregrossa *et al.*³⁷ demonstrated that variants in glial cell–derived neurotrophic factor (GDNF), a ligand for the tyrosine kinase receptor RET (HGCN-approved name: ret proto-oncogene), the most important inducer of the ureteric bud (UB) emergence and the branching process,³⁸ cosegregated with MSK in a few families.³⁷ However, although suggestive,³⁹ the role of the GDNF variants in MSK remains questionable, as the proportion of cases attributable to GDNF or RET mutations is small.^{40,41} In our family the proband underwent whole-exome sequencing, and no pathogenic variants were identified in the *GDNF* or *RET* gene. Whether HNF1b plays a direct role in the occurrence of MSK is currently unknown. Recent experimental studies revealed that HNF1B deletion can lead to defective expression of key regulators of UB branching, including GDNF–RET pathway components.⁴² Hence we might envisage *HNF1B* as an additional candidate for MSK. Although further studies are required to explore the role of the *HNF1B* gene in MSK, our data further suggest the genetic background of this developmental disorder

expanding the spectrum of renal malformation observed in the context of HNF1B nephropathy.

In summary, our data confirm the heterogenic presentation of the renal phenotype of HNF1B-associated disease. In addition, we describe 2 unusual renal phenotypes, namely, nephrogenic diabetes insipidus and MSK, enlarging the spectrum of HNF1B nephropathy. A considerable diagnostic delay is frequent, as demonstrated by our cases; in this context, extrarenal manifestations may be considered an important tool to make an early diagnosis.

We could also speculate that, in our era, clinical criteria could be surpassed by genetic testing availability. Nowadays, cost-effective NGS-based strategies allow simultaneous and rapid sequencing of a number of genes; thus, we suggest that all patients with familial tubulo-interstitial kidney disease or early-onset disease should be investigated for ADTKD genes including *HNF1B*. Diagnosing the syndrome is important, because this enable us to prevent unnecessary examinations, including renal biopsy and screening for diabetes and other extrarenal manifestations, and to offer genetic counseling to patients and family members.

DISCLOSURE

All authors declared no competing interests.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Materials and Methods.

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