

Cellular and Metabolic Effects of Renin-Angiotensin System Blockade  
on Glycogen Storage Disease Type I nephropathy

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**Table S1: Renal function and blood pressure in patients with GSDI before and after ARB /ACEi treatments.**

Patient number	GSD type	Treatment	Albumin/creatinine (mg/mmol)		% change in albuminuria	Proteinuria (g/24h)		% change in albuminuria	Serum creatinine (μmol/l)		eGFR (ml/min/1.73m <sup>2</sup> )		SBP (mmHg)/DPB (mmHg)		Plasma triglycerides (g/l)		Plasma cholesterol (mmol/l)		Other medications (dose/day) or therapeutic interventions	Biological data after OLT	
			Before	After		Before	After		Before	After	Before	After	Before	After	Before	After	Before	After			
1	la	ACEi	Ramipril 10mg/day	95.6	41.8	-56%	0.95	0.45	-53%	70	57	113	135	150/95	120/75	8.25	4.83	6.85	6.15	Fenofibrate 200mg Ezetimibe 10mg Allopurinol 200mg	
2	la	ACEi	Ramipril 10 mg/day for 3 years and then 20 mg/day	85.6	N/A	N/A	0.65	2.85	338%	81	211	78	24	115/70	120/69	11.44	7.33	6.65	6.12	Allopurinol 200 mg Pravastatine 20mg Kidney transplantation at 36 years old	
3	la	ACEi	Ramipril 5mg/day	32.8	0	-100%	0.28	0	-100%	77	83	110	95	121/69	118/76	15.21	9.33	7.23	6.15	Fenofibrate 200mg Pravastatine 40mg	
4	la	ACEi	Ramipril 2.5mg/day	35	31.3	-10%	0.32	0.29	-10%	45	49	164	139	123/68	120/72	4.2	7.7	5.05	6.12	Allopurinol 200mg Fenofibrate 67 mg OLT at 28 years old	After 8 years TG: 1.25g/l; chol 3.87 mmol/l; Creatinine 60 μmol/l; eGFR 138 ml/min/1.73m <sup>2</sup>
5	la	ACEi	Ramipril 5mg/day	44.6	0	-100%	0.34	0	-100%	46	53	158	125	130/69	127/66	4.87	3.04	5.98	5.52	Allopurinol 200mg	
6	la	ACEi	Ramipril 10mg/day	31	0	-100%	0.017	0	-100%	45	58	166	120	125/69	120/74	24.28	27.8	9.53	10.05	Allopurinol 200mg Pravastatine 20 mg	
7	la	ACEi	Ramipril 10mg/day	40.3	0	-100%	0.25	0	-100%	70	82	231	122	122/73	125/69	7.29	3.27	6.75	5.08	Allopurinol 100mg	
8	la	ACEi	Ramipril 12.5mg/day	46.2	71.6	55%	0.036	0.059	63%	40	64	202	102	126/64	121/62	10.32	4.13	6.85	5.75	Fenofibrate 200 mg Febuxostat 80mg	
9	la	ACEi	pril (5mg initially then 10mg)	55.7	5.8	-90%	N/A	N/A	N/A	63	67	>90	>90	130/80	135/86	3.88	3.23	9.5	7.4	Atorvastatin 40mg Allopurinol 300mg	
10	la	ACEi	Perindopril 2.5mg/day	6.6	2.8	-58%	N/A	N/A	N/A	83	90	>90	87	142/77	144/94	3.7	3.9	5.3	5.0	Fenofibrate 100mg Allopurinol 150mg	
11	lb	ACEi	Enalapril 5mg/day	N/A	693	persistant albuminuria	N/A	N/A	N/A	normal	72	165	121	99/61	123/85	4.14	9.3	3.3	5.9	Allopurinol 300mg	
12	lb	ACEi + ARB	Ramipril 10 mg/day and Ibesartan 75 mg/day	110.3	165.3	50%	1.35	1.43	6%	80	347	71	30	110/60	112/64	4.71	2.32	5.79	5.01	Amlodipine 5 mg Febuxostat 80mg G-CSF	
13	lb	ACEi	Ramipril 5 mg/day	95.8	0	-100%	1.1	0	-100%	43	47	233	187	122/68	129/70	9.78	8.69	6.22	6.15	Febuxostat 120mg Fenofibrate 200mg G-CSF	
14	lb	ACEi	Ramipril 1.25 mg/day	49.8	0	-100%	0.41	0	-100%	55	52	170	159	128/71	123/70	1.78	2.03	4.65	4.85	Allopurinol 300mg G-CSF	
15	la	ARB	Ibesartan 300mg/day	45.3	N/A	N/A	0.26	4.2	1515%	59	286	169	25	123/72	119/71	10.34	8.24	8.25	6.35	Allopurinol 200 mg Pravastatine 40 mg OLT + Kidney transplantation at 28 years old	After 3 years TG: 1.37g/l; chol 4.57 mmol/l; Creatinine 175 μmol/l; eGFR 42 ml/min/1.73m <sup>2</sup>
16	la	ARB	Ibesartan 150 mg/day	75.9	0	-100%	0.68	0	-100%	48	63	194	134	125/72	123/74	7.9	6.92	7.15	5.95	Febuxostat 80mg Pravastatine 20mg	After 4 years, TG: 0.7g/l; chol 3.55 mmol/l; Creatinine 77 μmol/l; eGFR 107 ml/min/1.73m <sup>2</sup>
17	la	ARB	candesartan 16mg/day	8.4	35.3	332%	N/A	N/A	N/A	86	154	83	35	110/80	135/89	16.3	0.73	5.9	3.73	Simvastatin 40 mg Allopurinol 100mg OLT at 36 years old	
18	la	ARB	candesartan 16mg/day	N/A	1.9	persistant albuminuria	N/A	N/A	N/A	N/A	110	N/A	66	N/A	114/72	N/A	3.53	N/A	3.7	Atorvastatin 40mg Allopurinol 300mg Hydrochlorothiazide 12.5 mg	
19	la	ARB	losartan 100mg/day	7	19.7	181%	N/A	N/A	N/A	55	72	>90	>90	132/80	129/76	3.67	2.9	5.81	5.68	Allopurinol 100mg OLT at 17 years old	
20	la	ARB	Ibesartan 150mg and since 1 year 100mg/day	N/A	74.9	persistant albuminuria	0.059	N/A	N/A	N/A	62	N/A	>90	124/68	159/103	6.4	3.37	6.70	4.16	Allopurinol 100mg	
21	la	ARB	Losartan 25mg/day and since 1 year 100 mg/day	2.9	4.9	69%	N/A	N/A	N/A	29	29	>90	>90	135/80	125/85	29.0	15.2	13.0	9.0	Fenofibrate 100mg	
22	la	ACEi	Lisinopril 5mg/day	7.2	9.92	N/A	N/A	N/A	N/A	30	28	>90	>90	133/83	N/A	27.4	19.9	11.2	9.99	Allopurinol 200mg	
23	la	ACEi	Lisinopril 5mg/day started on February 2021	42.2	N/A	N/A	N/A	N/A	N/A	37	N/A	>90	N/A	109/80	N/A	7.1	N/A	14.7	N/A	Allopurinol 400mg	
24	la	ARB	Candesartan 8mg/day	0	0	-	0	0	-	40	37	167	177	160/98	108/58	8.0	4.4	6.24	4.98	Febuxostat 120mg Rosuvastatine 5 mg Fenofibrate 200 mg Propranolol	
25	la	ACEi	Ramipril 2.5mg/day	0	0	-	0	0	-	68	49	130	186	152/90	129/77	4.21	2.66	4.55	4.25	Febuxostat 80mg	

Biological data of GSDI patients with nephropathy and/or hypertension were analyzed before starting and after ACEi/ARB treatment; 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 and additional biological data were also collected some years after transplantation, in 2020. Patients 24 and 25 were treated for

hypertension, without signs of CKD. The percentage in albuminuria was calculated as:  $(\text{value of albuminuria after the treatment} - \text{value of albuminuria before the treatment}) / \text{value of albuminuria before the treatment} \times 100$ . When no albuminuria/proteinuria was detected after treatment, the % change in albuminuria or proteinuria is indicated as -100%. N/A: not available data.

**Table S2: Plasma parameters of WT and K.G6pc<sup>-/-</sup> mice treated or not with ARB-or ACEi.**

	WT mice	K.G6pc <sup>-/-</sup> mice	ARB-treated K.G6pc <sup>-/-</sup> mice	ACEi-treated K.G6pc <sup>-/-</sup> mice
<b>Glucose (mg/dl)</b>	152 ± 6	164 ± 8	129 ± 5 <sup>*, ##, \$</sup>	152 ± 6
<b>TG (g/ml)</b>	0.66 ± 0.03	0.55 ± 0.04	0.58 ± 0.04	0.59 ± 0.03
<b>Cholesterol (g/l)</b>	0.92 ± 0.03	1.03 ± 0.05	1.09 ± 0.04	0.99 ± 0.06
<b>NEFA (mg/dl)</b>	25.2 ± 1.6	25.7 ± 2.3	27.8 ± 1.3	26 ± 1
<b>Uric acid (mg/l)</b>	10.5 ± 0.4	11.1 ± 0.9	10.6 ± 0.5	8 ± 0.3 <sup>*, ##, \$\$\$</sup>

Data were obtained from mice after 6 h of fasting and are expressed as the mean ± S.E.M. (n = 5–14). Significant differences between K.G6pc<sup>-/-</sup> and WT are indicated as \*  $P < 0.05$ . Significant differences between ARB/ACEi-treated K.G6pc<sup>-/-</sup> mice and untreated K.G6pc<sup>-/-</sup> mice are indicated as ##  $P < 0.01$ . Significant differences between ARB-treated K.G6pc<sup>-/-</sup> mice and ACEi-treated K.G6pc<sup>-/-</sup> mice are indicated as \$  $P < 0.05$ ; \$\$\$  $P < 0.001$ . Statistical test: One-way ANOVA followed by Tukey's *post hoc test*.

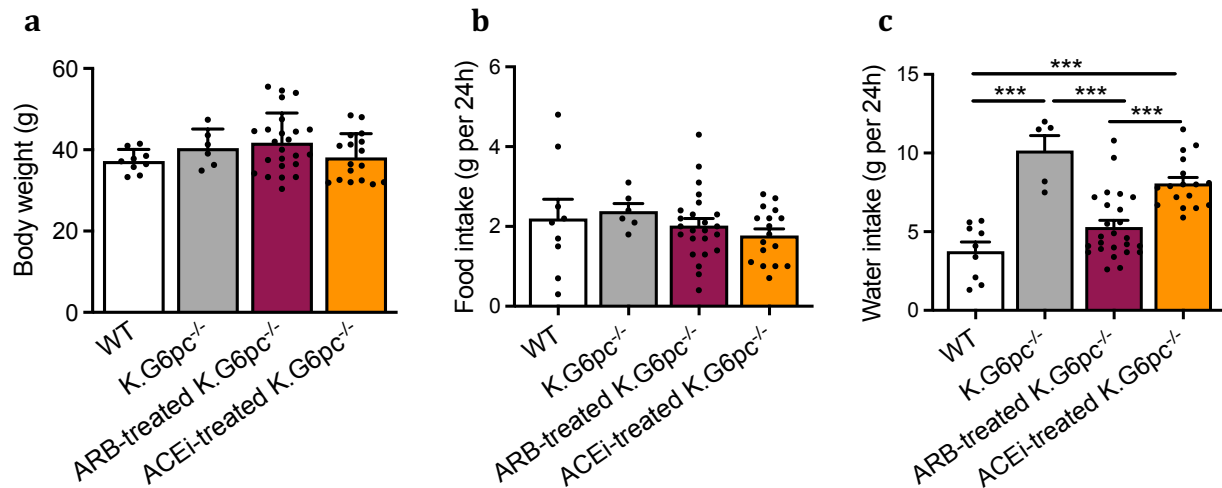
**Table S3: Antibody references and dilutions used for western blot analyses.**

<b>Name</b>	<b>Supplier</b>	<b>Cat no.</b>	<b>Clone no.</b>	<b>Dilution</b>
ATF4	NeoBiotech	NB22-18091	Polyclonal	1 :1000
ATF6	Novus biologicals	NBP1-40256	70B1413.1	1 :1000
CHOP (GADD153)	Cliniscience	OPA1336	Polyclonal	1 :1000
<i>Eukaryotic Initiation Factor 2</i> (eIF2 $\alpha$ )	Cell signaling	#9722	Polyclonal	1 :1000
Phospho-eIF2 $\alpha$ Ser 51	Cell signaling	#9721	Polyclonal	1 :1000
Fatty acid synthase (FAS)	Cell signaling	#3189	Polyclonal	1 :1000
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Cell Signaling	#5174	D16H11	1 :10,000
Glycogen synthase kinase (GSK3 $\beta$ )	Cell Signaling	#9315	27C10	1 :1000
Phospho-glycogen synthase kinase (GSK3 $\beta$ ) (Ser9)	Cell Signaling	#9322	D3A4	1 :1000
Goat secondary anti-rabbit IgG linked to peroxidase	BioRad	170-5046		1 :10,000
Inositol-requiring enzyme-1a (IRE1a)	Cell signaling	#3294	14C10	1 :1000
Mechanistic or mammalian target of rapamycin (mTOR)	Cell signaling	#2983	7C10	1 :1000
Mechanistic or mammalian target of rapamycin (mTOR Phospho	Cell signaling	#5536	D9C2	1 :1000
P62 (SQSTM1)	Abcam	ab56416	Unknown	1 :1000

**Table S4: Primer sequences used in qPCR.**

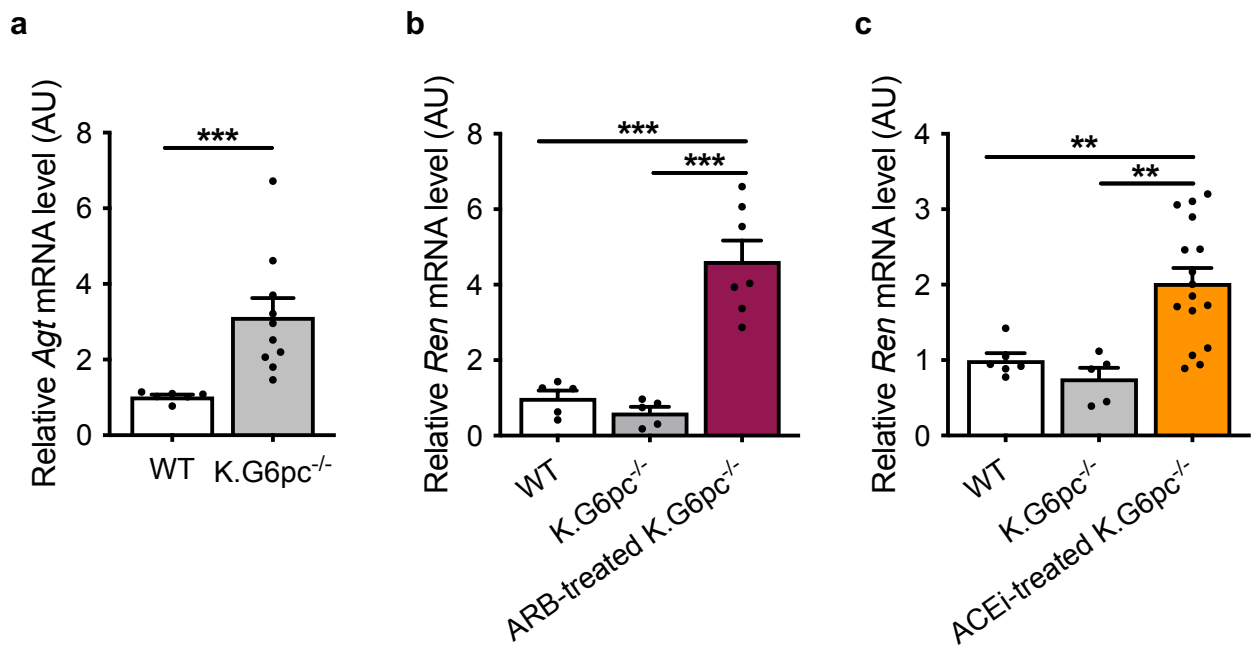
<b>Targeted gene</b>	<b>Primer sequences</b>
Acyl-CoA Oxidase 1 ( <i>Acox1</i> )	Fw : TGCCAAATTC CCTCATCTTC Rev : CTTGGATGGTAGTCCGGAGA
Angiotensinogen ( <i>Agt</i> )	Fw : TTCACTGCTCCAGGCTTTCGTCTA Rev : TTCTCAGTGGCAAGAACTGGGTCA
Carbohydrate-responsive element-binding protein, CHREBP ( <i>Chrebp</i> ), total	Fw : GAAGCCACCTATAGCTCCC Rev : CTGGGGACCTAAACAGGAGC
Carnitine palmitoyltransferase1 ( <i>Cpt1a</i> )	Fw : ACCTCCATGGCTCAGACAG Rev : AGCAGAGGCTCAAGCTGTTCA
Collagen, type I, alpha 1 ( <i>Col1a1</i> )	Fw : GCTCCTCTTAGGGGCCACT Rev : CCACGTCTCACCATTGGGG
Cytochrome P450 ( <i>Cyp4a10</i> )	Fw : TCCAGCAGTTC CATCACCT Rev : TTGCTTCCCCAGAACCATCT
Cytochrome P450 ( <i>Cyp4a14</i> )	Fw : TCAGTCTATTTCTGCTGTTC Rev : GAGCTCCTTGTCCTTCAGATGGT
Fatty acid elongase 6 ( <i>Elovl6</i> )	Fw : ACAATGGACCTGTCAGCAAA Rev : GTACCAGTGCAGGAAGATCAGT
Fatty acid synthase, FAS ( <i>Fasn</i> )	Fw : TTCCAAGACGAAAATGATGC Rev : AATTGTGGGATCAGGAGAGC
Fibronectin ( <i>Fn1</i> )	Fw : TGGCTGCCTTCAACTTCTCCT Rev : TGTTTGATCTGGACTGGCAGTTT
Interleukin 6 ( <i>Il6</i> )	Fw : GTGGCTAAGGACCAAGACCA Rev : AACGCACTAGGTTTGCCGA
Lipocalin 2 ( <i>Lcn2</i> )	Fw : GGACCAGGGCTGTCGCTACT Rev : GGTGGCCACTTGACATTGT

Monocyte chemoattractant protein 1 (Mcp1)	Fw : TTAAAAACCTGGATCGGAACCAA Rev : GCATTAGCTTCAGATTTACGGGT
Peroxisome proliferator-activated receptor alpha PPAR $\alpha$ (Ppara)	Fw : AGTTCACGCATGTGAAGGCTG Rev : TTCCGGTTCTTCTTCTGAATC
Plasminogen activator inhibitor (Pai1)	Fw : TTCAGCCCTTGCTTGCCTC Rev : ACACTTTTACTCCGAAGTCGGT
Renin (Ren)	Fw : GCCGCCTCTACCTTGCTTGTG Rev : GGGGCAGCTCGGTGACCTCT
Ribosomal protein mL19 (Rpl19)	Fw : GGTGACCTGGATGAGAAGGA Rev : TTCAGCTTGTGGATGTGCTC
Stearoyl-CoA desaturase 1 (Scd1)	Fw : TGGGTTGGCTGCTTGTG Rev : GCGTGGGCAGGATGAAG
Transforming growth factor $\beta$ 1 (Tgfb1)	Fw : CAACAATTCTGGCGTTACCTTGG Rev : GAAAGCCCTGTATTCCGTCTCCTT
Tumor necrosis factor $\alpha$ (Tnfx)	Fw : AGGCTGCCCCGACTACGT Rev : GACTTTCTCCTGGTATGAGATAGCAAA
Vimentin (Vim)	Fw : CGGCTGCGAGAGAAATTGC Rev : CCACTTTCCGTTCAAGGTCAAG
Spliced- X-box binding protein 1(sXbp1)	Fw : CTGAGTCCGAATCAGGTGCAG Rev : GTCCATGGGAAGATGTTCTGG
Unsliced- X-box binding protein 1 (uXbp1)	Fw : AGCACTCAGACTATGTGCA Rev : GTCCATGGGAAGATGTTCTGG



**Figure S1: Body weight and food/water intakes** of wild-type (WT, white bars, n=9), K.G6pc<sup>-/-</sup> (grey bars, n=10), ARB (pink bars, n=14) or ACEi (orange bars, n=124)-treated K.G6pc<sup>-/-</sup> mice at the end of treatment. Food and water intakes were measured during 24h in a metabolic cage. Data are expressed as the mean ± s.e.m. Significant differences are indicated as \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001. Groups were compared using two-way ANOVA followed by Tukey's *post hoc* test.





**Figure S2: Expression of renin-angiotensin system actors in the kidney** of wild-type (WT, white bars), K.G6pc<sup>-/-</sup> (grey bars), ARB (pink bars) or ACEi (orange bars)-treated K.G6pc<sup>-/-</sup> mice. *Agt* and *Ren* encode for angiotensin and renin, respectively. Data are expressed as the mean  $\pm$  s.e.m. Significant differences are indicated as \*\*, P < 0.01; \*\*\*, P < 0.001. In panel A, groups were compared using Student's test; and in panels B-C, groups were compared using two-way ANOVA followed by Tukey's *post hoc* test.