Targeted deletion of kidney glucose-6 phosphatase leads to nephropathy

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Renal failure is a major complication that arises with aging in glycogen storage disease type 1a and type 1b patients. In the kidneys, glucose-6 phosphatase catalytic subunit (encoded by *G6pc*) deficiency leads to the accumulation of glycogen, an effect resulting in marked nephromegaly and progressive glomerular hyperperfusion and hyperfiltration preceding the development of microalbuminuria and proteinuria. To better understand the end-stage nephropathy in glycogen storage disease type 1a, we generated a novel kidney-specific *G6pc* knockout (K-G6pc$^{-/-}$) mouse, which exhibited normal life expectancy. After 6 months, K-G6pc$^{-/-}$ mice showed glycogen overload leading to nephromegaly and tubular dilation. Moreover, renal accumulation of lipids due to activation of *de novo* lipogenesis was observed. This led to the activation of the renin–angiotensin system and the development of epithelial–mesenchymal transition process and podocyte injury by transforming growth factor β1 signaling. The K-G6pc$^{-/-}$ mice developed microalbuminuria caused by the impairment of the glomerular filtration barrier. Thus, renal *G6pc* deficiency alone is sufficient to induce the development of the early-onset nephropathy observed in glycogen storage disease type 1a, independent of the liver disease. The K-G6pc$^{-/-}$ mouse model is a unique tool to decipher the molecular mechanisms underlying renal failure and to evaluate potential therapeutic strategies.

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Glycogen storage disease (GSD) type 1 is an inherited disease characterized by severe hypoglycemia associated with hepatic, renal, and intestinal disorders. GSD type 1 is caused by a deficiency in glucose-6 phosphatase (G6Pase) activity, a key enzyme in endogenous glucose production.[1,2] G6Pase catalyzes the hydrolysis of glucose-6-phosphate (G6P), the terminal step of glucose production common to glycogenolysis and gluconeogenesis. GSD type 1a (GSD1a, OMIM #232200) is caused by mutations affecting the catalytic subunit (G6PC) of the G6Pase complex.[3,4] G6PC is specifically expressed in the liver, kidneys, and intestines, which are the only organs capable of producing glucose in the blood.[5–7] In the kidney, the gluconeogenic function is restricted to the proximal convoluted tubules of the cortex.[8] The clinical manifestations in GSD1 patients are fasting hypoglycemia, growth retardation, hypertriglyceridemia, hypercholesterolemia, hyperuricemia, hypoketonemia, and lactic acidemia.[9] Moreover, G6Pase deficiency leads to the accumulation of G6P and glycogen in hepatocytes and proximal renal tubules that results in hepatomegaly and nephromegaly, respectively. Since the 1980s, the life expectancy of GSD1 patients has been considerably improved by dietary management to achieve suitable metabolic control.[10,11] However, various complications occur with aging, such as gouty arthritis, osteoporosis, pulmonary hypertension, hepatic tumors, and progressive chronic renal disease.[12–14] Renal failure is one of the main causes of morbidity in GSD1 patients with aging.

Although renal disease was mentioned in von Gierke's original pathological description, the chronic renal disease of GSD1a was later recognized as a major complication.[15,16] Nearly all GSD1 patients above 25 years of age exhibit renal disease with microalbuminuria, and more than 50% exhibit proteinuria.[12] The first manifestation is glomerular hyperperfusion and hyperfiltration before the development of microalbuminuria and then proteinuria.[17] In addition, some patients also develop hypertension and nephrocalcinosis due to hyperuricemia, hypercalciuria, and hypocitraturia. At a later stage, kidney failure occurs, which can require dialysis or even renal transplantation.[12,13] In addition, renal biopsies reveal tubular atrophy, glomerulosclerosis, and

interstitial fibrosis.[17–20] Recent molecular studies suggest the involvement of the renin–angiotensin system in the development of renal fibrosis and renal oxidative stress in total *G6pc* knockout mice.[21,22] Accordingly, treatment with inhibitors of angiotensin converting enzyme and/or angiotensin II receptor antagonists partially corrects glomerular filtration in GSD1 patients, but no significant effect is observed on microalbuminuria and proteinuria.[23,24] Therefore, further investigations are needed to better understand the long-term mechanisms of the renal disease and to evaluate potential therapeutic strategies. Unfortunately, the long-term renal complications cannot be evaluated using total *G6pc* knockout mice because they rarely survive beyond weaning, which is likely due to the concomitant liver disease.[25]

To investigate the onset of GSD1 renal pathology independently of liver disease, we generated a novel GSD1a mouse model that exclusively targets G6PC deletion in the kidneys. We used a CRE-lox approach similar to that used to generate the liver-deficient model.[26] The kidney-specific *G6pc* knockout (K-G6pc$^{-/-}$) mice are viable and develop renal symptoms similar to the human pathology of the disease. This novel mouse model allows us to elucidate new molecular pathways involved in the impairment of the glomerular filtration barrier, such as podocyte alterations and ChREBP activation, which leads to lipid deposits.

RESULTS

Generation of mice with kidney-specific G6Pase deficiency

We generated mice lacking G6PC specifically in the kidneys by an inducible CRE-lox strategy. As the renal *G6pc* expression is restricted to the proximal tubules of the cortex region,[8] we used mice expressing the inducible CRE^{ERT2} recombinase under the control of the kidney androgen–regulated protein (Kap) promoter. The Kap promoter is known to be specifically active in the proximal tubules of the kidney where it is inducible by androgen.[27] Male B6.G6pc$^{lox/lox}$.Kap$^{creERT2/w}$ mice were treated with tamoxifen at 6–8 weeks of age to induce the excision of *G6pc* exon 3 in the kidneys.

PCR analysis of purified DNA showed a tissue-specific deletion of *G6pc* exon 3 in the kidneys of K-G6pc$^{-/-}$ mice (Figure 1a). The kidney-specific expression of CRE^{ERT2} recombinase under the control of the Kap promoter was confirmed by the presence of full-length *G6pc* in the liver, intestine, testis, and epididymis (Figure 1a). However, *G6pc* exon 3 deletion was only partial, as a floxed fragment of 1189 bp was also amplified in the kidneys of K-G6pc$^{-/-}$ mice (Figure 1a). This deletion was sufficient to inhibit G6Pase activity by about 50% in the kidneys of K-G6pc$^{-/-}$ mice compared with wild-type (WT) mice (Figure 1b). Western blot analyses confirmed the marked decrease in G6PC protein levels in K-G6pc$^{-/-}$ kidneys (Figure 1b). As expected, G6Pase expression is restricted to the proximal convoluted tubules of the cortex in WT mice (Figure 1c and e). In contrast, K-G6pc$^{-/-}$ kidneys showed a lower and heterogeneous immunostaining for G6PC in the cortex (Figure 1d and f), with the presence of only a few G6PC-positive cells identified in the proximal convoluted tubules (Supplementary Figure S1 online). G6PC was not detectable in the medulla, distal convoluted tubules, or glomeruli in both WT and K-G6pc$^{-/-}$ mice (Figure 1c and d).

Metabolic, morphological, and molecular characterization of the kidney disease in K-G6pc$^{-/-}$ mice

In contrast to the growth retardation reported in total *G6pc* knockout mice,[25] the 6-month renal *G6pc* deficiency that was induced at adulthood did not affect body weight (Table 1). K-G6pc$^{-/-}$ mice were viable without treatment and were normoglycemic after 6 h of fasting (Table 1). The liver weight was similar in K-G6pc$^{-/-}$ and WT mice, but hepatic G6Pase activity was increased by 40% in K-G6pc$^{-/-}$ mice compared with that in WT mice (Table 1). Biochemical analyses revealed normal plasma cholesterol, triglycerides, lactate, and blood urea nitrogen levels in K-G6pc$^{-/-}$ mice, indicating normal liver metabolism. Interestingly, plasma uric acid concentrations were slightly higher in K-G6pc$^{-/-}$ mice compared with WT mice (Table 1).

As expected, K-G6pc$^{-/-}$ mice showed enlarged kidneys (Figure 2a–c). After 6 months, K-G6pc$^{-/-}$ kidneys accounted for about $1.72 \pm 0.06\%$ of total body mass versus only $1.01 \pm 0.01\%$ in WT mice (Figure 2c). This was associated with a marked glycogen accumulation in the tubules in the external part of the kidney cortex, as revealed by a periodic acid–Schiff stain (Figure 2e). Indeed, the deposition of glycogen was nearly absent in the kidneys of WT mice (Figure 2d–f) compared with 22.7 ± 2.3 mg of glycogen per gram of tissue in the K-G6pc$^{-/-}$ kidneys (Figure 2f).

Histological observations of WT kidneys by hematoxylin and eosin staining revealed normal morphology with recognizable glomeruli (G), proximal convoluted tubules (PC), and distal convoluted tubules (DC) in the cortex (Figure 3a–c). In contrast, K-G6pc$^{-/-}$ mice showed morphological alterations in the kidney cortex with a clarification and enlargement of tubular epithelial cells (Figure 3b–d). Moreover, the abundance of renal mRNA for podocyte marker proteins such as podocin (*Nphs2*), synaptopodin (*Synpo*), and podocalyxin (*Podxl*) was decreased by 50–75% in K-G6pc$^{-/-}$ mice compared with WT mice (Figure 3e). Western blotting revealed a decrease in podocin (NPHS2) and nephrin protein levels in K-G6pc$^{-/-}$ kidneys. These alterations of renal morphology and podocyte damage in K-G6pc$^{-/-}$ mice were associated with disturbed urine parameters. The urinary pH of K-G6pc$^{-/-}$ mice was more acidic than that of WT mice. Uric acid levels were also increased by threefold in the urine of K-G6pc$^{-/-}$ mice compared with WT mice (Table 2). Interestingly, 6 months of renal *G6pc* knockout led to increased albuminuria but did not affect proteinuria. In addition, there was an imbalance in urinary excretion of electrolytes (Table 2). The urine magnesium and phosphate concentrations were increased in the urine of K-G6pc$^{-/-}$ mice compared with WT mice