

Supplemental Material

Serum protein biomarkers for estimated GFR decline – validated by iohexol clearance.

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Supplementary Tables

Table S1: Literature searches

Search	Search words	Papers (N)	Selected (N) based on heading and abstract
1	(gfr or "glomerular filtration rate")(biomarker or protein or "risk factor") (renal or kidney or "chronic kidney disease" or tubular) (aging or ageing or progression or incidence) (serum or plasma or urine) NOT (cancer or diabetes or transplant* or hiv or contrast* or "acute kidney injury" or "acute renal failure" or glomerulopathy or nephritis or glomerulonephritis or nephropathy)	391	32
2	(gfr or "glomerular filtration rate" or "kidney disease" or renal or "chronic kidney disease")(proteomics or biomarker or "risk factor")(progression or incidence or decline)(age-related or ageing or aging)(population or cohort or healthy or elderly or middle-aged or "general population") NOT (cancer or transplant* or hiv or contrast* or "acute kidney injury" or "acute renal failure")	307	10
3	(proteomics or biomarker)("kidney disease" or renal or GFR or "glomerular filtration rate" or "chronic kidney disease")(progression or "new onset" or incident or decline or aging or ageing)(serum or plasma or blood) NOT (T1D or ESRD or "type 1 diabetes" or glomerulonephritis or nephritis or polycystic or cancer or "acute kidney injury")	975	47

The three different searches used to find promising biomarkers for GFR decline in the PubMed database from March to April 2018, restricted to the last 5 years. Proteins were analyzed on a Luminex Machine from June to December 2018.

Table S2: Protein intra-and inter-assay % CV

Intra- and inter-assay % CV for the 12 serum proteins in the study measured with Luminex Multiplex BioRad 200, calculated based on two internal controls.

Protein:	Intra- assay % CV	Inter- assay % CV
MCP-1	6,2	4,9
TRAIL-R2	4,5	11,0
FABP4	6,1	8,0
CD40Lig	3,2	6,4
TNFR2	3,0	6,0
GDF-15	2,9	12,9
Tie-2	3,5	6,3
MMP7	3,1	19,3
suPAR	6,4	16,2
MMP2	5,0	6,2
UMOD	4,8	6,0
Gal-3	3,8	6,1

MCP-1: Monocyte Chemoattractant Protein-1, TRAIL-R2: TNF-Related Apoptosis-Inducing Ligand Receptor 2, FABP4: Fatty Acid Binding Protein 4, TNFR-2: Tumor Necrosis Factor Receptor 2, CD40Lig: CD40 Ligand receptor, GDF-15: Growth/Differentiation Factor 15, Tie2: TEK Tyrosin Kinase, MMP7: matrix metalloproteinase 7, suPAR: soluble urokinase-type Plasminogen Activator Receptor, MMP2: matrix metalloproteinase 2, Umod: Uromodulin, Gal-3: Galectin-3.

Table S3: Baseline characteristics

Baseline characteristics of the RENIS cohort and the study population. All 1627 participants at baseline and 1409 with baseline GFR and one or more follow-up GFR measurements included in the paper.

RENIS baseline population and the study population in the paper		
	1627	1409
Participants (n)	1627	1409
Male sex (n)	801 (49%)	696 (49%)
Age (years)	58.7 (54.7 - 61.4)	58.5 (54.5 - 61.4)
Height (cm)	170.6 (8.7)	170.7 (8.7)
Weight (kg)	79.7 (14.4)	79.6 (14.1)
BMI (kg/m ²)	27.3 (4.0)	27.2 (3.9)
mGFR (ml/min/1.73 m ²)	93.9 (14.4)	94.0 (14.3)
eGFR _{cre} (ml/min/1.73 m ²)	94.8 (9.5)	94.9 (9.4)
eGFR _{cys} (ml/min/1.73 m ²)	105.4 (12.4)	105.7 (12.1)
eGFR _{cyscre} (ml/min/1.73 m ²)	103.0 (11.4)	103.2 (11.2)
Urinary ACR (mg/mmol) ^a	0.23 (0.1 - 0.5)	0.2 (0.1 - 0.5)
Systolic BP (mmHg)	130 (18)	129 (17)
Diastolic BP (mmHg)	83 (10)	83 (10)
BP medication (n)	299 (18%)	250 (18%)
RAS- inhibitors	166 (10%)	143 (10%)
Fasting blood glucose (mmol/l)	5.34 (0.55)	5.3 (0.5)
LDL cholesterol (mmol/l)	3.67 (0.86)	3.7 (0.8)
HDL cholesterol (mmol/l)	1.53 (0.42)	1.5 (0.4)
Triglycerides (mmol/l)	1.0 (0.8 - 1.5)	1.0 (0.8 - 1.4)
Daily smoker (n)	1115 (69%)	949 (67%)
Current smoker (n)	344 (21%)	278 (20%)
Previously smoker (n)	771 (48%)	670 (48%)
Never smoker (n)	504 (31%)	456 (32%)
Serum protein biomarkers:		
CD40Lig (ng/ml)	6.77 (2.12)	6.8 (2.1)
GDF-15 (ng/ml)	0.80 (0.43)	0.8 (0.4)
MCP-1 (ng/ml)	0.5 (0.4 - 0.6)	0.5 (0.4 - 0.6)
Tie2 (ng/ml)	16.3 (5.6)	16.2 (5.7)
TRAIL-R2 (ng/ml)	0.03 (0.03 - 0.04)	0.03 (0.03 - 0.04)
FABP4 (ng/ml)	9.6 (6.7 - 13.8)	9.5 (6.6 - 13.8)
MMP7 (ng/ml)	2.01 (0.93)	1.9 (0.80)
suPAR (ng/ml)	0.30 (0.18)	0.3 (0.2)
MMP2 (ng/ml)	315.6 (59.1)	315.3 (58.7)
Umod (ng/ml)	237.3 (94.9)	237.8 (96.0)
Gal-3 (ng/ml)	7.50 (1.96)	7.5 (1.9)
TNFR2 (ng/ml)	2.7 (0.7)	2.7 (0.6)

Abbreviations: RENIS: The Renal Iohexol Clearance Survey, BMI: body mass index, mGFR: measured glomerular filtration rate, eGFR_{cre}/cys/cyscre: estimated GFR based on the CKD-EPI equation for creatinine, cystatin C or both, ACR: albumin to creatinine ratio, BP: blood pressure, RAS: renin-angiotensin system, LDL: low density lipoprotein, HDL: high density lipoprotein, CD40Lig: CD40 ligand receptor, GDF-15: growth/differentiation

factor 15, MCP-1: monocyte chemoattractant protein-1, Tie2: TEK tyrosine kinase, TRAIL-R2: TNF-related apoptosis-inducing ligand receptor 2, FABP4: fatty acid binding protein 4, MMP7: matrix metalloproteinase 7, suPAR: soluble urokinase-type plasminogen activator receptor, MMP2: matrix metalloproteinase 2, Umod: uromodulin, Gal-3: galectin-3, TNFR-2: tumor necrosis factor receptor 2.

Mean (SD) for normally distributed variables, median (IQR) for skewed variables, and number and percentages (%) for categorical variables.

Variables with missing baseline values (n):

The baseline cohort: ACR (5), triglycerides (4), smoke (8), TRAIL-R2 (1), FABP4 (3), TNFR2 (4), MMP7 (16) and suPAR (20).

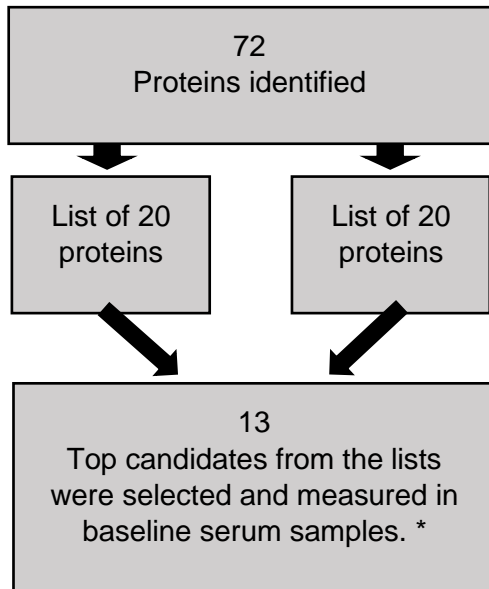
The study population: smoke (5), TRAIL-R2 (1), FABP4 (3), TNFR2 (3), MMP7 (14) and suPAR (18).

^a To convert ACR in mg/mmol to mg/g, multiply by 8.84.

Supplementary Figures
Figure S1: Literature search

Literature Search

Two researchers



*One protein (TNFR2) was already analyzed in an earlier project, thus not analyzed with the Luminex method. Another protein (KIM-1) analyzed with Luminex, were below the limit of detection, thus excluded in the statistical analysis.