Biochemical validation of a rat model for polycystic kidney disease: Comparison of guanidino compound profile with the human condition

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Polycystic kidney disease (PKD) accounts for 7–10% of all dialyzed renal insufficient patients. Accumulation of specific guanidino compounds (GCs) has been related to neurological, cardiovascular, hematological, and immunological complications of renal failure. In this study, we investigate whether the PKD/Mhm rat model can be used as a biochemical model for human PKD. For the validation of the rat model, we performed the first detailed evaluation of the concentrations of GCs in serum and urine of patients with PKD in addition to the GC patterns in the plasma, urine, and tissues of the PKD/Mhm rat model. The GCs were determined after separation on a cation exchange resin and fluorescence detection. The GC levels and changes observed in blood and urine of patients with PKD are comparable with those found in patients with renal insufficiency due to different etiologies. The PKD/Mhm rat model can be used as a biochemical model for human PKD as the obvious increases of urea, guanidinosuccinic acid, creatinine, guanidine, methylguanidine, and N^GN^G-dimethylarginine (symmetrical dimethylarginine) seen in blood of oldest heterozygous and younger homozygous PKD rats were largely within the same range as those found in the studied human PKD population, especially in patients with a glomerular filtration rate below 60 ml/min/1.73 m^2. The decreased levels of plasma guanidinoacetic acid seen at end-stage renal disease in homozygous and oldest heterozygous rats were also observed in serum of patients with a glomerular filtration rate below 20 ml/min/1.73 m^2. The PKD/Mhm rat model has, besides similar disease characteristics with human PKD, comparable GC alterations.

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Polycystic kidney disease (PKD) is a frequent cause of end-stage renal failure in adults. Autosomal dominant PKD is the most common form of PKD and presents in one of 800 live births. PKD accounts for 7 to 10% of all dialyzed patients with renal insufficiency. The disease, mainly caused by truncating mutations in the *PKD1* and *PKD2* genes, is characterized by cystic enlargement of both kidneys. In addition, cysts in the liver and pancreas, and increased risk for aortic and intracerebral aneurysms, are present.[1]

Different guanidino compounds (GCs) were reported as possible uremic toxins that could play a role in the symptomatology seen in patients with renal failure.[2–7] Guanidinosuccinic acid (GSA), guanidine (G), methylguanidine (MG), and creatinine (CTN) are significantly increased in the serum, urine, cerebrospinal fluid, and different brain regions of patients with renal failure and have been reported as potential uremic toxins.[8–10] Accumulation of GSA, MG, G, and CTN possibly contributes to neurological symptoms of uremia.[2,3,11] $N^GN^{G\prime}$-dimethylarginine (ADMA) was previously shown to be doubled in serum of non-dialyzed patients with chronic renal insufficiency.[12] This latter GC is an inhibitor of NO synthase and could play a role in hypertension and increased tension in cerebral arteries.[6,13–15] Some GCs have been related to leukocyte activation and might in this way be related to uremic vascular disease.[16] In addition, there is a decrease of some GCs like guanidinoacetic acid (GAA) and homoarginine (Harg) in patients with renal failure.[12]

In several animal models for renal disease, altered GC concentration patterns, corresponding more or less to those found in man, have been reported.[17–19] Several mouse strains, in which PKD is transmitted in an autosomal recessive pattern, provided insights into the pathogenesis of renal cyst formation.[20,21] The PKD/Mhm rat model is related to a spontaneous mutation in the Sprague–Dawley strain, with autosomal dominant transmission.[22] PKD in the PKD/Mhm rat appeared to be due to mutation in a novel gene, *PKDr1*.[23] Homozygous PKD/Mhm animals develop massive renal enlargement leading to death within the first postnatal

month. The two kidneys are usually injured to the same extent. Heterozygous animals of both sexes display slowly progressive renal cystic disease, but male heterozygous animals manifest a significantly more severe form of the disease than female animals.[21,24] Previous studies reported different features of this rat model, which are similar to the characteristics of human with PKD.[1,22,25,26] However, there are no data available showing that complex biochemical processes, such as the uremic retention pattern of GCs, are similar to the human equivalent.

This is the first detailed study evaluating the concentrations of GCs in a population of renal insufficient individuals consisting exclusively of non-dialyzed PKD patients. In addition, we studied for the first time the GC patterns in the plasma, urine, and tissues of the PKD rat model PKD/Mhm. The observed profiles of the PKD rats were compared with those obtained in patients with PKD. The validation of the PKD rat model in this area is useful for future research on pathobiochemistry and pathophysiology of the PKD, considering the importance of these compounds in symptoms of renal failure.

RESULTS

Concentration of GCs in PKD patients

Serum. Significantly increased levels of urea, GSA (which is metabolically related to urea), CTN, ADMA, and N^GN^G-dimethylarginine (SDMA) were found in the serum of PKD patients (Table 1a). Increased levels of G, MG, α-keto-δ-guanidinovaleric acid (α-Keto-δ-GVA), α-*N*-acetylarginine (α-NAA), and γ-guanidinobutyric acid (γ-GBA) were also observed in patients. Serum GAA was significantly decreased in patients with a glomerular filtration rate (GFR) below 20 ml/min/1.73 m^2.

The latter increases progressed with decreasing GFR: urea ($R=-0.767$; $P<0.001$), SDMA ($R=-0.751$; $P<0.001$), CTN ($R=-0.740$; $P<0.001$), G ($R=-0.726$; $P<0.001$), GSA ($R=-0.700$; $P<0.001$), argininic acid ($R=-0.695$; $P<0.001$), and α-NAA ($R=-0.510$; $P<0.01$). Although no significant differences in Harg were observed as compared with controls, Harg decreased progressively with increasing renal dysfunction ($R=0.605$; $P<0.001$).

Urine. Practically, only in the studied PKD patients with a GFR below 20 ml/min/1.73 m^2, significant changes were observed: significantly increased levels of GSA, G, and MG next to decreased urinary excretion levels of GAA, ADMA, α–Keto-δ-GVA, α-NAA, and γ-GBA. The urinary excretion levels of the other GCs did not change (Table 1b).

The urinary excretion levels of GSA and MG increased progressively with decreasing GFR, whereas those of GAA and ADMA decreased progressively with renal dysfunction (respectively, $R=-0.589$, -0.670, 0.611, 0.525 and $P=0.002$, <0.001, $=0.001$, $=0.01$)

Concentration of GCs in PKD rats

Plasma. Urea and GSA were progressively retained in plasma during renal failure in homozygous and heterozygous PKD rats (Table 2a and b). Further, a clear retention of CTN and γ-GBA was observed in all homozygous and heterozygous animals. Plasma concentration of G, MG, α-Keto-δ-GVA, and SDMA was increased in homozygous and in oldest heterozygous rats. Plasma GAA was clearly decreased in homozygous rats with end-stage renal failure and in oldest heterozygous rats. Creatine (CT) was significantly lower in plasma of oldest homozygous rats and in heterozygous rats aged 10 and 18 months. A progressive decrease of plasma arginine (Arg) was observed in homozygous animals.

Table 1a | GCs (μM) and urea (mM) in serum of PKD patients and controls

Serum	Controls *n*=9	GFR: normal–60 ml/min/1.73m² *n*=9	Controls *n*=7	GFR: 20–60 ml/min/1.73 m² *n*=7	Controls *n*=11	GFR: <20 ml/min/1.73 m² *n*=11
αKetoδGVA	<0.035	<0.035	<0.035	0.15±0.04	<0.035	0.22±0.03
GSA	0.28±0.09	0.36±0.04	0.29±0.06	1.30±0.41*	0.28±0.05	11.0±1.8***,[a]
CT	40.5±10.4	38.4±5.5	36.3±7.5	34.8±6.4	36.3±8.3	34.4±9.4
GAA	2.77±0.33	2.07±0.14	2.29±0.30	2.22±0.29	2.45±0.12	1.83±0.19*
α-NAA	<0.025	0.55±0.06	<0.025	0.73±0.03	<0.025	0.88±0.12
ArgA	<0.025–0.12	0.05±0.01	0.16±0.05	0.15±0.03	0.18±0.04	0.28±0.04
β-GPA	<0.013	<0.013	<0.013	<0.013	<0.013	<0.013
CTN	68.0±5.9	66.9±6.0	72.6±6.6	153±27*	66.8±4.4	519±66***,[a]
γ-GBA	<0.013	<0.013–0.05	<0.013	0.06±0.01	<0.013	<0.013–0.1
Arg	103±7.4	107±7.8	112±8.2	125±6.6	116±5.9	114±9.9
Harg	1.40±0.22	2.07±0.24	1.55±0.20	1.36±0.09	1.63±0.22	1.16±0.11
G	<0.06	0.22±0.03	<0.06	0.41±0.06	<0.06	1.34±0.16
MG	<0.02	<0.02	<0.02	0.08±0.02	<0.02	1.01±0.46
ADMA	0.39±0.03	0.68±0.05***	0.49±0.04	0.70±0.05**	0.45±0.02	0.85±0.06***
SDMA	0.39±0.04	0.66±0.05***	0.45±0.05	0.93±0.14**	0.40±0.04	2.27±0.17***,[a]
Urea	4.06±0.41	5.29±0.38	4.77±0.42	9.76±1.71*	4.28±0.40	24.2±2.2***,[a]

no–60 ml/min/1.73 m^2, GFR range from normal to 60 ml/min/1.73 m^2; comparisons were performed by means of a *t*-test.
ADMA, asymmetrical dimethylarginine; Arg, arginine; ArgA, argininic acid; CT, creatine; CTN, creatinine; G, guanidine; GAA, guanidinoacetic acid; GSA, guanidinosuccinic acid; γ-GBA, γ-guanidinobutyric acid; β-GPA, β-guanidinopropionic acid; Harg, homoarginine; αKetoδGVA, α-keto-δ-guanidinovaleric acid; MG, methylguanidine; α-NAA, α-*N*-acetylarginine; SDMA, symmetrical dimethylarginine.
*$P<0.05$; **$P<0.01$; ***$P<0.001$.
[a]When the normality of data distribution failed, a Mann–Whitney rank sum test was run.

Table 1b | GCs (μmol/24 h) and urea (mmol/24 h) in urine of PKD patients and controls

Urine	Controls n=8	GFR: no-60 ml/min/1.73 m^2 n=8	controls n=7	GFR: 20–60 ml/min/1.73 m^2 n=7	controls n=10	GFR: <20 ml/min/1.73 m^2 n=10
αKetoδGVA	13.0±1.71	8.28±1.72	11.2±2.71	10.2±2.23	10.1±1.11	3.91±0.68***
GSA	29.6±2.6	29.3±4.6	28.9±2.12	53.6±10*	29.4±4.4	150±24***
CT	842±375	674±227	342±108	624±380	601±274	212±64
GAA	382±73	324±56	305±48	255±72	337±56	38.4±7.1***,[a]
α-NAA	29.2±3.6	23±3.1	29.9±3.5	29.3±8.4	23.8±2.8	12.0±2.0**
ArgA	6.7±0.9	5.3±1.0	7.3±1.5	9.7±2.0	6.0±0.8	4.4±0.9
β-GPA	<DL	<DL-0.3	<DL	<DL-1.7	<DL	<DL-1.4
CTN	10587±1001	7644±1411	11058±1454	12695±3402	9993±881	9465±1195
γ-GBA	9.6±3.1	7.8±1.7	17.5±3.6	14.1±4.9	12.1±3.0	3.71±0.68*
Arg	13±2.7	11±2.2	25±5.3	20±5.9	24±4.4	25±5.4
Harg	<DL-5.8	<DL-12	<DL-2.2	<DL-6.7	<DL-2.9	<DL-2.3
G	13±1.0	10±1.4	13±1.3	20±6.6	11±1.2	18±2.5*
MG	4.2/−0.34	3.7±0.55	8.1±1.9	11±2.8	5.0±0.8	52±8.0***
ADMA	44.4±1.9	53.3±9.5	41.8±7.0	43.9±16	43.2±3.2	9.64±1.7***
SDMA	38.6±2.4	50.4±9.1	39.8±4.7	65.0±23	37.2±2.5	31.5±4.8
Urea	284±35	233±34	308±36	367±65	277±42	262±37

no-60 ml/min/1.73 m^2, GFR range from normal to 60 ml/min/1.73 m^2; comparisons were performed by means of a t-test.
ADMA, asymmetrical dimethylarginine; Arg, arginine; ArgA, argininic acid; CT, creatine; CTN, creatinine; DL, detection limit; G, guanidine; GAA, guanidinoacetic acid; GSA, guanidinosuccinic acid; γ-GBA, γ-guanidinobutyric acid; β-GPA, β-guanidinopropionic acid; Harg, homoarginine; αKetoδGVA, α-keto-δ-guanidinovaleric acid; MG, methylguanidine; α-NAA, α-*N*-acetylarginine; SDMA, symmetrical dimethylarginine.
*P<0.05; **P<0.01; ***P<0.001.
[a]When the normality of data distribution failed, a Mann–Whitney rank sum test was run.

Table 2a | GCs (μM) and urea (mM) in plasma of homozygous PKD/Mhm rats and controls

Age	15 days		20 days		23 days	
Genotype	CO n=11	*HO n=11*	*CO n=11*	HO n=11	CO n=12	HO n=12
αKetoδGVA	0.17±0.01	0.68±0.07***,[a]	0.23±0.03	1.31±0.16***	0.37±0.03	0.83±0.06***
GSA	0.09±0.01	3.03±1.4***,[a]	0.09±0.01	8.23±2.4***,[a]	0.07±0.01	19.2±1.2***,[a]
CT	236±18	288±29	162±11	123±17	190±14	132±17*
GAA	5.80±0.22	7.66±0.71*,[a]	5.97±0.31	5.39±0.61	5.53±0.32	2.27±0.22***
α-NAA	2.12±0.08	1.81±0.16	2.13±0.08	2.01±0.15	1.92±0.12	1.75±0.10
ArgA	<0.007–0.08	0.10±0.01	0.06±0.00	0.13±0.01***	<0.007–0.08	0.20±0.02
β-GPA	0.05±0.00	0.06±0.01	0.04±0.00	<0.007-0.07	<0.007–0.07	<0.007–0.03
CTN	15.3±0.63	39.2±2.4***	18.6±1.1	79.7±7.3***,[a]	13±0.55	163±11***,[a]
γ-GBA	0.21±0.03	0.51±0.10*	0.47±0.04	1.22±0.23***,[a]	1.07±0.14	2.32±0.32**
Arg	205±5.2	225±16	218±10	163±8.8***	209±11	111±6.1***
Harg	7.85±0.49	7.70±0.77	7.76±0.20	5.50±0.56***	3.80±0.45	2.41±0.41*
G	<0.03-0.6	0.45±0.08	0.14±0.01	0.79±0.09***	0.29±0.02	1.26±0.05***
MG	<0.01	<0.01–0.1	0.05±0.00	0.19±0.04***	<0.01	0.65±0.08
ADMA	1.49±0.07	1.77±0.07**	1.60±0.03	1.53±0.04	1.50±0.07	1.23±0.07*
SDMA	0.69±0.04	2.12±0.08***	0.88±0.03	2.67±0.14***	0.60±0.05	3.68±0.09***
Urea	5.35±0.16	39.7±6.8***,[a]	4.85±0.17	53.5±5.6***,[a]	5.24±0.19	88.5±4.8***,[a]

CO, controls; HO, homozygous.
Full name of the compounds studied can be found in Table 1b.
*P<0.05; **P<0.01; ***P<0.001 difference between genotypes per age group; comparisons were performed by means of a t-test.
[a]When the normality of data distribution failed, a Mann–Whitney rank sum test was run.

Urine. Urinary excretion of GSA and MG was progressively increased in homozygous and heterozygous rats (Table 3a and b). Urinary excretion of GAA, however, was progressively decreased in homozygous and heterozygous animals. ADMA and SDMA excretion was decreased in homozygous rats, but increased in oldest heterozygous rats. CTN excretion was increased in youngest heterozygous rats but decreased in homozygous rats toward end-stage renal disease. The excretion of the other GCs had a tendency to decrease in homozygous rats toward end-stage renal failure; in heterozygous rats, however, they tended to be unchanged.

Kidney. In kidneys of homozygous PKD rats, urea, GSA, CTN, and MG increased progressively; α-Keto-δ-GVA was also increased in younger rats (Table 4a and b). Within the studied heterozygous groups, retention of these compounds was mostly seen in kidneys of oldest rats. γ-GBA and to a lesser extent G were increased in kidney tissue of most heterozygous rats, which was not observed in the homozygous group.

Clearly, decreased levels of kidney CT, GAA, β-guanidinopropionic acid (β-GPA), Arg, Harg, and ADMA during renal failure were observed in homozygous rats. Similar observations were made for CT, GAA, β-GPA, and Harg in all heterozygous rats and for Arg only in younger heterozygous rats. A trend for a decrease of ADMA in kidney of heterozygous rats was also seen.

Table 2b | GCs (μM) and urea (mM) in plasma of heterozygous PKD/Mhm rats and controls

Age	2 months		10 months		18 months	
Genotype	CO *n*=6	HE *n*=8	CO *n*=6	HE *n*=7	CO *n*=6	HE *n*=6
αKetoδGVA	0.21±0.02	0.30±0.01**	<0.01-0.2	0.23±0.03	0.13±0.01	0.45±0.12**,a
GSA	0.07±0.01	0.17±0.01***	0.12±0.01	0.32±0.02***	0.15±0.02	5.01±2.6**,a
CT	215±20	204±6.0	506±23	224±17***	409±31	179±73*
GAA	5.2±0.19	5.0±0.16	5.3±0.43	3.9±0.30*	5.6±0.65	2.6±0.64**
α-NAA	1.7±0.12	1.7±0.07	1.5±0.05	1.4±0.05	1.4±0.12	1.7±0.18
ArgA	<0.007	<0.007-0.05	<0.007-0.04	0.03±0.00	<0.007	0.12±0.03
β-GPA	<0.007-0.07	0.07±0.00	0.08±0.01	0.12±0.00**	0.12±0.02	0.16±0.03
CTN	15±0.75	31±0.70***	34±1.8	47±2.0***	34±2.2	165±44*
γ-GBA	0.59±0.06	1.5±0.19**	0.28±0.09	1.1±0.16**	0.32±0.06	3.5±0.46***
Arg	166±4.2	204±4.7***	138±13	170±7.4*	138±11	145±26
Harg	1.6±0.10	1.7±0.06	1.3±0.13	1.0±0.03*	0.84±0.14	0.81±0.11
G	0.27±0.03	0.36±0.02*	<0.03-0.2	0.30±0.02	0.24±0.03	1.29±0.27**
MG	<0.01	<0.01	<0.01	<0.01-0.03	<0.01	0.27±0.14
ADMA	1.09±0.05	0.81±0.03***	0.55±0.04	0.56±0.02	0.56±0.03	0.73±0.12
SDMA	0.54±0.01	0.36±0.01***	0.34±0.04	0.35±0.02	0.39±0.03	0.93±0.17*
Urea	3.9±0.34	9.8±0.37***	6.3±0.44	14±0.46***	5.2±0.35	30±7.7**,a

Full name of the compounds studied can be found in Table 1b.
CO, controls; HE, heterozygous.
*$P<0.05$; **$P<0.01$; ***$P<0.001$ difference between genotypes per age group; comparisons were performed by means of a *t*-test.
[a]When the normality of data distribution failed, a Mann–Whitney rank sum test was run.

Table 3a | GCs (μM) and urea (mM) in urine of homozygous PKD/Mhm rats and controls

Age	15 days		20 days		23 days	
Genotype	CO *n*=11	*HO n=11*	*CO n=11*	HO *n*=10	CO *n*=10	HO *n*=9
αKetoδGVA	13.9±1.6	18.9±2.7	78.8±14	13.6±2.0***	86.3±17	6.23±0.53***
GSA	4.75±0.46	43.5±20***,a	9.54±1.1	48.9±7.5***,a	8.10±1.3	70.3±4.5***
CT	170±37	596±143**	65.7±10	26.4±3.5**	165±103	22.3±2.3
GAA	365±49	141±14***	585±88	31.5±6.3***,a	559±102	10.2±2.2***,a
α-NAA	14.9±1.8	23.9±2.2**	27.9±5.4	14.4±1.2*	36.6±6.3	7.21±1.2***
ArgA	0.58±0.08	0.98±0.09**	2.09±0.33	0.86±0.06**	3.78±0.67	0.94±0.07***,a
β-GPA	<DL-0.30	<DL-0.73	<DL-0.4	<DL-0.72	0.34±0.05	<DL-0.86
CTN	909+/+84	882±70	2491±371	898±33**,a	1569±248	951±65*
γ-GBA	21.8±3.1	13.4±1.9*	160±27	17.4±0.85***,a	394±71	18.0±2.5***,a
Arg	26.0±2.4	34.1±7.1	74.4±12	29.4±6.0**	95.7±20	55.1±16
Harg	<DL-1.6	0.48±0.03	2.62±0.30	0.51±0.03***	<DL-3.6	0.53±0.03
G	5.89±0.52	4.31±0.26*	28.7±5.4	6.53±0.71**	38.8±6.6	6.18±0.49***,a
MG	1.44±0.13	3.70±0.46***	2.2±0.5	8.80±0.86***,a	2.01±0.31	15.7±1.3***,a
ADMA	37.0±4.9	3.23±0.44***,a	45.7±8.3	3.00±0.06***,a	9.01±1.3	2.06±0.2***
SDMA	58.0±7.0	14.5±1.1***	90.9±14	14.5±0.7***,a	28.1±5.1	12.2±0.62*
Urea	126±12	232±8.7***	230±27	234±6.8	278±39	242±5.8

Full name of the compounds studied can be found in Table 1b.
CO, controls; DL: detection limit; HO, homozygous.
*$P<0.05$; **$P<0.01$; ***$P<0.001$ difference between genotypes per age group; comparisons were performed by means of a *t*-test.
[a]When the normality of data distribution failed, a Mann–Whitney rank sum test was run.

Liver. As in the kidney, increased levels of urea, GSA, and CTN were also observed in the liver of homozygous and heterozygous rats (Table 5a and b). The increases of α-Keto-δ-GVA, argininic acid, γ-GBA, G, MG, ADMA, and SDMA seen in the liver of homozygous PKD animals were also observed, to a certain extent, in the liver of heterozygous rats. Decreased GAA levels were seen only in the liver of homozygous rats with end-stage renal failure. The other studied GC levels in the liver of homozygous and heterozygous rats are similar to those observed in controls.

Brain. Increase of urea and GSA with renal failure was also present in brains of homozygous and heterozygous animals (Table 6a and b). Brain concentration of SDMA increased progressively with kidney dysfunction in homozygous rats. On the other hand, it should be stressed that the ADMA levels of the homozygous and heterozygous rats were not significantly higher than those found in control rats. In contrast to the kidney and liver, brain CTN concentration was unchanged in homozygous and decreased with renal failure in heterozygous rats. Brain concentration of Arg was decreased in homozygous rats with end-stage renal failure and oldest heterozygous rats. The levels of the other GCs found in homozygous and heterozygous rats are comparable (metabolically) with corresponding control levels.

Table 3b | GCs (μmol/24 h) and urea (mmol/24 h) in urine of heterozygous PKD/Mhm rats and controls

Age	2 months		10 months		18 months	
Genotype	CO *n*=6	HE *n*=8	CO *n*=6	HE *n*=7	CO *n*=6	HE *n*=5
αKetoδGVA	0.98±0.08	0.55±0.03***	0.81±0.07	0.55±0.10	0.71±0.15	0.44±0.12
GSA	0.15±0.01	0.44±0.02***	0.40±0.03	0.93±0.05***	0.40±0.07	1.58±0.29**
CT	0.89±0.08	1.13±0.08	2.51±0.74	1.62±0.17	3.60±2.1	1.10±0.07
GAA	8.86±0.88	5.14±0.37***	5.34±0.96	4.01±0.28	5.64±1.3	1.65±0.33*
α-NAA	0.77±0.15	1.31±0.04**	1.32±0.20	1.29±0.07	0.82±0.26	0.93±0.11
ArgA	0.06±0.00	0.07±0.00*	0.07±0.01	0.06±0.01	0.04±0.01	0.09±0.01***
β-GPA	0.02±0.00	0.03±0.00**	0.07±0.01	0.06±0.00	0.05±0.01	0.03±0.00
CTN	37.6±2.4	69.9±1.7***	136±7.9	163±7.9*	129±16	138±3.7
γ-GBA	6.42±0.31	6.02±0.12	5.98±0.76	5.72±0.36	5.23±0.90	4.72±0.27
Arg	1.30±0.17	2.03±0.37	0.60±0.12	0.77±0.13	0.67±0.14	0.62±0.03
Harg	0.03±0.01	0.01±0.00*	<DL-0.05	0.02±0.00	<DL-0.06	<DL
G	1.13±0.06	1.41±0.02***	0.67±0.10	1.08±0.12*	0.65±0.12	1.29±0.11**
MG	0.05±0.00	0.18±0.00***	0.09±0.01	0.18±0.01***	0.09±0.01	0.47±0.10**
ADMA	0.008± 0.003	0.013±0.001*,a	0.03±0.00	0.06±0.01*	0.04±0.005	0.22±0.05**
SDMA	0.16±0.04	0.22±0.01	0.08±0.01	0.44±0.04***	0.08±0.03	0.51±0.04***
Urea	6.13±0.45	9.82±0.24***	10.2±0.65	11.8±0.82	7.71±1.1	12.8±0.96**

Full name of the compounds studied can be found in Table 1b.
CO, controls; DL: detection limit; HE, heterozygous.
*$P<0.05$; **$P<0.01$; ***$P<0.001$ difference between genotypes per age group; comparisons were performed by means of a *t*-test.
[a]When the normality of data distribution failed, a Mann–Whitney rank sum test was run.

Table 4a | GCs (nmol/g tissue) and urea (μmol/g tissue) in kidney of homozygous PKD/Mhm rats and controls

Age	15 days		20 days		23 days	
Genotype	CO *n*=8	HO *n*=11	CO *n*=8	HO *n*=11	CO *n*=8	HO *n*=12
αKetoδGVA	1.47±0.12	13.1±0.79***	1.69±0.22	9.71±0.36***	4.87±0.59	6.78±1.17
GSA	0.95±0.04	23.3±8.0***,a	0.70±0.09	35.1±7.5***,a	0.88±0.08	47.5±2.9***,a
CT	1473±65	474±48***	864±32	208±10***	969±48	197±15***
GAA	299±16	61±6.9***	275±9.0	22.2±2.2***	257±28	12.1±1.2***a
α-NAA	24.4±0.82	16.7±1.3***	18.8±1.5	14.4±0.61*	23.3±1.1	8.47±1.0***
β-GPA	1.85±0.08	0.41±0.07***	2.10±0.08	0.45±0.09***	2.35±0.11	0.41±0.08***
CTN	154±5.1	385±40***	206±12	505±16***	112±6.5	519±28***
γ-GBA	3.27±0.37	5.61±0.96	10.5±0.80	8.20±0.42*	21.7±1.7	8.64±1.3***
Arg	415±18	110±4.3***	562±31	99±2.0***,a	515±26	93±6.3***
Harg	8.25±0.57	2.19±0.13***	9.60±0.36	1.99±0.13***	5.22±0.61	1.27±0.18***
G	2.72±0.75	2.19±0.29	2.01±0.08	2.83±0.12***	4.16±0.44	3.48±0.27
MG	<DL	1.38±0.19	0.37±0.05	4.28±0.64***,a	<DL	6.17±0.35
ADMA	6.44±0.45	1.91±0.07***	11.0±1.7	2.12±0.15***,a	7.94±0.83	1.43±0.09***,a
SDMA	6.16±0.38	7.77±0.49*	9.88±0.70	10.3±0.50	6.74±0.37	7.61±0.64**
Urea	19.8±1.9	52.2±8.8**	16.7±1.6	64.0±6.0***,a	30.4±3.8	87.1±4.5***

ArgA could not be determined in kidney as its concentration was around detection limit; comparisons were performed by means of a *t*-test.
Full name of the compounds studied can be found in Table 1b.
CO, controls; DL: detection limit; HO, homozygous.
*$P<0.05$; **$P<0.01$; ***$P<0.001$ difference between genotypes per age group.
[a]When the normality of data distribution failed, a Mann–Whitney rank sum test was run.

DISCUSSION

Patients with end-stage renal failure show altered GC concentrations that probably contribute to their symptoms.[4,11,12,27] This is the first study evaluating concentrations of GCs in a population of renal insufficient individuals consisting exclusively of PKD patients. In addition, alterations of GC concentrations were studied for the first time in a rat model for PKD and compared with the observations in patients. As seen in humans with PKD, the PKD rat model shows loss of renal function as a consequence of renal cyst formation.[22]

Our observations in patients with PKD largely confirm previous findings in patients with uremia due to different etiologies.[12] The increase of GSA to about 39 times in PKD patients' serum is comparable to the 38 times GSA increase found by Marescau *et al.*[12] Further, also the range of MG, G, CTN, α-NAA, ADMA, and SDMA increases reported by those authors were confirmed in the PKD patients studied here. The significant decrease of serum GAA reported in the uremic patient group including different etiologies[12] was also seen in the serum of the PKD patients studied here.

Urea and GSA, which is metabolically related to urea,[28–30] were shown to be important retention solutes in the PKD rat model. Their concentrations increased progressively with renal failure in plasma and all tested tissues from homozygous rats and in most heterozygous rats. The decreased

Table 4b | GCs (nmol/g tissue) and urea (μmol/g tissue) in kidney of heterozygous PKD/Mhm rats and controls

Age	2 months		10 months		18 months	
Genotype	CO *n*=6	HE *n*=8	CO *n*=6	HE *n*=7	CO *n*=6	HE *n*=6
αKetoδGVA	3.45±0.22	1.12±0.08***	1.86±0.42	2.38±0.39	1.72±0.13	7.78±1.4***
GSA	0.93±0.07	1.20±0.04**	1.49±0.20	2.62±0.36*	1.42±0.12	16.2±4.5**,a
CT	1299±129	806±24***	4081±581	532±14***	2933±375	634±131***
GAA	265±16	106±7.2***	257±5.5	81.4±3.2***	243±14	39.0±13***
α-NAA	12.2±0.43	6.20±0.40***	6.94±0.88	7.76±1.04	5.10±1.35	15±2.91*
β-GPA	4.34±0.19	2.78±0.14***	9.23±0.74	2.66±0.13***	10.1±1.3	1.72±0.15***
CTN	186±11	186±8.1	405±72	307±40	328±19	686±90**
γ-GBA	12.8±1.3	20.1±1.4**	7.44±2.4	19.1±2.2**	7.55±2.3	35.6±6.3**
Arg	583±35	331±46**	436±23	262±25***	434±39	472±69
Harg	3.59±0.21	2.28±0.09***	3.43±0.40	1.42±0.10***	2.73±0.56	1.10±0.13*
G	4.23±0.61	3.59±0.30	2.41±0.33	4.73±0.46**	3.67±0.62	5.28±0.35*
MG	<DL-0.23	0.18±0.01	0.29±0.04	0.36±0.06	0.28±0.04	3.65±1.00**
ADMA	9.30±0.58	3.76±1.2**	3.52±0.35	2.22±0.54	4.63±0.84	2.34±0.16*
SDMA	5.50±0.46	2.09±0.20***	1.72±0.25	1.62±0.13	2.34±0.44	4.56±0.68*
Urea	19.9±2.9	22.1±1.3	24.9±4.3	24.5±2.4	26.6±3.3	40.2±6.1

ArgA could not be determined in kidney as its concentration was around detection limit; comparisons were performed by means of a *t*-test.
Full name of the compounds studied can be found in Table 1b.
CO, controls; DL: detection limit; HE, heterozygous.
*$P<0.05$; **$P<0.01$; ***$P<0.001$ difference between genotypes per age group.
[a]When the normality of data distribution failed, a Mann–Whitney rank sum test was run.

Table 5a | GCs (nmol/g tissue) and urea (μmol/g tissue) in liver of homozygous PKD/Mhm rats and controls

Age	15 days		20 days		23 days	
Genotype	CO *n*=8	HO *n*=11	CO *n*=8	HO *n*=11	CO *n*=8	HO *n*=12
αKetoδGVA	<DL-0.3	0.76±0.07	0.33±0.02	0.83±0.06***	0.47±0.05	0.89±0.07***
GSA	2.21±0.23	53.3±17***,a	6.35±1.1	82.5±10***,a	4.57±0.32	126±7.4***,a
CT	408±23	497±88	221±18	198±21	262±21	239±43
GAA	16.0±0.84	16.1±1.2	11.1±0.62	10.2±0.68	14.7±2.5	5.08±0.39***
ArgA	<DL-0.2	0.72±0.05	0.27±0.01	1.13±0.10***,a	<DL-0.2	2.14±0.08
β-GPA	9.32±0.43	17.2±1.4***	8.44±0.42	9.68±1.1	12.4±0.93	14.6±1.0
CTN	45.8±2.8	58.7±4.8	23.1±0.69	82.2±11***,a	44.6±4.9	160±8.7***
γ-GBA	52.3±2.8	81.7±13	92.4±9.0	231±25***	266±13	501±65**
Arg	49.6±5.2	33.4±5.6	18.1±0.92	19.6±1.9	21.7±1.8	19.7±2.0
Harg	11.1±0.64	13.9±1.8	9.21±0.47	12.6±1.7	5.36±0.82	7.92±1.3
G	1.48±0.25	10.9±4.6*,a	1.15±0.14	11.4±3.4***,a	1.81±0.48	16.1±5.7***,a
MG	<DL	0.24±0.03	0.26±0.02	0.67±0.15**,a	<DL	2.49±0.23
ADMA	1.02±0.09	2.01±0.23**	1.54±0.11	1.45±0.10	0.95±0.04	1.63±0.09***,a
SDMA	2.49±0.08	6.53±0.59***	1.85±0.06	8.31±0.51***,a	1.27±0.06	16.2±0.66***
Urea	5.31±0.20	27.6±4.7***,a	5.50±0.25	40.8±4.9***,a	5.34±0.17	64.4±3.3***,a

α-NAA could not be determined in liver because of analytical restrictions; comparisons were performed by means of a *t*-test.
Full name of the compounds studied can be found in Table 1b.
CO, controls; DL: detection limit; HO, homozygous.
*$P<0.05$; **$P<0.01$; ***$P<0.001$ difference between genotypes per age group.
[a]When the normality of data distribution failed, a Mann–Whitney rank sum test was run.

removal of GSA as well as an increased synthesis because of increased urea retention are probably involved. Also, CTN was observed as an important retention product, with increased concentrations in the plasma, kidney, and liver of homozygous rats and in most heterozygous rats. Other possibly important retained solutes (although not significantly increased in all tissues) were G, MG, SDMA, and α-Keto-δ-GVA. In addition to decreased MG removal by the kidneys, it is known that the formation of the CTN degradation product MG is favored with a lowered GFR in renal failure.[31] Although the ADMA levels in the liver of homozygous and heterozygous rats were approximately doubled, ADMA was not retained in the plasma, kidney, and brain of the model. Decreased concentrations of Arg and GAA in the kidneys of PKD rats are probably the consequence of their disturbed synthesis, which is known to take place in the proximal tubules of the kidneys.[32,33] Harg, CT, ADMA, and β-GPA were the other solutes decreased in the kidney of PKD rats, which could also be directly or indirectly related to the lowered kidney synthesis.

Can the rat model be used as a biochemical model for human PKD? Increase of urea was observed in plasma and

Table 5b | GCs (nmol/g tissue) and urea (μmol/g tissue) in liver of heterozygous PKD/Mhm rats and controls

Age	2 months		10 months		18 months	
Genotype	CO *n*=6	HE *n*=8	CO *n*=6	HE *n*=7	CO *n*=6	HE *n*=6
αKetoδGVA	<DL-0.5	0.38±0.04	0.30±0.04	0.59±0.05***	<DL-0.4	1.17±0.16
GSA	4.42±0.51	11.1±0.42***	15.3±1.43	23.8±1.5**	12.7±1.3	53.6±14**,a
CT	356±35	324±11	886±70	283±17**,a	726±59	628±399
GAA	8.51±0.57	8.20±0.65	6.58±1.4	10.1±2.1	4.33±0.70	4.63±0.63
ArgA	<DL-0.3	0.53±0.04	<DL-0.2	0.53±0.05	<DL-0.3	1.03±0.25
β-GPA	17.7±1.1	25.4±1.0***	43.1±3.7	38.1±3.3	50.8±6.5	79.5±33
CTN	43.9±2.4	72.0±4.6***	43.9±2.5	76.8±6.2***	63.3±6.4	179±46*
γ-GBA	106±11	163±11**	51.7±15	228±15***	71.8±28	816±182**
Arg	13.9±1.6	11.7±1.2	10.4±1.1	14.4±1.2*	12.1±1.0	16.5±2.3
Harg	2.63±0.19	3.51±0.17**	3.90±0.32	2.68±0.19**	2.23±0.24	2.07±0.22
G	3.38±1.4	7.59±1.6	1.61±0.16	5.74±2.4**,a	1.44±0.19	4.23±0.99*
MG	<DL	<DL	<DL	<DL	<DL	<DL-3.8
ADMA	0.80±0.08	1.95±0.16***	0.85±0.09	2.65±0.38**	1.06±0.24	2.30±0.38*
SDMA	1.25±0.19	1.91±0.10**	2.06±0.29	4.97±1.3	2.16±0.26	4.17±1.1
Urea	4.33±0.27	8.39±0.28***	6.05±0.26	11.6±0.83***	5.21±0.32	22.8±6.0**,a

α-NAA could not be determined in the liver because of analytical restrictions; comparisons were performed by means of a *t*-test.
Full name of the compounds studied can be found in Table 1b.
CO, controls; DL: detection limit; HE, heterozygous.
*$P<0.05$; **$P<0.01$; ***$P<0.001$ difference between genotypes per age group.
[a]When the normality of data distribution failed, a Mann–Whitney rank sum test was run.

Table 6a | GCs (nmol/g tissue) and urea (μmol/g tissue) in brain of homozygous PKD/Mhm rats and controls

Age	15 days		20 days		23 days	
Genotype	CO *n*=8	HO *n*=11	CO *n*=11	HO *n*=11	CO *n*=8	HO *n*=12
GSA	0.22±0.02	1.27±0.50***,a	0.36±0.03	1.84±0.47***,a	0.21±0.01	2.89±0.26***
CT	7545±134	7869±373	7148±211	8170±333*,a	8729±157	9071±162
GAA	5.41±0.37	4.75±0.39	5.18±0.20	3.52±0.21***	4.09±0.25	4.03±0.20
α-NAA	2.31±0.18	2.95±0.21*	2.30±0.08	2.82±0.30	5.15±0.29	2.19±0.10***
ArgA	0.23±0.01	0.37±0.04**	0.29±0.02	0.43±0.02***	0.61±0.04	0.52±0.02*
β-GPA	0.40±0.03	0.37±0.02	0.39±0.01	0.34±0.02	0.45±0.03	0.34±0.02**
CTN	81.7±12	68.8±10	92.2±8.1	75.4±6.5	87.7±8.7	86.2±12
γ-GBA	18.6±2.4	21.9±0.45	19.5±0.83	20.3±0.85	22.9±1.7	19.0±0.57*
Arg	172±11	162±9.1	170±3.7	163±5.1	293±9.6	149±11***
Harg	5.30±0.49	6.05±0.55	5.60±0.18	5.86±0.72	4.62±0.53	3.30±0.46
G	<DL-1.8	3.04±1.2	0.54±0.03	2.63±0.66*,a	<DL-5.3	6.46±2.3
MG	<DL	<DL-0.2	0.32±0.06	<DL-0.2	<DL	0.31±0.05
ADMA	0.54±0.03	0.58±0.03	0.55±0.02	0.47±0.03*	0.45±0.04	0.42±0.05
SDMA	0.85±0.06	1.52±0.06***	0.65±0.03	2.19±0.16***	0.56±0.04	3.79±0.21***
Urea	4.48±0.19	26.4±4.6***,a	4.54±0.31	38.7±4.0***,a	4.43±0.09	58.8±2.8***,a

α-Keto-δ-GVA could not be determined in brain because of coelution of an unknown compound; comparisons were performed by means of a *t*-test.
Full name of the compounds studied can be found in Table 1b.
CO, controls; DL: detection limit; HO, homozygous.
*$P<0.05$; **$P<0.01$; ***$P<0.001$ difference between genotypes per age group.
[a]When the normality of data distribution failed, a Mann–Whitney rank sum test was run.

tissues of PKD rats. Range of increase of urea in plasma of 15-day-old homozygous (7×) and heterozygous (2–6×) rats was comparable with increase in serum of patients with a GFR below 60 ml/min/1.73 m^2 (2–6×). Increase of GSA was obvious in plasma, urine, and tissues of homozygous and heterozygous animals and was also observed in serum and urine of PKD patients. GSA increased about 33 times in the plasma of youngest homozygous and oldest heterozygous rats, which was similar to the 39 times increase of GSA in serum of patients with GFR below 20 ml/min/1.73 m^2. The increase of plasma GSA seen in heterozygous rats aged 2 and 10 months was in the same order of magnitude as the increase in serum of patients with a GFR between 20 and 60 ml/min/1.73 m^2. CTN increase in the plasma, kidney, and liver of homozygous and heterozygous PKD rats was also present in patients' serum. The 2 and 8 times increase of CTN in serum of patients with a GFR of 20–60 and <20 ml/min/1.73 m^2, respectively, was comparable to the increase in plasma of heterozygous and homozygous rats (2× to 12×). Increased MG and G in homozygous and heterozygous rats was observed in serum of patients. Increased SDMA values in the plasma, liver, and brain of homozygous and oldest heterozygous rats were also seen in serum of patients, and degrees of increase in blood were in the same order of

Table 6b | GCs (nmol/g tissue) and urea (μmol/g tissue) in brain of heterozygous PKD/Mhm rats and controls

Age	2 months		10 months		18 months	
Genotype	CO n=6	HE n=8	CO n=6	HE n=7	CO n=6	HE n=6
GSA	0.11±0.00	0.25±0.01***	0.10±0.01	0.20±0.01***	0.12±0.01	0.71±0.26**,a
CT	8891±184	9695±205*	9620±208	8614±319*	9231±245	7112±91***
GAA	3.26±0.10	2.90±0.09*	4.40±0.82	3.97±0.16	3.11±0.20	6.63±0.79**
α-NAA	3.88±0.26	3.49±0.08	2.03±0.13	8.53±0.43***	2.42±0.23	1.69±0.08*
ArgA	1.19±0.10	1.15±0.05	1.44±0.17	1.64±0.07	1.70±0.16	1.49±0.05
β-GPA	0.51±0.04	0.32±0.02***	0.43±0.04	0.54±0.03	0.63±0.04	0.67±0.08
CTN	108±8.2	66.0±5.3***	90.0±9.0	56.0±2.9**	177±21	52.7±5.8***
γ-GBA	11.7±1.1	10.9±0.22	7.58±0.66	9.28±0.16*	8.66±0.60	10.7±0.70*
Arg	215±5.6	231±25	160±8.7	135±5.1*	191±9.5	123±23*
Harg	1.61±0.10	1.57±0.05	1.012±0.086	1.009±0.088	0.84±0.08	0.66±0.06
G	1.86±1.2	5.67±2.0*,a	0.52±0.05	2.71±1.1**,a	1.89±0.97	1.34±0.40
MG	<DL-0.24	<DL-0.33	<DL-0.22	0.10±0.01	0.25±0.02	0.18±0.05
ADMA	0.28±0.03	0.28±0.03	0.17±0.01	0.17±0.03	0.20±0.02	0.16±0.03
SDMA	0.44±0.10	0.33±0.03	0.25±0.01	0.29±0.01*	0.33±0.03	0.61±0.16
Urea	2.62±0.16	6.30±0.32***	3.02±0.11	6.26±0.48***	2.97±0.29	10.8±2.8**,a

α-Keto-δ-GVA could not be determined in brain because of coelution of an unknown compound; comparisons were performed by means of a t-test.
Full name of the compounds studied can be found in Table 1b.
CO, controls; DL: detection limit; HE, heterozygous.
*P<0.05; **P<0.01; ***P<0.001 difference between genotypes per age group.
[a]When the normality of data distribution failed, a Mann–Whitney rank sum test was run.

Table 7 | GCs (μM) and urea (mM) in serum of non-dialyzed PKD patients with a GFR <20 ml/min/1.73 m^2 and in dialyzed PKD patients

Serum	Non-dialyzed PKD patients with GFR <20 ml/min/1.73 m^2 n=11	Dialyzed PKD patients n=12
αKetoδGVA	0.22±0.03	0.38±0.06
GSA	11.0±1.8	9.96±1.5
CT	34.4±9.4	50.1±12.4
GAA	1.83±0.19	2.08±0.23
α-NAA	0.88±0.12	1.43±0.15
ArgA	0.28±0.04	0.393±0.09
β-GPA	<0.013	<0.013-0.05
CTN	519±66	854±76
γ-GBA	<0.013-0.1	0.12±0.02
Arg	114±9.9	105±9.5
Harg	1.16±0.11	0.92±0.14
G	1.34±0.16	1.7±0.1
MG	1.01±0.46	3.4±0.7
ADMA	0.85±0.06	0.77±0.05
SDMA	2.27±0.17	2.86±0.27
Urea	24.2±2.2	18.5±1.0

Full name of the compounds studied can be found in Table 1b.

magnitude. The increases of blood α-Keto-δ-GVA and γ-GBA levels observed in the rat model were seen, as well in the studied patients. The significantly decreased concentration of plasma GAA seen at end-stage renal disease in homozygous and oldest heterozygous rats was also observed in serum of patients with lowest GFR. Moreover, decreased excretion of GAA observed in homozygous and heterozygous rats did correspond with decreased excretion of GAA in patients with lowest GFR. Furthermore, the serum Harg levels seen in the PKD patient group decreased progressively with decreasing GFR, similar to the gradual decrease of the Harg plasma levels found in homozygous and heterozygous rats. It should also be stressed that PKD patients with renal insufficiency to a degree comparable to that of homozygous rats of 23 days (just before dying) were not included into our study group as end-stage renal failure patients are usually dialyzed at this stage. This could be the reason why the significantly decreased concentration of Arg and CT seen in plasma of homozygous rats at end-stage renal failure was not observed in the PKD patients studied here. Furthermore, differences between rat and human metabolism have also to be kept in mind and can possibly be related to the consistently increased levels of ADMA seen in serum of PKD patients, which were not observed in plasma of homozygous and heterozygous rats.

Although not being the goal of this study (comparison of the GC profile of a PKD rat model with the human condition), the serum GC levels observed in dialyzed PKD patients, just before a new dialysis session, are comparable to those found in our non-dialyzed PKD patient group with a GFR below 20 ml/min/1.73 m^2 (Table 7). However, this does not implicate that the retained GCs would not further increase when the patients would not be dialyzed.

In general, increases of urea and some GCs (GSA, CTN, G, MG, and SDMA) in blood of oldest heterozygous and younger homozygous PKD rats were comparable with those found in the studied human PKD population, especially in patients with GFR below 60 ml/min/1.73 m^2. Decreased levels of plasma GAA seen at end-stage renal disease in homozygous and oldest heterozygous rats were also observed in serum of patients with GFR below 20 ml/min/1.73 m^2.

Previously, our group reported GC levels in a surgical rat model (80% nephrectomy) for renal failure.[17,19] The degree of increases (urea, GSA, CTN, G, MG, SDMA, α-Keto-δ-GVA, and γ-GBA) seen in plasma of this surgical model is

similar to the one observed in plasma of older (10–18 months) heterozygous PKD rats. Referring to the decreased CT, GAA, Arg, and Harg levels in plasma of our PKD rat model, only the levels of CT were decreased in the nephrectomized rat model.[17] On the other hand, decreased GAA levels in blood have been described in a nephrectomized mouse[18] and rabbit[34] model for renal failure.

In conclusion, PKD patients show GC concentration changes similar to those found in patients with uremia owing to different etiologies. The PKD rat model shows, besides similar disease characteristics with PKD patients, comparable GC alterations. GC concentration changes in older heterozygous rats and younger homozygous rats were largely within the same range of those seen in humans with PKD. Studying the progression of pathobiochemistry of GCs in PKD in a relative short time is possible in homozygous rats; however, a slower progression of this pathobiochemistry can be seen in heterozygous rats.

MATERIALS AND METHODS

Clinical protocols were approved by the Institutional Review Boards. All patients gave their written informed consent according to the Declaration of Helsinki Principles. Animal experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC) and the protocol was approved by the Animal Ethics Committee of the University of Antwerp.

Sample collection and preparation

Patients. Fasting morning blood and 24 h urine was collected from non-dialyzed patients with PKD. Blood was centrifuged ($1000 \times g$, 4°C, 10 min) to obtain serum. Patients were divided into three different groups: GFR from normal to 60 ml/min/1.73 m^2 (mean ± standard error of the mean (s.e.m.): 105 ± 11 ml/min/1.73 m^2), including two male and seven female patients with age ranging from 31 to 50 years; GFR from 20 to 60 ml/min/1.73 m^2 (mean ± s.e.m.: 43 ± 5.7 ml/min/1.73 m^2), including three male and four female patients with age ranging from 48 to 65 years; and finally, GFR <20 ml/min/1.73 m^2 (mean ± s.e.m.: 12 ± 1.5 ml/min/1.73 m^2), including eight male and three female patients with age ranging from 37 to 71 years. Four patients had a GFR lower than 10 ml/min/1.73 m^2. GFR was calculated with the abbreviated Modification of Diet in Renal Disease Study equation presented by Levey *et al.*[35] and Levy.[36] For each group, age- and gender-matched control subjects were included. Mean ± s.e.m. GFR values of the used control groups were, respectively, 130 ± 13, 102 ± 11, and 116 ± 9 ml/min/1.73 m^2. Control subjects were healthy volunteers and individuals presenting with transient neurologic complaints in whom after performing clinical and chemical diagnostic tests, no neurologic, renal, hepatic, or metabolic disease was diagnosed.

Rat model. A Sprague–Dawley rat model of PKD (Han:SPRD), obtained from a breeding colony from Dr F Deerberg (Central Institute for Laboratory animal breeding, Hannover, Germany) and maintained by Dr Gretz (Medical Research Center, Mannheim, University of Heidelberg),[21,22] was used. Since 1997, the strain is registered in the list of inbred strains of rats by MFW Festing as PKD/Mhm (http://www.informatics.jax.org/external/festing/rat/docs/PKD.shtml). Groups were aged 15, 20, and 23 days for the homozygous animals and 2, 10, and 18 months for the heterozygous PKD rats. Homozygous groups consisted of males as well as females and heterozygous groups consisted of male rats only as they manifest a significantly more severe form of the disease than females.[21,24] For each group, a comparable amount of Sprague–Dawley control rats with the same age and sex were included. All animals had free access to tap water and were kept on a same standard laboratory diet (ssniff® R/M, Soest, Germany) containing 19% protein. Animals were housed in a room maintained at 20°C with a 12-h light and 12-h dark cycle.

Plasma and 24 h urine, brain, kidney, and liver of all rats were collected. Twenty-four hours before starting the urine collection, one drop of a pentachlorophenol solution (5 mg/ml in 95% ethanol) was added to the collection tube. Urine collection was done after 24 h stay in individual metabolic cages for heterozygous and age-matched control rats. Afterwards, animals were anesthesized (Nembutal: 60 mg/kg) and blood was collected by heart punction in heparinized tubes and centrifuged to obtain plasma. The liver, kidney, and brain were dissected. Free blood has been eliminated as much as possible by washing in ice-cold saline and adsorption by blotting paper. Thereafter, tissue was put immediately in liquid nitrogen and kept together with the other samples at −70°C until analysis. From homozygous and age-matched control animals, urine was taken from the bladder using an injection needle after anesthesia (Nembutal), because they were too young to be kept in metabolic cages.

For determination of GCs, plasma, serum, and urine were deproteinized with 10% trichloroacetic acid and centrifuged for 10 min (20 800 *g*, 4°C). For ADMA and SDMA determination, the plasma, serum, and urine were deproteinized with 10% sulfosalicylic acid and centrifuged for 10 min (20 800 *g*, 4°C). Tissues were homogenized in 30% trichloroacetic acid and centrifuged for 30 min ($20\,800 \times g$, 4°C).

Determination of GCs and urea

Samples were analyzed on a cation exchange liquid chromatograph (Biotronik Amino Acid Analyser (LC5001) Biotronik, Eppendorf, Hamburg, Germany) with post-column ninhydrine fluorescence detection as described earlier.[28] With this method 13 GCs are detected: α-Keto-δ-GVA, GSA, CT, GAA, α-NAA, argininic acid, β-GPA, CTN, γ-GBA, Arg, Harg, G, and MG. ADMA and SDMA were determined with a cation exchange liquid chromatograph (Biotronik Amino Acid Analyser (LC6001) Biotronik, Eppendorf, Hamburg, Germany) with post-column OPA fluorescence detection as described earlier.[12]

Urea was determined using the method of Ceriotti.[37]

Statistics

Results were presented as mean ± s.e.m. Comparison between GC concentrations of PKD patients and age- and gender-matched controls was made with *t*-test using the statistical software program Sigma Stat® version 2.0 (Jandel GmbH, 40699 Erkrath, Germany). Statistical analysis of GC concentrations in rats was also performed with *t*-test comparing controls and PKD/Mhm rats per age group. When the normality test of data distribution failed, a Mann–Whitney rank sum test was run. Correlations between GFR and concentrations of GCs in patients were determined with Pearson's product moment correlation. Compounds with concentrations below the detection limit could not be included in the statistical analysis and correlation studies. A *P*-value of <0.05 was considered statistically significant. Statistical differences are depicted in tables as follows: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

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