PLASMA MOLECULAR SIGNATURES IN HYPERTENSIVE PATIENTS WITH RENIN ANGIOTENSIN SYSTEM SUPPRESSION: NEW PREDICTORS OF RENAL DAMAGE AND *DE NOVO* ALBUMINURIA INDICATORS

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SUPPLEMENTARY MATERIAL (TABLES)

Table S1. Differentially Expressed Proteins Identified by iTRAQ Analysis

	Protein name	Abbreviation	Nº of unique peptides	Zq (dnA/N)	Zq (SA/N)
P01876	Ig alpha-1 chain C region	IGHA1	26	6.916	1.973
P01860	Ig gamma-3 chain C	IGHG3	10	6.026	2.069
P01857	Ig gamma-1 chain C	IGHG1	15	5.927	2.359
P01877	Ig alpha-2 chain C	IGHA2	4	5.24	2.21
P01859	Ig gamma-2 chain C	IGHG2	5	5.17	2.119
P0CG05	Ig lambda-2 chain C	LAC2	6	4.689	1.301
B9A064	Immunoglobulin lambda-like polypeptide 5	IGLL5	11	4.025	0.932
Q96K76	Ubiquitin carboxyl-terminal hydrolase 47	UBP47	2	3.652	0.408
P01834	Ig kappa chain C	IGKC	12	3.256	0.956
Q7RTR0	NACHT, LRR and PYD domains-containing protein 9	NALP9	2	3.032	0.239
Q12756	Kinesin-like protein KIF1A	KIF1A	2	2.957	1.116
P80748	Ig lambda chain V-III region LOI	LV302	2	2.816	1.346
Q9Y619	Mitochondrial ornithine transporter 1	ORNT1	2	2.516	1.888
Q5VT25	Serine/threonine-protein kinase MRCK alpha	MRCKA	3	2.289	0.243
P00739	Haptoglobin-related protein	HPTR	40	2.201	4.312
Q70CQ4	Ubiquitin carboxyl-terminal hydrolase 31	USP31	2	2.197	1.593
Q8N6K4	Putative uncharacterized protein MGC34800	YP021	2	2.149	-0.157
P18031	Tyrosine-protein phosphatase non-receptor type 1	PTPN1	2	2.094	0.79
Q6F5E8	Leucine-rich repeat-containing protein 16C	RLTPR	2	2.076	0.105
Q00610	Clathrin heavy chain 1	CLTC	4	2.055	2.561
Q8WXX5	DnaJ homolog subfamily C member 9	DNJC9	9	2.054	-0.195
Q8NCT1	Arrestin domain-containing protein 4	ARRDC4	2	1.339	2.105
P02656	Apolipoprotein C-III	APOC3	10	0.869	2.934
P02655	Apolipoprotein C-II	APOC2	11	0.615	2.928
P52945	Pancreas/duodenum homeobox protein 1	PDX1	4	-0.708	-4.022
P37837	Transaldolase	TALDO1	2	-0.715	-3.003
Q8IVF2	Protein AHNAK2	AHNAK2	5	-1.082	-2.237
Q9NY74	Ewing's tumor-associated antigen 1	ETAA1	2	-1.976	-4.207
Q15772	Striated muscle preferentially expressed protein kinase	SPEG	3	-2.257	-1.663
O60503	Adenylate cyclase type 9	ADCY9	2	-2.329	-3.033
Q6NUI6	Chondroadherin-like protein	CHADL	2	-2.38	-0.565
P20742	Pregnancy zone protein	PZP	66	-2.723	-1.455
P21108	Ribose-phosphate pyrophosphokinase 3	PRPS1L1	2	-2.987	-0.146
P04279	Semenogelin-1	SEMG1	2	-3.002	-1.46
Q9Y2R2	Tyrosine-protein phosphatase non-receptor type 22	PTPN22	2	-3.033	-1.239
P02787	Serotransferrin	TF	105	-3.445	-0.768

Zq: mean of log2-ratios expressed in form of the standardized variables for 4 replicates *versus* group of normoalbuminuria. Zq ± 2. p values ≤ 0.05. N: normoalbuminuria; dnA: *de novo* albuminuria; SA: sustained albuminuria

Table S2. Differentially Expressed Proteins Identified by 2D-DIGE experiment.

Position	Spot number	Protein name	Abbreviation	dnA *vs* N		SA *vs* dnA		SA *vs* N	
				Av. Ratio	Anova	Av. Ratio	Anova	Av. Ratio	Anova
1	322	Vitamin D-binding protein	VTDB	-1.4	0.03				
2	767	Complement factor B	CFAB	1.5	0.036				
3	880	Complement factor B	CFAB			-1.4	0.036		
4	996	Coagulation factor XIII B chain	F13B			1.6	0.018		
5	1035	Inter-alpha-trypsin inhibitor heavy chain H4	ITIH4					1.5	0.022
6	1248	Hemopexin	HEMO	-1.3	0.001				
7	1273	Hemopexin	HEMO					-1.4	0.041
8	1484	Kininogen-1	KNG1			1.4	0.033		
9	1493	Kininogen-1	KNG1	-1.5	0.035	1.5	0.023		
10	1565	Alpha-2-HS-glycoprotein	FETUA					3.1	0.011
11	1614	Leucine-rich alpha-2-glycoprotein	A2GL	-1.8	0.018				
12	1659	Leucine-rich alpha-2-glycoprotein	A2GL	-1.6	0.007				
13	1666	Leucine-rich alpha-2-glycoprotein	A2GL					1.6	0.037
14	1687	Leucine-rich alpha-2-glycoprotein	A2GL			2.3	0.041	2.9	0.01
15	1762	Serum paraoxonase/arylesterase 1	PON1					2.7	0.047
16	1789	Serum paraoxonase/arylesterase 1	PON1			2.5	0.016	2.1	0.033
17	1846	Serum amyloid P-component	SAMP			1.5	0.01		
18	1892	Sex hormone-binding globulin	SHBG	1.9	0.006				
19	1980							1.7	0.022
20	2186					1.6	0.007		
21	2196	Complement factor H-related protein 2	FHR2	1.4	0.045				
22	2257					1.7	0.022	1.8	0.013
23	2430			-3.2	0.022				

N: normoalbuminuria; dnA: *de novo* albuminuria; SA: sustained albuminuria

Table S3. Parameters of SRM experiment for 17 target proteins selected for validation phase.

Accesion no.	Protein name	Peptide sequence	parent (m/z) (Q1)	transition (m/z) (Q3)	CE (V)	Dwell time	Fragment Ion
P00751	Complement factor B	EAGIPEFYDYDVALIK	921.96	1099.57	45.6	40	y9
P05160	Coagulation factor XIII B chain	YPLCTR	405.2	436.2	22.8	120	y3
P02790	Hemopexin	NFPSPVDAAFR	610.81	579.29	31.9	40	y5
P01877	Ig alpha-2 chain C region	HYTNPSQDVTVPCPVPPPPPCCHPR	970.45	1214.56	53	200	y10
P01859	Ig gamma-2 chain C region	CCVECPPCPAPPVAGPSVFLFPPKPK	970.14	1384.8	52.5	60	y13
		VVSVLTVVHQDWLNGK	897.5	1296.67	44	60	y11
P01860	Ig gamma-3 chain C region	TPEVTCVVVDVSHEDPEVQFK	805.39	747.4	44.3	60	y6
P01834	Ig kappa chain C region	SGTASVVCLLNNFYPR	899.45	1295.66	45	40	y10
		VYACEVTHQGLSSPVTK	938.47	1253.68	46	40	y12
P80748	Ig lambda chain V-III region LOI	FSGSNSGNTATLTISR	806.9	1033.56	41	40	y10
P27169	Serum paraoxonase/arylesterase	LIALTLLGMGLALFR	801.49	1191.69	40.3	60	y11
P02774	Vitamin D-binding protein	HQPQEFPTYVEPTNDEICEAFR	903.07	1351.59	49	40	y11
		VPTADLEDVLPLAEDITNILSK	789.43	841.39	43.5	40	b8
P02743	Serum amyloid P-component	GYVIIKPLVWV	643.9	854.55	33	60	y7
Q9Y619	Mitochondrial ornithine transporter 1	GLTDCCLK	483.72	260.2	26.3	100	y2
Q6NUI6	Chondroadherin-like protein	EVPTGALEGLPALLELQLSGNPLR	829.8	643.35	45.5	80	y6
P02750	Leucine-rich alpha-2-glycoprotein	YLFLNGNK	484.76	692.37	26	80	y6
		VAAGAFQGLR	495.28	819.45	27	80	y8
Q96K76	Ubiquitin carboxyl-terminal hydrolase 47	QHLEPFVGVLSSHFK	862.96	294.18	43	80	y2
Q7RTR0	NACHT. LRR and PYD domains-containing protein 9	HCQHLTTLR	583.3	389.25	30.7	80	y3
		HLSLVENPLR	589.34	628.34	30.9	80	y5
Q8WXX5	DnaJ homolog subfamily C member 9	ELGLDEGVDSLK	637.83	561.32	33.1	80	y5
		ISLEDIQAFEK	646.84	423.22	33.5	100	y3

Q1 and Q3 (*m/z*) represent the Q1 and Q3 transitions for proteotypic peptide, respectively; CE represents collision energy.

Table S4. Clinical characteristics of the study population for discovery phase.

Variable	N (n=8)	dnA (n=8)	SA (n=8)	P-value
Age (years)	62 ± 6	63 ± 10	60 ± 10	0.806
Male sex (%)	50	50	63	0.845
BMI (kg/m2)	30 ± 4	29 ± 3	30 ± 4	0.957
Current smoking (%)	0	13	25	0.355
Albumin/creatinine (mg/g)	3.6 ± 2	66 ± 36	498 ± 380	0.001**
Creatinine clearance rate (mg/mL)	88 ± 22	105 ± 42	84 ± 40	0.485
eGFR (ml/min/1.73m^2)	72 ± 30	79 ± 24	84 ± 13	0.601
Total cholesterol (mg/dL)	194 ± 27	160 ± 17	188 ± 43	0.083
Triglycerides (mg/dL)	86 ± 22	93 ± 30	136 ± 72	0.089
HDL cholesterol (mg/dL)	56 ± 14	53 ± 17	46 ± 11	0.411
LDL cholesterol (mg/dL)	121 ± 28	84 ± 11	114 ± 33	0.032*
Glycaemia (mg/dL)	100 ± 15	100 ± 11	107 ± 29	0.709
Uric acid (mg/dL)	4.6 ± 1.1	5.9 ± 2	5.9 ± 1.6	0.195
Systolic blood pressure (mmHg)	126 ± 9	134 ± 8	128 ± 16	0.385
Diastolic blood pressure (mmHg)	75 ± 10	81 ± 9	78 ± 13	0.549
ACEi (%)	25	13	13	0.741
ARB (%)	75	87	87	0.741
Diuretic (%)	88	63	50	0.269
Calcium channel blocker (%)	75	25	63	0.113
Beta blocking agent (%)	25	25	38	0.817
Alpha blocking agent (%)	13	50	25	0.244
Anticoagulant agent (%)	0	25	25	0.331
Lipid lowering agents (%)	88	75	75	0.8
Antidiabetic agent (%)	0	13	38	0.133

Values expressed as mean ± SD or percentages (%). BMI: body mass index; eGFR: estimated glomerular filtration rate; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; ACEi: angiotensin converting enzyme inhibitors; ARB: angiotensin receptor blockers. N: normoalbuminuria; dnA: *de novo* albuminuria; SA: sustained albuminuria.

Statistical significance was accepted at *$p < 0.05$, **$p < 0.01$

Table S5. Experimental design iTRAQ

Label	Experiment 1	Experiment 2
113	C1	SA4
114	N1	dnA4
115	dnA1	N4
116	SA1	C4
117	C3	SA2
118	N3	dnA2
119	dnA3	N2
121	SA3	C2

N: normoalbuminuria; dnA: *de novo* albuminuria; SA: sustained albuminuria.

Table S6. Experimental design 2D-DIGE.

Gel	Cy3	Cy5	Cy2
1	C1	N1	Standard
2	dnA1	C2	Standard
3	N2	SA1	Standard
4	SA3	dnA2	Standard
5	C3	SA2	Standard
6	SA4	N3	Standard
7	dnA4	C4	Standard
8	N4	dnA3	Standard

N:normoalbuminuria; dnA: *de novo* albuminuria; SA: sustained albuminuria.