The urine proteome/peptidome mirroring kidney health: application in guiding intervention

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Conflict of interest: co-founder and –owner of Mosaiques Diagnostics

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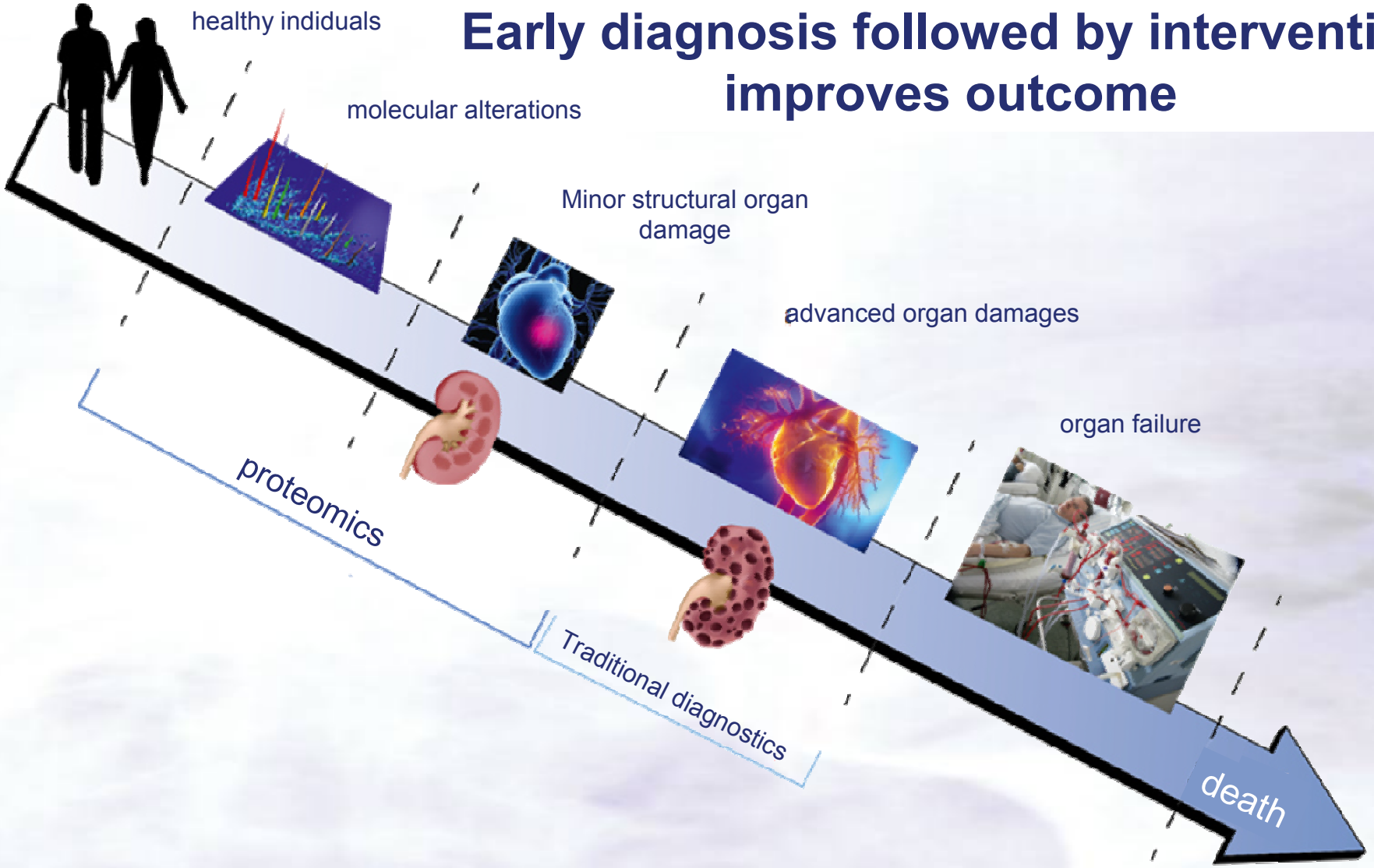
Background

- **Proteins and peptides** are active key players in every organism that enable and control life, normal and pathological development.
- Proteins **are responsible for disease-specific processes**, and are THE target for drugs
- **Knowledge of the Proteome/Peptidome,** the entirety of all proteins/peptides, enables accurate assessment of (patho)physiology on an individual level, in the context of disease enabling optimal and personalized patient management.

Early diagnosis followed by intervention improves outcome



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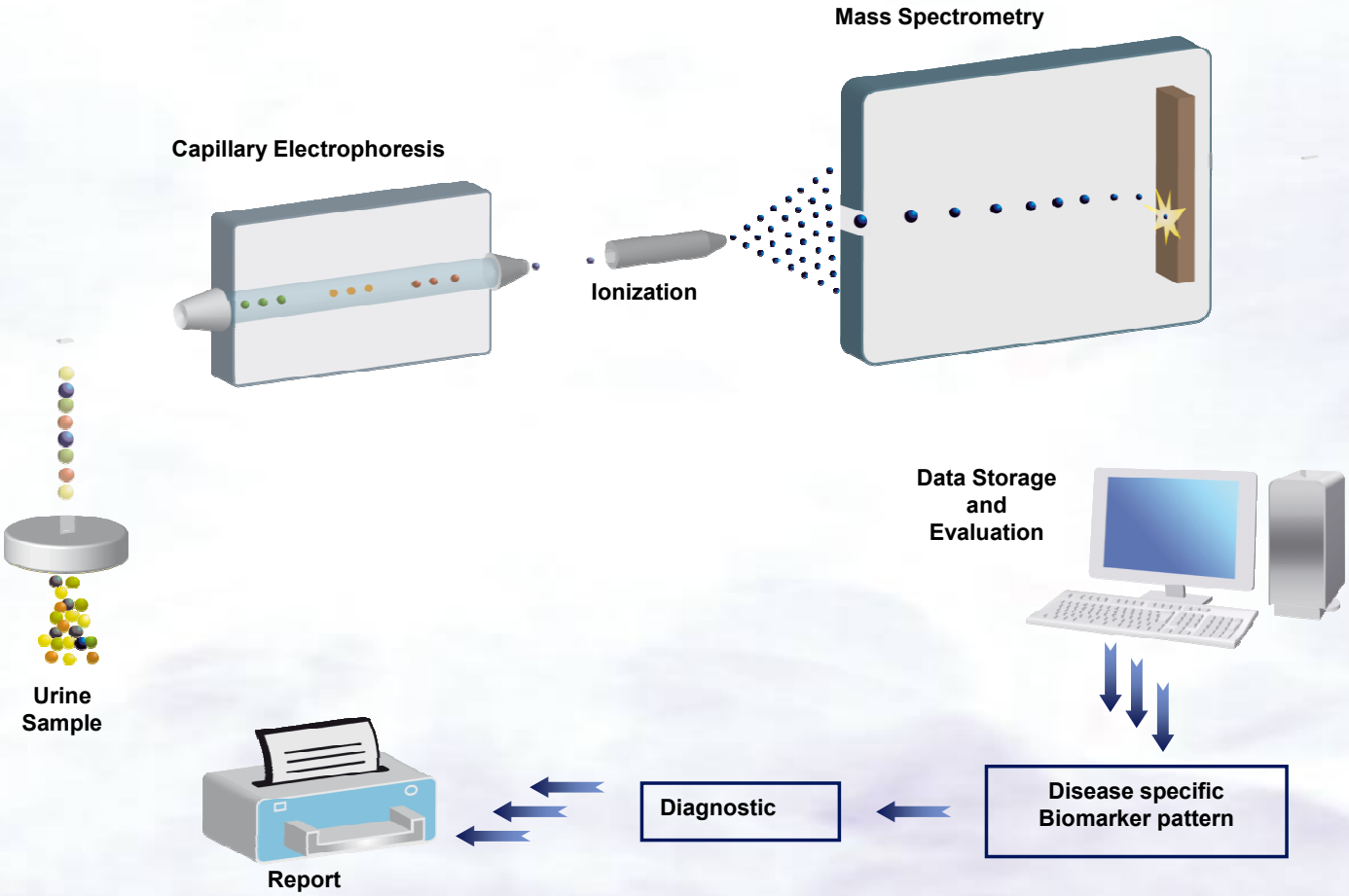
Why urine?

- ✓ Easily accessible
- ✓ Obtained non invasively
- ✓ Available in large quantities
- ✓ Urinary polypeptides are stable, yielding comparable datasets
- ✓ Mirrors the “status” especially of the kidney, the extracellular matrix and the vasculature

Proteomics Technology platform: CE/MS Technology
Capillary Electrophoresis coupled to Mass Spectrometry



Separation and analysis of proteins and peptides (typically 2000-5000)

Run time **~60 min**

CE
- fast
- robust
- inexpensive
- reproducible

MS
- resolution
- scan speed

Pontillo and Mischak. Clin Kidney J. 2017 Apr;10(2):192-201

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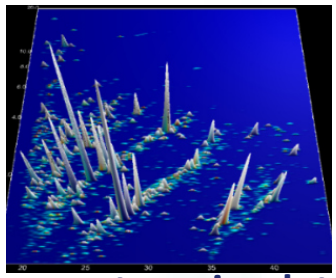
Human urinary proteome database

Clinical data, Patient history

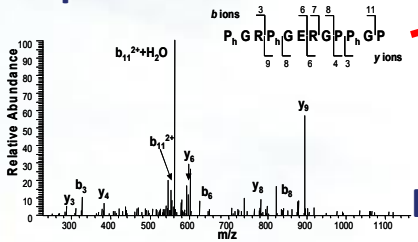
Age
Gender
Urinary albumin/creatinine
Cholesterole (mmol/l)
Creatinine (micromol/l)

Database

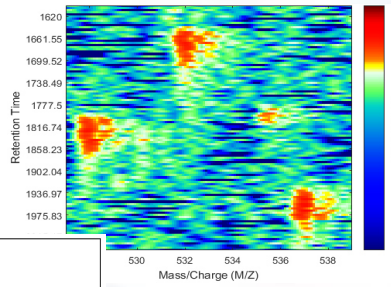
Urinary proteomics data



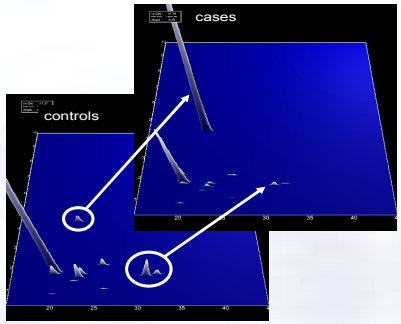
Sequence information

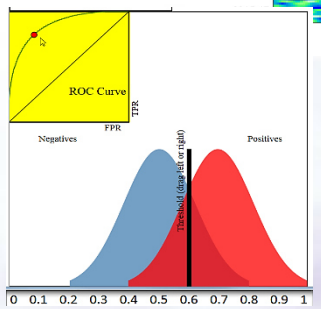


Tissue proteomics data

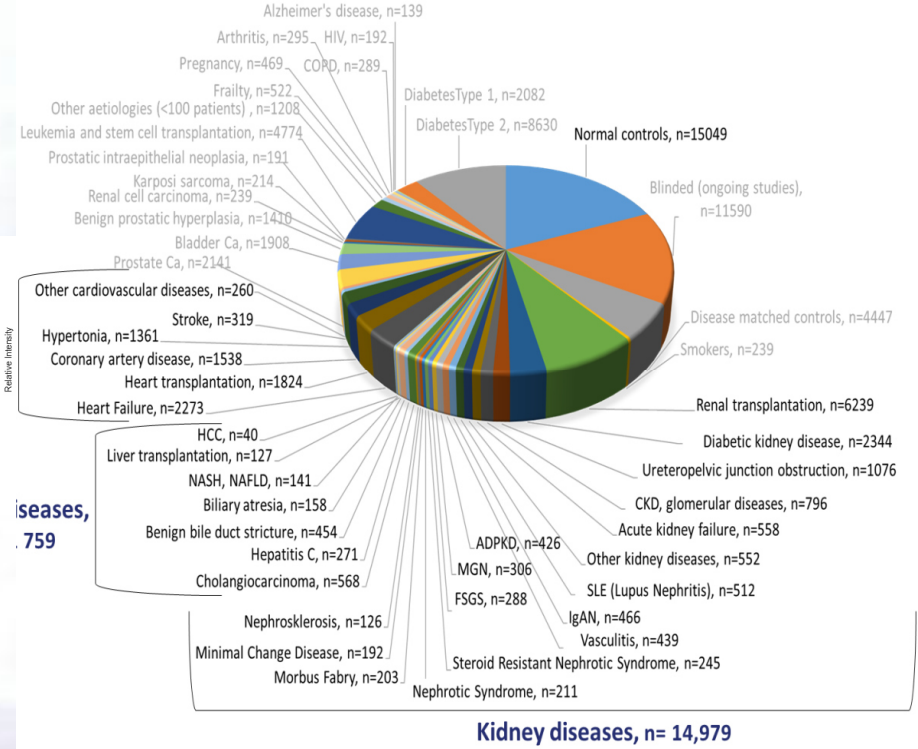


Biomarker selection





Statistics



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1 (Early) detection of CKD

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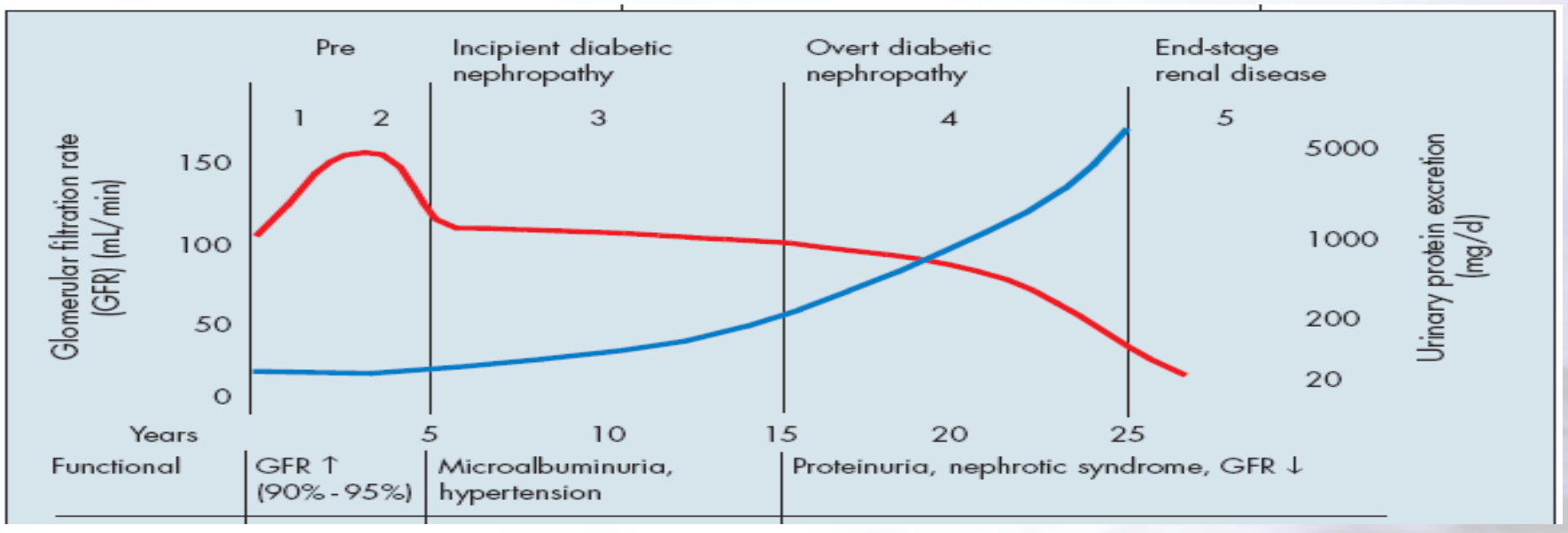
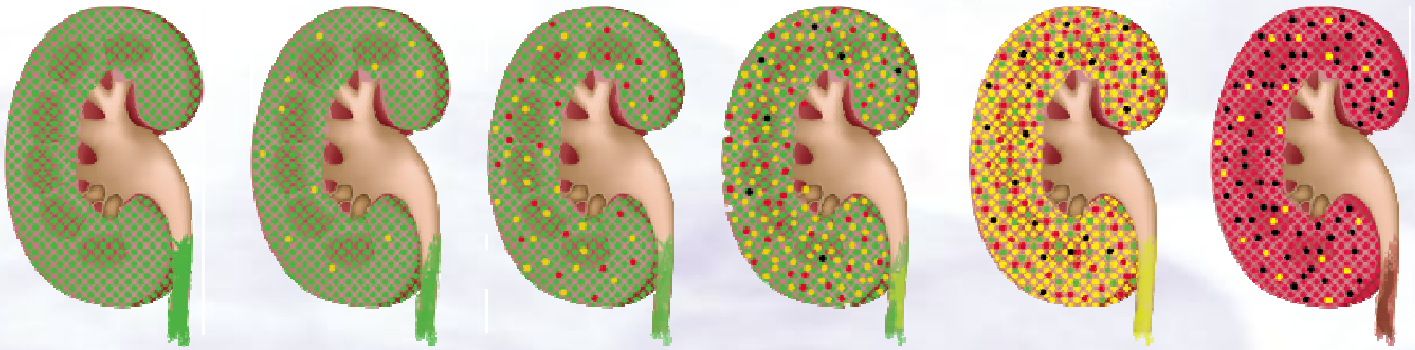
Disease onset and progression in CKD





Pontillo and Mischak Clinical Kidney Journal 2017

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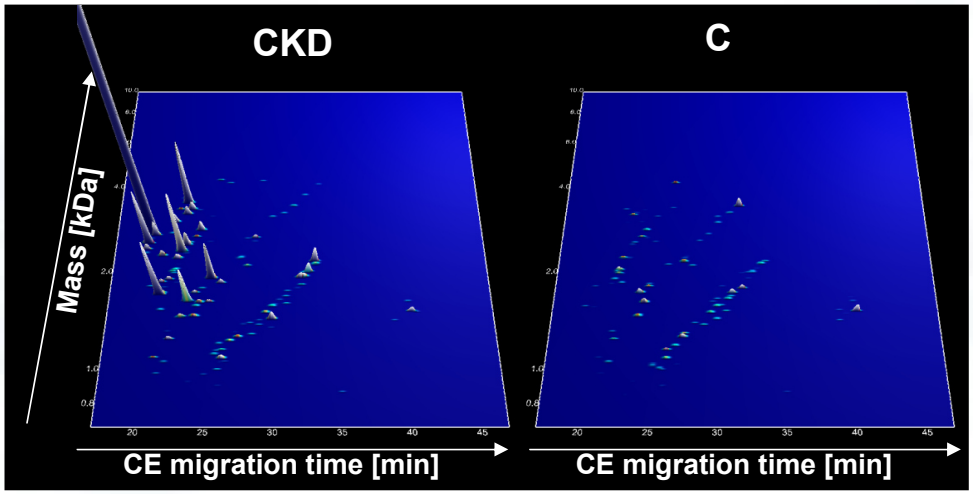
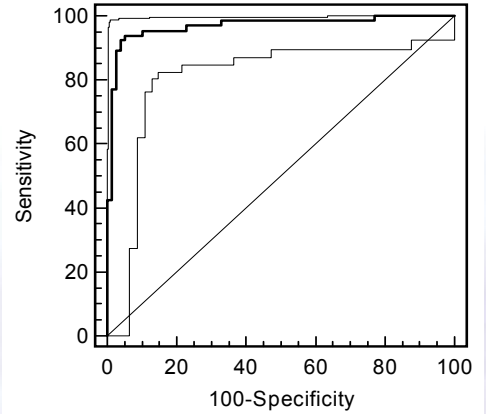
CKD biomarker discovery



Training set	
CASE	**CONTROL**
Σ: 230 **30 ANCA,** **30 MGN,** **22 MCD,** **44 IgAN,** **25 FSGS,** **58 DN,** **21 SLE**	**Σ: 379** **379 C**



CKD pattern (n=273 biomarker): Peptides derived from

- different collagens
- plasma proteins (serum albumin, transthyretin, alpha-1-antitrypsin, alpha- 1B-glycoprotein, alpha-2-HS-glycoprotein, antithrombin-III, apolipoprotein A-I, beta-2-microglobulin, fibrinogen alpha)
- clusterin
- uromodulin
- Na/K-transporting ATPase gamma chain
- psoriasis susceptibility 1 candidate gene 2
- prostaglandin-H2 D-isomerase
- proprotein convertase subtilisin/kexin type 1 inhibitor
- polymeric-immunoglobulin receptor
- osteopontin
- neurosecretory protein VGF
- Membrane associated progesterone receptor component 1
- CD99 antigen

- Ig lambda chain C regions

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Good et al., MCP 2010

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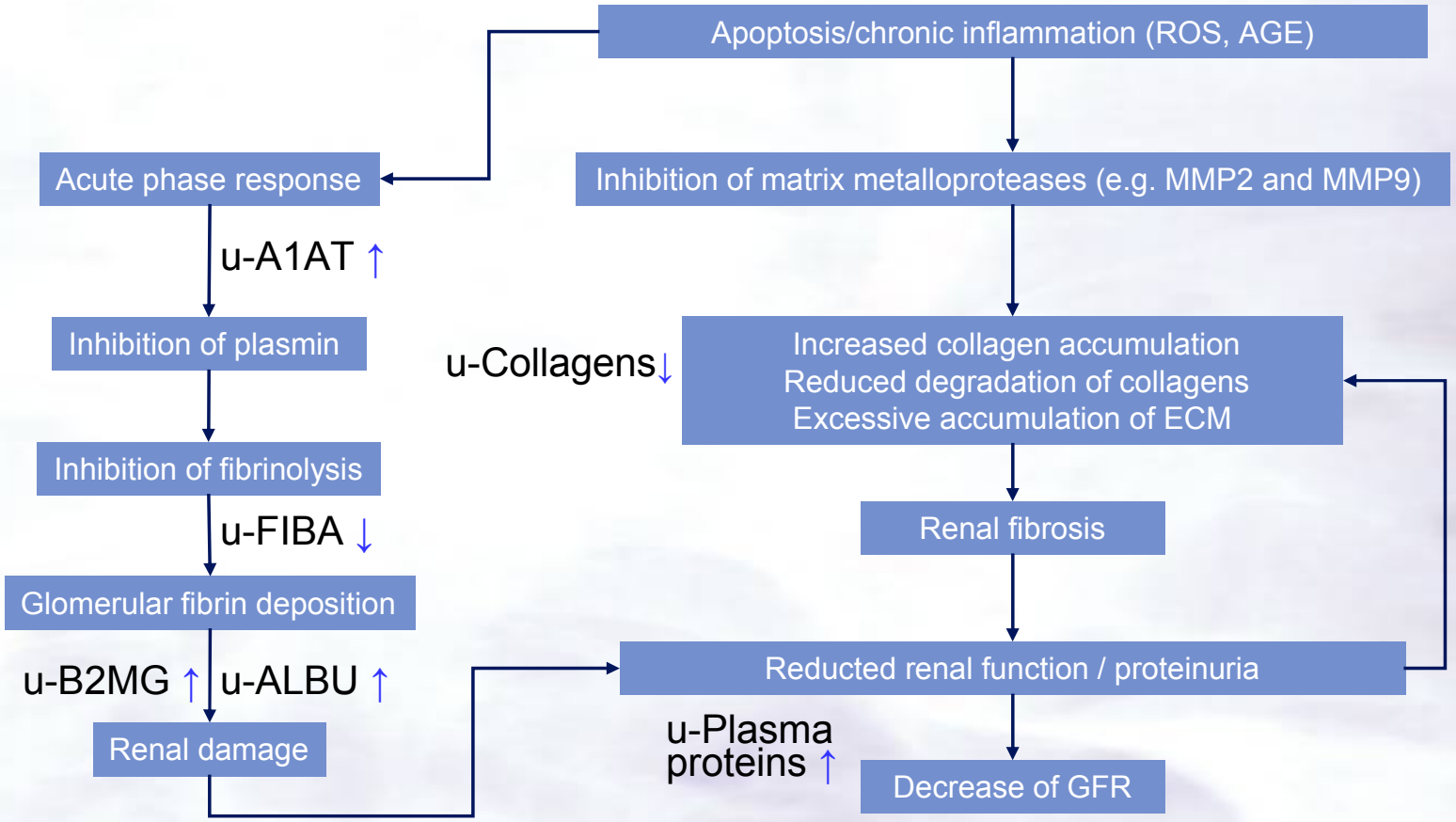
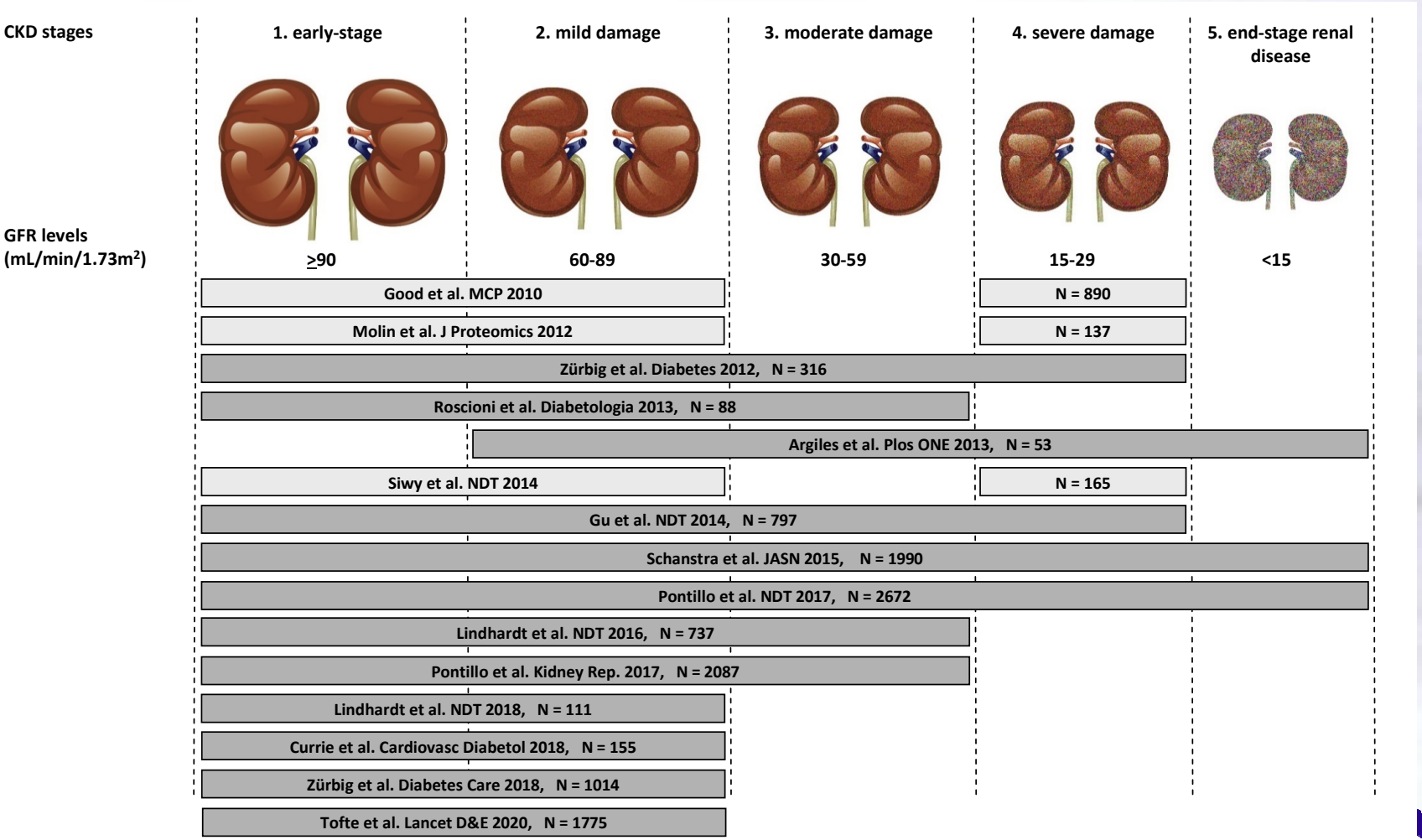
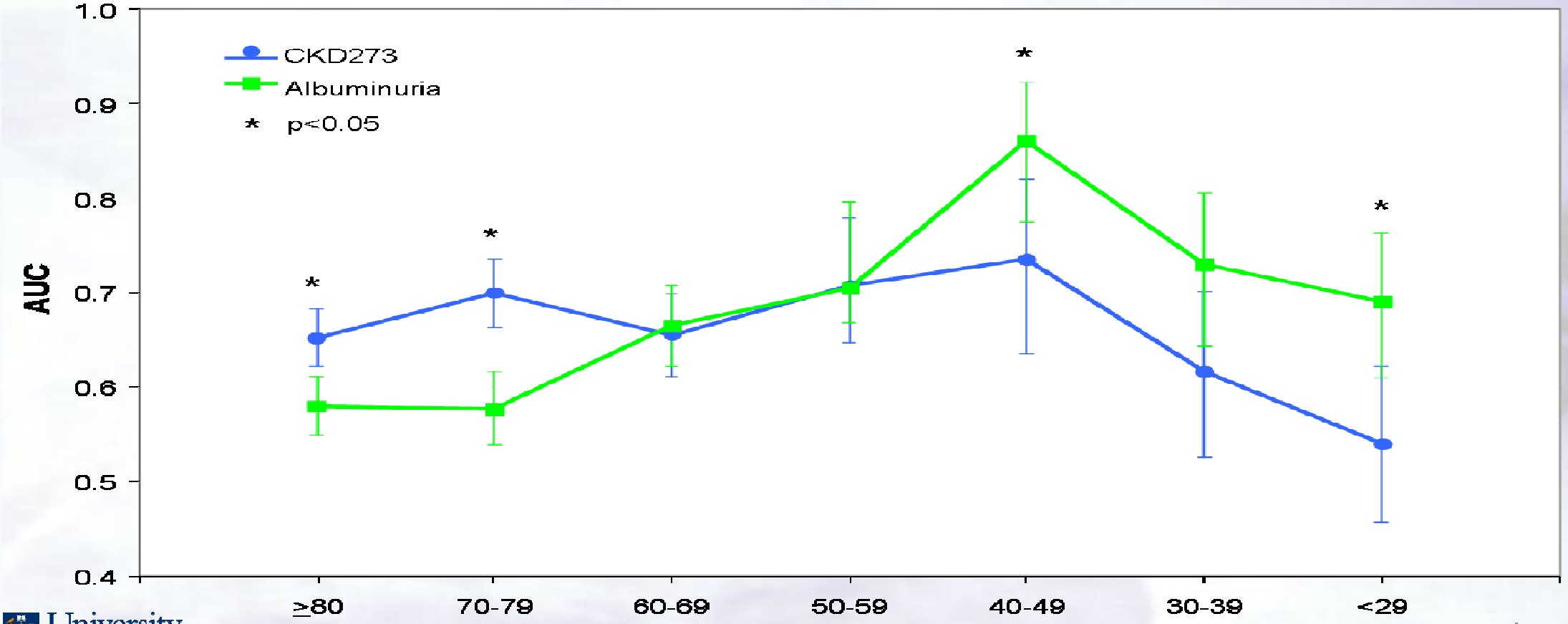
Pathophysiological relevance



Application of CKD273 according to disease stage



Comparison of albuminuria and CKD273 in predicting CKD progression in 2672 patients



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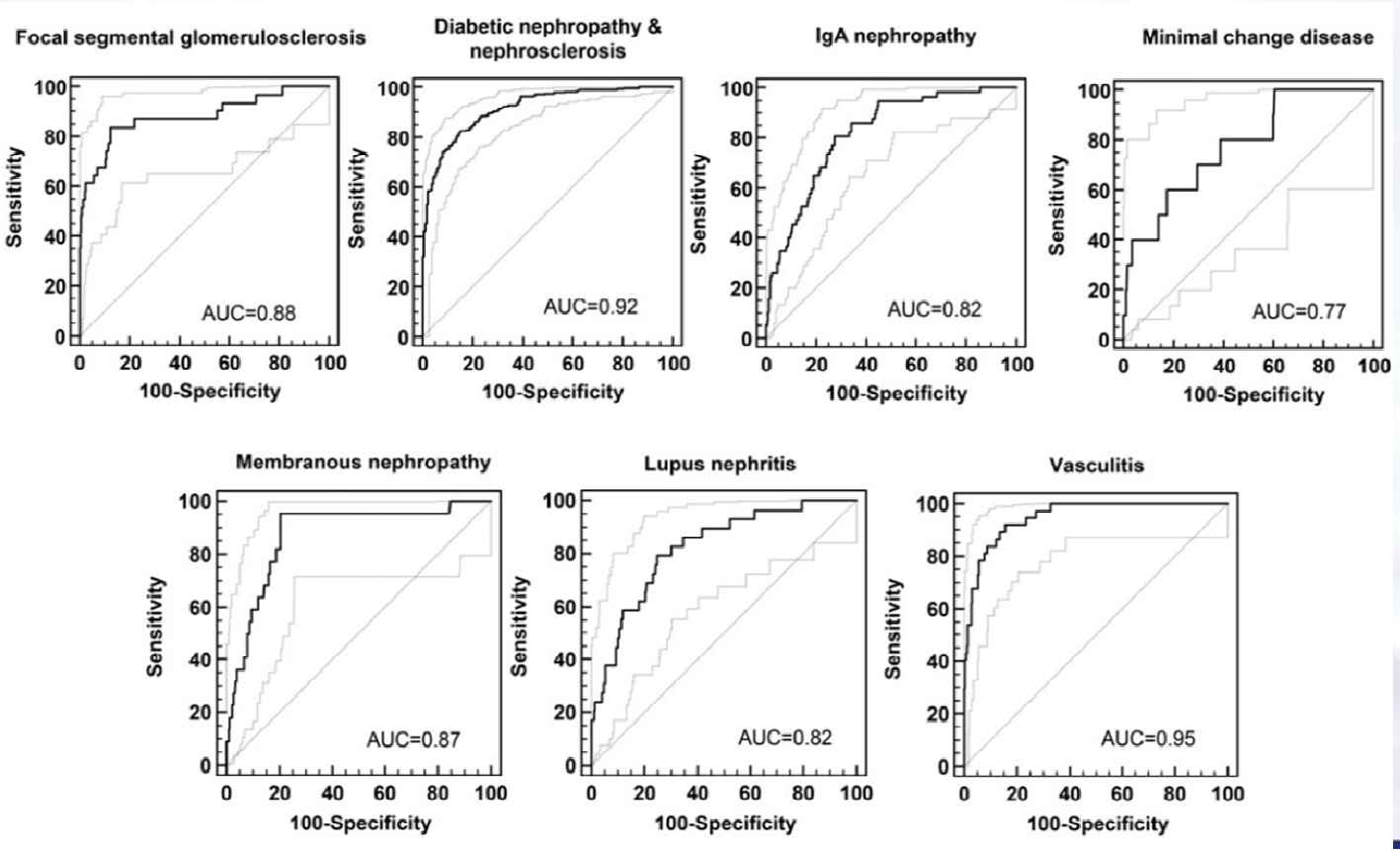
	Sample number	Gender (% male)	Age (years)	eGFR (mL/min/1.73 m^2)	Sample number	Gender (% male)	Age (years)	eGFR (mL/min/1.73 m^2)
FSGS	79	62	41.3 ± 21.8	45.1 ± 26.7	31	55	29.1 ± 23.2	46.9 ± 32.7
DN&N	288	66	65.4 ± 13.8	40.0 ± 22.9	288	57	64.7 ± 10.7	55.6 ± 22.8
IgAN	122	65	42.6 ± 16.0	50.8 ± 29.8	57	63	37.0 ± 14.2	94.7 ± 30.0
MCD	25	72	35.1 ± 15.2	85.8 ± 35.9	10	40	45.7 ± 23.2	103.4 ± 53.9
MN	55	74	52.0 ± 15.2	68.5 ± 32.4	22	67	50.9 ± 16.4	89.6 ± 22.3
LN	63	17	39.8 ± 12.6	57.1 ± 23.5	29	13	35.6 ± 13.4	99.3 ± 17.6
Vasculitis-induced kidney	74	58	64.5 ± 10.3	41.3 ± 22.4	37	44	58.8 ± 14.6	70.2 ± 13.7



Siwy al., NDT 2016

Disease	Number of sequencing
FSGS	287 (107)
DN&N	619 (248)
IgAN	116 (71)
MCD	291 (121)
MN	311 (107)
LN	172 (70)
Vasculitis-induced kidney disease	509 (203)

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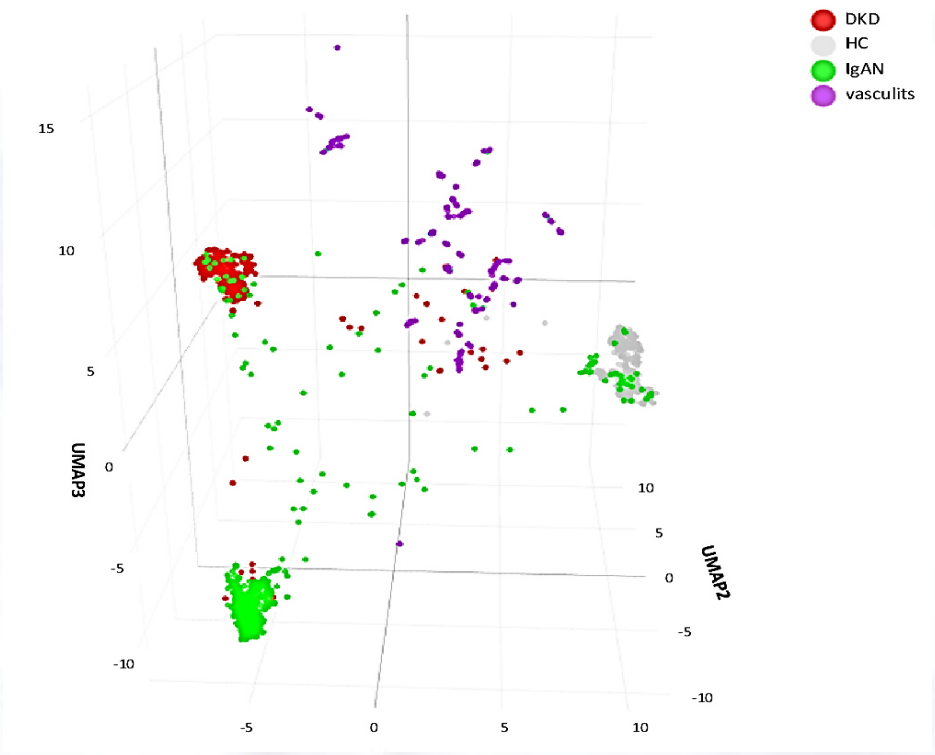
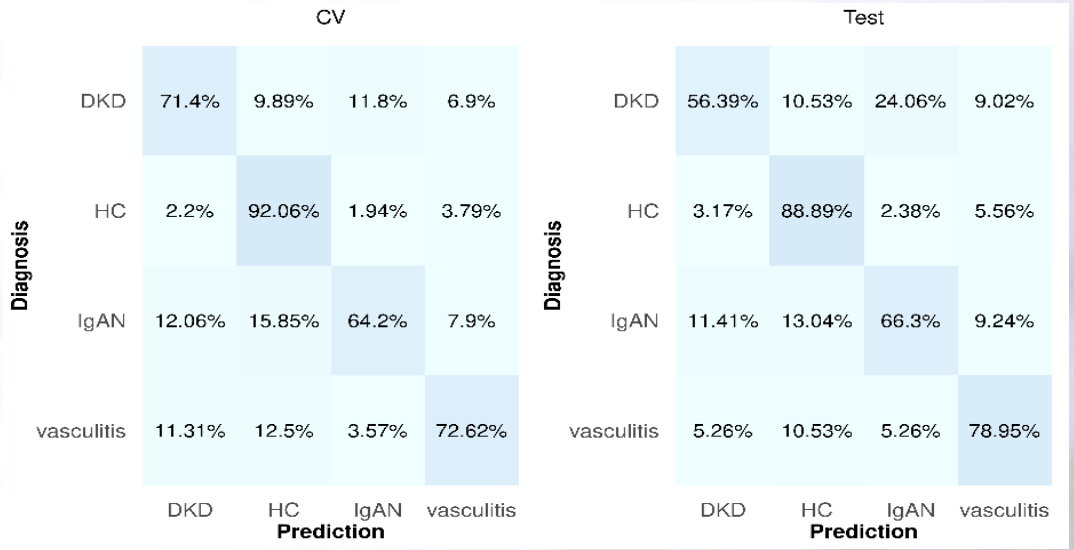
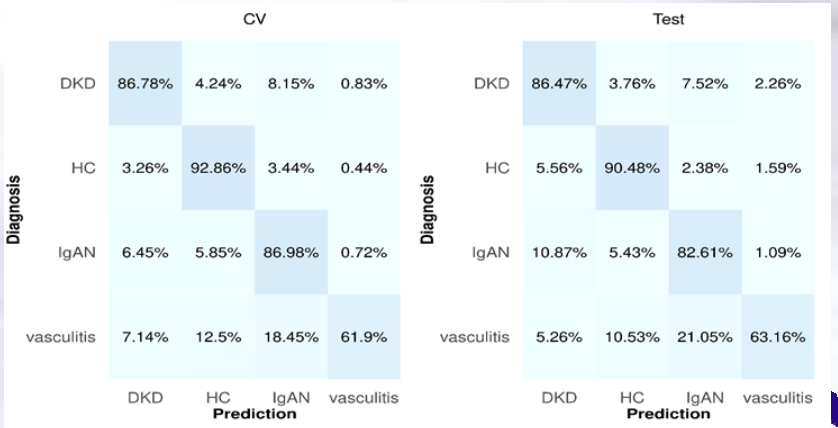
Umap for differential diagnosis based on urine proteome



The whole peptidomic profiles (N=1850) in the 3-D space were used as a basis in which the UMAP algorithm was applied (default parameters). Cluster formation of UMAP with tuned parameters as observed by the training set of DKD (red), HC (grey), IgAN (green) and Vasculitis (purple) participants.



Confusion matrices for predictions using the train and test sets (classification accuracies are displayed in percentages).



SVM based result

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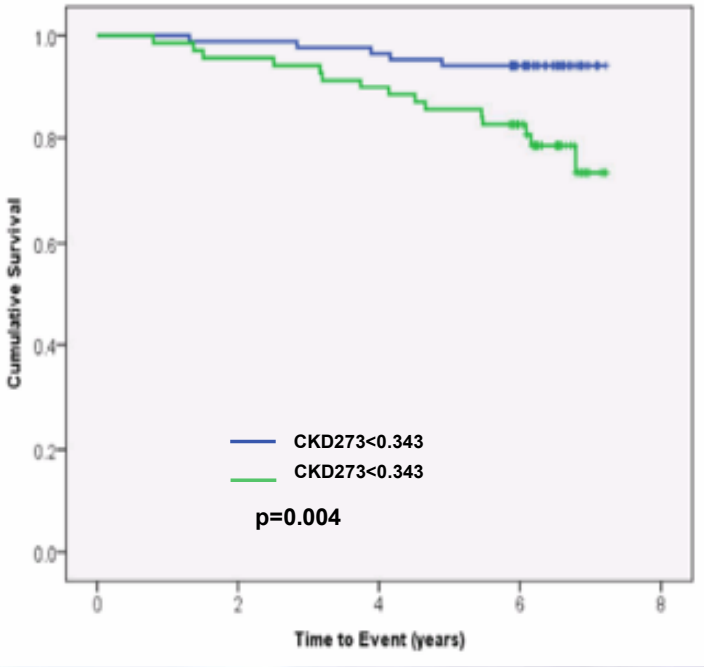
Prediction of patient-relevant outcome by CKD273

Parameter	CKD273>0.343 (n=71)	CKD273<0.343 (n=86)	P-value
Age (years)	61 (59-71)	61 (60-70)	0.573
Gender (M/F)	61/10	59/27	*0.010
Diabetes duration (years)	10 (1-35)	13 (1-36)	0.153
Retinopathy (Y/N)	42/29	53/33	0.752
Smokers (Y/N)	27/44	16/70	*0.007
BMI (kg/m^2)	31.3 (22.5-55.6)	31.7 (21.6-45.6)	0.662
SBP (mmHg)	130.4±17.4	128.8±15.3	0.547
DBP (mmHg)	75±11.2	73.8±11.3	0.543
HbA1c (mmol/mol)	59 (41-86)	59 (39-123)	0.118
Cholesterol (mmol/l)	3.9 (2-7)	3.8 (2.2-6.1)	0.549
UAE (mg/24hrs)	141 (9-1372)	57 (3-980)	*<0.001
eGFR (ml/min/1.73m^2)	87.6±18	89.6±16	0.452
CKD273 score	0.527 (-1.078 -1.231)	0.140 (-1.004 - 0.780)	*<0.001



All-cause mortality in patients with CKD273 score above and below treshold for diagnosis of DN

Currie et al., CARDIOVASCULAR DIABETOLOGY 2018

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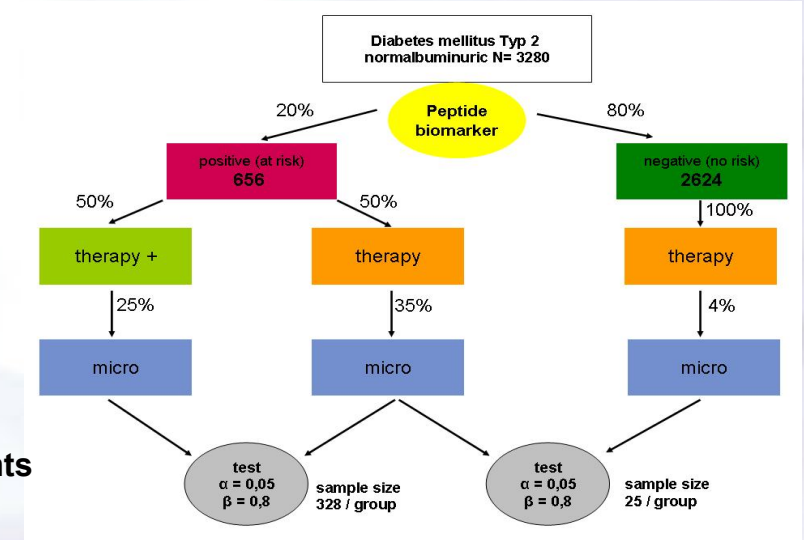
CKD273 improves patient stratification

EU PRIORITY

RCT employing CKD273 for stratification Targeted therapy/personalized medicine in Nephrology

Early prediction of diabetic nephropathy through urinary proteome analysis

- Multicenter study
- 15 partners in Europe
- 6 years



- **1770 normoalbuminuric type 2 diabetic patients**
- **Stratification into low and high risk patients**
- **High risk patients were randomly assigned to aldosterone blocker spironolactone 25 mg or placebo therapy on top of optimal standard therapy**

Lindhardt et al., BMJ open 2016

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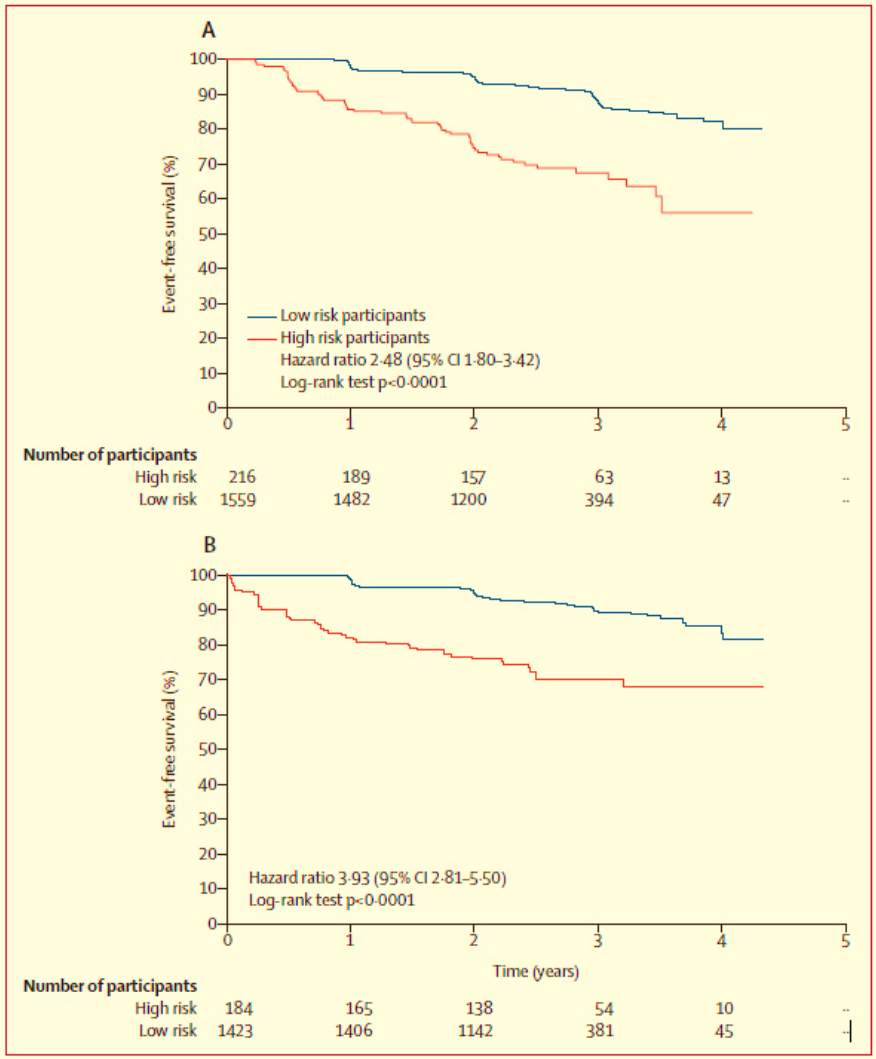
Progression to renal endpoints according to CKD273 risk score status in the observational cohort



(A) Microalbuminuria in the observational cohort.
(B) Decrease in renal function and progression to chronic kidney disease stage 3

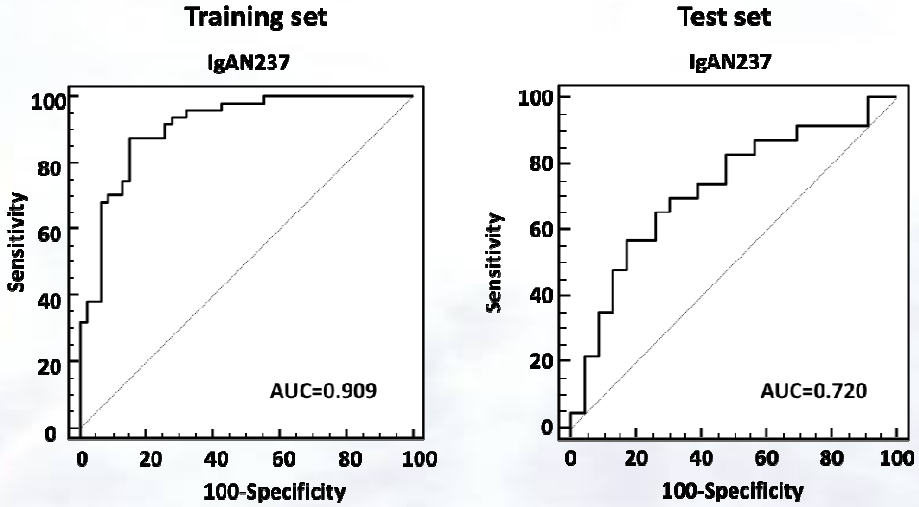
	Low-risk participants (n=1559)	High-risk participants (n=216)	Endpoint measure (95% CI)	p value
Primary endpoint				
Microalbuminuria (confirmed)	139 (8·9%)	61 (28·2%)	HR 3·92 (2·90–5·30)	<0·0001
Secondary endpoints				
Microalbuminuria (single value)	288 (18·5%)	99 (45·8%)	HR 3·68 (2·93–4·62)	<0·0001
Macroalbuminuria (confirmed)	22 (1·4%)	2 (0·01%)	HR 0·66 (0·15–2·81)	0·57
Chronic kidney disease stage 3 (eGFR <60 mL/min per 1·73 m²)*	119 (7·6%)	48 (22·2%)	HR 3·50 (2·50–4·90)	<0·0001
Fatal and non-fatal cardiovascular outcome†	53 (3·4%)	12 (5·6%)	HR 1·77 (0·92–3·22)	0·089
Ischaemic heart disease	24 (1·5%)	7 (3·2%)	HR 2·22 (0·96–5·2)	0·063
Stroke	15 (0·96%)	4 (1·9%)	HR 1·99 (0·66–6·0)	0·22
Congestive heart failure	8 (0·51%)	2 (0·93%)	HR 1·96 (0·42–9·21)	0·72
All-cause mortality	11 (0·62%)	2 (0·93%)	HR 1·41 (0·31–6·37)	0·65
Development of retinopathy or laser treatment (self-reported)	144 (9·2%)	21 (9·7%)	HR 1·02 (0·65–1·62)	0·93
Retinopathy	101 (6·5%)	14 (6·5%)	HR 0·96 (0·55–1·68)	0·89
Laser treatment for retinopathy	54 (3·5%)	9 (4·2%)	HR 1·21 (0·56–2·44)	0·60
Change in UACR, % per year	2·6 (0·85)	7·1 (1·14)	4·50 (2·70–6·20)	<0·0001
Change in eGFR, mL/min per 1·73 m² per year	0·47 (0·19)	1·37 (0·34)	0·90 (0·14–1·67)	0·206

Data are n (%) or mean (SE), unless otherwise indicated, and endpoint measures are either HRs or differences. p values are calculated from χ^2 test. eGFR=estimated glomerular filtration rate. HR=hazard ratio. UACR=urine albumin-to-creatinine ratio. *For patients with eGFR >60 mL/min per 1·73 m² at baseline. †Comparison of composite fatal and non-fatal cardiovascular outcome (myocardial infarction, stroke, coronary artery bypass graft, percutaneous transluminal coronary angioplasty, hospital admission for heart failure or cardiovascular disease) and all-cause mortality during the study.

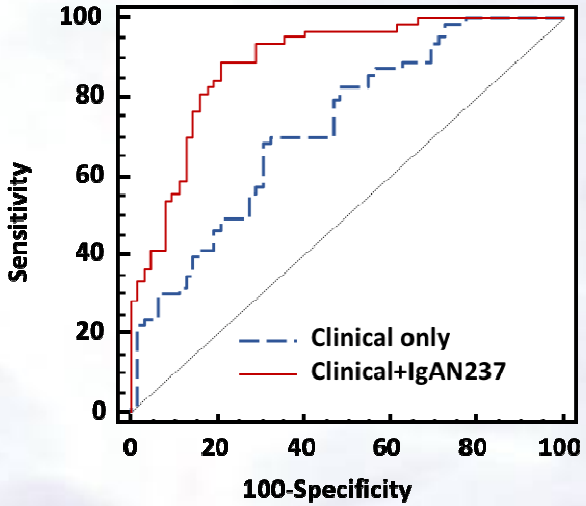
Table 2: Primary and secondary endpoints in the observational cohort

Prediction of disease progression in patients with IgA nephropathy (PERSTIGAN)

ROC for prediction of IgAN progression by the IgAN237 biomarker panel.
A)N-1 cross validated training set (n=94).
B)B) Test set (n=46).



Added value of proteomics IgAN237 classifier for prediction of disease progression



[a] proteomics classification score; [b] baseline clinical data include age, gender, eGFR, proteinuria, mean arterial pressure; [c] SE standard error of the mean; [d] 95% confidence interval

	AUC	SE [c]	95% CI [d]
IgAN237 [a] + clinical [b]	0.887	0.0294	0.830 to 0.945
Clinical only [b]	0.724	0.0449	0.636 to 0.812

Rudnicki et al., NDT 2020

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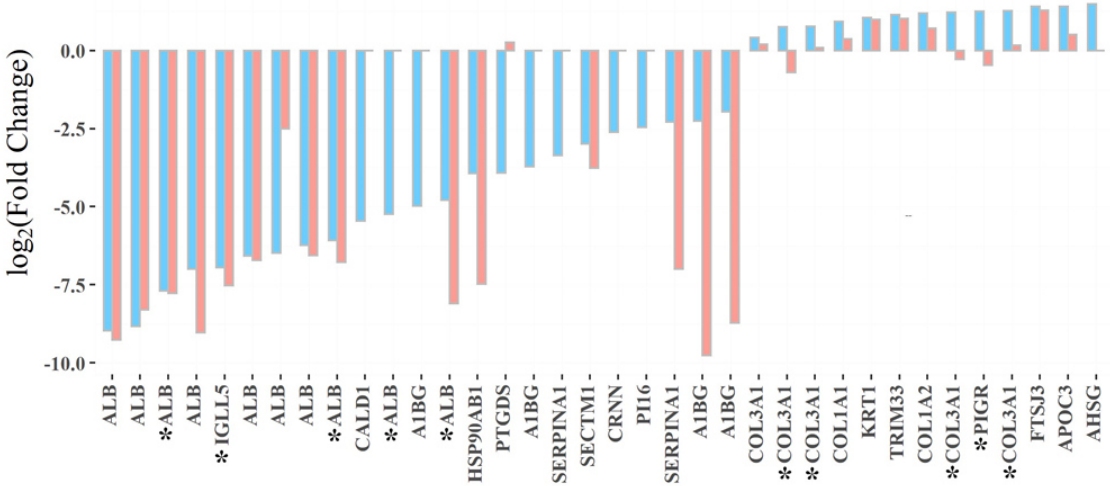
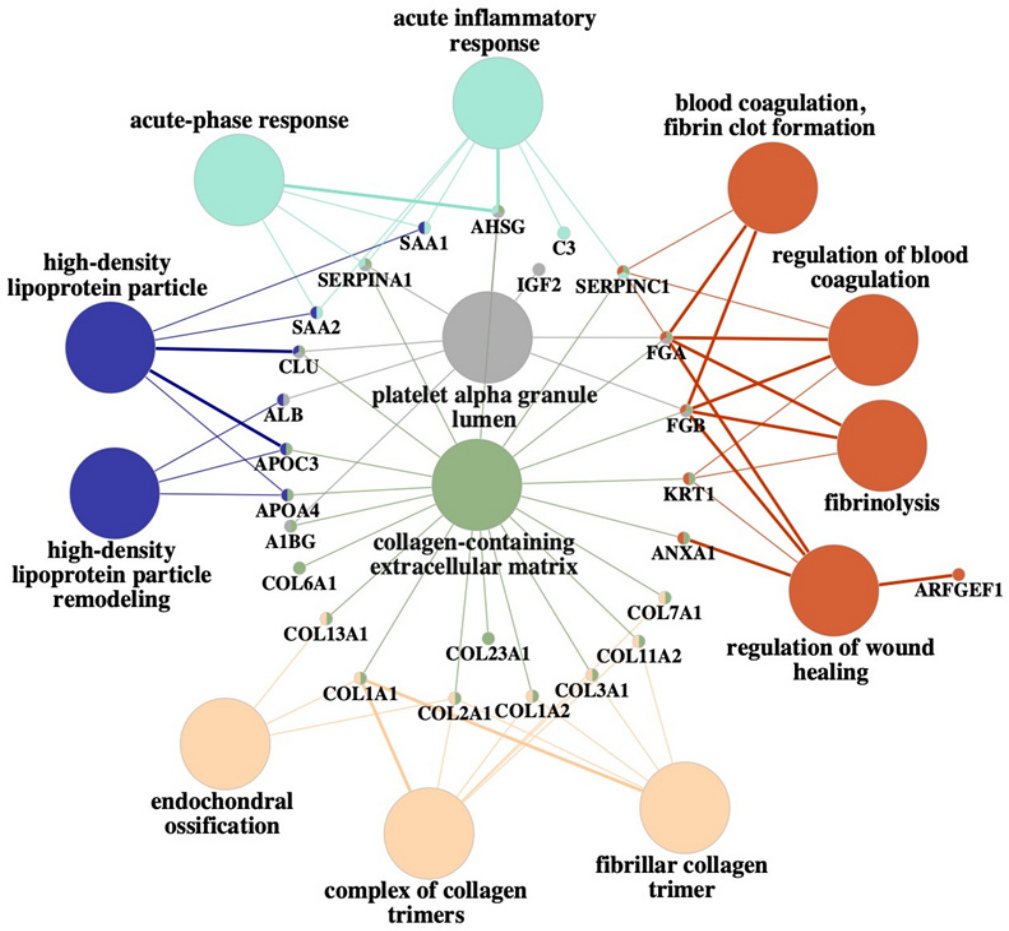
Impact of SGLT2 inhibition on urinary peptides indicates anti-fibrotic mechanism

Symbol	Protein	Change	Molecular weight (Da)	Potential Role in DKD
A1BG	α-1B-glycoprotein	-	54,254	Proteinuria biomarker *(44)*
ALB	Serum albumin	-	69,367	Proteinuria biomarker *(44)*
AHSG	α-2-HS-glycoprotein	+	39,341	Tissue development, endocytosis, inflammation *(23)*, proteinuria biomarker *(44)*
APOC3	Apolipoprotein C-III	+	10,852	Lipoprotein metabolism & inhibitor of lipolysis *(47)*
CALD1	Caldesmon	-	93,231	Actin cytoskeleton organization *(27, 28)*, regulation of mesangial cell activation *(29)*
COL1A1	Collagen α-1(I) chain	+	138,941	Extracellular matrix organisation *(23, 44)*
COL3A1	Collagen α-1(III) chain	+	138,564	Extracellular matrix organisation *(44)*
COL1A2	Collagen α-2(I) chain	+	129,314	Extracellular matrix organisation *(23, 44)*
CRNN	Cornulin	-	53,533	Repair *(44)*, positive regulation of NF-κB transcription factor activity *(42)*
FTSJ3	pre-rRNA processing protein FTSJ3	+	96,558	Unknown
HSP90AB1	Heat shock protein HSP 90-β	-	83,264	Regulation of TGF-β signaling *(32)*, immunity & inflammation *(62)*, stress response in wound healing *(36)*
IGLL5	Immunoglobulin λ-like polypeptide 5	-	23,063	Inflammation *(63–65)*
KRT1	Keratin; type II cytoskeletal 1	+	66,039	Inflammation *(44)*, coagulation*, extracellular matrix*, and wound healing*
PI16	Peptidase inhibitor 16	-	49,471	Extracellular matrix organisation *(44)*
PIGR	Polymeric immunoglobulin receptor	+	83,284	Tubular response to injury *(66, 67)*
PTGDS	Prostaglandin-H2 D-isomerase	-	21,029	Inhibition of TGF-β induced EMT *(35, 37)*, GFR biomarker *(44)*
SECTM1	Secreted and transmembrane protein 1	-	27,039	Inflammation *(41)*, NF-κB pathway activation*
SERPINA1	α-1-antitrypsin	-	46,737	Inflammation, coagulation & proteinuria biomarker *(23, 44)*, renal fibrosis *(45)*
TRIM33	E3 ubiquitin-protein ligase TRIM33	+	122,533	Regulation of TGF-β pathway *(33, 34)*, inflammation & fibrosis *(33)*





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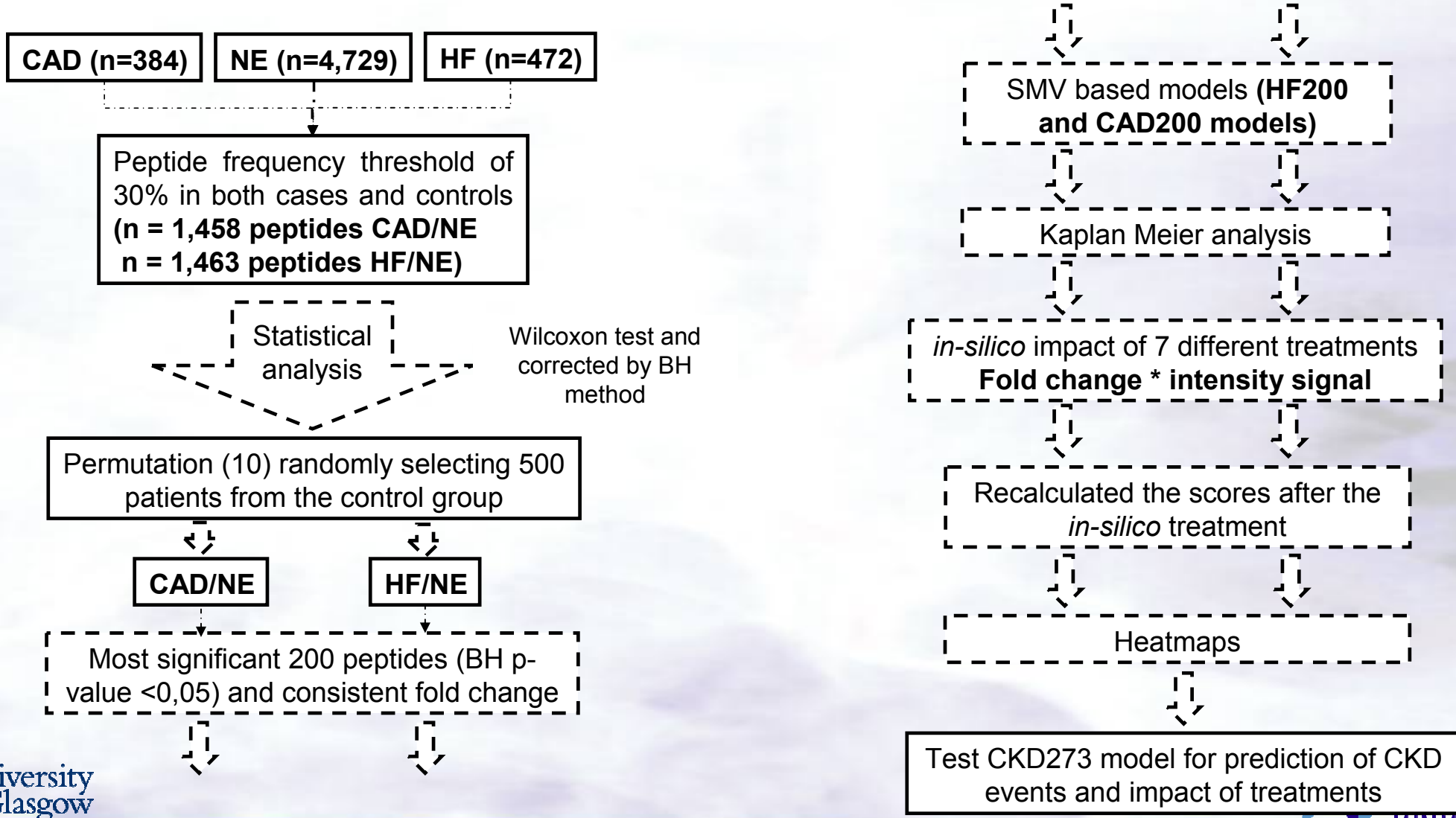
Prediction of CVD and CKD events and *in-silico* impact of treatments



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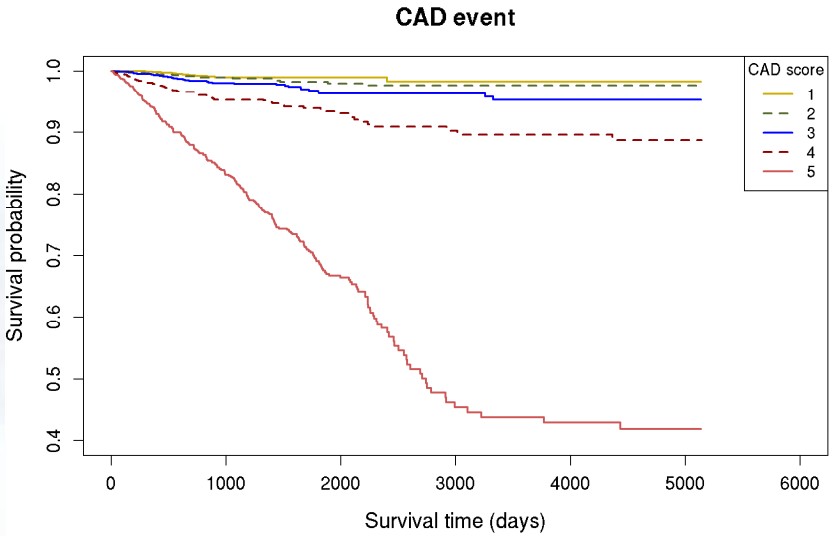
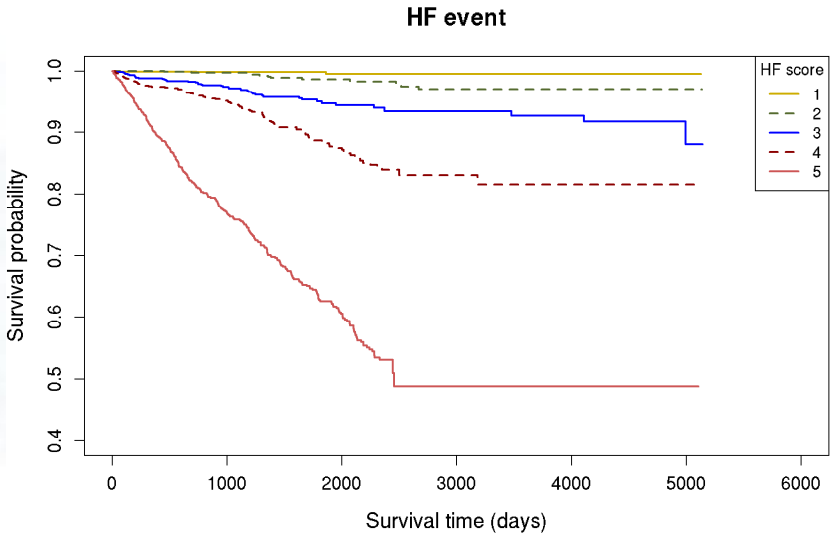
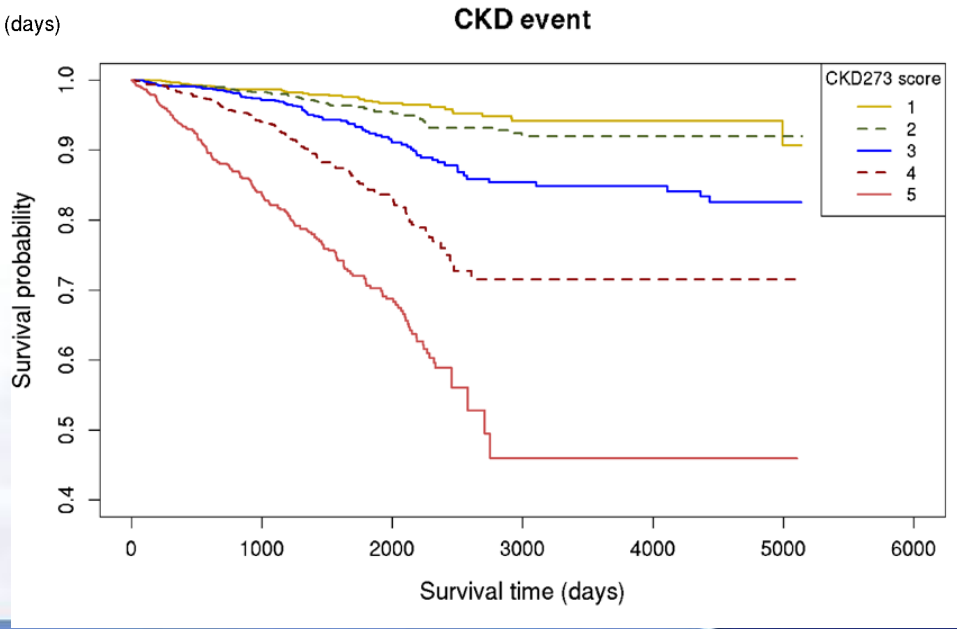
Events in quintiles according to scoring







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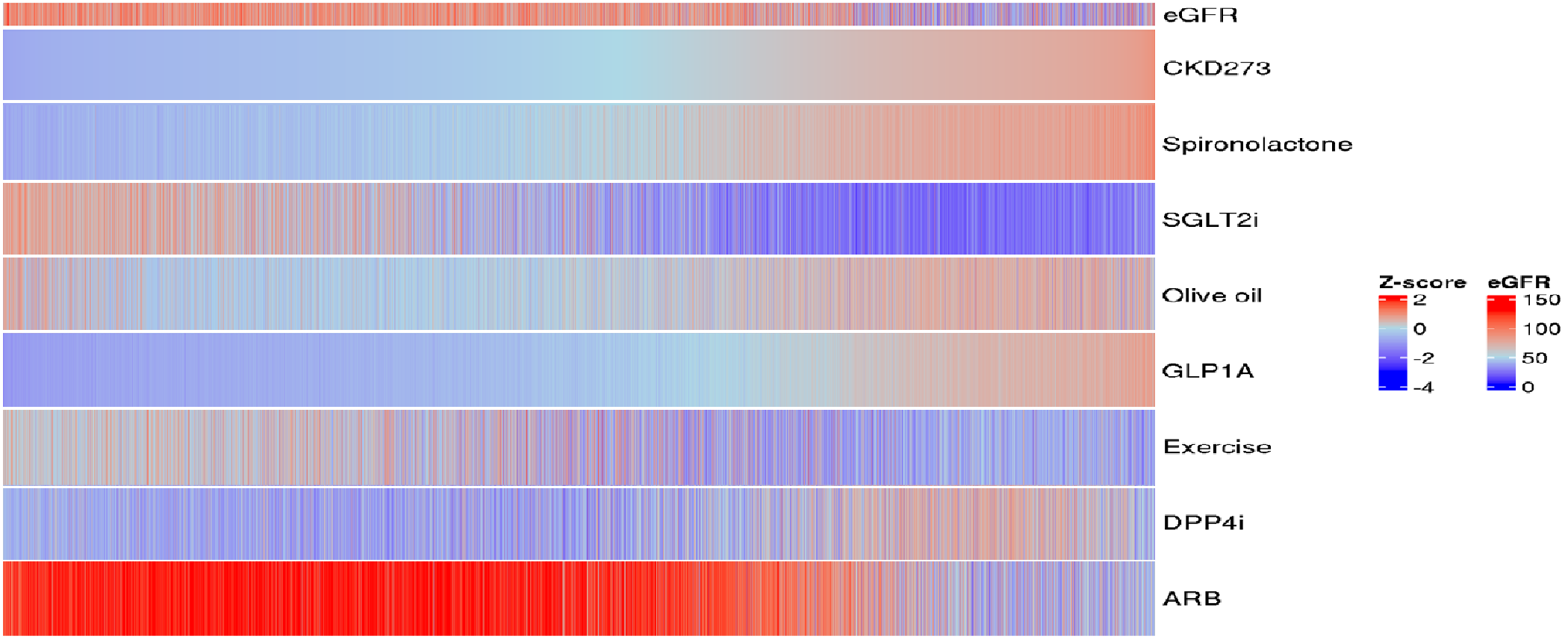
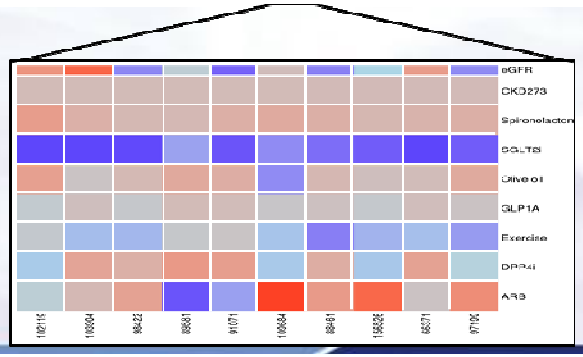
IMPACT OF TREATMENTS-CKD273 MODEL





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1 (Early) detection of CKD
2 CKD differential diagnosis
3 prognosis of disease progression
4 assessment and prediction of therapeutic response
5 associated consideration and applications

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The urinary proteome informs about biological age and risk of death

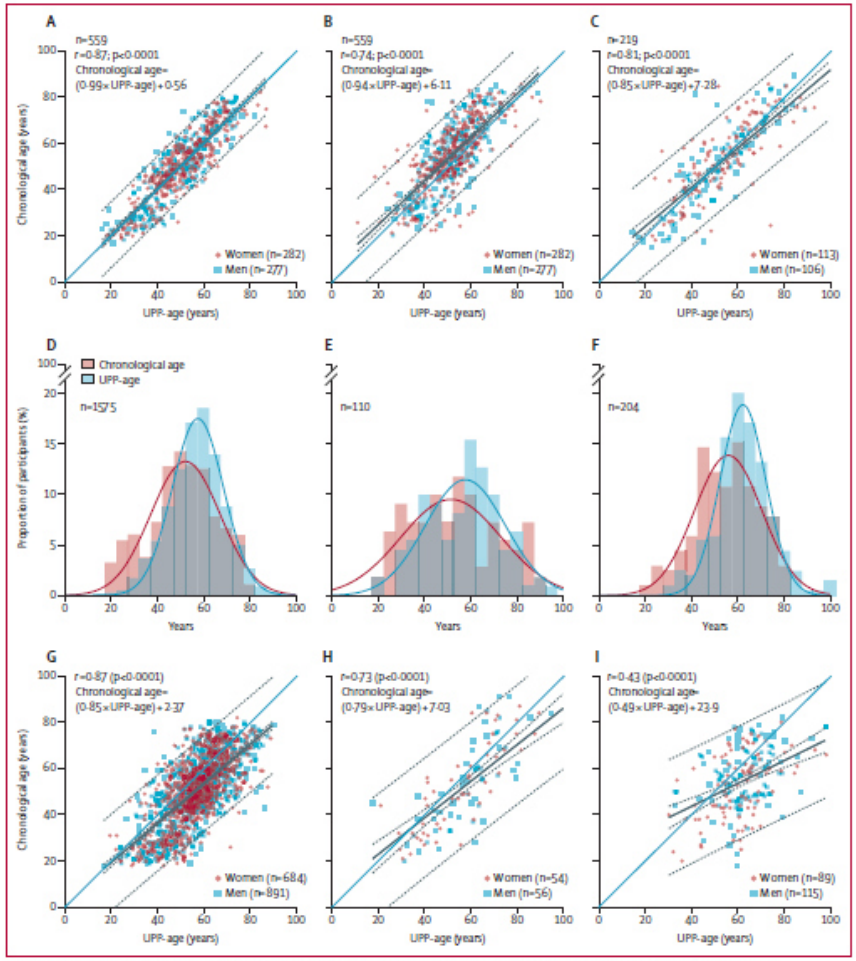


Figure 2: **Correlations between observed chronological age and age predicted by the urinary proteome in FLEMENGHO participants and patients**
Correlations for the derivation dataset (2005–10; A), time-shifted internal validation dataset (2009–13; B), and synchronous internal validation dataset (2005–10; C) in FLEMENGHO participants. Distributions of chronological age and UPP-age (superimposed on the UPP-age distribution in the FLEMENGHO derivation dataset [grey bars]) for patients with diabetes (D), COVID-19 (E), and chronic kidney disease (F). Correlations between chronological age and UPP-age for patients with diabetes (G), COVID-19 (H), and chronic kidney disease (I). Regression lines (solid black) are given with 95% CIs (dotted lines) for predicting mean chronological age (narrow band) and chronological age in individual participants (broad band). The blue line in the correlation plots is the identity line. FLEMENGHO=Flemish Study on Environment, Genes, and Health Outcomes. UPP=urinary peptidomic profile. UPP-age=age as predicted by the UPP.

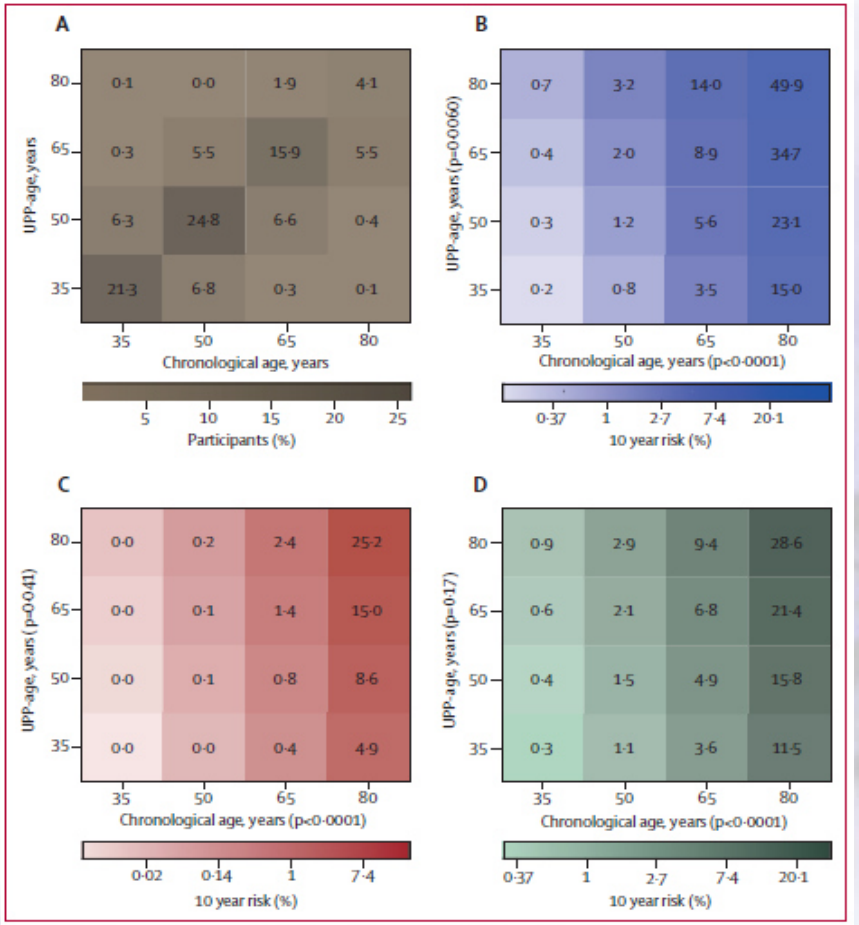
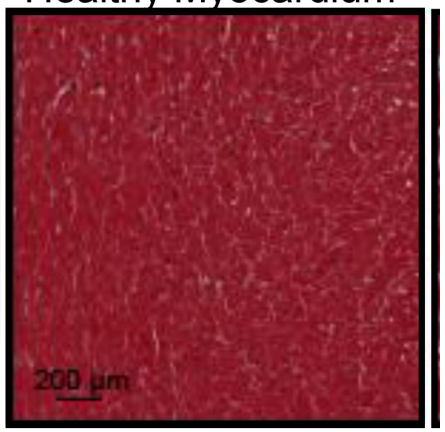


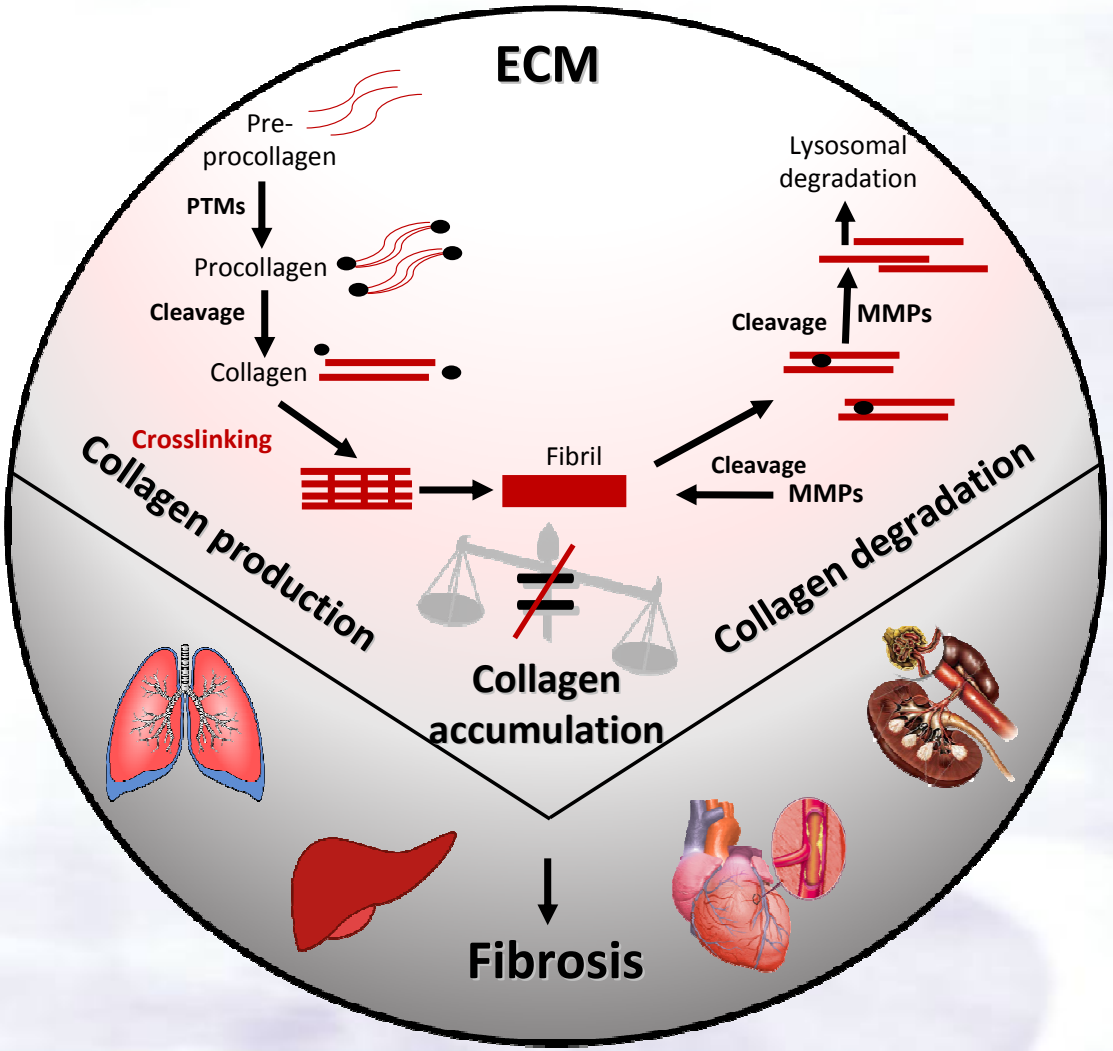
Figure 3: **Heat maps relating mortality to age in 778 FLEMENGHO participants**
Proportion of participants by UPP-age and chronological age cross-classification (A). Cox proportional hazards regression showing 10 year risk of death for total (B), cardiovascular (C), and non-cardiovascular (D) mortality in relation to chronological age and UPP-age, derived from the 2005–10 baseline examinations (appendix p 33). FLEMENGHO=Flemish Study on Environment, Genes, and Health Outcomes. UPP=urinary peptidomic profile. UPP-age=age as predicted by the UPP.

Martens et al. The Lancet Healthy Longevity, 2021

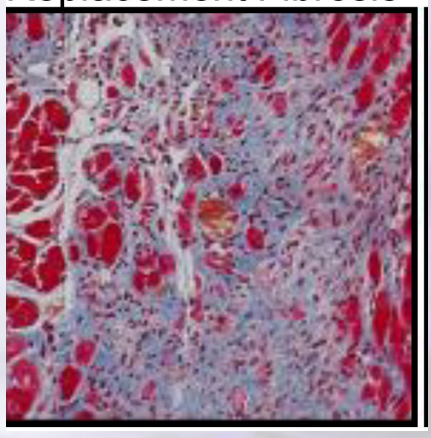
Fibrosis as a major cause for CKD and CVD onset and progression

Healthy Myocardium





Replacement Fibrosis



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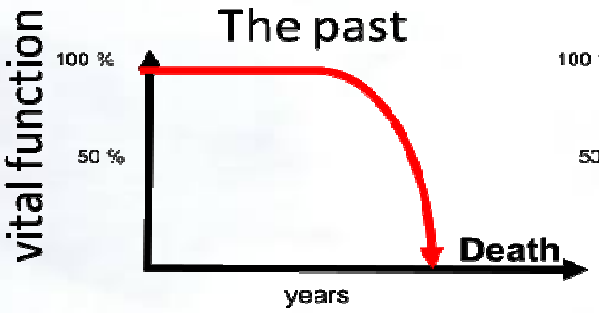
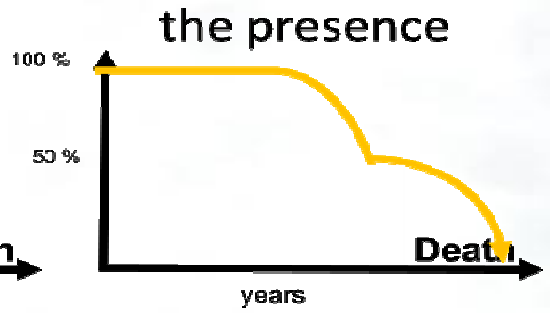
Potential impact of urinary proteomics on patient relevant outcome



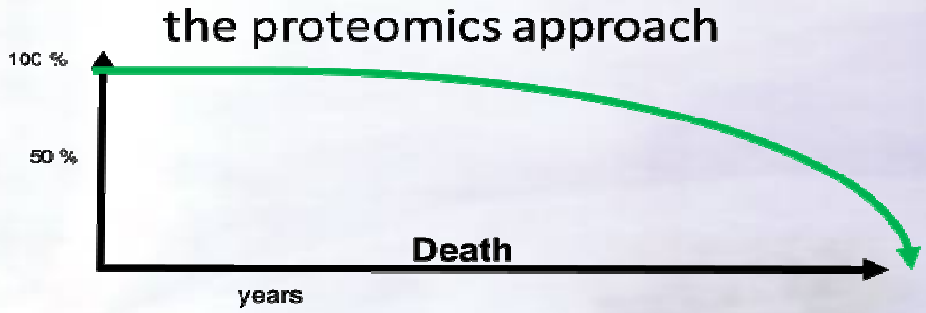
In the past individuals died quickly from diseases.

Transition from healthy to sick to death occurred fast.



Current patient care aims towards delaying death - but not preventing disease.

Disease treatments prolong life, but cannot stop or heal irreversible organ damage



Personalized medicine with proteome analysis enables early detection of cellular disease-specific changes, as the diseases are the results of proteome changes.

Cellular changes are controlled by proteins. Decoding this information at the proteome level enables the timely detection and prevention of the effects of diseases prior to irreversible organ damage.

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Conclusions

The urinary proteins and peptides biomarkers …

- ✓are associated with disease onset and progression
- ✓Indicate the underlying disease etiology
- ✓indicate response to therapy
- ✓can be combined into highly selective high dimensional classifiers
- ✓A key feature of most urinary peptide based classifiers is the inclusion of specific collagen peptides, reflecting fibrosis
- ✓Further major pathologies depicted appear endothelial dysfunction and inflammation
- ✓Assessment of disease based on urinary proteins/peptides is superior to other established parameters (e.g. albuminuria, eGFR)
- ✓Urinary proteome analysis enables early disease detection and differential diagnosis, can guide early intervention, resulting in improvement of outcome.

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