Tubular proteinuria in patients with HNF1α mutations: HNF1α drives endocytosis in the proximal tubule

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Hepatocyte nuclear factor 1α (HNF1α) is a transcription factor expressed in the liver, pancreas, and proximal tubule of the kidney. Mutations of HNF1α cause an autosomal dominant form of diabetes mellitus (MODY-HNF1A) and tubular dysfunction. To gain insights into the role of HNF1α in the proximal tubule, we analyzed *Hnf1a*-deficient mice. Compared with wild-type littermates, *Hnf1a* knockout mice showed low-molecular-weight proteinuria and a 70% decrease in the uptake of β_2-microglobulin, indicating a major endocytic defect due to decreased expression of megalin/cubilin receptors. We identified several binding sites for HNF1α in promoters of *Lrp2* and *Cubn* genes encoding megalin and cubilin, respectively. The functional interaction of HNF1α with these promoters was shown in C33 epithelial cells lacking endogenous HNF1α. Defective receptor-mediated endocytosis was confirmed in proximal tubule cells from these knockout mice and could be rescued by transfection of wild-type but not mutant HNF1α. Transfection of human proximal tubule HK2 cells with HNF1α was able to upregulate megalin and cubilin expression and to increase endocytosis of albumin. Low-molecular-weight proteinuria was consistently detected in individuals with *HNF1A* mutations compared with healthy controls and patients with non–MODY-HNF1A diabetes mellitus. Thus, HNF1α plays a key role in the constitutive expression of megalin and cubilin, hence regulating endocytosis in the proximal tubule of the kidney. These findings provide new insight into the renal phenotype of individuals with mutations of *HNF1A*.

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Hepatocyte nuclear factor 1α (HNF1α) is a homeodomain-containing transcription factor that activates the transcription of a large set of genes in the liver, pancreas, and intestine.[1] HNF1α binds to a consensus DNA sequence of 15 nucleotides as a homodimer or a heterodimer with the closely related HNF1β.[2] In addition to the liver and pancreas, HNF1α and HNF1β are also expressed in epithelial cells lining various segments of the mammalian nephron. At variance with HNF1β, which is expressed in most nephron segments, HNF1α is only found in the cells lining the proximal tubule segments.[3,4]

Heterozygous mutations of the *HNF1A* gene that encodes HNF1α cause maturity- onset diabetes of the young type 3 (MODY-HNF1A; MIM #600496), an autosomal dominant form of noninsulin-dependent diabetes mellitus. MODY-HNF1A belongs to a single-gene disorder family (MODY) responsible for 1% to 2% of type 2 diabetes worldwide, and it is considered the most common form of MODY. The *HNF1A* mutations are strongly but not completely penetrant, and there is large variability in the severity or the age at onset of diabetes in individuals. In fact, diabetes develops in only half of the *HNF1A* mutation carriers before 25 years of age, with a strong insulin secretion defect and preserved sensitivity to sulfonylureas.[5] Diabetic nephropathy frequently develops in patients with MODY-HNF1A, with occasional glomerular proteinuria[6,7] and aminoaciduria.[8] Possible links with urolithiasis[7] and developmental abnormalities[9] have also been suggested.

Mice knockout for HNF1α are diabetic and exhibit hepatic defects (hyperphenylalaninemia and hypercholesterolemia), together with manifestations of proximal tubule dysfunction (polyuria due to glycosuria, generalized aminoaciduria, and phosphaturia) which affect their growth and life span.[3]

HNF1α was shown to regulate the expression of the sodium-glucose transporter SGLT2/SLC5A2[4] and the sodium-phosphate transporters NPT1/SLC17A1 and NPT4/SLC17A3,[10] providing a basis for the glucosuria/polyuria observed in *Hnf1α*$^{-/-}$ mice. HNF1α also regulates the expression of the chloride-proton exchanger ClC-5, which operates in the endosomes of the cells lining the proximal tubule.[11]

In addition to glucose, amino acids, phosphate, and other ions, a significant amount of filtered albumin and low-molecular-weight (LMW) plasma proteins is reabsorbed by the epithelial cells lining the proximal tubule. The uptake of LMW proteins by these cells essentially involves receptor-mediated endocytosis, mediated by the multiligand receptors megalin and cubilin that are expressed at the brush border.[12] Upon ligand binding, both receptors interact and are internalized into coated vesicles to further progress to endosomes and lysosomes for ligand degradative processing and receptor recycling. Defects in receptor-mediated endocytosis play a major role in proximal tubule dysfunction (i.e., renal Fanconi syndrome), as observed in patients and corresponding mouse models with inactivating mutations in the *LRP2* gene coding for megalin (Donnai-Barrow syndrome) or the *CUBN* gene coding for cubilin (Imerslund-Gräsbeck syndrome).[13] The molecular basis of the transcriptional regulation of endocytic receptors involved in proximal tubule cells and the potential link between HNF1α and receptor-mediated endocytosis have not been previously investigated.

In this study, we used well-characterized cellular systems, mouse models, and human samples to investigate the role of HNF1α in the regulation of receptor-mediated endocytosis in the proximal tubule. We show that HNF1α knockout mice and derived proximal tubule cells show a severe defect in endocytosis caused by a reduced expression in megalin/cubilin. We demonstrate several binding sites for HNF1α in *Lrp2* and *Cubn* and show that the overexpression of HNF1α per se is sufficient to rescue (HNF1α knockout) or enhance (HK2 cells) the endocytic activity in proximal tubule cells. MODY-HNF1A patients consistently show LMW proteinuria, confirming the clinical relevance of these findings.

RESULTS

LMW proteinuria and defective endocytosis in *Hnf1α*$^{-/-}$ mice

In order to evaluate the role of HNF1α in PT cells, we first investigated renal and tubular function parameters in the *Hnf1α* mice. Compared with wild-type *Hnf1α*$^{+/+}$ littermates, *Hnf1a1*$^{-/-}$ mice showed a severe growth defect, higher urea and plasma creatinine corrected for body weight, polyuria, glucosuria, and hypercalciuria (Table 1). They also showed LMW proteinuria, as evidenced by the detection of Clara cell protein (CC16), vitamin D binding protein, and transferrin in the urine (Figure 1a). The urinary loss observed in *Hnfa1*$^{-/-}$ versus *Hnf1α*$^{+/+}$ mice concerned both ligands of megalin (β_2-microglobulin: 187 ± 97 vs. 13 ± 4 g/L respectively, n = 5 pairs, $P < 0.01$) and cubilin (CC16: 1769 ± 457 vs. 53 ± 36 g/g creatinine respectively, n = 5 pairs, $P < 0.01$). The defect in receptor-mediated endocytosis was substantiated by the major decrease in the apical uptake of the LMW tracer horseradish peroxidase in proximal tubule cells of *Hnf1a*$^{-/-}$ mice in absence of gross structural defects (Figure 1b). *In vivo* capture studies confirmed an ~80% reduction in the endocytic uptake of ^{125}I-labeled β_2-microglobulin by HNF1α-null kidneys compared with wild-type controls at 7 minutes after perfusion (Figure 1c). These data confirm that the deletion of HNF1α is associated with a major defect in receptor-mediated endocytosis in proximal tubule cells, with ensuing loss of LMW ligands of megalin and cubilin.

Table 1 | Clinical and biochemical parameters in *Hnf1a* mice

	Hnf1a$^{+/+}$	*Hnf1a*$^{-/-}$
Body weight (g)	24.3 ± 2 (12)	10.5 ± 1 (10)[a]
Plasma		
Creatinine (mg/dl)	0.09 ± 0.004 (6)	0.09 ± 0.008 (6)
Urea (mg/dl)	49.5 ± 3.3 (6)	59.7 ± 1.9 (6)[a]
Na^+ (mmol)	147 ± 2.4 (6)	150 ± 1.7 (6)
K^+ (mmol)	5.7 ± 0.7 (6)	6.1 ± 0.5 (6)
Cl^- (mmol)	109 ± 2.9 (6)	106 ± 1.6 (6)
Urine		
Urinary flow rate (L/min/BW)	0.06 ± 0.006 (12)	0.11 ± 0.023 (12)[a]
Creatinine (mg/dl)	10.7 ± 1.1 (12)	8.2 ± 2.7 (12)
Na^+ (mmol)	190 ± 21 (12)	167 ± 12 (12)
K^+ (mmol)	56 ± 11 (12)	47 ± 6 (12)
Cl^- (mmol)	176 ± 24 (12)	156 ± 14 (12)
Phosphate (mg/dl)	51 ± 9.8 (12)	59 ± 8.2 (12)
Calcium (mg/dl)	6.59 ± 0.6 (12)	50.5 ± 10 (12)[a]
Glucose (mg/dl)	16 ± 2 (12)	4019 ± 1010 (9)[a]

Values are mean ±SEM; (N), number of mice in each group shown in parentheses.
BW, body weight.
[a]$P < 0.05$ versus wild type.

Defective expression of endocytic receptors in *Hnf1α*$^{-/-}$ kidneys

We next investigated whether the endocytic defect in the *Hnf1α*$^{-/-}$ mice is due to altered expression of essential components of the receptor-mediated endocytic pathway in the proximal tubule (Figure 2). Immunostaining of *Hnfa1*$^{-/-}$ kidneys revealed a significant decrease in the expression of megalin and cubilin at the apical pole of proximal tubule cells. These modifications were not associated with detectable morphologic changes at the optical and ultrastructural level, including the brush border and the apical area of the cells (Figure 2a). Immunoblotting analyses confirmed a major reduction in the expression of both megalin and cubilin in the HNF1α-null kidneys (residual level ~52% and 30% of wild type, respectively), whereas constituents of the brush border (aminopeptidase N) and endosomes catalysts (Rab5a) were unchanged (Figure 2b). Real-time quantitative reverse transcriptase polymerase chain reaction (PCR) analyses showed a significant decrease in the mRNA expression of megalin and cubilin in *Hnf1α*$^{-/-}$ kidneys, along with other known targets of HNF1α (SGLT2/SLC5A2), whereas membrane transporters without HNF1α binding sites were unchanged (Na^+-K^+-ATPase) or even increased (aquaporin-1 [AQP1]). Of note, the *Hnf1α*$^{-/-}$ kidneys showed a significant increase in the expression of HNF1β (Figure 2c).