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Original Article

ORIGINAL ARTICLE

Cellular and metabolic effects of renin-angiotensin system blockade on glycogen storage disease type I nephropathy

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Abstract

Glycogen Storage Disease Type I (GSDI) is an inherited disease caused by glucose-6 phosphatase (G6Pase) deficiency, leading to a loss of endogenous glucose production and severe hypoglycemia. Moreover, most GSDI patients develop a chronic kidney disease (CKD) due to lipid accumulation in the kidney. Similar to diabetic CKD, activation of renin-angiotensin system (RAS) promotes renal fibrosis in GSDI. Here, we investigated the physiological and molecular effects of RAS blockers in GSDI patients and mice. A retrospective analysis of renal function was performed in 21 GSDI patients treated with RAS blockers. Cellular and metabolic impacts of RAS blockade were analyzed in K.G6pc$^{-/-}$ mice characterized by *G6pc1* deletion in kidneys. GSDI patients started RAS blocker treatment at a median age of 21 years and long-term treatment reduced the progression of CKD in about 50% of patients. However, CKD progressed to kidney failure in 20% of treated patients, requiring renal transplantation. In K.G6pc$^{-/-}$ mice, CKD was associated with an impairment of autophagy and ER stress. RAS blockade resulted in a rescue of autophagy and decreased ER stress, concomitantly with decreased fibrosis and improved renal function, but without impact on glycogen and lipid contents. In conclusion, these data confirm the partial beneficial effect of RAS blockers in the prevention of CKD in GSDI. Mechanistically, we show that these effects are linked to a reduction of cell stress, without affecting metabolism.

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Introduction

Initially used as a class of drugs for treating arterial hypertension, renin-angiotensin system (RAS) blockers are the current gold-standard drug therapies to improve renal outcomes, especially for diabetic nephropathy, which is the most common chronic kidney disease (CKD) (1,2). In diabetes, treatment with RAS inhibitors, namely angiotensin-converting inhibitors (ACEi) or angiotensin receptor blockers (ARBs), is often initiated at an early stage of CKD, associated or not with hypertension (1,3,4). Blockade of RAS delays the progression of renal injuries, by reducing renal fibrosis and proteinuria and controlling blood pressure. However, their efficacy is often limited, possibly due to the compensatory increase of intrarenal renin after ACEi and ARB treatments (5).

Similarly, in patients with Glycogen Storage Disease Type I (GSDI), treatment with ACEi is effective in decreasing glomerular hyperfiltration and delaying microalbuminuria, but not in improving microalbuminuria and proteinuria (6,7). GSDI is a rare metabolic disease caused by glucose-6-phosphatase (G6Pase) deficiency, a key enzyme of endogenous glucose production. It consists of two subtypes, GSDIa and GSDIb, due to mutations in *G6PC1*, encoding the catalytic subunit, or *SLC37A*, encoding glucose-6-phosphate (G6P) translocase of G6Pase, respectively (8,9). Due to the lack of G6Pase activity, patients with GSDI are prone to severe hypoglycemia and G6P accumulates in kidneys. This leads to the activation of glycogenesis and *de novo* lipogenesis that is responsible for renal glycogen and lipid accumulation (10,11). Interestingly, we recently evidenced that renal lipids activate RAS, resulting in the development of interstitial fibrosis and glomerulosclerosis (12). With time, most adult GSDI patients over the age of 20–25 years develop CKD, which currently represents one of the main causes of morbidity (8). In addition to a strict diet to prevent hypoglycemia and to control secondary metabolic abnormalities, GSDI patients are generally treated with RAS blockers when the first signs of nephropathy, i.e. microalbuminuria, are detected (8).

To better determine the specific impact of RAS inhibitors on GSDI nephropathy, we report the effect of RAS blockade in French and Swiss cohorts of GSDI patients on CKD progression and blood pressure control. In addition, we investigated the effect of RAS blockade on renal function, metabolism and cellular damages in GSDIa mice treated by ACEi or ARB. We specifically analyzed fibrosis development and cellular defenses, such as endoplasmic reticulum stress (ER stress) and autophagy.

Results

Renal function in GSDI patients treated with RAS blockers

Renal function was retrospectively analyzed in a cohort of 73 GSDI patients. Before the introduction of renoprotective treatment, 27 patients (i.e. 37%) exhibited signs of renal disease, i.e. persistent microalbuminuria or proteinuria associated or not with glomerular hyperfiltration. Among them, 23 patients (19 GSDIa and 4 GSDIb) were treated with ACEi (15/23 i.e. 65%), ARB (7/23 i.e. 30%) or both (1/23 i.e. 4%). The duration of the treatment was at least 3 years (average duration ≈10 years), except for two patients treated for less than 1 year for whom RAS blocker effects were not analyzed (patients 22 and 23, Table 1). Two additional patients have recently started treatment in the last months of the study and two others have not accepted RAS blocker medication yet (not included). The median age of ACEi/ARB treatment initiation was 21 years, with a range from 11 to 44 years (Table 1). In this study, two patients with GSDIa (Patients 24 and 25) were also treated with RAS blockers for hypertension, without albuminuria (Supplementary Material, Table S1). Conversely to what is generally observed in patients with type 2 diabetes, the majority of GSDI patients were not hypertensive; only four out of 25 treated patients, i.e. less than 20%, had mild or moderate hypertension (Fig. 1a-b, Supplementary Material, Table S1). For these, the RAS blockade was effective to decrease SBP and DBP, except for one patient who developed hypertension under ARB treatment (patient 20) (Fig. 1A-B; Supplementary Material, Table S1). Among the 21 GSDI patients with nephropathy and treated with RAS blockers, albuminuria/proteinuria was decreased in 52% (11 out of 21) of patients (72% of patients treated with ACEi and 17% of patients treated with ARB) (Fig. 1c; Table 1). More precisely, it was normalized in six out of 14 patients treated with ACEi (42%), while it was only reduced and normalized in one out of six patients treated by ARB (17%). Yet, the degree of proteinuria or microalbuminuria progressed in four of the 14 patients treated with ACEi (28%), and in six of the seven patients treated with ARB (85%) (Table 1), suggesting a trend for better renoprotective effect of ACEi treatment in comparison to ARB treatment in GSDI patients. In addition, before the start of the therapy, 10 patients (out of the 21) exhibited a glomerular hyperfiltration (eGFR$\geq$135 ml/min/1,73m^2), which was slightly decreased or normalized in nine patients of which only one was treated with ARB (Table 1). Nevertheless, Patients 2 and 15 already received a kidney transplant at the age of 36 and 28 years, respectively, i.e. about 10 years after the start of treatment with RAS blockers. Moreover, one patient treated with both ARB and ACEi for 14 years developed a renal failure, also requiring kidney transplantation, despite the treatment (Table 1). Renal function also highly decreased in Patient 17 after more than 10 years of ARB treatment, but his clearance remained stable during the last years (Table 1). In these last four cases, high levels of serum creatinine (over 150 µmol/l) were associated with end-stage CKD (Fig. 1d-e, Supplementary Material, Table S1). It is noteworthy that two patients also developed lithiasis/nephrocalcinosis, another typical renal complication of GSDI (Table 1), despite ACEi treatment. Despite dietary treatment indicated in Table 1, all GSDI patients of this cohort showed hypertriglyceridemia and hypercholesterolemia before and after RAS blockade, as a typical secondary metabolic abnormality in GSDI (Supplementary Material, Table S1). Thus, many of GSDI patients were also treated with lipid-lowering drugs such as statins and/or fibrates, but not systemically (see Supplementary Material, Table S1). Moreover, most of them also showed hyperuricemia (data not shown), requiring treatment with allopurinol or febuxostat (Supplementary Material, Table S1). Furthermore, five patients had undergone liver transplantation because of the presence of hepatic tumors. This permitted to normalize plasma TG and cholesterol levels, despite the stop of specific diet, but liver transplantation did not improve kidney function (Supplementary Material, Table S1).

These results confirm that glomerular hyperfiltration and persistent microalbuminuria/proteinuria develop at early age in patients with GSDI, since half of the patients had to be treated with ACEi/ARB at an age under 21. ACEi/ARB treatment reduced progression of CKD in 57% of patients of this cohort. Interestingly, our data suggest a trend for better renoprotective effect of ACEi treatment in comparison to ARB treatment in GSDI patients, even though many other factors may influence the course of nephropathy. However, CKD progressed to kidney failure in 20% of patients of the cohort, requiring kidney transplantation.

Table 1. Data of patients with GSDI treated with RAS blockers

Patient number	GSDI type	Gender	Genotype	Age at the beginning of treatment (years)	Duration of treatment (years)	Treatment	% change in albuminuria or proteinuria	eGFR (ml/min/1.73m^2) before	after	Observations	Dietary treatment
1	Ia	M	c.79delC c.1039C > T	43	15	ACEi	−56%	113	135		Frequent meals Galactose and fructose restriction Cornstarch
2	Ia	F	c.328G > A c.1039C > T	27	9	ACEi	338%	78	24	Kidney transplantation at 36 years old	Frequent meals Galactose and fructose restriction Cornstarch
3	Ia	M	c.208 T > C c.1072 T > C	29	15	ACEi	−100%	110	95		Frequent meals Galactose and fructose restriction Glycosade®
4	Ia	F	No mutation in coding sequence	20	8	ACEi	−10%	164	139	OLT at 28 years old No sign of CKD 6 years after OLT	No more specific diet since liver transplantation
5	Ia	F	c.247C > T c.1039C > T	21	9	ACEi	−100%	158	125		Frequent meals Galactose and fructose restriction Glycosade®
6	Ia	M	c.809G > T c.809G > T	19	13	ACEi	−100%	166	120	Lithiasis Nephrocalcinosis	Glycosade® No specific diet
7	Ia	M	c.209G > A c.323C > T	16	14	ACEi	−100%	231	122		Frequent meals Galactose and fructose restriction Glycosade®
8	Ia	F	c.247C > T c.734_735insG	14	14	ACEi	63%	202	102	Recurring lithiasis	Frequent meals Galactose and fructose restriction Glycosade®
9	Ia	M	c.328G > A c.563G > C	28	9	ACEi	−90%	>90	>90		Galactose and fructose restriction Cornstarch
10	Ia	M	N/A	44	8	ACEi	−56%	>90	87		Galactose and fructose restriction Cornstarch
11	Ib	M	c.81 T > A c.81 T > A	11	16	ACEi	Persistent albuminuria	165	121		Galactose and fructose restriction Glycosade® and Cornstarch

Continued

Table 1. Continued

Patient number	GSDI type	Gender	Genotype	Age at the beginning of treatment (years)	Duration of treatment (years)	Treatment	% change in albuminuria or proteinuria	eGFR (ml/min/1.73m^2) before	after	Observations	Dietary treatment
12	Ib	F	c.82C > T c.1015G > T	34	14	ACEi +ARB	50%	71	30	Waiting for kidney transplantation-Hemodialysis	Frequent meals Galactose and fructose restriction Glycosade®
13	Ib	M	c.59G > A c.59G > A	20	15	ACEi	−100%	233	187		Frequent meals Glycosade®
14	Ib	M	c.148G > C c.1015G > T	23	9	ACEi	−100%	170	159		Frequent meals Galactose and fructose restriction Glycosade®
15	Ia	M	c.1039C > T c.1039C > T	16	11	ARB	1515%	169	25	OLT + Kidney transplantation at 28 years old	Frequent meals Galactose and fructose restriction Cornstarch No more specific diet since transplantations
16	Ia	M	c.79delC c.840C > G	26	8	ARB	−100%	194	134	OLT at 34 years old	Frequent meals Galactose and fructose restriction Glycosade®
17	Ia	F	N/A	N/A	>10	ARB	332%	83	35	OLT at 36 years old ARB treatment not stopped after OLT	Galactose and fructose restriction Cornstarch
18	Ia	M	N/A	N/A	>7	ARB	Persistent albuminuria	N/A	66	Patient already under treatment when first seen	Galactose and fructose restriction Cornstarch
19	Ia	M	N/A	14	3	ARB	181%	>90	>90	OLT at 17 years old Persistent albuminuria after OLT	Continuous gastric tube feeding (Glucose polymer and maltodextrin)
20	Ia	F	N/A	12	17	ARB	Persistent albuminuria	N/A	>90		Galactose and fructose restriction Glycosade®

Continued

Table 1. Continued

Patient number	GSDI type	Gender	Genotype	Age at the beginning of treatment (years)	Duration of treatment (years)	Treatment	% change in albuminuria or proteinuria	eGFR (ml/min/1.73m^2) before	after	Observations	Dietary treatment
21	Ia	F	N/A	15	3	ARB	69%	>90	>90	Treatment was stopped in 2013 Stable biological parameters since 2013	Galactose and fructose restriction Glycosade®
22	Ia	F	c.247C > T (2nd mutation undeter-mined)	20	<1	ACEi	N/A	>90	N/A		Galactose and fructose restriction Cornstarch
23	Ia	F	c.79delC c.247C > T	22	<1	ACEi	N/A	>90	N/A		Galactose and fructose restriction Cornstarch
24	Ia	F	c.1039C > T c.1039C > T	36	6	ARB	No sign of CKD	167	177	Treated for hypertension	Frequent meals Galactose and fructose restriction Glycosade®
25	Ia	M	c.527A > G c.1039C > T	26	3	ACEi	No sign of CKD	130	186	Treated for hypertension Kidney carcinoma at 26 years old	Frequent meals Galactose and fructose restriction Glycosade®

Biological data of GSDI patients with nephropathy and/or hypertension were analyzed before starting and after ACEi/ARB treatment. Details of treatments (doses, medications) are available in Supplementary Material, Table S1. The data after treatment were obtained in 2019–2021, including data of Patients 17 and 19 who have continued ARB treatment after orthotopic liver transplantation (OLT). For other patients who received liver and/or kidney transplant, the data after treatment were obtained just before the transplantation. Patients 24 and 25 were treated for hypertension, without signs of CKD. All biological data (albuminuria, proteinuria, serum creatinine, eGFR, SBP/DBP, plasma triglyceride and cholesterol levels) are available in Supplementary Material, Table S1. The percentage in albuminuria was calculated as: (value of albuminuria after the treatment—value of albuminuria before the treatment)/value of albuminuria before the treatment x100. When no albuminuria/proteinuria was detected after treatment, the % change in albuminuria or proteinuria is indicated as −100%. Diagnosis was based on enzymatic assay when the genotype is not available (N/A). F: female; M: male. Reference sequences are NM_000151.4 and NM_001467.6 for *G6PC1* and *SLC37A4* genes, respectively.

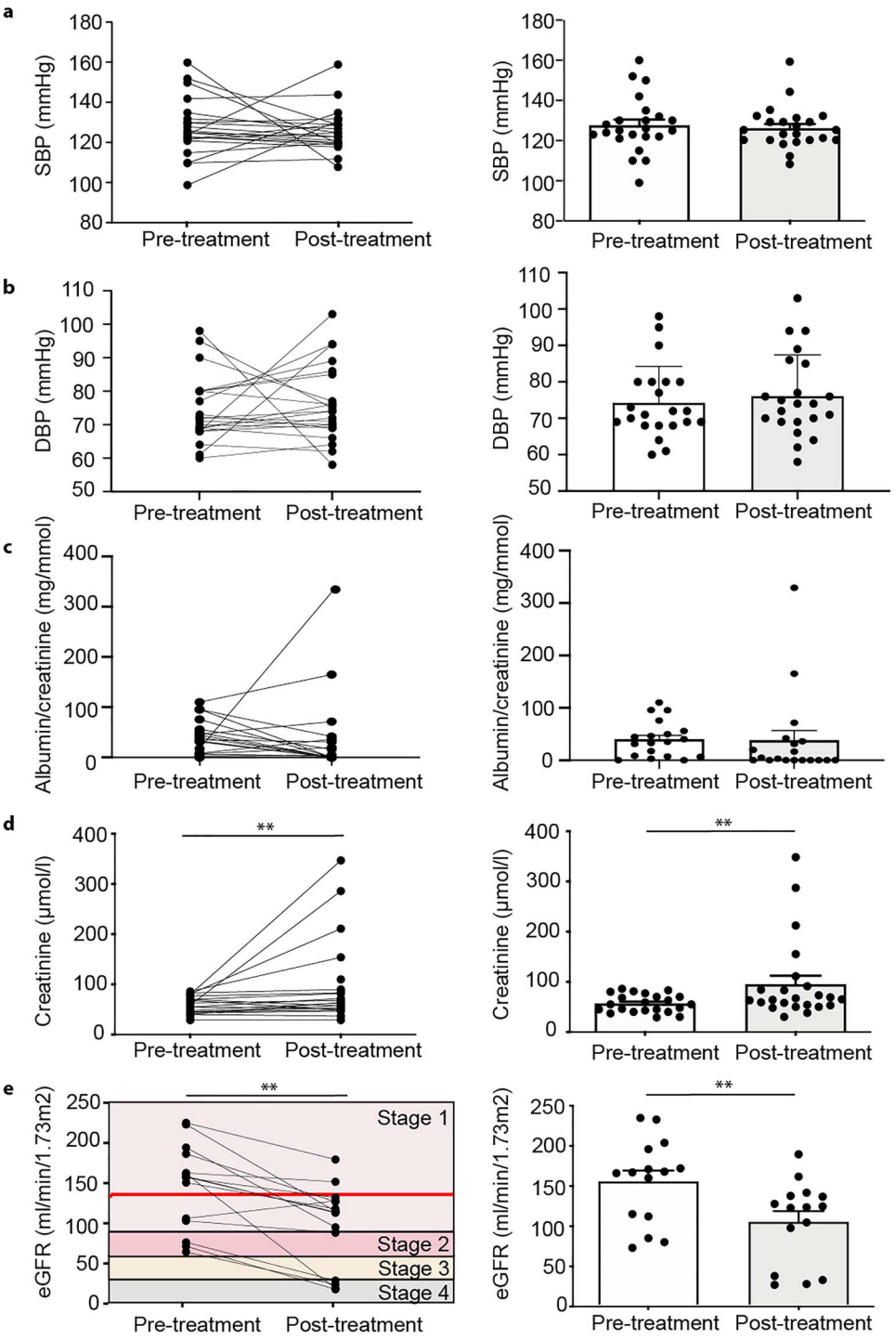

Figure 1. Clinical and biochemical parameters of GSDI patients who developed a chronic kidney disease before (pre-treatment) and after treatment (post-treatment) with RAS blockers. (a) Systolic blood pressure (SBP) and (b) Diastolic blood pressure (DBP). (c) Albumin/creatine ratio measured in urine. (d) Creatinine measured in the plasma. (e) Estimated Glomerular Filtration Rate (eGFR) calculated using CKI-EPI formula. Value above the red line shows glomerular hyperfiltration. Stages of CKD are defined by values of eGFR. All the individual data are indicated in Supplementary Material, Table S1. Significant differences are indicated as $^{*}P < 0.05$; $^{**}P < 0.01$. Patient data were compared using a Wilcoxon matched pairs test.

RAS blockade slows down the CKD progression in GSDIa mice

We previously developed a mouse model with a renal *G6pc1* deletion (K.G6pc$^{-/-}$ mice) that recapitulates the macroscopic and molecular aspects of the GSDI nephropathy observed in patients (11,13). To study the efficiency of RAS blockade on GSDI nephropathy, K.G6pc$^{-/-}$ mice were treated with Irbesartan (ARB) or Imidapril (ACEi) for 6 months. The treatment started 6 months after *G6pc1* deletion, the time of onset of CKD in K.G6pc$^{-/-}$ mice (14). ARB and ACEi treatments had no effect on body

weight and food intake, but allowed the normalization of water intake, which was increased in K.G6pc$^{-/-}$ mice (Supplementary Material, Fig. S1).

As previously observed (11), K.G6pc$^{-/-}$ mice exhibited a strong renal RAS activation reflected by the increase in the expression of the intra-renal angiotensinogen gene (*Agt*), compared to WT mice (Supplementary Material, Fig. S2). This was not associated with a modification of SBP or DBP (Fig. 2a). ARB/ACEi treatments efficiently blocked RAS in the kidneys of K.G6pc$^{-/-}$ mice, as illustrated by the compensatory increase in renal renin (*Ren*) mRNA expression (Supplementary Material, Fig. S2). ACEi treatment had no effect on SBP and DBP in K.G6pc$^{-/-}$ mice, while ARB slightly decreased SBP compared to WT mice (Fig. 2a).

As expected, K.G6pc$^{-/-}$ mice exhibited signs of CKD, illustrated by a tendency to increase albuminuria and a significant increase in urine excretion and renal expression of LCN2, a specific biomarkers of CKD (14), compared to WT mice (Fig. 2b-c). This was associated with an increase in urinary uric acid and urea excretion and in BUN level (Fig. 2d-e). Interestingly, renal function was improved after ARB and ACEi treatments in K.G6pc$^{-/-}$ mice, with a concomitant decrease of LCN2 urinary excretion and renal mRNA expression, as well as uric acid and urea urinary excretion (Fig. 2c-d). Albuminuria and BUN levels were also significantly decreased in ACEi-treated K.G6pc$^{-/-}$ mice but not in ARB-treated K.G6pc$^{-/-}$ mice (Fig. 2b & d), suggesting a better renoprotection with ACEi treatment. Nevertheless, at the histological levels, proximal convoluted tubules were still dilated in both RAS-treated and untreated mice compared to control mice (Fig. 2f).

Focal segmental glomerulosclerosis and interstitial fibrosis, frequently reported in GSDI patients with nephropathy, were highly advanced in untreated K.G6pc$^{-/-}$ mice, and were also observed in RAS-treated mice (Fig. 3a), despite a reduction of pro-fibrotic gene expression, such as *Tgfb1* (coding for the profibrotic Transforming Growth Factor β1, TGFβ1) and *Col1a1* (Collagen Type 1 α 1 chain) in kidneys of ARB- and ACEi- treated K.G6pc$^{-/-}$ mice. *Pai* (Plasminogen activator inhibitor-1) and *Vim* (Vimentin) expression was also decreased by ACEi treatment but not by ARB treatment, in accordance with a better efficiency of ACEi compared to ARB treatment (Fig. 3b). Interestingly, the increased phosphorylation of Glycogen Synthase Kinase 3β (GSK3β) at Ser9 observed in untreated K.G6pc$^{-/-}$ mice, which could contribute to the development of fibrosis, was decreased by both RAS blockers (Fig. 3c). Moreover, RAS blockade were associated with lower residual inflammation, as suggested by the decrease in the renal *Mcp1* and *Il6* mRNA expression in ARB- and ACEi-treated K.G6pc$^{-/-}$ mice. The expression of the renal *Tnfa* mRNA was also normalized in ACEi- treated K.G6pc$^{-/-}$ mice (Fig. 3d).

Therefore, altogether these results show an improvement of renal function in ARB- and ACEi-treated K.G6pc$^{-/-}$ mice, which seems independent of the potential hemodynamic effects of the drugs. RAS blockade would slow down the progression of GSDI nephropathy by reducing the renal inflammation, the TGFβ1 pathway activation and consequently the development of fibrosis, with a better efficacy of ACEi treatment compared to ARB treatment.

RAS blockade does not prevent glycogen and lipid accumulation in the kidneys of GSDIa mice

GSDI is characterized by the accumulation of glycogen in the kidney that leads to the development of nephromegaly, as observed in K.G6pc$^{-/-}$ mice, while glycogen was not detected in the kidneys of WT mice (Fig. 4a-b). ARB/ACEi-treated K.G6pc$^{-/-}$ mice exhibited similar renal glycogen levels as those measured in untreated K.G6pc$^{-/-}$ mice (Fig. 4a-b). Consequently, nephromegaly was not reduced by RAS blockade. In concordance, renal glucose concentration, which was reduced in K.G6pc$^{-/-}$ mice compared to WT mice, was not normalized by either treatment (Fig. 4a). Thus, these results suggest that the improvement of renal function by ARB- and ACEi-treatments was not due to modifications of the renal glycogen content.

In addition, the lack of G6Pase in kidneys leads to a discrete accumulation of lipids (14). As also highlighted in this study, this is due to the activation of *de novo* lipogenesis and the decrease of lipid oxidation in K.G6pc$^{-/-}$ kidneys (Fig. 4c–e). However, ARB- or ACEi- treatments did not modify the expression of the key genes involved in lipid synthesis or oxidation in K.G6pc$^{-/-}$ kidneys, with the exception of *Cpt1* expression (Fig. 4c–e). Thus, these results indicate that RAS blockade did not modify lipid and glycogen metabolism in K.G6pc$^{-/-}$ mice, suggesting that the renoprotective effects of these treatments are likely secondary to the anti-inflammatory and antifibrotic effects in GSDIa kidneys.

RAS blockade improves cellular defenses in the kidneys of K.G6pc$^{-/-}$ mice

Besides metabolic perturbations, it has been reported that autophagy downregulation could participate in the pathogenesis of GSDI nephropathy (15). As previously observed, the intracellular levels of p62, an autophagy substrate that is generally used as a reporter of autophagy activity, was highly increased in K.G6pc$^{-/-}$ mice compared to WT mice, confirming the autophagy downregulation in GSDI nephropathy (Fig. 5Aa). Interestingly, p62 protein quantity decreased in both ARB- and ACEi-treated K.G6pc$^{-/-}$ mice in comparison to untreated K.G6pc$^{-/-}$ mice, but remained higher than in WT mice, suggesting a partial rescue of autophagy by RAS blockade (Fig. 5a). To go further, we assessed the phosphorylation of the mammalian target of rapamycin (mTOR) at Ser^{2448} residue, which is known to activate mTOR, a central negative regulator of autophagy. In line with autophagy downregulation in K.G6pc$^{-/-}$ mice, mTOR phosphorylation at Ser^{2448} residue was increased in K.G6pc$^{-/-}$ kidneys compared to WT kidneys (Fig. 5b). Interestingly, ACEi-treatment, but not ARB-treatment, reduced phosphorylation of mTOR on Ser^{2448} in K.G6pc$^{-/-}$ mice (Fig. 5b). These results suggest that the inhibition of renal autophagy in K.G6pc$^{-/-}$ mice is dependent on the activation of mTOR pathway, and that ACEi, but not ARB, could lead to an activation of autophagy by mTOR inhibition.

In addition to the loss of autophagy, ER stress activation could promote CKD in GSDI by increasing fibrosis, as it was previously shown in various renal diseases (16,17). First, we analyzed ER stress by assessment of the three unfolded protein response (UPR) branches, IRE1/XBP1, ATF6 and PERK/ATF4 in K.G6pc$^{-/-}$ mice. Although the expression of IRE1 was not significantly increased (increasing tendency), the mRNA spliced/unspliced *Xpb1* ratio was strongly induced in the kidneys of K.G6pc$^{-/-}$ mice, compared to WT mice (Fig. 6a). On the other hand, the PERK/eiF2/ATF4 branch was not affected in K.G6pc$^{-/-}$ mice compared to WT mice (Fig. 6b). Finally, the cleaved form of ATF6 had a tendency to increase in K.G6pc$^{-/-}$ kidneys (Fig. 6c). These data confirm the presence of a chronic ER stress that results in a sustained activation of IRE1 and ATF6 branch of UPR in K.G6pc$^{-/-}$ kidneys. This ER stress activity could be responsible

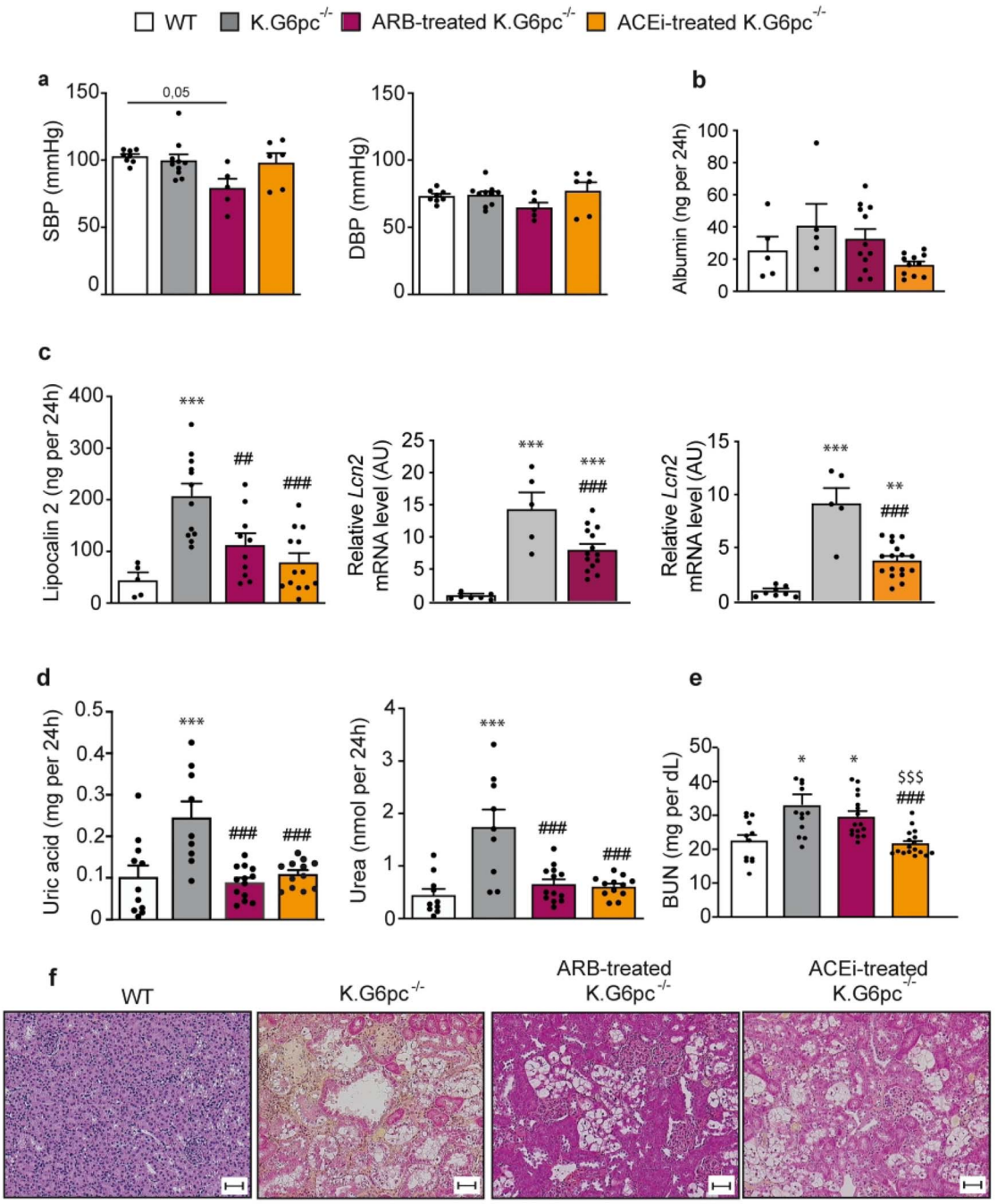

Figure 2. Irbesartan and Imidapril treatments reduce chronic kidney disease development in K.G6pc $^{-/-}$ mice. (a) Systolic blood pressure (SBP) and Diastolic blood pressure (DBP) were measured in WT (white bars), K.G6pc$^{-/-}$ (gray bars), ARB-treated (pink bars), ACEi-treated (orange bars) K.G6pc$^{-/-}$ mice, 2 weeks before the end of experiments. **(b)** Albuminuria, **(c)** Urinary LCN2 concentration and renal *Lcn2* gene expression **(d)** Uric acid and urea concentration in the 24-h collected urine. **(e)** Blood urea nitrogen (BUN). **(f)** Histological analyses hematoxylin and eosin staining of the kidneys of WT, K.G6pc$^{-/-}$ and ARB- or ACEi-treated K.G6pc$^{-/-}$ mice. Bars represent 50 µm. Data are expressed as the mean ± sem. Significant differences are indicated as $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Panels A and D: Groups were compared two-way ANOVA followed by Kruskal–Wallis *post hoc* test. Panels B and C: Groups were compared two-way ANOVA followed by Tukey's *post hoc* test.

for the activation of pro-apoptotic signals that was illustrated by the increased expression of C/EBP homologous protein (CHOP) in K.G6pc$^{-/-}$ mice compared to WT mice (Fig. 6d). Secondly, we analyzed the impact of RAS blockade on ER stress in K.G6pc$^{-/-}$ mice. ARB- and ACEi-treatments normalized the IRE1/Xbp1 and ATF6 branches of UPR (Fig. 6a–c), but without decreasing CHOP expression (Fig. 6d). Surprisingly, ARB and ACEi, which drastically declined serine 51 phosphorylation rate of eif2 protein in K.G6pc$^{-/-}$ kidneys, did not reduce the level of its direct target ATF4 (Fig. 6b).

Taken together these results suggest that RAS blockade allowed attenuation of CKD in K.G6pc$^{-/-}$ mice by blocking pro-fibrotic pathways and by activating cell defenses, in particular by increasing autophagy and reducing ER stress.

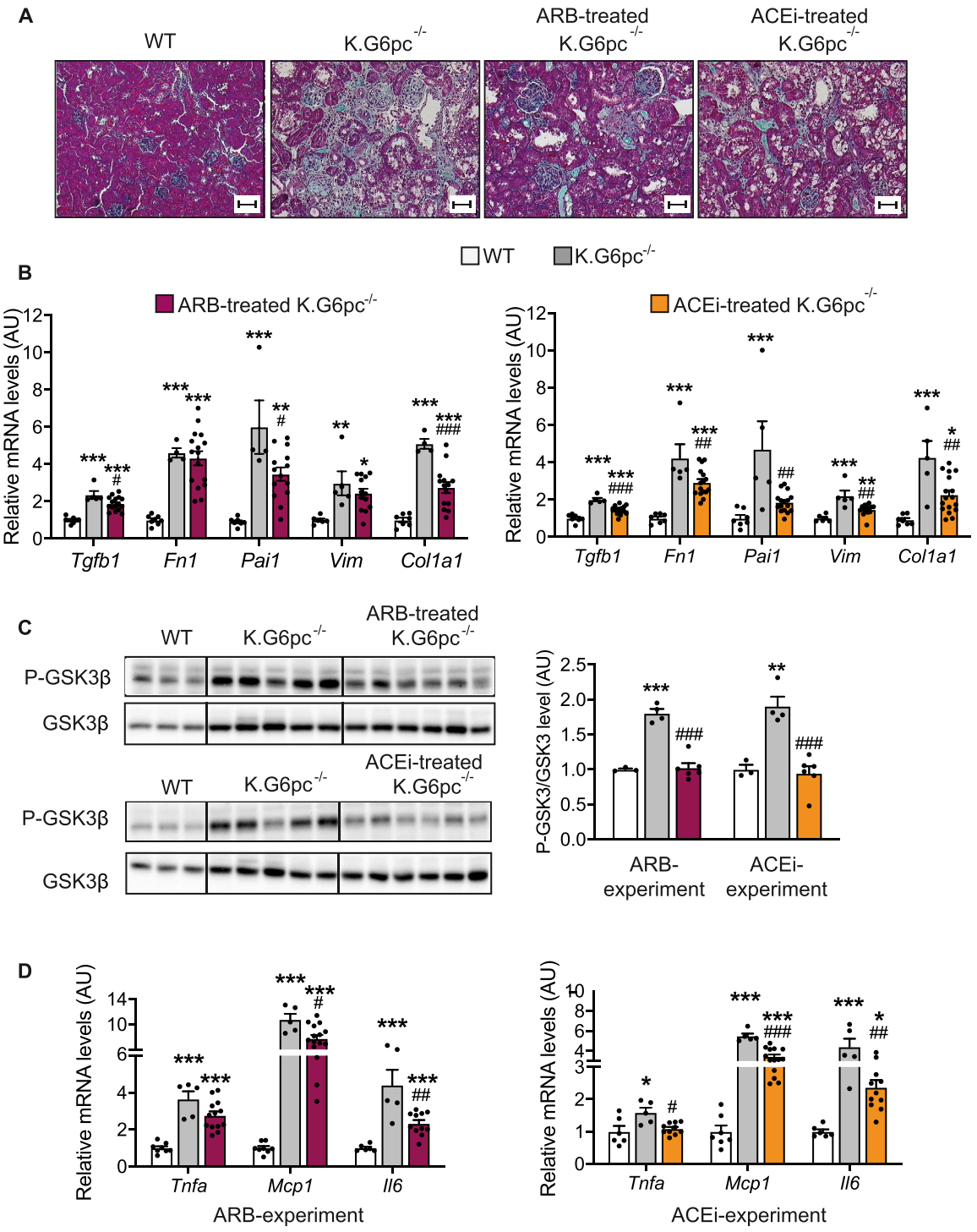

Figure 3. Irbesartan and Imidapril treatments reduce renal fibrosis in K.G6pc $^{-/-}$ mice. (a) Histological analyses of Masson's Trichrome stained kidneys of WT, K.G6pc$^{-/-}$ and ARB- or ACEi-treated K.G6pc$^{-/-}$ mice. Bars represent 50 µm. **(b)** Relative renal expression of pro-fibrotic/fibrotic genes in the kidneys of WT (white bars), and untreated (gray bars), ARB-treated (pink bars), ACEi-treated (orange bars) K.G6pc$^{-/-}$ mice. The renal expression of target mRNA in K.G6pc$^{-/-}$ mice treated or not with ARB/ACEi is expressed relatively to WT mice. *Tgfb1:* Transforming Growth Factor β1; *Pai-1:* Plasminogen activator inhibitor-1; *Fn1:* Fibronectin; *Col1a1:* Collagen Type 1 α 1 chain; *Vim* Vimentin. **(c)** Quantitative analyses of renal GSK3 phosphorylation by western blot of WT, and untreated K.G6pc$^{-/-}$, and ARB- or ACEi-treated K.G6pc$^{-/-}$ mice. The ratio of P-GSK3/GSD3 is shown on the right panel, relatively to that of WT mice. **(d)** Relative renal expression of inflammation genes in the kidneys of WT (white bars), and untreated (gray bars), ARB-treated (pink bars), and ACEi-treated (orange bars) K.G6pc$^{-/-}$ mice. *Tnfa:* Tumor necrosis factor α; *Mcp1:* Monocyte chemoattractant protein 1; *Il6:* interleukin 6. Data are expressed as the mean ± sem. Significant differences between WT and K.G6pc$^{-/-}$ mice are indicated as $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Significant differences between untreated and treated K.G6pc$^{-/-}$ mice are indicated as $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$. Groups were compared two-way ANOVA followed by Tukey's *post hoc* test.

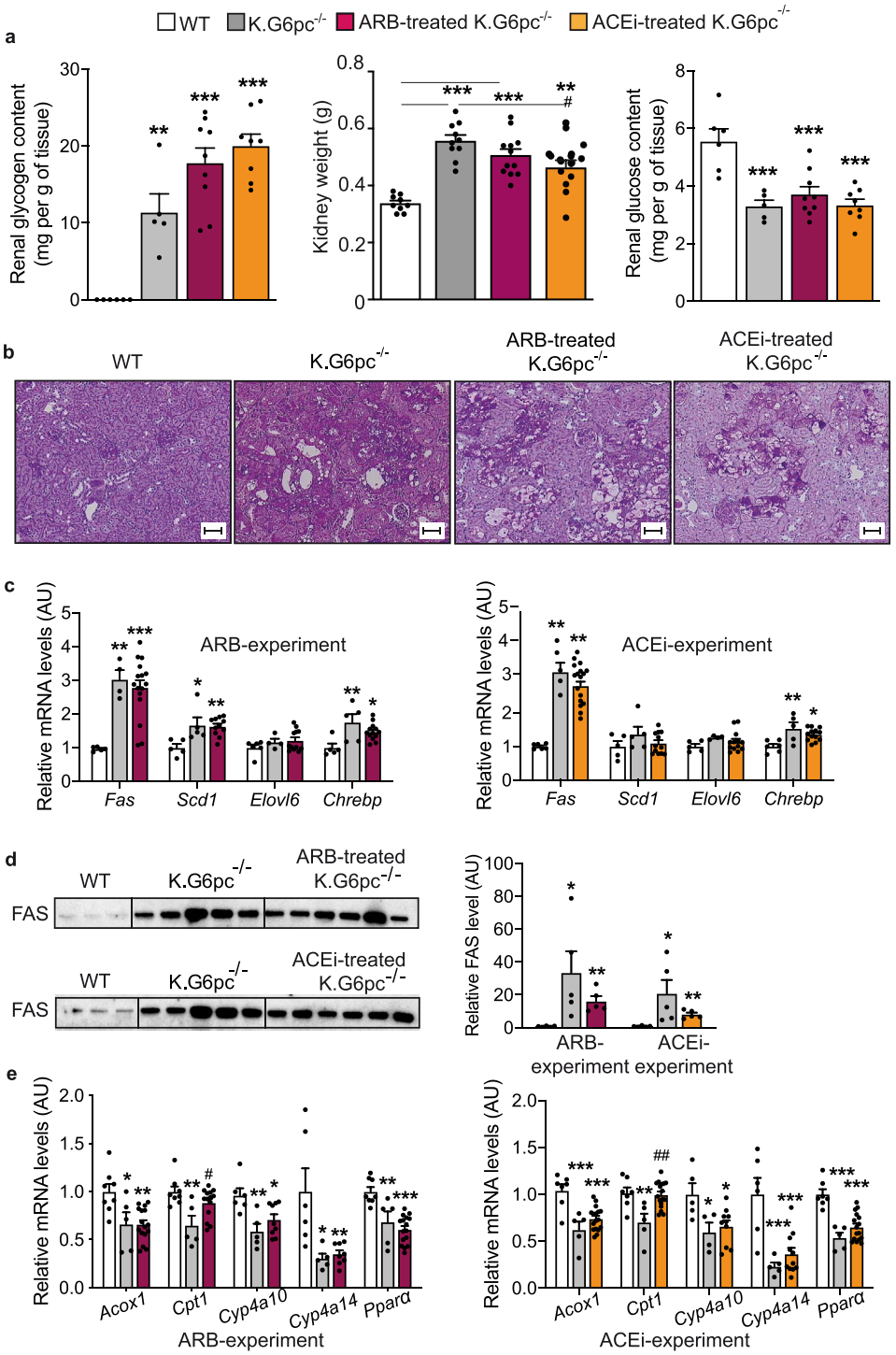

Figure 4. Irbesartan and Imidapril treatments do not modify glucose and lipid metabolism in the kidney of K.G6pc $^{-/-}$ mice. (a) Renal glycogen content, kidney weight and renal glucose level in WT (white bars), K.G6pc$^{-/-}$ (gray bars), ARB-treated (pink bars) and ACEi-treated (orange bars) K.G6pc$^{-/-}$ mice. **(b)** Histological analyses of PAS-stained kidneys. Bars represent 50 µm. **(c)** Quantitative analyses of renal *de novo* lipogenesis by RT-qPCR. *Fasn*: Fatty acid synthase, *Scd1*: Stearoyl-CoA desaturase, *Elov6*: Elongation of very long chain fatty acids protein 6, *Chrebp*: Carbohydrate Response Element Binding Protein. **(d)** Quantitative analyses of FAS expression by western blot in the kidney of WT, and untreated, ARB-treated, ACEi-treated K.G6pc$^{-/-}$ mice. Images of blots (on the left) and quantification graph (on the right) are shown. The quantification was performed relatively to total amount of proteins using stained-free imaging technology. **(e)** Relative renal expression of genes involved in lipid oxidation in WT. *Acox1*: Acyl-CoA oxidase, *Cpt1*: Carnitine palmitoyltransferase 1, *Cypa10* and *Cypa14*: Cytochrome P450, and *Ppara*: Peroxisome proliferator-activated receptor α. Data are expressed as the mean ± sem. Significant differences between WT and K.G6pc$^{-/-}$ mice are indicated as $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Significant differences between untreated and treated K.G6pc$^{-/-}$ mice are indicated as $^{\#}P < 0.05$; $^{\#\#}P < 0.01$. Groups were compared two-way ANOVA followed by Tukey's *post hoc* test.

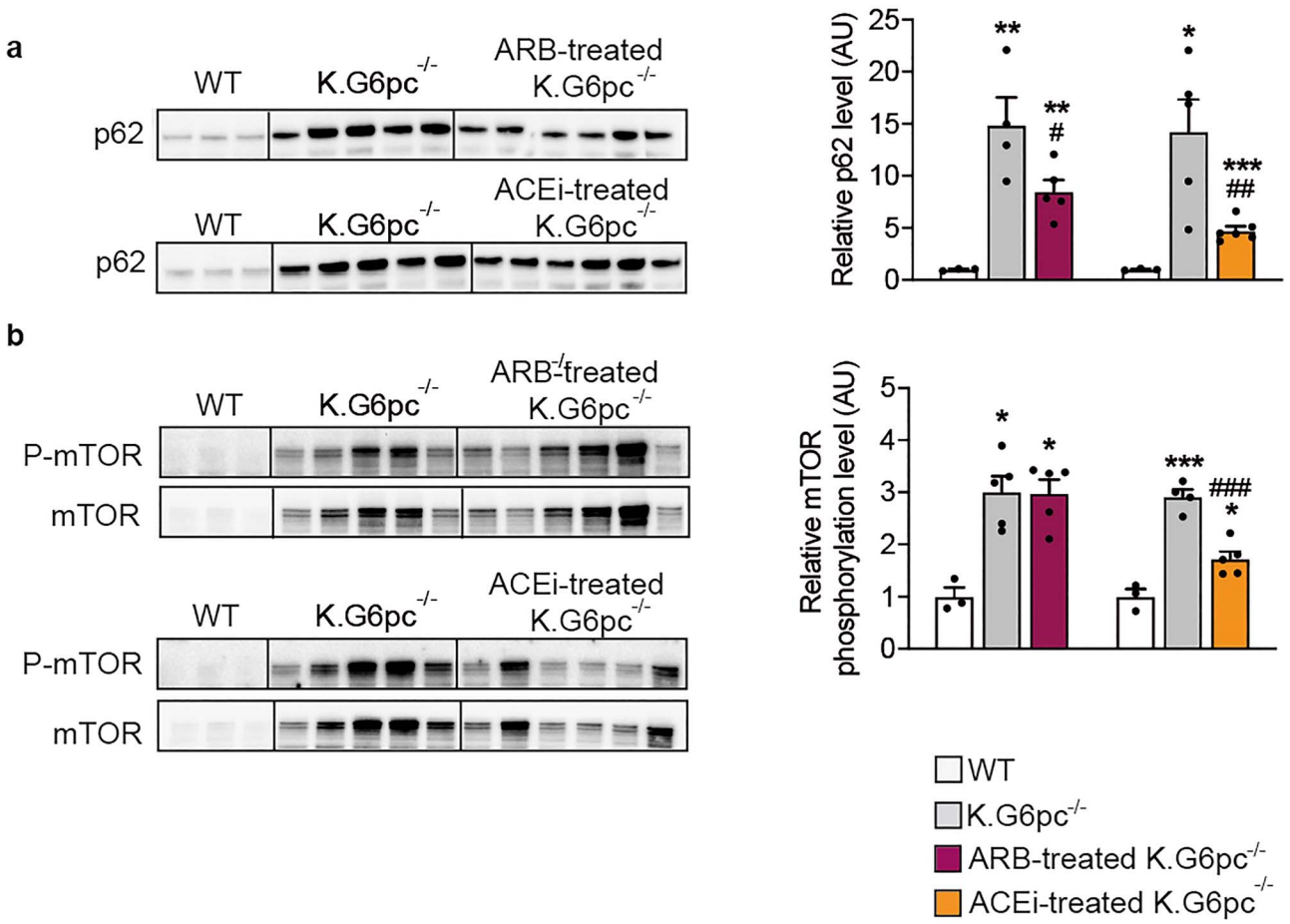

Figure 5. Irbesartan and Imidapril treatments 're-activate' autophagy in the kidneys of K.G6pc $^{-/-}$ mice. Western blot analyses of p62 **(a)** phospho mTOR (Ser2448) compared to mTOR **(b)** in the kidney of WT (white bars), and untreated (gray bars), ARB-treated (pink bars), ACEi-treated (orange bars) K.G6pc$^{-/-}$ mice. Images of blots (on the left) and quantification graph (on the right) are shown. Data are expressed as the mean ± sem. Significant differences between WT and K.G6pc$^{-/-}$ mice are indicated as $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Significant differences between untreated and treated K.G6pc$^{-/-}$ mice are indicated as $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$. Groups were compared two-way ANOVA followed by Tukey's *post hoc* test.

Discussion

CKD is a frequent long-term complication of GSDI that represents one of the main causes of morbidity of this pathology. In addition to nephromegaly due to glycogen accumulation in the kidneys, CKD starts with a long period of silent hyperfiltration, followed by the development of microalbuminuria, proteinuria and progressively fibrosis that can ultimately evolve into renal failure.

Here, the retrospective study of two French and Swiss cohorts of 73 GSDI patients confirmed the early development of CKD in more than 40% of GSDI patients, at a median age of 21 years, as previously described in ESGSDI study (6,7,13). Thus, the incidence of microalbuminuria/proteinuria in GSDI is highest between 12 and 25 years of age. The care of these patients is primarily based on dietary therapy, to avoid hypoglycemia and to control secondary metabolic abnormalities (such as hyperlactatemia, hypertriglyceridemia), but also to prevent renal metabolic disorders and consequently GSDI nephropathy (8,18,19). In our cohort, most patients followed the diet recommended in the general guidelines (8), consisting of limited consumption of fruits, juice and other products containing sucrose, fructose and lactose, and appropriate doses of cornstarch (or modified cornstarch, i.e. Glycosade®). Nevertheless, in the pubertal and adolescent age, poor adherence to diet recommendations may promote early development of renal and hepatic complications (20,21). It should be noted that liver-transplanted patients no longer followed a specific diet. To improve and delay the progression of renal damage, the guidelines for GSDI recommend to initiate pharmacological treatment based on classic RAS blockers at the first signs of glomerular hyperfiltration (7,8). It is recommended to initiate RAS blocker treatment in GSDI patients with a low dosage of RAS blocker and then to increase it progressively until therapeutic targets are reached in agreement to nephrologist. Interestingly, the efficiency of RAS blockade was observed in more than half of our cohort patients, allowing to decrease or normalized microalbuminuria/proteinuria, over a median period of 10 years. Moreover, most of GSDI patients with glomerular hyperfiltration showed a normal eGFR after treatment. These data strongly suggest that RAS blockage is an effective long-term treatment to attenuate progression of CKD. It should be noted that the effectiveness is not 100%, but it was already showed that renal damages systematically worsen in GSDI patients in the absence of ACEi treatment and may progress to renal failure requiring renal transplantation (7,9). However, our data do not allow us to show any association of the

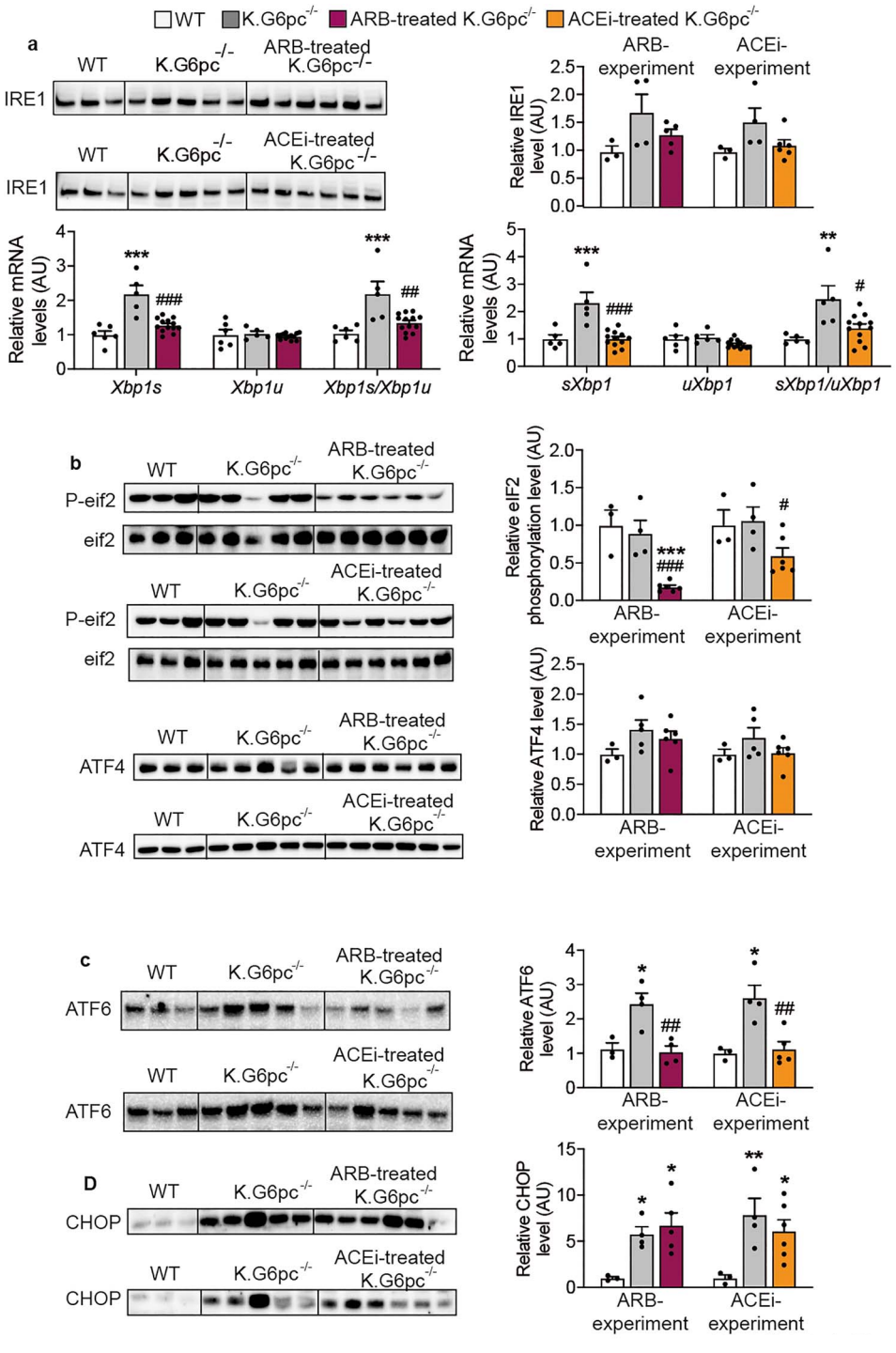

Figure 6. Irbesartan and Imidapril treatments prevent ER stress pathways activation in the kidneys of K.G6pc$^{-/-}$ mice. Western blot analyses of IRE1 (a), P-eif2/eif2 and ATF4 (b) ATF6 (c) and CHOP (d) of WT (white bars), and untreated (gray bars), ARB-treated (pink bars), ACEi-treated (orange bars) K.G6pc$^{-/-}$ kidneys. Images of blots (on the left) and quantification graph (on the right) are shown. The mRNA expression of *Xbp1S* and *Xbp1U* was analyzed in panel a. Data are expressed as the mean ± sem. Significant differences between WT and K.G6pc$^{-/-}$ mice are indicated as $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. Significant differences between untreated and treated K.G6pc$^{-/-}$ mice are indicated as $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$. Groups were compared two-way ANOVA followed by Tukey's *post hoc* test.

dosage of ACEi/ARB and the change in proteinuria and GFR in patients. Interestingly, our results suggest that ACEi could have better renoprotective effects than ARB, particularly in K.G6pc$^{-/-}$ mice. This is consistent with other available human data showing superior benefits of ACEi than ARB on the renal outcomes (22–25). Nevertheless, despite RAS blocker treatment, CKD progressed to renal insufficiency in about 20% of treated patients. A limitation of our retrospective study is the inability to relate the effects of other renoprotective treatments that may have been associated with ACEi/ARB, such as tight metabolic

control, as well as the prescription of lipid-lowering drugs or liver transplant. This could explain the heterogenic response to ACEi/ARB treatment with regard to albuminuria/proteinuria in patients. Finally, no association between the genotype and the response to ARB/ACEi treatment was observed. Thus, the relative renoprotective effect of ACEi/ARB treatment in GSD1 could be explained by the sustained metabolic disturbances induced by GSDI, i.e. lipid accumulation, which are not impacted by RAS blockade (20).

Interestingly, the pattern of CKD symptoms and renal injury-inducing events in GSDI are usually associated with RAS activation but not arterial hypertension, a main risk factor in nephropathy development (1,4). Accordingly, in our GSDI cohort, less than 20% of patients were hypertensive (two of them without signs of CKD), suggesting that hypertension does not seem to play a role in the development of nephropathy in GSDI. This was confirmed in K.G6pc$^{-/-}$ mice, which exhibit kidney dysfunction but normal SBP and DBP. It should be noted that ACEi/ARB treatments have been effective in reducing SBP/DBP in hypertensive GSDI patients of this cohort, except one who was treated by ARB for 17 years.

In search of the molecular mechanisms involved in the improvement of renal function by ACEi/ARB treatments, we focused on metabolism, fibrosis-leading pathways and cellular defenses that are known to be deregulated in GSDI kidneys in mice (11,14). First, we showed that both ACEi/ARB treatments effectively blocked RAS and partially prevented the development of CKD in K.G6pc$^{-/-}$ mice, by decreasing the expression of RAS-induced profibrotic factors such as TGFβ1 and components of the extracellular matrix such as collagen 1α1, resulting in delayed fibrosis. Nevertheless, it is noteworthy that ACEi was more effective in normalizing markers (i.e. BUN) and actors (i.e. pro-fibrotic and inflammatory genes) of CKD than ARB in K.G6pc$^{-/-}$ mice, which is in accordance with data obtained in the GSDI patient cohort. We also show that GSK-3β, which is closely related to the occurrence of renal fibrosis by affecting EMT (26), was activated by phosphorylation in K.G6pc$^{-/-}$ kidneys and inactivated during ACEi/ARB treatment that could also participate in the delay of fibrosis.

Interestingly, in a previous study, we showed that lipid accumulation in K.G6pc$^{-/-}$ kidneys, caused by the concomitant activation of *de novo* lipogenesis and decrease in lipid oxidation, triggers the activation of the renal RAS, which in turn activates the TGFβ1 pathway and renal fibrosis (12). Here, RAS blockade did not impact renal lipid metabolism in K.G6pc$^{-/-}$ mice, suggesting a persistent activation of molecular pathways triggering RAS. This could explain the partial efficiency of ACEi/ARB treatment observed in GSDI and diabetic patients. Similarly RAS blockade did not impact renal glycogen metabolism in K.G6pc$^{-/-}$ mice.

As observed in the liver of GSDI mice (27), the metabolic disturbances induced by *G6pc* deletion in the kidneys also induced cellular stress, characterized by a decreased autophagy and a chronic ER stress in K.G6pc$^{-/-}$ mice. Interestingly, a chronic ER stress and increased apoptosis was also observed in a human kidney cell model characterized by mutations in *SLC37A4* that can represent what happens in the GSDIb kidney (28). This was also described in diabetic CKD and proposed as a possible contributor to the pathogenesis by favoring cell death, inflammation and extracellular matrix accumulation (16,29,30). Interestingly, RAS blockade resulted in the partial 'reactivation' of autophagy and the normalization of UPR pathways in K.G6pc$^{-/-}$ mice, probably participating in the renoprotective effects of ACEi/ARB treatments. This is in line with the decrease of LCN2 expression in the kidneys, as a consequence of decreasing ER stress. Indeed, LCN2 plays a crucial role in CKD progression by triggering apoptosis (17). Thus, altogether these results suggest that targeting autophagy and UPR by pharmacological drugs could be an efficient treatment for preserving renal function and could be a new therapeutic strategy used in patients who develop adverse side effects on RAS inhibitors.

In conclusion, this study shows that RAS blockade delays renal damages associated with renal fibrosis in K.G6pc$^{-/-}$ mice by decreasing pro-fibrotic factors expression, ER stress and increasing autophagy. These data support the beneficial, but only partial, effects of RAS blockers on CKD of patients with GSDI. Thus, in the absence of curative treatment, RAS blockers, especially ACEi, should be used to delay the progression of kidney damage in patients with GSDI. As CKD appears in young adults or earlier in GSDI patients, this work emphasizes the introduction of RAS blockers, in addition to diet recommendations and optimal tight metabolic/glycemic control, as soon as the first signs of the renal pathology are detected.

Materials and Methods

Patients

Two cohorts of GSDI patients were reviewed retrospectively. The first cohort contains 42 patients (36 GSDIa and 6 GSDIb), followed at Antoine Béclère Hospital (Clamart, France). The second cohort contains 31 patients (27 GSDIa and 4 GSDIb) from the Swiss hepatic GSD registry (21). The approval number by the responsible ethics committee is KEK ZH 2013-0387,PB_2021-00013. Patients were seen at the clinic at least once a year for metabolic evaluations. None of the patients had used anti-hypertensive drugs before starting ARB/ACEi treatment. Albumin/creatinine ratio and proteinuria were assessed in 24 h-urine collections in the French cohort. Albumin/creatinine ratio was assessed in spot urine for Swiss patients. The estimated Glomerular Filtration Rate (eGFR) was calculated using CKI-EPI formula (31). Urine and plasma biology data and systolic/diastolic blood pressure (SBP/DBP) were obtained at the annual medical visit prior to initiation of ARB/ACEi therapy and at the last visit in 2020 (shown in Fig. 1/Table 1/Supplementary Material, Table S1 as pre-treatment and post-treatment, respectively), or just prior to liver/renal transplantation.

Animal models

K.G6pc$^{-/-}$ mice were obtained by the deletion of *G6pc1* exon 3 specifically in proximal tubules, using an inducible CRE-lox strategy, as previously described (11). Briefly, 6–8 weeks old B6.G6pc1$^{ex3lox/ex3lox}$.Kap$^{CreERT2/wt}$ and wild-type (WT, C57Bl6/J from Charles Rivers Laboratories, Saint-Germain-Nuelles, France) mice received 0.2 mg of tamoxifen/day for five consecutive days to generate K.G6pc$^{-/-}$ and control mice, respectively. Tamoxifen treatment was performed only in male mice, since Kap promoter is under androgenic control.

Mice were housed in the animal facility of University Lyon 1, in groups in an enriched environment, at 21–23°C and with light/darkness cycle (7 a.m./7p.m). Six months after tamoxifen treatment, ARB-treated K.G6pc$^{-/-}$ mice were fed a standard diet (A04, Safe, Augy, France) supplemented with Irbesartan (Mylan, Saint Priest, France) at an average dose of 50 mg/kg/day and ACEi-treated K.G6pc$^{-/-}$ mice were fed a standard diet and water was supplemented with Imidapril (Prilium®, Vetoquinol, Paris, France) at an average dose of 1 mg/kg/day, for 6 months. Untreated K.G6pc$^{-/-}$ and WT mice were fed a standard diet for

12 months. Systolic and diastolic blood pressures were measured (10-repeated measurements) with a CODA computerized tail-cuff system in conscious mice (Kent Scientific Corporation, Torrington, USA). Mice were killed at 6 h of fasting (with continuous access to water). Half of one kidney was included in paraffin after a 48 h-fixation in a 10% formaldehyde solution and the rest of kidneys was rapidly frozen in liquid nitrogen. All the procedures were performed in accordance with the principles and guidelines established by the European Convention for the Protection of Laboratory Animals and were approved by the local animal ethics committee and the French Ministry of National Education, Higher Education and Research (Apafis Permit: #8959).

Histological analyses

Kidney sections (4 µm thick) were stained with hematoxylin and eosin (H&E stain), periodic acid and Schiff's reagent (PAS) or Masson's Trichrome.

Biological parameters

Mice were housed in individual metabolic cages (Ugo Basile, Gemonio, Italy) for urine collection during 24 h. Blood was withdrawn by sub-mandibular bleeding and collected into EDTA tubes.

Albumin and lipocalin 2 (LCN2) concentrations were measured using mouse albumin ELISA kit (Clinisciences, Nanterre, France) and mouse LCN2/NGAL ELISA kit (R&D Systems, Lille, France). Uric acid and urea concentrations were assessed using colorimetric kits (Diasys, Holzheim, Germany and Hayward, CA, USA). Glycogen and glucose contents were determined on tissue homogenate by colorimetric assay as previously described (32).

Gene and protein analyses

Antibody references and dilutions used for western blot analyses are listed in Supplementary Material, Table S2. Target proteins were detected by chemiluminescence using Bio-Rad's ClarityTM Western ECL substrate and ChemiDocTM Touch Imaging System. Quantification was performed by using Bio-Rad Image LabTM software and stained-free imaging technology (33). Phosphorylated or cleaved proteins were normalized with respect to the non-phosphorylated/non-cleaved form.

Total RNAs were extracted according to the Trizol protocol (Invitrogen Life Technologies). Reverse transcription and real-time PCR were performed as previously described (12). Sequence-specific primers are listed in Supplementary Material, Table S3. The expression of mRNA was normalized to the *mL19* transcript expression using the $2^{\Delta\Delta Ct}$ method. Gene expression analyses were performed independently for each treatment and were presented in two different panels on the figures.

Statistical analyses

The results are reported as the mean ± s.e.m. (standard error of the mean). Patient data obtained before and after ARB/ACEi treatment were compared using a Wilcoxon matched pairs test. Mouse data were compared using a one-way ANOVA analysis followed by Tukey's *post hoc* test (against all groups) or a Kruskal–Wallis test. Statistical analysis was performed using the GraphPad Prism software. Differences were considered statistically significant at P-value < 0.05.

Supplementary Material

Supplementary Material is available at *HMG* online.

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Conflict of interest statement. No conflict of interest has to be disclosed relating to the work described in this paper.

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