

PROJECT FINAL REPORT

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1 Final publishable summary report

1.1 Executive summary

The clinical focus – background and challenges: Renal disease remains clinically silent in many affected individuals until an advanced stage, and diabetes, hypertension or cardiovascular disease confers the highest risk for developing chronic kidney disease (CKD). Hence, CKD comes along with highly prevalent conditions like diabetes mellitus leading to diabetic nephropathy, seeing significant morbidities and mortality. Challenges include optimized diagnosis and prognosis together with further improving therapy, all on the background of preventive measures.

The project – aims and objectives: SysKid proposed an integrative, Systems Biology-motivated strategy for i) identifying persons at risk of developing chronic kidney disease utilizing epidemiology as well as molecular tools, ii) understanding the molecular processes triggering early stage chronic kidney disease and identifying associated biomarkers, iii) developing novel diagnostic and therapeutic strategies to control progression of chronic kidney disease, and iv) performing pre-clinical verification of novel therapy approaches and perform clinical testing of novel diagnostics.

Tools for assessing onset and development of CKD: SysKid executed biomarker discovery studies covering genetic background, transcripts, proteins and metabolites, complemented by large scale validation of biomarker candidates and multi-marker classifiers regarding onset and progression of disease. SysKid brought forward novel tools for profiling of progressive chronic kidney disease.

Risk factors, healthcare recommendations and epidemiological perspective: SysKid executed comprehensive screening for risk factors, further integration in high-dimensional models and practical implementation in risk scores. Analysis of life style parameters brought forward recommendations on optimizing modifiable parameters from a preventive perspective, and added to optimized use of given diagnosis and therapy practise. Significant activities allowed comparative analysis of CKD prevalence on a European level.

Novel approaches for halting disease progression: SysKid executed molecular mechanistic analysis of CKD on human and disease model systems, integrating all in a computational Systems Biology framework. On such basis the project added to our understanding of the pathophysiology involved in CKD, provided models for improving given therapy approaches, and added novel targets promising beneficial interference with progressive disease.

Contributions to State-of-the-Art: SysKid assembled a broad molecular and clinical repository characterizing CKD, executed quantitative assessment of risk factors, models and classifiers characterizing onset and progression of disease, performed analysis of disease models utilizing explorative as well as hypothesis-driven approaches, combined with disease models linking biomarkers and molecular mechanisms in the background of clinical parameters.

Socio-economic impact: SysKid added to improving clinical and molecular biomarker-based characterization of CKD, educated on associated molecular processes and mechanisms, and together with comparative analysis of disease model systems provided key elements for improving CKD drug development in the realm of precision medicine.

Scientific impact: SysKid brought forward more than 170 peer-reviewed publications, another about 30 publications at present submitted to scientific journals, complemented by more than 300 presentations at scientific conferences.

Dissemination: SysKid established multiple channels for educating on project background, progress and results, including monthly updates on the web site, newsletter and social media throughout the project.

Exploitation: SysKid brought forward patent applications on molecular assets, validation data on a biomarker-based classifier for disease progression being discussed with regulatory agencies, and analysis routines for high dimensional data being included in a range of software products.

1.2 Description of project context and objectives

SysKid, a project executed within HEALTH-2009-2.4.5-2 (Cellular and molecular mechanisms of the development of chronic kidney disease (CKD)) was set out to utilize an integrative, Systems Biology-motivated R&D approach. Key aims focused on deepening our understanding of molecular mechanisms and associated biomarkers and therapy targets afflicted with progressive renal disease in the context of diabetes and hypertension, further combined with risk factors and embedded in an epidemiological context.

As stated in the project summary defined at project start, motivation for doing this research lies in the significant prevalence of CKD in western industrialized nations, associated cardiovascular mortality, and massive impact of end stage renal disease on a patient as well as healthcare budget level.

On these grounds a systematic analysis of the molecular basis of the disease is deemed essential for bringing forward novel biomarkers allowing improved clinical phenotyping of progressive disease, novel therapeutic approaches (or more tailored utilization of given treatment) aligned with improved phenotyping, and equivalently essential a better characterization of risk factors from a preventive perspective was included in SysKid project work.

1.2.1 Chronic kidney disease, epidemiological background

In Europe some general population based studies are available holding information on the prevalence of chronic kidney disease (CKD) based on the KDOQI classification scheme (vide infra). In the Dutch city of Groningen all 85,421 inhabitants aged 28 – 75 years were invited to participate in the “Prevention of Renal and Vascular Endstage Disease (PREVEND)” prospective study. 40,856 responded and a sub-cohort enriched for the presence of high albuminuria was selected. 6,000 patients had a urinary albumin excretion > 10 mg/l and 2,592 below this threshold. After excluding patients with missing data 6,905 subjects had no renal disease (81.2%), 2.9% had CKD stage I (eGFR > 90 ml/min/1.73m² but other abnormalities present like e.g. albuminuria), 10% stage II (eGFR > 60-89 ml/min/1.73m² and other abnormalities) and 5.8% stage III (eGFR 30-59 ml/min/1.73m²); 8 and 3 patients respectively were in stage IV (eGFR 15-29 ml/min/1.73m²) and V (eGFR <15 ml/min/1.73m²). The mean age of the population was 49 years and there was a strong association between increasing age and CKD class (the group without CKD had a mean age of 47.4 years, the one with stage I 48.2 years, stage II 56.5 years and stage III patients were 63.2 years old on average) (Brantsma AH et al., *Nephrol Dial Transplant* 2008). When extrapolating the prevalence data to the general population in the Netherlands the numbers changed only marginally (1.3, 3.8 and 5.3 % for stages I, II and III respectively, giving a total of 10.4 %) (de Zeeuw D et al., *Kidney Int* 2005). In Norway 70.4% of 92,939 individuals invited participated in a large scale general health survey (second Health Survey of North Trondelag, HUNT II) (Hallan SI et al., *J Am Soc Nephrol* 2006). The mean age of the population was 49 years. Whereas serum creatinine was determined in all participants urinary albumin excretion was measured only in a 5% random sample. The prevalence of CKD stages I to IV was 3.1, 3.4, 4.6 and 0.16 % (total 11.3 %) and thus comparable to the results obtained in Groningen. Otero et al. (Otero A et al., *Nefrologia* 2010) published the results of a cross sectional study performed in Spain. 13,013 individuals stratified by age, sex and residence were targeted, 6,464 were contacted and 2,746 completed a questionnaire. The mean age was 50 years and the prevalence of CKD stages I-IV was 0.99, 1.3, 6.5 and 0.27% respectively. Viktorsdottir et al. (*Nephrol Dial Transplant* 2005) used data from 19,381 subjects of the Reykjavik Heart Study, a population based cohort study conducted in the years 1967 – 1996. 1.6% had stages I and II, 7.4 % stage III and 0.2% stage IV CKD. The prevalence of eGFR levels below 60 ml/min/1.73 m² increased dramatically with age in males and even more pronounced in females with more than 53% of female participants aged over 80 years being affected. Finally, Gambaro et al. (Gambaro G et al., *Clin J Am Soc Nephrol* 2010) studied 6,200 Caucasians > 40 years of age in northern Italy chosen randomly from patient lists of general practitioners. In total 1.7% had CKD stage I, 4.3% stage II, 6.4% stage III and 0.1% stage IV.

When European data are compared to US studies the overall prevalence of CKD in the general population is comparable (Mayer G. *Nephrol Dial Transplant* 2013). This fact is interesting, as the incidence of end stage renal disease (ESRD) is much higher in the latter (De Jong et al., *Clin J Am Soc Nephrol* 2008) calculated that the incidence of ESRD as % of prevalence of CKD stages III and IV in the Norway (based on HUNT data) and The Netherlands (based on PREVEND) is 0.24 and 0.19 respectively but 0.61 in the US (this calculation being based on white participants in NHANES). The authors discussed that renal disease might progress at a faster rate in US patients. In summary, all these studies support the conclusion that the prevalence of CKD dramatically increases with age and thus the number of renal patients is very likely to grow in the coming decades in parallel with the expected increase in life expectancy.

1.2.2 Chronic kidney disease, risk factors

As elaborated by Levey et al. (Kidney Int 2007) a history of diabetes, hypertension or cardiovascular disease confers the highest risk for developing CKD, and individuals who have such a history should be screened. In addition, obesity, a family history of CKD or prolonged treatment with potentially nephrotoxic drugs should provoke formal screening. From an epidemiological and economical perspective diabetes mellitus deserves special attention. In contrast to type 1 the prevalence of type 2 diabetes mellitus is increasing rapidly worldwide (van Dieren S et al., Eur J Cardiovasc Prev Rehabil 2010). The emerging pandemic is driven by population ageing, rising levels of obesity and inactivity and greater longevity of diabetic patients due to improved management. By the year 2025 380 million individuals will have type 2 diabetes and 418 million impaired glucose tolerance. In Europe 48.4 million adults had diabetes in 2003 (7.8% of the total population); by the year 2025 58.6 million will be affected (9.1%) but the growth rate and prevalence are quite different among various countries. In Germany the percentage will increase within this time period from 10.2 to 11.9%, in Italy from 6.6 to 7.9%, in the Netherlands from 3.7 to 5.1% and in the UK from 3.9 to 4.7% (www.heartstats.org). Diabetes is a widely underestimated cause of death as only a minority of patients die directly from severe hypo- or hyperglycemia. However, premature cardiovascular and renal disease account for about 50% and 10% of the total mortality respectively and the annual excess mortality attributable to diabetes is estimated to be 3.8 million (van Dieren S et al., Eur J Cardiovasc Prev Rehabil 2010). Ethnicity is a major risk factor for the development of diabetic renal disease. In a cross sectional survey among more than 24,000 individuals with an overall prevalence of normo-, micro- (MIA), and macroalbuminuria (typical “stages” of diabetic renal disease) of 51, 39, and 10%, respectively microalbuminuria was found more frequently in Asians and Hispanics (55%) than in Caucasians (41%) (Parving HH et al., Kidney Int 2006). Other risk factors for MIA are the genetic background, glycemic control and hypertension. For example in the UKPDS diabetics with hypertension were significantly more likely to have MIA than those with normotension (24% versus 14%) (HDS study, J Hypertens 1993). However, one has to acknowledge that MIA also is a frequent finding in hypertension per se and thus it is questionable whether MIA is indeed a marker for early diabetic renal disease.

1.2.3 Chronic kidney disease, clinical status

Chronic kidney disease not only leads to end stage renal failure with the necessity to start renal replacement therapy but also is a potent risk factor for cardiovascular events. The Chronic Kidney Disease Consortium analysed data from more than 2 million participants of general or vascular high risk population and CKD cohort studies on an individual level meta-analysis basis. Hazard ratios of mortality and end stage renal disease according to eGFR and albuminuria were determined across age categories after adjusting for sex, race, cardiovascular disease, diabetes, systolic blood pressure, cholesterol, body mass index and smoking status. Low eGFR and high albuminuria were associated with mortality and ESRD regardless of age. In general and high risk populations the mortality risk associations with eGFR were weaker on the relative (probably due to the higher risk in the elderly reference population due to higher comorbidities) but stronger on the absolute scale at older ages. For albuminuria the relative risk attenuation was smaller but the absolute increase in risk with age was even more pronounced. In cohorts specifically selected for CKD age did not modify the risk of mortality (Hallan SI et al., JAMA 2012).

1.2.4 Chronic kidney disease, patient perspective

Renal disease remains clinically silent in many affected individuals until very late in the course. Thus laboratory tests are essential in early diagnosis and progression prevention. Chudek et al. reported that only 3.2 % of the elderly subjects with CKD were aware of their disease (Nephrol Dial Transplant 2014). A similar low percentage has been reported in some US studies. For example in the KEOP study, a free screening program, which was targeting risk patients for CKD 60% tested positive for microalbuminuria, but less than 4% had ever been told that they had kidney disease (Harward DH et al. N C Med J 2009). Platinga et al. analysed data from the NHANES survey covering the years 1999 to 2004. Awareness improved over time in those with CKD stage 3 only from 4.7% in 1999 to 9.2% in 2003-2004. The numbers were significantly better for men and those with proteinuria, diabetes and hypertension (Platinga et al., Arch Int Med 2008). The presence of albuminuria markedly increased CKD awareness in a study by Tuot et al. (Clin J Am Soc Nephrol 2011, who also used NHANES data whereas other complications of CKD like hyperkalemia, hyperphosphatemia, elevated blood urea nitrogen or anemia did only to a smaller extent. Nonetheless 90 % of individuals with two to four markers and 84% with more than 5 markers of CKD were unaware of their disease. Clearly more advanced renal disease also increases awareness (Nickolas TL et al. Am J Kidney Dis 2004). Better awareness was demonstrated by Gorini et al., who assessed eGFR in 573 volunteers (aged 21 - 62 years) in

central Italy. 55% of the subjects had $\text{eGFR} < 90 \text{ ml/min/1.73m}^2$ and approximately 45% showed an awareness of CKD (J Nephrol 2012). Low level of awareness may reflect poor health care provider recognition and confusion regarding appropriate diagnosis and intervention, particularly at earlier stages of kidney disease leading to a lack of education of patients. It has been shown in other areas of medicine that increasing awareness improves treatment pattern (Burt VL et al. Hypertension 1995; Schucker B et al. Arch Int Med 1991) and thus one might speculate that achieving higher level of awareness might also improve outcome in CKD. The Kidney Early Evaluation Program assessed awareness of subjects at high risk for CKD at the time of the initial screening and followed the subjects longitudinally (Whaley-Connell A et al., Am J Med 2012). Among those with $\text{eGFR} < 60 \text{ ml/min/1.73m}^2$ 9% were aware of their disease. Compared with those unaware these individuals had a lower eGFR (49 vs. 62), a higher prevalence of albuminuria, diabetes, cardiovascular disease and cancer. Hence it was not surprising that aware subjects were more likely to progress to ESRD and die when compared to those unaware (4 vs. 17% and 22 vs. 19%). Surprisingly however even after statistical adjustment for demographics, socioeconomic factors, comorbidity, and severity of CKD at screening aware participants continued to demonstrate this increased risk (HR 1.37; 95% CI 1.07-1.75; $p < 0.0123$ for ESRD and HR 1.27; 95% CI 1.07-1.52 $p < 0.0077$ for mortality). Even though the authors clearly state that they do not conclude that unawareness conveys a survival advantage they nonetheless suggest that awareness on its own is not necessarily sufficient to reduce the poor outcomes associated with CKD.

1.2.5 Chronic kidney disease, molecular basis

For many years chronic kidney disease progression was considered a purely hemodynamically mediated process. Loss of functionally active nephrons by any disease process was accused to lead to counter-regulatory mechanisms in order to maintain glomerular filtration rate (GFR). Alterations in glomerular hemodynamics were considered of utmost importance. Both afferent arteriolar glomerular vasodilation and efferent vasoconstriction increase intra-glomerular filtration pressure thus leading to hyperfiltration, which on the short term stabilizes GFR but on the long term leads to progressing glomerular sclerosis thereby initiating a vicious cycle (Klahr S et al. N Engl J Med. 1988). However, during the last decade a large variety of molecular pathways has been described to be involved in chronic renal disease especially in diabetic subjects. For example oxidative stress caused by increased free radical production is believed to play a central role in the development of microvascular complications of diabetes, including diabetic kidney disease (Badal SS et al. Am J Kidney Dis. 2014). Under physiologic conditions, steady-state concentrations of oxidants are maintained at nontoxic levels by antioxidant defense and repair enzymes. Disturbance of the delicate balance between reactive oxygen species (ROS) that promote oxidative stress and antioxidant defense systems may play a critical role in DN pathogenesis. Mitochondria are the main source of ROS, while the NOX protein family, the catalytic component of the multiprotein enzyme complex NADPH (reduced nicotinamide adenine dinucleotide phosphate) oxidase, represents the primary nonmitochondrial source. Changes in mitochondrial morphology and dynamics under hyperglycemic conditions contribute to increasing mitochondrial ROS, while increased ROS secondary to upregulation of certain NOX subunits may underlie microvascular damage and progression of DKD. Animal studies suggest that mitochondrial-targeted therapies may reduce oxidation-induced cell damage, interstitial fibrosis, and glomerular damage and improve tubular and glomerular function. Further examination of mitochondrial machinery, as well as increased understanding of the role of ROS in vascular complications of diabetes, may help identify therapeutic targets for intervention. A number of local endogenous factors that retard the progression of diabetic nephropathy have recently been identified and include angiopoietin-1 (Angpt1), Smad7 and nuclear factor (erythroid-derived 2)-like 2 (Nrf2). Calcium-dependent regulation of podocyte actin dynamics involving transient receptor potential canonical (TRPC) channels and the Rho and Rac small GTPases has been shown to play important functions in glomerular health and disease. A central role for mammalian target of rapamycin (mTOR) activation in the development of diabetic nephropathy and regulation of autophagic flux in podocytes during aging has been demonstrated. Discovery of a circulating factor (suPAR) that can modulate outside-in $\beta 3$ integrin signaling in recurrent focal segmental glomerulosclerosis provides exciting therapeutic possibilities. Another secreted factor, the hyposialylated form of angiopoietin-like-protein 4 (ANGPTL4) was found to favor albuminuria in rats and in minimal change disease. Therapeutic sialylation of ANGPTL4 could limit albuminuria. Finally, neutralization of de novo paracrine activation of glomerular epithelial cells by heparin-binding epidermal growth factor (EGF)-like growth factor or EGF receptor antagonists could limit crescent formation and renal failure in immune-mediated vasculitis (Henique C et al., Curr Opin Nephrol Hypertens. 2012). Flux through the hexosamine pathway has been implicated in diabetic glomerulopathy. Under normal physiological conditions, a small percentage (1–3%) of glucose transported into the cells is metabolized through the hexosamine pathway. The rate-limiting enzyme glutamine:fructose-6-phosphate amidotransferase converts fructose-6-phosphate to glucosamine-6-phosphate favouring the accumulation of uridine diphosphate *N*-acetylglucosamine, substrate

for O- and N-protein glycosylation. In mesangial cells, the flux of glucose through the hexosamine pathway has been implicated in activation of p38MAPK, increased expression of TGF- β 1 and NF- κ B activation. AGEs represent a heterogeneous group of proteins, lipids and nucleic acids, irreversibly cross-linked with reducing sugars that directly or via receptor-mediated mechanisms (receptor for AGEs—RAGE) determine alteration in gene expression and activation of cellular signalling proteins that results in increased oxidative stress, increased inflammation and cytokines release. Blockade of RAGE in experimental animal models of diabetes ameliorates both functional (albuminuria) and structural diabetes-mediated renal alterations (glomerulosclerosis). Similarly, studies in RAGE null mice support a key role for RAGE in glomerular perturbations in diabetes (Gnudi L; Nephrol Dial Transplant. 2012).

1.2.6 SysKid aims and objectives

Based on background as outlined above SysKid defined the following specific aims:

- Aim 1:** Identify **persons at risk** of developing chronic kidney disease utilizing epidemiology as well as molecular tools.
Aim 2: Understand the **molecular processes** triggering early stage **chronic kidney disease** and **identify associated biomarkers**.
Aim 3: Develop **novel diagnostic** and **therapeutic strategies** to control progression of chronic kidney disease.
Aim 4: Perform **pre-clinical verification** of novel **therapy approaches** and perform **clinical testing** of **novel diagnostics**.

For tackling these aims the following objectives were defined:

- Objective 1:** *Integrate existing, and extend clinically well-defined sample cohorts of patients with chronic kidney disease.*
Objective 2: *Establish and unify a broad 'omics' repository characterizing CKD.*
Objective 3: *Decipher processes, molecular pathways and associated CKD biomarkers utilizing a Systems Biology approach.*
Objective 4: *Use cell cultures and animal models to deepen our understanding of identified processes associated with early CKD.*
Objective 5: *Delineate novel therapeutic strategies and pre-clinical evaluation for prevention and slowing of progression of chronic renal disease.*
Objective 6: *Clinical validation of identified biomarkers for generating early stage diagnosis and prognosis IVD kits*
Objective 7: *Delineate a novel risk score for the development of chronic kidney disease*
Objective 8: *Deepen our understanding of the epidemiological aspects of early CKD with particular focus on consequences for healthcare policies*

SysKid followed a bottom-up approach for allowing an integrative view: biomarkers and drug targets embedded in a landscape of molecular mechanisms seen with CKD as derived from large scale human, in vitro and in vivo studies, combined with risk factors in high-dimensional models for assessing risk for development and progression of disease, in consequence allowing provision of healthcare recommendations for prevention and improved treatment in case of developed disease.

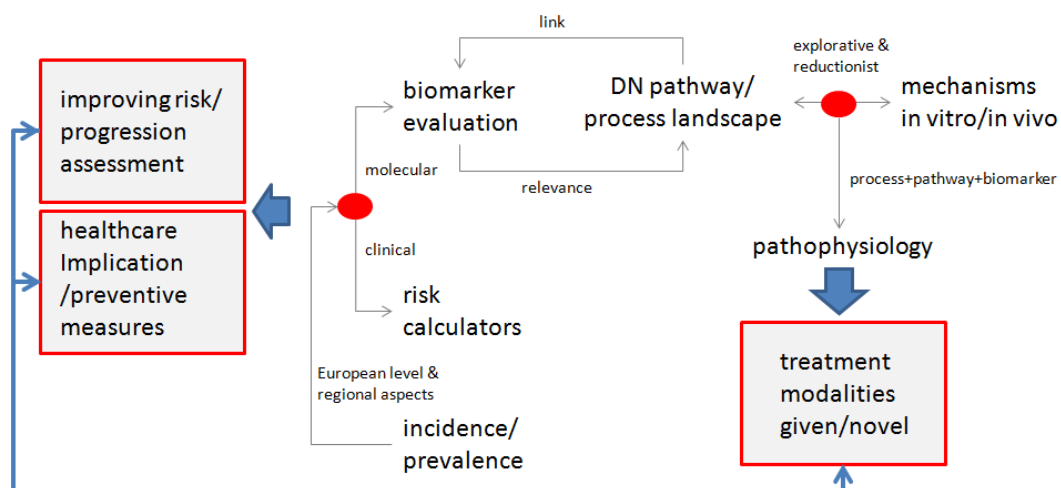


Figure: Systems Biology-centric implementation of SysKid.

From this perspective SysKid defined three tangible deliverables:

- Have (a) clinically tested kit(s) ready for identifying CKD risk/progression
- Have (a) CKD risk calculator(s) and healthcare recommendations ready
- Have novel concepts on hindering CKD progression ready utilizing given/novel drugs

1.3 Description of main S&T results/foreground

1.3.1 Kits for identifying CKD risk/progression

The primary goal of SysKid activities in the scope of prognostic assays is providing a better estimate on risk for onset as well as progression of CKD with specific focus on the most prevalent cause of ESRD, diabetic nephropathy (DN), compared to present clinical routines.

Such improvements are pivotal for assessing the value of preventive measures, but also for optimizing therapeutic strategies.

SysKid executed screening for biomarker candidates indicative of CKD risk/progression on the genetic, transcript, peptide/protein and metabolite level. Experimental procedures involved execution of nested case-control discovery studies for identifying candidates (first project phase), followed by large scale validation of promising candidates (second project phase). The SysKid team had the unique opportunity of combining top-level experimental capacities in Omics profiling, and assay development with large biobanks and clinical databases, complemented by dedicated statistics teams offering novel approaches for analysis of high-dimensional data.

On top, the biomarker-centric screening studies provided a rich molecular data basis for computational Systems Biology efforts aimed at integrating molecular mechanisms associated with the clinical presentation of diabetic nephropathy. The biomarker validation results in turn allowed to also assess the effective relevance of molecular mechanism at the various stages of disease development and progression.

The genetic level

Genetic analysis aims at identifying determinants on the DNA level with impact on predisposition for developing the clinical presentation, or adding to the pace of disease progression.

By making use of two prominent clinical trials in renal and diabetes medicine, ONTARGET and ORIGIN, the consortium together with the Population Health Research Institute (PHRI, Hamilton, Canada) discovered a single nucleotide polymorphism in the CERS2 gene (ceramide synthase 2) in a GWAS (genome-wide association study) to be significantly associated with incidence and progression of CKD.

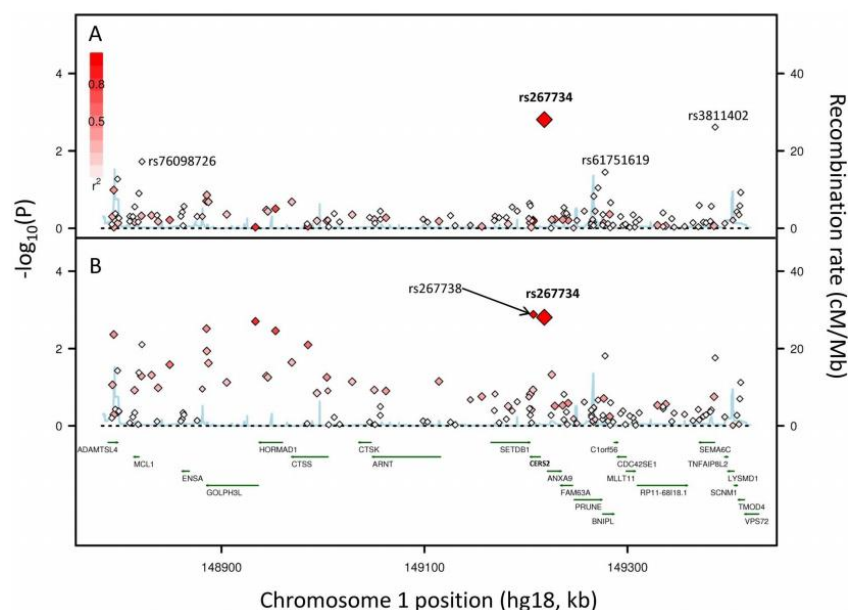


Figure: Regional plot for the CERS2 locus. SNPs are plotted by association P value of linear mixed models adjusted for age, sex, and principal components of genetic variation for the association between SNP and annual rate of change in albuminuria.

The annual change in urinary albumin to creatinine ratio was found to be highest in homozygous carriers of the A risk allele compared to homozygous G non-risk allele carriers as reference. The A risk allele frequency was found to be 0.79.

This CERS2 SNP could be confirmed in an independent validation study also performed together with PHRI in the ORIGIN study (N=1,916). Multivariable mixed linear regression model adjusted for age, sex and population structure showed the same significant increase in albuminuria as in the discovery cohort.

The transcript level

As second molecular level of analysis SysKid evaluated tissue transcript changes associated with progressive CKD, including both RNA from protein coding genes as well as regulatory RNA elements, at the first time combined from the very same sample base enabling integrative analysis of transcript composition.

For the discovery setting total RNA was isolated from renal biopsies from patients with diabetic nephropathy, hypertensive nephrosclerosis and other proteinuric kidney diseases (n=43), split into stable and progressive disease. Furthermore, we also collected zero hour pre-transplant wedge biopsies which served as control tissue.

Differentially regulated transcripts were identified further allowing correlation of miRNAs and mRNAs from the same subjects. In the progressive phenotype we identified down-regulation of 7 miRNAs (miR-30d, miR-140-3p, miR-532-3p, miR-194, miR-190, miR-204, and miR-206), and up-regulation of 29 target mRNAs involved in inflammatory response, cell-cell-interaction, apoptosis, and intracellular signaling.

mRNA targets	miRNAs							BIOLOGICAL PATHWAYS					
	hsa-miR-30d	hsa-miR-140-3p	hsa-miR-532-3p	hsa-miR-194	hsa-miR-190	hsa-miR-204	hsa-miR-206	Inflammation	CA	A	SIG		
Gene Symbol								Natural killer cell mediated cytotoxicity					
CCL19								Chemokine signaling pathway					
CLDN4								Chronic myeloid leukemia					
CTTN								Leukocyte transendothelial migration					
CXCL1								T cell receptor signaling pathway					
F11R								Acute myeloid leukemia					
IFNAR2								Cytokine-cytokine receptor interaction					
IKBKB								Tight junction					
ITPR3								Gap junction					
KRAS								Cell adhesion molecules (CAMs)					
LAMC2								Apoptosis					
MAP3K1								GnRH signaling pathway					
MAP3K14								RIG-I-like receptor signaling pathway					
NCK2								Pathways in cancer					
PLCB2													
PLD2													
PML													
PTK2B													
PTPRC													
PTPRF													
RASGRP1													
RUNX1													
TANK													
TNFRSF10B													
TNFRSF10D													
TNFRSF25													
TNFSF10													
TRADD													
TRAF2													
TUBB3													

Table: miRNA-mRNA pairs identified as relevant in progressive disease, including associated molecular pathway information.

Reduced expression of miR-206 in progressive disease correlated with the upregulation of target mRNAs of CCL19, CXCL1, IFNAR2, NCK2, PTK2B, PTPRC, RASGRP1, and TNFRSF25, all participating in inflammatory pathways. Progressive cases also showed a lower expression of miR-532-3p and an increased expression of cognate target transcripts MAP3K14, TNFRSF10B/TRAIL-R2, TRADD, and TRAF2, all being involved in apoptosis pathways.

To validate these results we collected an independent cohort of n=29 cases (renal diseases). We confirmed the results from the discovery cohort regarding decreased expression of miR-206 and miR-532-3p in progressive subjects, and the inverse correlation of these miRNAs with the expression of 9 of the 12 target genes identified in the discovery run.

The peptides, protein and metabolite level

A third level of biomarker analysis aimed at the effector levels, hence proteins and metabolites, where the consortium composition allowed utilization of various technologies and platforms for studying single markers as well as marker combinations.

We assessed a series of individual proteins either alone or in combination for their predictive performance of transition in albuminuria stage, examples including GDF-15 and TroponinT.

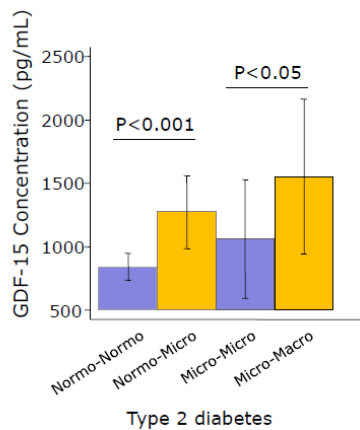


Figure A: GDF15 concentration, albuminuria stage transitions.

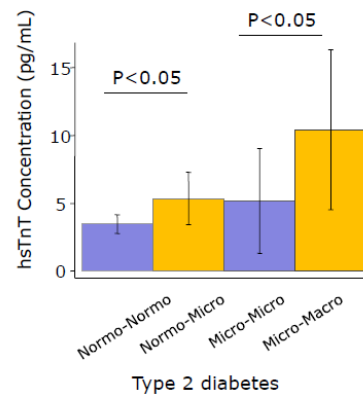


Figure B: hsTnT concentration, albuminuria stage transitions.

Growth-Differentiation Factor-15 (GDF-15), a member of the TGF- β family has been implicated as a predictor for cardiovascular and all-cause mortality. GDF-15 was found to predict transition in albuminuria stage in type 2 diabetes (n=66) beyond conventional risk markers. The findings were replicated in a non-diabetic hypertensive cohort (n=150), implying that GDF-15 is not specifically related to diabetes.

The necrosis marker high-sensitivity TroponinT (hs-TnT) is increasingly recognized as a strong predictive marker for vascular events. In hypertensive patients (n=150) without diabetes, the odds for transition in albuminuria stage significantly increased per SD increase in hs-TNT (p=0.02), independently of conventional risk markers. Similar, non-significant associations of hs-TNT were observed in type 2 diabetes (n=66).

Since type 2 diabetes is a complex disease involving multiple pathophysiological processes of renal function decline, we speculated that a combination of multiple biomarkers capturing different disease pathways of renal damage may provide a more realistic picture of a patient's actual pathophysiological status, yielding a better assessment of disease prognosis. We therefore assessed the predictive ability of a protein biomarker panel for eGFR decline. A novel panel of biomarkers representing different pathways of renal disease progression including inflammation, fibrosis, angiogenesis, and endothelial function improved prediction of eGFR decline on top of established risk markers in type 2 diabetes (n=82): When modeled on top of established risk markers, the biomarker panel including matrix metalloproteinases, tyrosine kinase, podocin, CTGF, TNF-receptor-1, sclerostin, CCL2, YKL-40, and NT-proCNP improved the explained variability of eGFR decline (R² increase from 37.7% to 54.6%; p=0.018) and improved prediction of accelerated eGFR decline (C-index increase from 0.835 to 0.896; p=0.008).

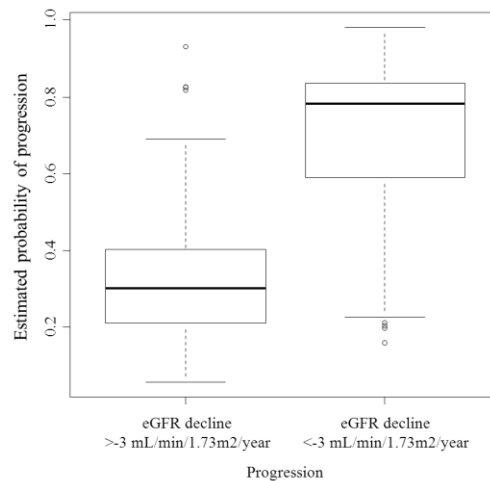


Figure: Predicted probability, multi-marker panel, of accelerated renal function decline (eGFR decline <-3 or >-3 mL/min/1.73m2/year) in patients with type 2 diabetes.

The measurement of multiple biological molecules has advanced significantly over the past years with the introduction of high-throughput Omics screening platforms. Proteomics and metabolomics have emerged as strong tools in biomarker discovery, and SysKid had the opportunity to utilize these approaches in discovery and validation studies.

In a case-control study, the high-dimensional urinary proteomic classifier CKD273 was shown to be independently associated with transition to micro- or macroalbuminuria (OR 1.35 [95% CI 1.02, 1.79], $p=0.035$).

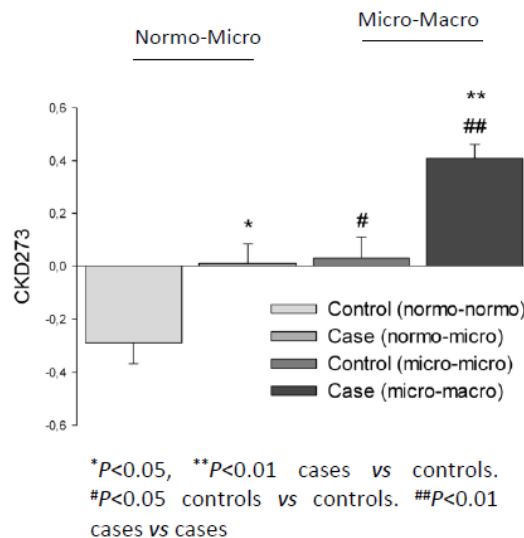


Figure: Scoring values of the CKD273 classifier for comparison of normo-microalbuminuria, and micro-macroalbuminuria.

The classifier predicted the development and progression of albuminuria on top of albuminuria and estimated GFR (eGFR, area under the receiver operating characteristic [ROC] curve increase of 0.03, $p=0.002$; integrated discrimination index [IDI]: 0.105, $p=0.002$). Fragments of collagen and α -2-HS-glycoprotein showed significantly different expression between cases and controls.

In a discovery study in type 2 diabetes ($n=82$) and hypertension ($n=125$), plasma proteomics classifiers were developed and cross-validated for prediction of transition in albuminuria stage. Improvement in risk prediction was tested on top of a reference model of baseline albuminuria, eGFR, and renin-angiotensin-aldosterone system intervention.

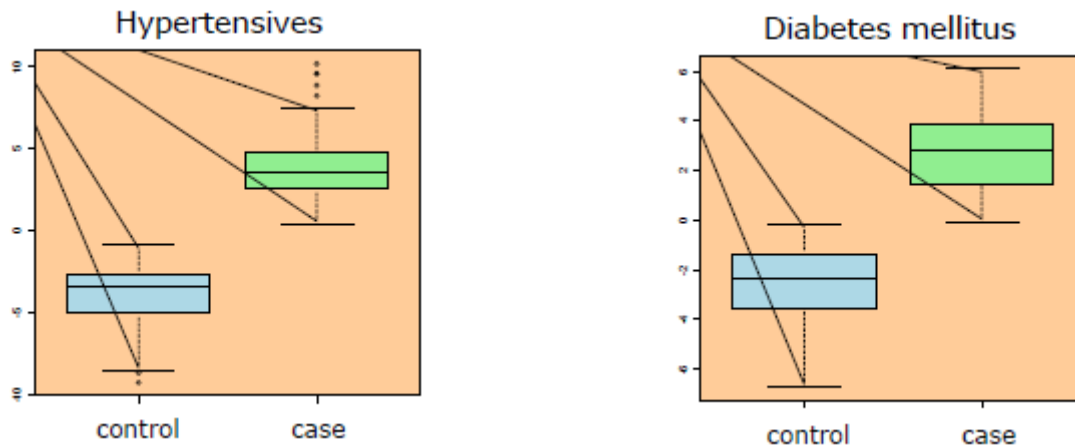


Figure A: plasma proteomics-based classifier, hypertensive cohort. **Figure B:** plasma proteomics-based classifier, diabetes cohort.

In hypertensive patients, the classifier improved risk prediction for transition in albuminuria stage on top of the reference model (C-index from 0.69 to 0.78; $p < 0.01$). In type 2 diabetes, the classifier improved risk prediction for transition from micro- to macroalbuminuria (C-index from 0.73 to 0.80; $p = 0.04$). In both diseases, the identified peptides were linked to pathways recognized to contribute to nephropathy, including fibrosis, inflammation, angiogenesis, and mineral metabolism.

The plasma metabolites histidine and butenoylcarnitine and urine metabolites hexose, glutamine, and tyrosine were discovered to predict the transition from microalbuminuria to macroalbuminuria ($n = 42$) beyond established renal risk markers. The metabolites were not predictive for transition from normo- to microalbuminuria ($n = 48$). These metabolites appear specific to type 2 diabetes as the metabolites were not predictive in hypertension without diabetes ($n = 150$).

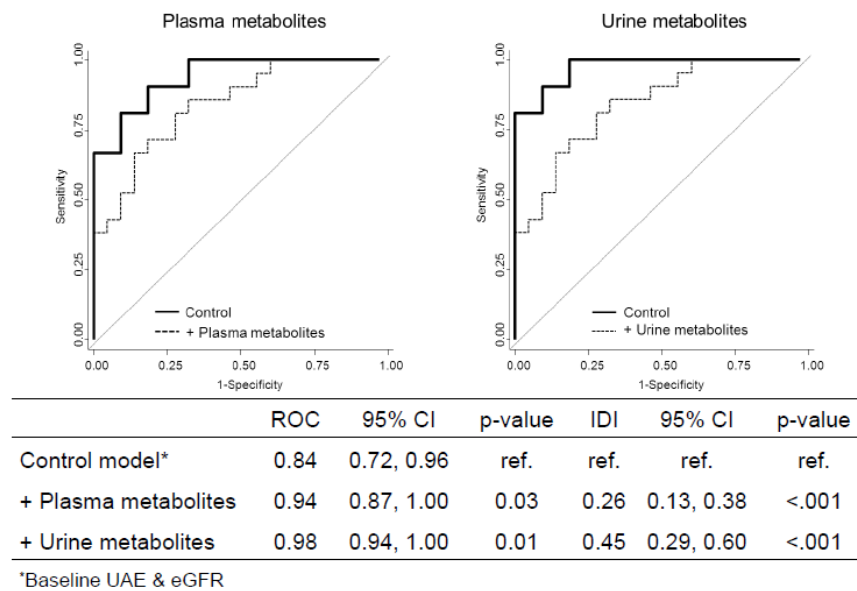


Table: Area under the Receiver Operating Characteristic curve (AUROC) and Integrated Discrimination Index (IDI) for conditional logistic regression models predicting transition in albuminuria stage in patients with type 2 diabetes mellitus: Micro- to macroalbuminuria subgroup ($n = 42$).

Using the knowledge gained from the SysKid Discovery Studies, we then proceeded to design a study to validate our findings (V/ED, Validation/Extended Discovery study).

Serum and urine samples from patients from 4 distinct studies (DIRECT-2 ($n = 790$), PREVEND ($n = 266$), SUN-Micro ($n = 225$), SUN-Macro ($n = 930$)) were selected for the SysKid extended discovery / validation study. The

studies were selected to include patients with type 2 diabetes at early stage of nephropathy (normoalbuminuria and eGFR>60); mid stage nephropathy (microalbuminuria) and late stage nephropathy (macroalbuminuria and eGFR<60).

CKD Stage			Albuminuria		
			Normo-	Micro-	Macro-
GFR	I	>90	<i>DIRECT PREVEND_{T2DM}</i>	→	
	II	60-89		→	
	III	30-59		<i>SUN-Micro PREVEND_{T2DM}</i>	
	IV	<30			<i>SUN-Macro</i>
	V	ESRD			

Figure: sample matrix for the SysKid V/ED study.

The aims of this validation study were to assess the validity of individual protein biomarkers, protein biomarker panels, and omics- based biomarkers in predicting the development and progression of CKD in a large type 2 diabetes population. Additionally, we aimed to evaluate the predictive performance of these biomarkers at early mid and late stage CKD. Validation provided positive results on both, individual biomarker as well as biomarker panel level.

The single markers CHI3L1 (YKL-40), HGF, TNF-R1, TEK (TIE-2), Metallo-Matrix Proteases (MMP)1, MMP2, MMP7, MMP8, MMP13, and Growth Hormone independently predicted eGFR decline.

The urinary proteomics marker panel/classifier CKD273 performed significantly better than the currently available clinical standards in predicting progression of CKD, hence further underscoring the validity of utilizing proteomics in the clinical setting.

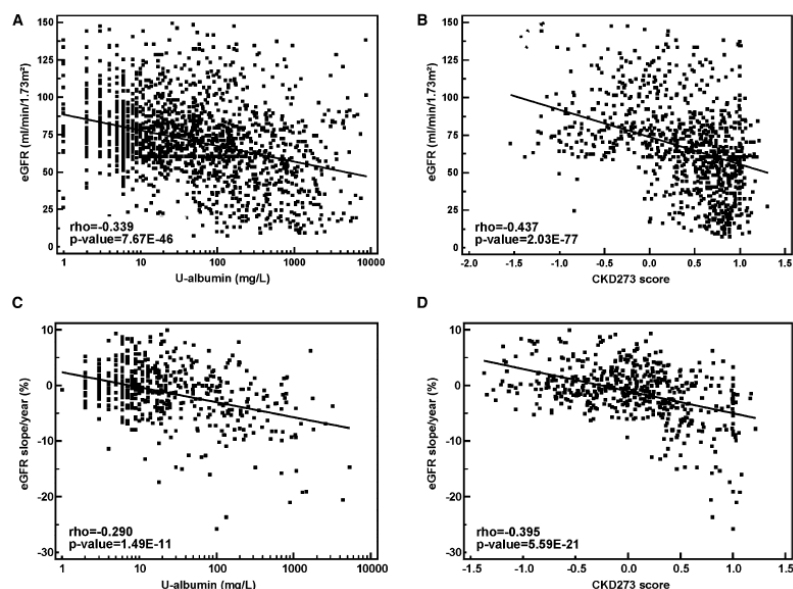


Figure: Correlation analysis. Scatter diagrams of regression analysis with baseline eGFR and log urinary albumin (A) or CKD273 classifier (B) or with slope of eGFR per year and log urinary albumin (C) or CKD273 classifier (D).

At present status the CKD273 classifier has been verified in multiple, in some instances multicentre studies, and a significant improvement over the current state of the art in early detection and prognosis of CKD could be demonstrated.

Refining eGFR/GFR

Within SysKid, there was the opportunity to associate novel metabolomics biomarkers with measured GFR. SysKid had access to plasma samples available from type 1 and type 2 diabetic patients in whom GFR was measured with plasma clearance of 51Cr-EDTA. These plasma samples allowed dedicated investigation of the association and predictive ability of novel markers with renal function impairment or loss of renal function for early renal disease assessment. The aim was to identify novel filtration biomarkers using metabolomics that are associated with renal function impairment or loss of renal function for early renal disease assessment. We measured metabolites in plasma samples from patients with type 1 or type 2 diabetes in whom measured GFR was assessed at regular time-intervals.

Findings suggest that Kynurenine (category: biogenic amine), C5-DC / C6-OH, C4, C5:1-DC, and C8 (category: acylcarnitines), SDMA (category: biogenic amine), and Cit (category: amino acid) may be potential makers for GFR decline in type-1 and type-2 diabetes.

The integration perspective

SysKid executed screening for biomarkers on the genetic, transcript, protein and metabolite level following three generic strategies: **1.** explorative screening, **2.** targeted screening and **3.** hypothesis driven screening. For the first two approaches the consortium implemented Omics-based technologies, the third approach being realized by single analyte assays in part complemented by multiplexed assays.

Explorative approaches were implemented for genetic background, transcripts and serum-based proteomics, seeing positive validation of SNPs as well as of transcripts (including miRNAs), further including novel serum-based signatures. Targeted approaches included background knowledge on candidates of interest as implemented in urinary proteomics and metabolomics work, leading to discovery of novel candidates as well as validation of high-dimensional classifiers. Hypothesis-driven work rested on background mechanisms known to be involved in DN, on this basis following a guided selection of biomarker candidates.

A molecular biomarker in its generic definition serves as a proxy for educating on the status/activity of a molecular process. In case such biomarker is identified as relevant in a specific clinical context, e.g. serving for prognosis, such biomarker becomes a clinical biomarker.

We in SysKid focused on identifying and validating biomarker towards clinical utility, but further aimed at complementing the clinical perspective with exploring biological links between biomarker candidates and molecular mechanisms. Such reference of biomarkers and mechanisms is pivotal in developing novel and optimizing given therapeutic measures. For retrieving a comprehensive annotation of DN on a molecular pathway and process level we established a dedicated data infrastructure for handling and integration of molecular profiles characterizing DN from a) public domain, b) background from project partners, and c) foreground generated in the project. With these tools in hand we consolidated 48 Omics studies characterizing DN, providing us with a most comprehensive molecular characterization of the disease.

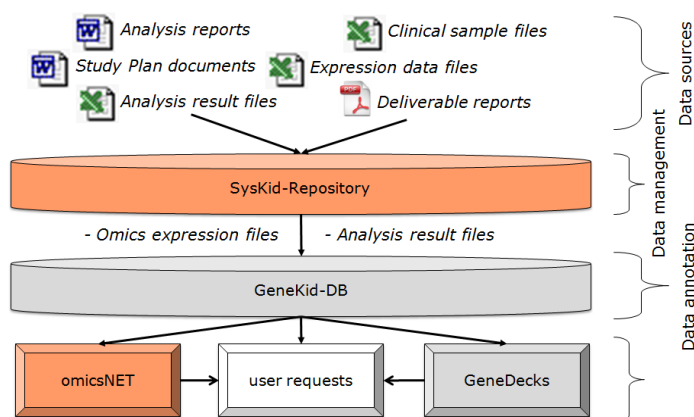


Figure: SysKid data organization for enabling integrative analysis.

Utilizing the huge data space on CKD organized in the SysKid data environment allowed delineation of molecular mechanism representations, including **1.** a standard molecular pathway model, **2.** various molecular mechanism models, **3.** a novel concept on superpaths, and **4.** a novel concept on molecular process models.

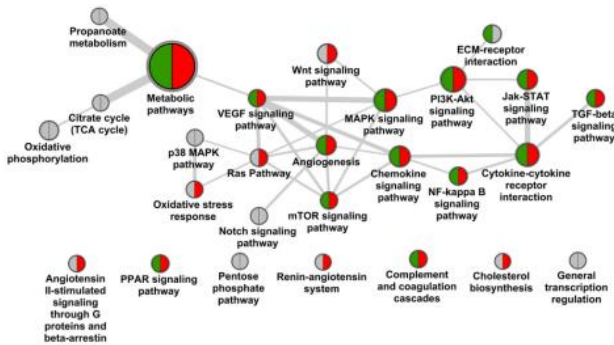


Figure A: DN – the molecular pathway level.

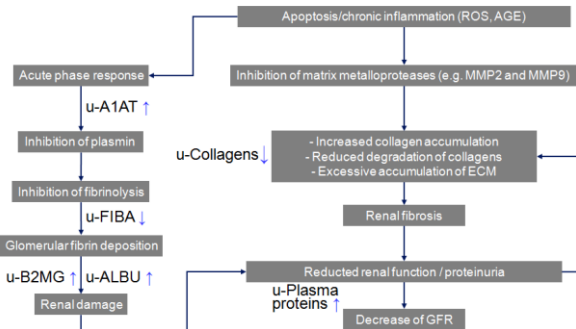


Figure B: DN – the molecular mechanism level.

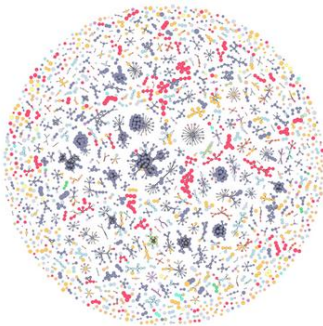


Figure C: DN – the molecular superpath level.

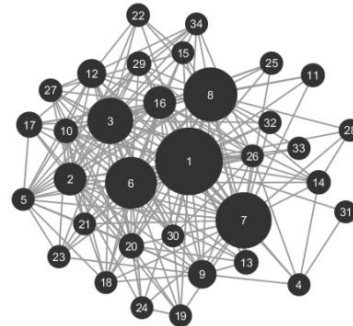


Figure D: DN – the molecular model level.

The different molecular mechanism representations were then used for i) assigning all biomarker candidates identified/validated in SysKid studies (in total including 393 features evaluated on the various platforms), and ii) proposing novel biomarker candidates out of a molecular process and mechanism perspective.

With this approach we established and along the V/ED study also validated a novel concept for rational provision of biomarker candidates, and integrating molecular processes with valid biomarkers with regard to disease progression directly provided us with knowledge on molecular mechanisms of relevance in disease progression.

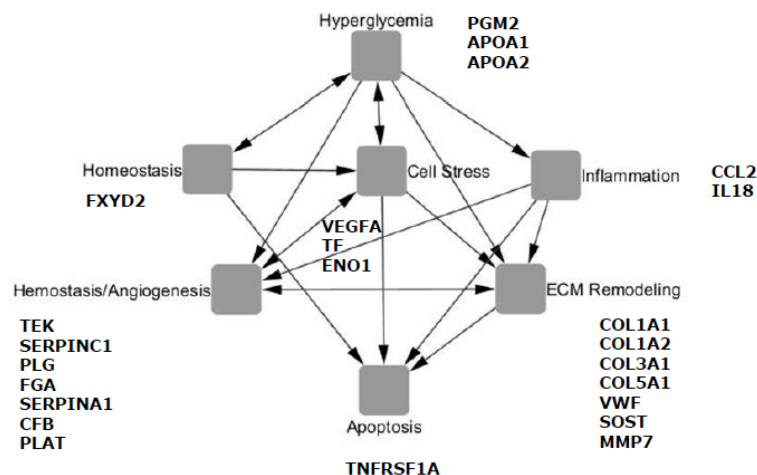


Figure: top-level mechanism perspective on early stage CKD, and candidate biomarkers assigned to specific mechanisms.

1.3.2 CKD risk calculator(s) and healthcare recommendations

SysKid executed significant activities on identifying risk factors for disease development and progression culminating in developing risk scores and calculators. This on the one hand offers the baseline regarding novel risk assessment approaches as provided by biomarkers, on the other hand allows identification of parameters eventually serving for preventive measures. Here we in SysKid specifically focused on life style factors for delineating healthcare recommendations.

A second major activity was on identifying CKD prevalence estimates on a European level following a large scale data collection effort (European CKD burden consortium) together with a significantly sized prospective study including five European countries.

Data analysis was encountering numerous data aggregation and statistical analysis challenges. The SysKid team composition further allowed adding to guidelines for clinical statistics in the field, combined with developing improved data analysis methodologies.

Guidelines

A number of SysKid activities focused on epidemiological analyses, e.g. identifying clinical risk factors involved in the development and progression of CKD. While the key risk factors can be assumed to be known, it is less clear which functional relationship exists with the outcome variables (i.e. type of relationship – e.g. linearity or non-linearity, and how risk factors interplay with each other).

For this reason the SysKid consortium has written 10 guidelines on epidemiological and statistical issues arising in the epidemiological analyses within the SysKid project (and in any project addressing such questions). In order to integrate knowledge from epidemiologists and statisticians, the guidelines were written in collaboration with investigators from at least two research groups from the SysKid consortium. The guidelines first dealt with some general considerations, and later turned to peculiarities of the data sources available to SysKid. Furthermore, where possible, the guidelines summarized newest methodological literature on the respective topic.

Finally, the guidelines were implemented in a suite of packaged software for routine use.

The topics of the 10 guidelines on epidemiological and statistical issues are:

- | | |
|---|-------------------------------------|
| 1. Sample size calculations | 6. Time-dependent effects |
| 2. Representation of exposures in regression models | 7. Etiological vs prognostic models |
| 3. Analysis of longitudinal measurements | 8. Missing values |
| 4. Type of outcome: time-to-event (survival analysis) | 9. Measures to assess model fit |
| 5. Type of outcome: continuous and dichotomous | 10. Risk calculator |

Novel high dimensional data analysis methodologies

In SysKid, we analyzed urine and plasma samples from diabetic and hypertensive patients, taken at a time point where it was not yet known whether and how fast these patients would develop chronic kidney diseases, or whether first signs of kidney problems, i.e., albuminuria, would progress towards more advanced stages. Follow-up data was available for these patients and so we could use their samples to identify, using high-throughput technologies, biomarkers that may be related with incidence or progression of chronic kidney disease.

High-throughput analyses such as plasma proteomics produce a large amount of data, in particular, the expression of thousands of peptides in a sample can be measured. However, many of these peptides are not detectable in a considerable proportion of samples, and so non-expression could be related to progression of chronic kidney disease. The combination of these two problems, having far more potential biomarkers to evaluate than samples, and having a high percentage of non-expressed biomarkers poses challenges to data analysis which had to be tackled. We reviewed literature on biomarker identification in case of such zero-inflated variables and added a novel approach, the left-inflated mixture model likelihood ratio test, to the body of statistical tests that specifically address excess non-expression. In a comprehensive simulation study, the method showed favorable properties across a variety of possible data constellations. The test was implemented in an R package `lim.lrt` and provided to the scientific community.

In order to derive a biomarker panel, we also elaborated and validated (in different data sets) a procedure that filters and selects biomarkers subject to excess non-expression and estimates their association with progression or incidence of CKD. The procedure is based on penalized logistic regression models (Lasso) and involves, for each biomarker and each sample, the expression/non-expression status and the magnitude of expression. The procedure typically arrives at a biomarker panel which contains 18-44 markers. We assessed the importance of each biomarker in the panel by evaluating the drop in percentage of explained variation if a single biomarker were removed from the panel.

By using resampling techniques, a cross-validated CKD progression prediction score based on the biomarker panel can be devised which can then be used to validate the findings, assessing the added value of the biomarker panel score in prediction of CKD progression.

A further frequent challenge, also of relevance in SysKid data, was the application of the Lasso technique in the presence of occasionally missing biomarker values, which had to be imputed using multiple imputation with chained equations. While for this particular situation a group-Lasso approach has been recently proposed, we developed a stratified resampling approach which offered major computational advances over the group Lasso.

Yet another data analysis challenge was tackled in SysKid, namely handling of different statistical approaches (an illness-death model for interval-censored data and some standard competing risk models) to assess progression of mGFR to some specific CKD stages.

As example, one of the developed statistical software products, the SAS macro PSHREG, has been made available online in 2012 and is since then highly demanded by registered users all over the world. It received a recommendation by Paul Allison, who is a well-known author of numerous data analysis textbooks and course instructor.

Risk factors and healthcare recommendations

A SysKid study demonstrated that tight control of risk factors was proven to significantly lower morbidity and mortality by comparing progression of loss of GFR in diabetic nephropathy patients including patients with type 2 diabetes (n=286) as well as type 1 diabetes n=315). With data on patients followed before 2000 a reduced rate of decline in GFR of 14-20% in the most recent decade was found, associated with better control of BP, more use of RAS blocking treatment, and improved cholesterol and diabetes treatment. More importantly the analysis showed an almost 50% reduction in mortality, after adjustment for age, and other risk factors.

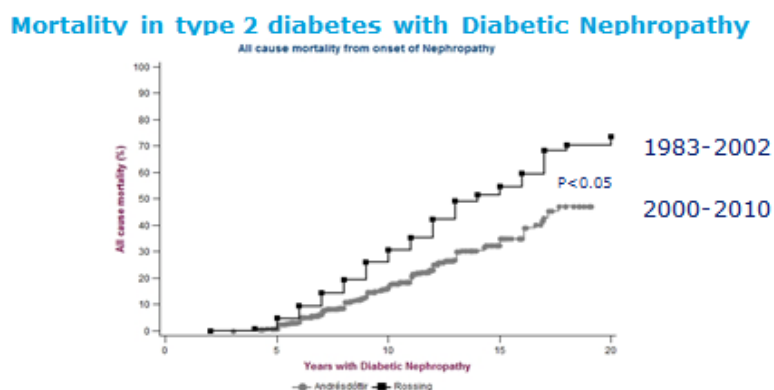


Figure: comparative analysis, mortality in T2DM with DN.

In another study the fact of monitoring kidney function (laboratory measurements) was identified as the most important prognostic factor of adequate health care. Despite the fact that kidney function is often monitored

without evidence of reduced kidney function, we could also see a significantly higher monitoring rate in patients with such evidence.

Analysis of the GIANTT database (which is a regional initiative in Groningen of health care providers and researchers focusing on the care delivered to ambulant patients with type 2 diabetes mellitus in the north of The Netherlands) was executed to assess guideline adherence and factors associated with albuminuria screening and treatment in patients with type 2 diabetes mellitus (T2DM) in primary care. In this cohort it turned out that approximately 60% of type 2 diabetes patients are annually screened for the presence of micro- or macroalbuminuria and if confirmed, treatment with ACE-inhibitors or angiotensin receptor blockers was only started in 13% of patients (who were not already treated with such drugs).

In addition to the well-established risk factors for advanced CKD such as albuminuria and GFR we in SysKid specifically evaluated potentially modifiable lifestyle and nutritional factors for incident and very early stages of CKD. This is especially important because of the relative paucity of data in this much larger patient group. The two large RCTs ONTARGET and TRANCEND were used for the discovery of risk factors and the very similar ORIGIN study was used to validate the findings.

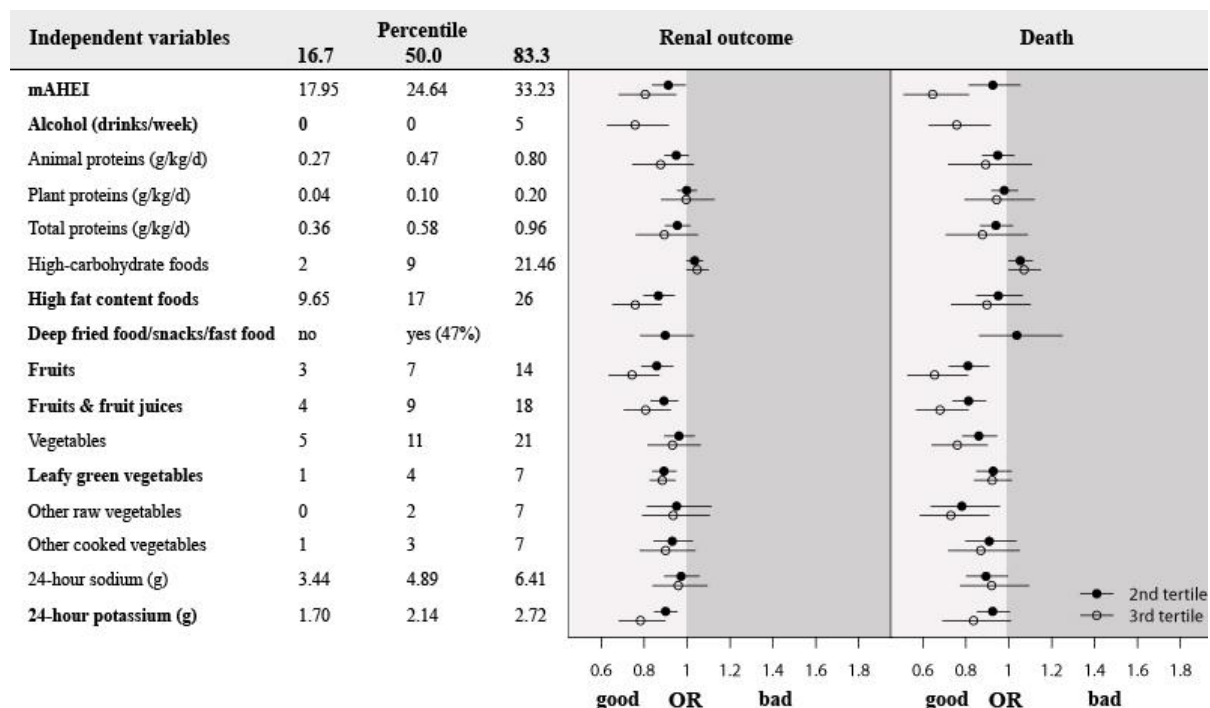


Figure: Forest plot of adjusted single variable multinomial logit models on the association of nutrition and lifestyle variables with renal outcome and death.

In a first analysis we found that a healthier diet measured by the mAHEI score was highly associated with a reduced risk for CKD incidence and progression. In this cohort 32% of participants developed CKD defined as new micro- or macro-albuminuria or GFR decline of more than 5% per year within the 5.5 years follow up period and eight percent died. Compared with participants in the least healthy tertile of mAHEI score, participants in the healthiest tertile had lower risk of CKD (adjusted OR 0.75, 95%CI 0.65-0.85) and lower risk of death (0.62, 0.49-0.78).

Participants consuming more than three servings of fruits and leafy green vegetables per week had a lower risk of CKD compared to participants consuming these food items less frequently. Participants in the lowest tertile of total and animal protein intake had an increased risk of CKD compared to participants in the highest tertile (total protein OR 1.16, 1.05-1.30). Moderate alcohol intake reduced the risk of CKD (0.76, 0.66-0.90) and mortality (0.70, 0.54-0.89).

Based on these associational findings it sounds reasonable to advocate a healthier diet rich in fruits and vegetables for the prevention of and slowing the progression of established renal disease.

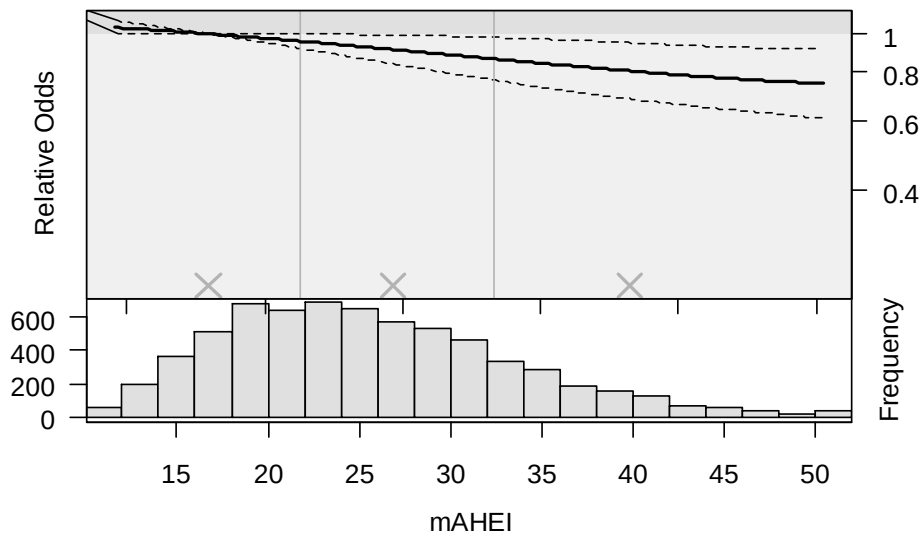


Figure: Relative odds of CKD incidence or progression in patients with diabetes according to mAEI, a measure of healthy diet. The analysis accounted for the competing risk of death and was adjusted for established confounders.

Besides nutrition, lifestyle sub-summarizes a whole set of behavioural items such as alcohol intake, smoking, bodily appearance, physical activity, sleeping duration and quality, stress, worries, work, wealth, education, mobility and social contacts/networks. We evaluated these parameters in their association to CKD incidence and progression using the same large RCTs as above. The main finding was that moderate alcohol consumption and physical activity significantly decreased CKD risk compared to non-users. The size of the social network, i.e. the number of friends and social interactions measured by the SNS score was a significant inverse associated with the risk of CKD and mortality.

The relative risk reduction was 11% and 22% when comparing the 3rd to the 1st tertile of the SNS, respectively. Education showed an association with CKD ($p=0.036$). Stress and financial worries were not associated with CKD. Validation of these results by analysis of all, predominantly non-diabetic, participants of ONTARGET yielded very similar results. Based on these findings is intuitive that an intact social network is vital for older patients with diabetes.

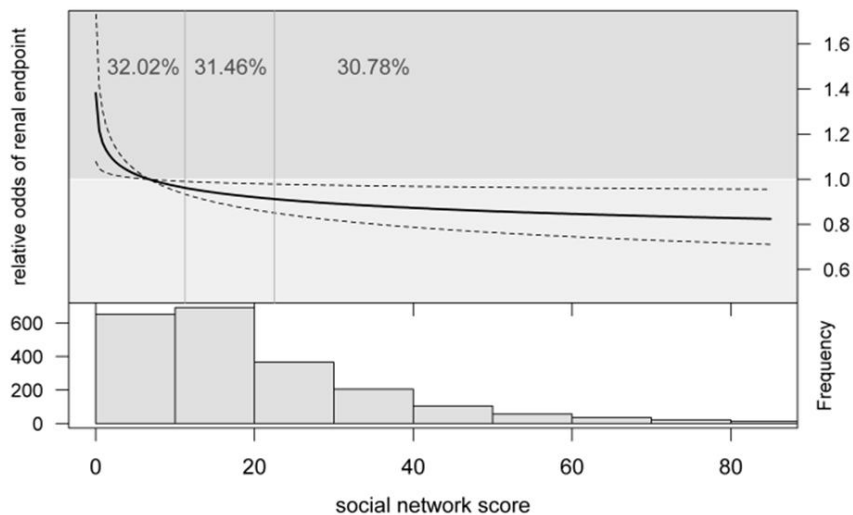


Figure: Plots of Relative Odds for the Social Network Score (SNS) and CKD incidence or progression in patients with diabetes adjusted for a set of established confounders.

In order to determine the impact of findings not only for the individual patient but also on a population level the PAF (population attributable fraction) of changing nutrition and lifestyle behavior was analyzed. Our results showed that in case physical activity would be performed daily roughly 5% of cases with CKD and 12% deaths

could be avoided within five years. Furthermore, increasing the vegetables consumption would have the largest impact on population health. Adding two servings of leafy green vegetables per week could reduce 2% of cases and 5% deaths. Considering diet, weight, physical activity, use of tobacco and social network, exposure to less than optimum levels was addressing 17% of CKD cases and 38% deaths in the five years observation period.

HLS	%	%CKD	%Death	Adj. OR _{CKD} (95% CI)
0-1	11.9	35.0	19.0	1
1.5	12.6	34.2	18.2	0.88 (0.83-0.92)
2	16.8	31.2	15.3	0.82 (0.76-0.89)
2.5	18.9	29.6	15.1	0.76 (0.69-0.85)
3	16.6	32.0	14.8	0.72 (0.63-0.82)
>3.5	23.2	29.1	9.8	0.58 (0.47-0.72)

Table: Healthy lifestyle score and Population Attributable Fraction (PAF) for the incidence and progression of CKD.

Multi-dimensional risk models and scores

We developed two risk prediction models for incidence or progression of CKD and mortality; a parsimonious model containing only laboratory markers of kidney function and an extended model where typically available clinical information is used.

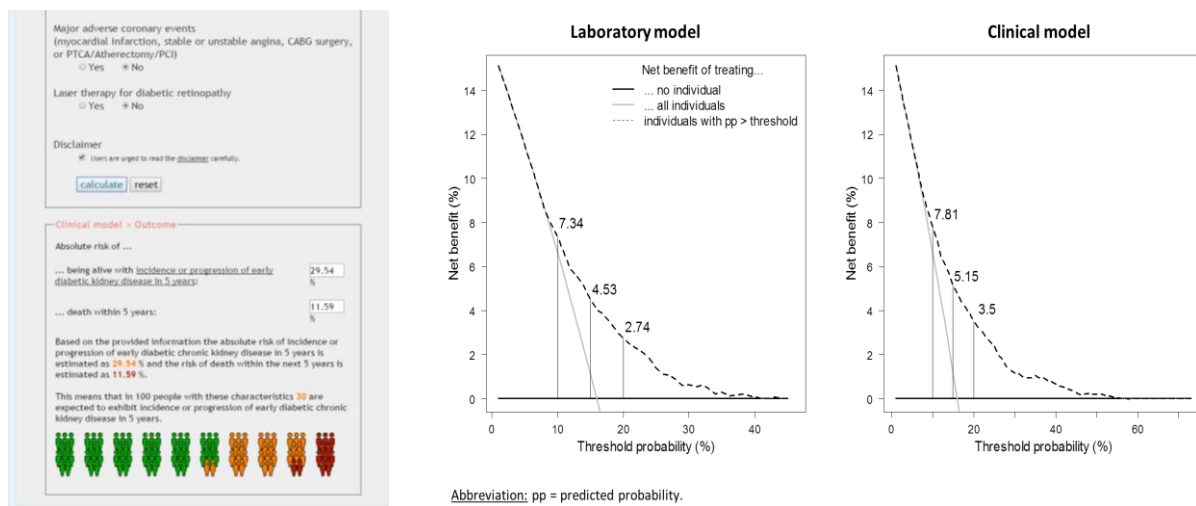


Figure A: user front end, renal risk calculator.

Figure B: Decision curve analysis of the renal risk calculator.

Prevalence of CKD in Europe

Based on published data in European countries, we projected the prevalence of CKD by stage over the next decade in the patient population with diabetes.

Utilizing these baseline data we applied the Lee Carter algorithm and national population data to predict the rates of CKD in twelve countries. The estimated prevalence of chronic kidney disease in patients with diabetes is expected to increase in all 12 countries up to the year 2025. For CKD stage 3, we estimate for Austria in 2025 a prevalence of 215,000 per million population (pmp) (95 % confidence interval 169,000, 275,000), for CKD4 18,600 pmp (14,500, 23,700) and for CKD5 6,900 pmp (5,400, 8,900). The median prevalence in the considered countries is 132,900 pmp (IQR: 118,500, 195,800), 11,500 (10,200, 16,900) and 4,300 (3,800, 6,300) for CKD stages 3, 4 and 5, respectively. Altogether these data predict an annual increase of 3.2% in the prevalence rates of diabetic CKD stage 5.

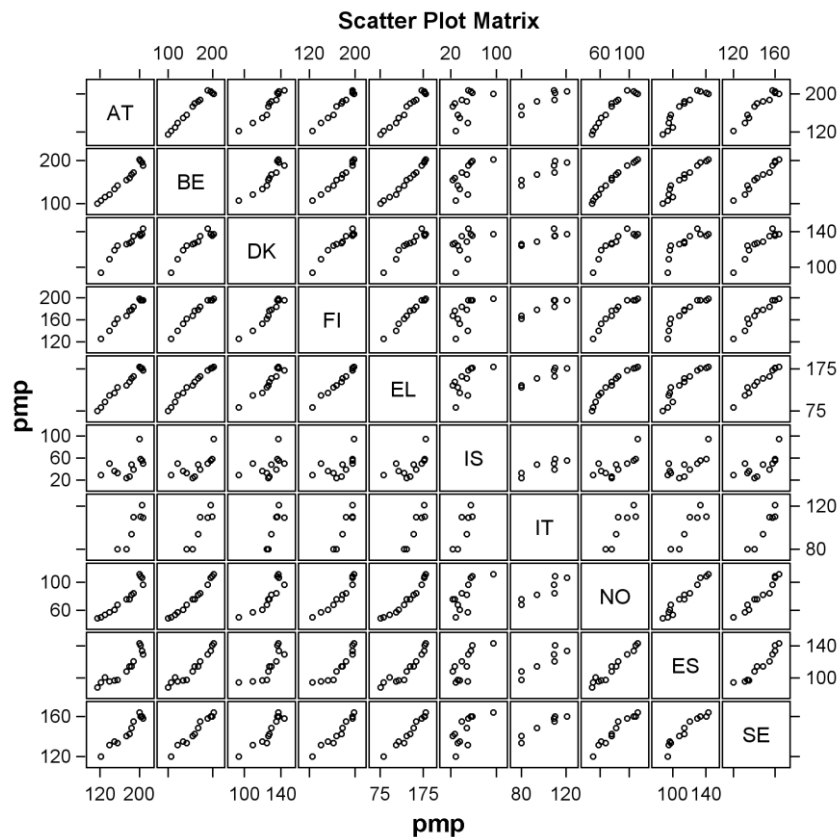


Figure: observed prevalence rates of CKD stage 5 in patients with diabetes in the twelve analyzed countries (AT, Austria; BE, Belgium; DK, Denmark; FI, Finland; EL, Greece; IS, Iceland; IT, Italy; NO, Norway; ES, Spain; SE, Sweden; NL, Netherlands; UK, United Kingdom).

The prediction was validated by using a split sample approach. Available data were divided into two parts according to period (1998 – 2004 and 2005 - 2011). The first period was taken to predict prevalences in the second time window. Observed prevalences from 2005 to 2011 and predicted data were compared, and observed-to-expected ratios did not diverge much.

In an effort focused on Italy the period prevalence of stages 3-5 CKD [eGFR (CKD-EPI formula) <60 ml/min/1.73 m²] was investigated in 3,780 individuals (Men: n=1,937, age ranging from 20 to 80 years; Women: n=1,843, age ranging from 21-81 years) randomly extracted by electoral rolls of Lazio Region (Middle Italy) in 1993-1996. Calibrated creatinine was measured in 2011 samples previously collected (1993-1996). The temporal stability of serum creatinine was tested in 30 individuals who underwent twice a fasting blood sampling, 5 years apart. This analysis showed a very strong correlation between the two creatinine measurements with the regression lines between them starting from the origin. The prevalence of stages 3-5 CKD in the MATISS cohort was 1.5% (1.1% in men and 1.8% in women). The prevalence of CKD in Italy was also previously assessed by the INCIPE investigators in 2006. In this study, 3,629 subjects, all Caucasians and older than 40 years, were randomly enrolled from the lists of 62 general practitioners (GP) randomly selected in the area of the North-Eastern Italy. In the INCIPE study the prevalence of stages 3-5 CKD was 6.7%. In another epidemiological study (CARHES study) performed on 9,000 Italian individuals, the prevalence of stages 3-5 CKD was about 3%. Overall, the prevalence of stages 3-5 CKD in Italy ranged from 1.5% to 6.7%. Differences in enrolment strategies (GP versus electoral rolls), in the year of data collection and in inclusion/exclusion criteria adopted by investigators (mainly age) may play a role in the heterogeneity of CKD prevalence data in Italy.

SysKid team members established the European CKD Burden Consortium, including nephrologists and epidemiologists and/or other study representatives, to investigate the differences in chronic kidney disease (CKD) prevalence within Europe, executing two studies on behalf of the European CKD Burden Consortium.

In the first study of the European CKD Burden Consortium, a systematic literature review was performed to identify all studies reporting on CKD prevalence in the European adult general population and to describe the methodology used in these studies. The systematic literature search identified 48 eligible original research articles reporting the prevalence of CKD in the adult general population between January 2002 and November 2014. The findings from this systematic review showed considerable variation in methods for sampling the general population and for assessment of kidney function across studies reporting on CKD prevalence (e.g. 67% used a Jaffe assay and 13% used the enzymatic assay for creatinine determination, whereas 29% used isotope dilute mass spectrometry calibration). Moreover, considerable differences exist in the reporting of the results (e.g. CKD prevalence was reported by sex and age strata in 54% and 50% of studies, respectively). These results were utilized to provide recommendations to help optimize both the design and the reporting of future CKD prevalence studies, which will enhance comparability of results across studies.

In a second study of the European CKD Burden Consortium, CKD prevalence was assessed in 19 adult general-population studies from 13 European countries, overall and by age, sex, and presence of diabetes, hypertension, and obesity. To enhance comparability across studies, the same CKD definitions were used (i.e. CKD stage 1-5 was defined as estimated Glomerular Filtration Rate (eGFR) < 60 ml/min/1.73m² or albuminuria >30mg/g and CKD stage 3-5 was defined as eGFR < 60 ml/min/1.73m², as calculated by the CKD-EPI equation). Moreover, CKD prevalence was age and sex standardized to the EU 27 population. The findings of this study show considerable differences in both CKD stage 1-5 and CKD stage 3-5 prevalence across European study populations. The adjusted prevalence of CKD stage 1-5 varied between 3.31% (95%CI 3.30-3.33) in Norway and 27.2% (95%CI 26.2-28.2) in Northeast Germany, whereas the adjusted prevalence of CKD stage 3-5 varied between 1.0% (95%CI 0.7-1.3) in central Italy and 5.9% (95%CI 5.2-6.6) in Northeast Germany. The variation of CKD prevalence is consistently seen in subgroups according to diabetic status, hypertensive status and obesity status. It cannot be determined to what extent these differences in CKD prevalence across studies can be explained by true differences (human and environmental factors (e.g. diet, smoking, physical activity, socio-economic status and public health policies) or by heterogeneity of studies (e.g. laboratory methods, non-response bias). The results of this study may be used to guide future projections of the CKD burden in Europe. Additionally, this study is a first step in monitoring the impact of strategies designed to reduce the burden of CKD in Europe, and thereby assisting the medical community and policy makers in the further development of these strategies.

For further adding to CKD prevalence we in SysKid initiated the PROVALID study, initiated in four European countries. PROVALID recruits patients with diabetes mellitus type II at the primary level of healthcare and follows them for the incidence of renal as well as cardiovascular endpoints. Additionally an extensive biobanking effort was undertaken. PROVALID fosters epidemiological research, provides healthcare recommendations and allows validation of risk calculators as well as biomarkers as developed within SysKid. While in SysKid 799 patients were recruited into the PROVALID study (centers in Austria, Hungary, Poland and the UK) we were able of further expanding in a SysKid-associated effort to 4,000 patients (and additionally including centers in The Netherlands).

1.3.3 Novel concepts on hindering CKD progression

SysKid had the opportunity of executing explorative as well as hypothesis-driven analysis on cell line and animal model level allowing on the one hand side deepening mechanistic analysis of molecular mechanisms of interest in CKD, on the other hand enabling comparative analysis from models to human.

in vitro - explorative

miRNA profiling was done on RPTEC/TERT1 cells exposed to different extracellular conditions (TGF- β , Angiotensin ii, Albumin, AGE-Albumin). Exposure to TGF β 1 caused differential expression of 17 miRNAs. Angiotensin ii exposure caused 16 miRNAs to be differentially expressed. Albumin exposure resulted in 12 dysregulated miRNAs. AGE-Albumin caused differential expression of 31 miRNAs. There was no overlap between results of D-glucose and additional treatments. miR-222 was differentially expressed after exposure to Albumin, Ang ii and TGF β 1. miR-872 and miR-571 were dysregulated in AGE-Albumin, Ang ii and TGF β 1.

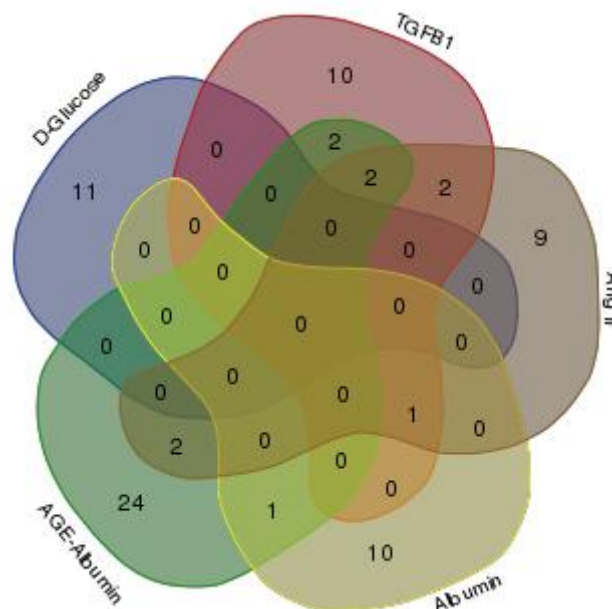


Figure: Venn diagram showing overlap between dysregulated miRNAs after exposure of human proximal tubular cells to different extracellular conditions mimicking diabetic nephropathy.

Comparative analysis with human CKD tissue samples and ZDF rats identified one miRNA being upregulated in all three experiments (human, animal, cell culture high glucose treatments), namely miR-155. Further studies could lead to verification of miR-155 as a biomarker and potential therapeutic target for diabetic nephropathy.

Biomarker candidates identified in SysKid were utilized to assess the effect of high glucose. Biomarkers that were affected by high glucose included TGF β 1, IL8, NRCAM, IL1B and CTGF. As expected, exposure to TGF-beta caused an increase in TGF β 1 expression. AGE-Albumin caused expression of CCL2 (aka MCP1) to be altered. Aside from the genes in the biomarker candidate set, several other interesting genes were changed in response to these additional treatments. Exposure of cells to TGF-beta caused significant changes in genes including MMP1 and MMP10 (involved in degradation of collagen and other extracellular matrix proteins), SERPINE2 (aka PAI-1, a promoter of fibrosis), COL7A1 and ITGB6 (an adhesion receptor that functions in signaling from the extracellular matrix). Exposure to AGE-Albumin caused changes in expression of genes including HIF1A, LCN2 (aka NGAL, which has previously been demonstrated as a biomarker of kidney injury), IL4I1, and SERPINA1 (aka alpha 1 antitrypsin, a biomarker of CKD).

For analysis on the protein level RPTEC/TERT1 cells were exposed to several pathophysiological conditions including high glucose, TGF β 1, high albumin and angiotensin II for 72 hours. Western blotting was used to

assess the effects of these conditions on novel biomarker protein expression. For example, MMP2 (a matrix metalloproteinase) expression was increased after exposure to high glucose and TGFB1. NOS1 expression was increased in high glucose and TGFB1, and even more so after exposure to angiotensin ii. Expression of CTGF, NRCAM and PLA2G6 all increased after exposure to TGFB1. TNF expression was elevated after exposure to high glucose, high albumin, TGFB1 and angiotensin ii. PRCKD expression increased after exposure to high glucose, TGFB1 and angiotensin ii, but not albumin. IL1B expression increased after exposure to high glucose and TGFB1. This agrees with the result from array studies of high glucose that showed elevated IL1B gene expression. PLA2G4A expression increased in high glucose samples.

in vitro - targeted

Targeted functional studies have been performed in human proximal tubular cell (PTC) lines HK-2 and RPTEC/TERT1 using pro-inflammatory and pro-fibrotic mediators such as interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), and transforming growth factor- β 1 (TGF- β 1) as well as the pleiotropic IL-6 family member oncostatin M (OSM). Besides target genes such as OSM, leukemia inhibitory factor (LIF), IL-6, IL-8, IL-6 receptor (IL-6R), OSM receptor (OSMR), and LIF receptor (LIFR), which have been derived from an experimental PTC model of high glucose, the focus was kept on four pro-fibrotic target genes (CTGF, SPARC, TNC, TSP-1), which have been identified as being relevant in the context of early stage diabetic nephropathy (DN) based on the consolidation of omics studies in a molecular model representation of DN. Moreover, the targeted cellular experiments concentrated on the expression of two pro-inflammatory genes CCL2/MCP-1 and CCL5/RANTES. Gene silencing approaches have been established and utilized in order to study the regulation of target gene expression in human PTCs.

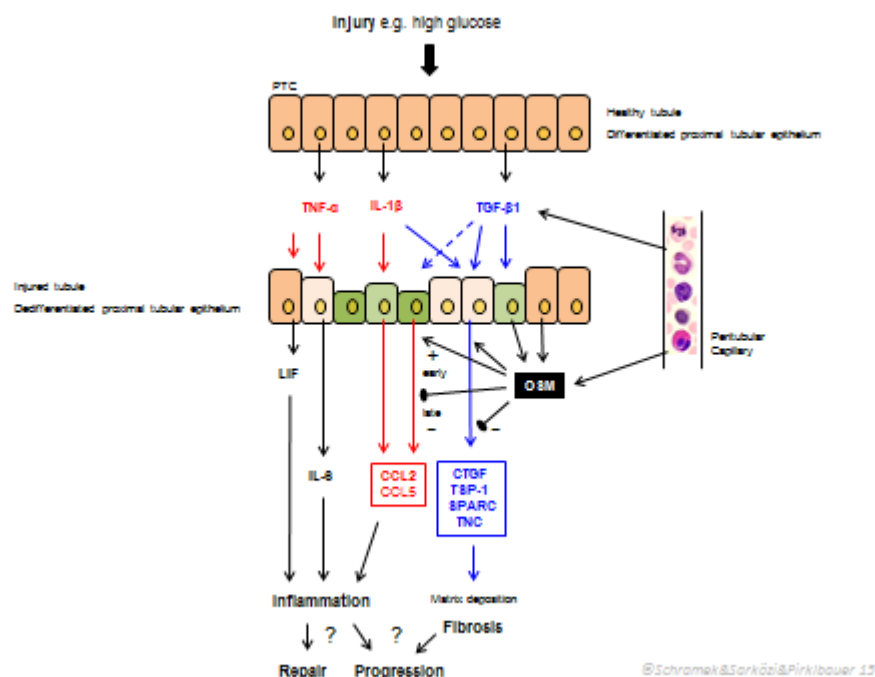


Figure: OSM may stimulate acute inflammation (e.g. expression of CCL2 and CCL5) via synergistic effects with other pro-inflammatory cytokines (e.g. IL-1 β , TNF- α , eventually TGF- β 1) early after injury, but may attenuate chronic inflammation and fibrogenesis (e.g. expression of CCL2 and matricellular proteins such as CTGF, TSP-1, SPARC, and TNC) at later time points.

OSM attenuated the expression of epithelial marker proteins and increased the expression of mesenchymal markers in human PTC. These effects were associated with OSM-induced human PTC scattering in three-dimensional collagen matrices after long-term incubation, all together suggesting that OSM is able to induce cellular events indicative of tubular epithelial-mesenchymal transition (EMT). In contrast to these potential pro-fibrotic effects, OSM was shown to inhibit TGF- β 1-induced matricellular protein expression, namely the expression of connective tissue growth factor (CTGF), SPARC, tenascin C (TNC) and thrombospondin-1 (TSP-1). Its inhibitory effect on TGF- β 1-induced CTGF mRNA expression started after 2 h of cytokine administration and lasted for at least for 24 h. When OSM was administered as a single ligand, it exerted a

time-dependent dual effect on CTGF mRNA expression in human proximal tubular HK-2 cells. At early time-points (between 15 min and 1 h of ligand administration) this cytokine led to a robust but transient induction of CTGF mRNA expression followed by a strong and long-lasting inhibition of basal and TGF- β 1-mediated upregulation of CTGF mRNA, which was mainly driven by STAT3.

Although OSM inhibited IL-1 β - and TNF- α -mediated mRNA expression of matricellular proteins TSP-1 and TNC, it acted synergistically with these two pro-inflammatory cytokines to induce CCL2 mRNA expression for up to 24 h. Stimulation of two independent human PTC lines with OSM alone led to a rapid and strong induction of this chemokine within the first hour of ligand administration, which subsequently returned towards basal levels in between 3 h and 24 h and finally switched into a significant OSM-mediated 70% inhibition of basal CCL2 mRNA expression after 48 h of incubation. In contrast to OSM, which stimulated both STAT1/3 and ERK1/2 signaling, IL-1 β led to a strong phosphorylation of p65 NF κ B/RelA, SMAD2/3 and p38 MAPK in human PTC. Selective silencing of these signaling molecules revealed that p65 NF κ B/RelA is involved in IL-1 β -mediated stimulation of CCL2 mRNA, and that superinduction of CCL2 mRNA expression in the presence of both OSM and IL-1 β at least partially depends on STAT3 signaling.

Similar results have been obtained for the second pro-inflammatory target gene CCL5. IL-1 β and TNF- α but not OSM or TGF- β 1 are strong and long-lasting stimulators of CCL5 mRNA expression in HK-2 cells when administered as a single ligand. When incubated together with IL-1 β , OSM has a potent additive effect on CCL5 mRNA expression, which is more pronounced when compared with OSM's synergistic effect in the presence of TNF- α . While silencing of STAT1 or STAT3 further stimulated IL-1 β -mediated CCL5 mRNA expression, NF κ B signaling through p65 NF κ B/RelA is involved in CCL5 mRNA superinduction in the presence of both OSM and IL-1 β .

Thus, OSM may stimulate acute inflammation (e.g. expression of CCL2 and CCL5) via synergistic effects with other pro-inflammatory cytokines (e.g. IL-1 β , TNF- α , eventually TGF- β 1) early after injury, but may attenuate chronic inflammation and fibrogenesis (e.g. expression of CCL2 and matricellular proteins such as CTGF, TSP-1, SPARC, and TNC) at later time points.

in vivo-explorative

In vivo studies in a rat model of diabetic nephropathy (DN) have been performed with the objective of identifying processes/mediators contributing to diabetic disease and provide potential targets for therapy. We used explorative approaches on mRNA and miRNA profiling as well as on specific mediators of kidney damage and related pathways. Being aware that there is still a lack of animal models that fully replicate changes found in human DN, we selected Zucker diabetic fatty (ZDF) rats, a type 2 diabetes model that within certain limitations can be considered as a reliable model for mimicking the human disease, both in terms of its relevance to the pathophysiology of the disease as well as its response to therapy. ZDF rats carry a spontaneous mutation in the leptin receptor gene phenotypically expressed as hyperphagia and obesity. They develop hyperglycemia, altered lipid profile, and proteinuria that increases over time. Renal structural changes including glomerular sclerosis, interstitial inflammation and fibrosis are evident, in association with cardiac abnormalities. Building a renal tissue, blood and urine sample bank throughout the time course of the disease in ZDF rats and age-matched control lean rats was instrumental to collaborative studies with Syskid partners.

The similarity between ZDF rats, as a model of type 2 diabetes-associated vascular complications and human disease, was evaluated by urinary proteome analysis. In the urine of ZDF rats compared to lean rats, 180 peptides were identified as potentially associated with diabetes complications. Overlaps with human CKD and cardiovascular disease (CVD) biomarkers were observed, corresponding to proteins marking kidney damage (albumin, alpha-1-antitrypsin) or related to disease development (collagen). Concordance in regulation of these peptides in rats versus humans was more pronounced in the CVD compared to the CKD panels. In addition, disease-associated predicted protease activities in ZDF rats showed higher similarities to the predicted activities in human CVD. Thus ZDF rats may represent a useful tool for studying cardiovascular complications in type 2 diabetes.

Transcriptomic mRNA analysis in the renal tissue from ZDF and lean rats showed 133 genes at early phase of disease (2 months) and 366 genes at late phase of the disease (8 months) that were differentially expressed between diabetic and lean rats. Comparison between ZDF rat and human DN mRNA profiles showed a modest overlap that mainly consisted of inflammatory transcripts. The expression of these transcripts was confirmed by real-time PCR both in rat and human DN.

Renal miRNA profiling revealed that 15 miRNA were significantly altered between ZDF and lean rats at 8 months of age. We focused on the most upregulated miRNA, miR-184 (18-fold increase), and through *in situ* hybridization experiments found that it localized in tubular epithelial cells. Using bioinformatic algorithms a potential target gene was identified which is involved in fibrosis. *In vitro* settings allowed us to define the mediators of kidney damage involved in the regulation of this specific miRNA, thus providing further understanding of molecular mechanisms underlying renal disease progression in DN.

We evaluated renal mRNA expression of candidate biomarkers, among 32 proposed within the consortium, and showed that mRNA expression of monocyte chemoattractant protein-1, plasminogen activator inhibitor-1 and fibroblast growth factor 23 (FGF23) increased during progression of the disease in ZDF rats compared to lean rats. FGF23 is a phosphaturic hormone mainly produced by bone that acts in the kidney through FGF receptors requiring Klotho as co-receptor. We provided the novel evidence of FGF23 production by the kidney in experimental type 2 diabetes. While the prevailing paradigm proposes the bone as the main tissue that senses changes in phosphate balance and produces FGF23, finding that the kidney is a source of FGF23 would pinpoint it as a direct sensor organ that may interfere in phosphate homeostasis via its own FGF23 production during renal disease progression in diabetes.

In ZDF rats renal Klotho expression decreased over time and serum phosphate levels increased parallel with a decline of fractional phosphorus excretion. Treatment with ACE inhibitor besides limiting proteinuria and renal damage, attenuated time-dependent FGF23 upregulation and ameliorated Klotho expression. The recovery of renal Klotho after ACE inhibitor allowed the re-engagement of serum and residual renal FGF23 to exert phosphaturic activity, leading to normalization of serum phosphate. Thus in experimental diabetes there is a complex interaction between angiotensin II-FGF23-Klotho-phosphorus and disturbances of this axis may contribute to disease progression. Interfering pharmacologically with this delicate balance might have clinical implications.

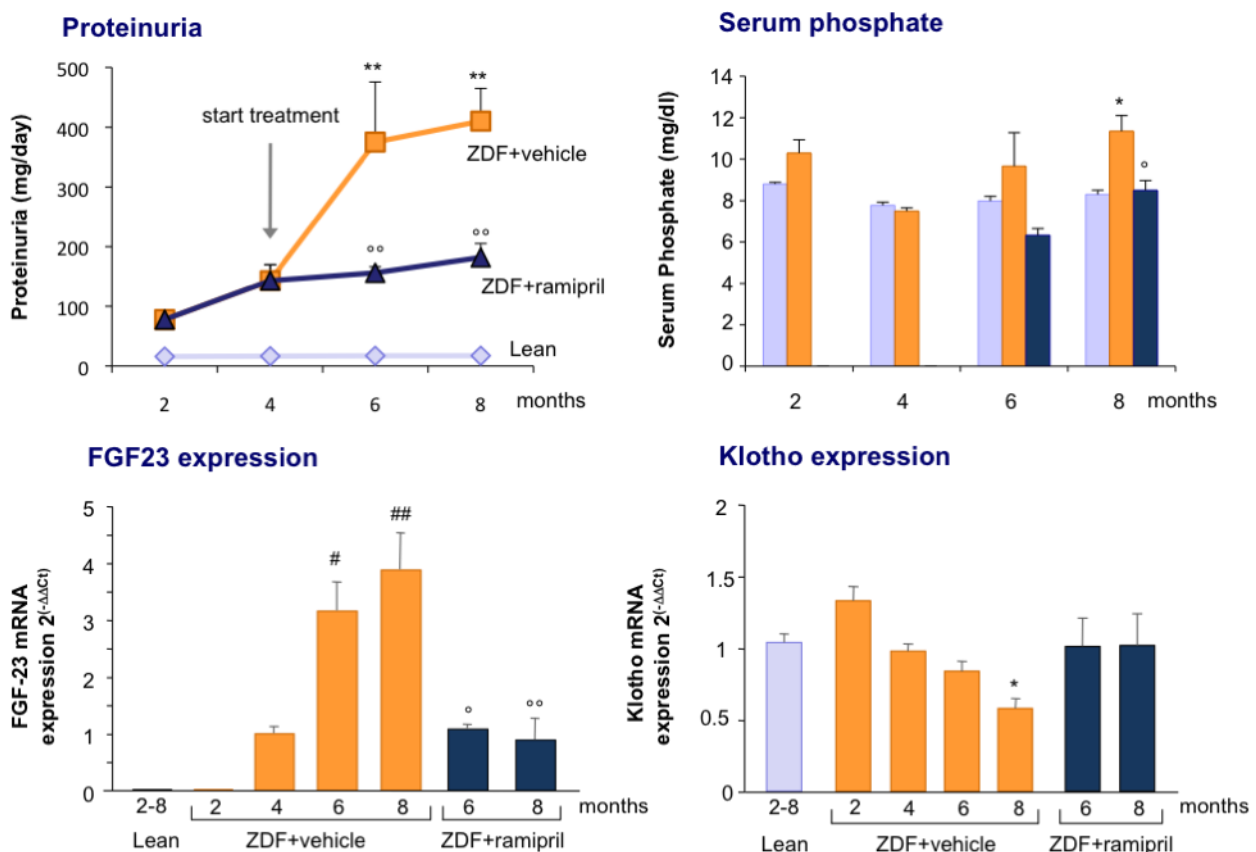


Figure: Effect of ACE inhibitor therapy on proteinuria, renal expression of FGF23 and Klotho, and serum phosphate levels in ZDF rats * P<0.05, **P<0.01 vs age-matched lean rats; #P<0.05, ##P<0.01 vs ZDF rats at 4 months; °P<0.05, °°P<0.01 vs ZDF+vehicle rats at corresponding age.

Through a multimodal approach including isolated perfused rat kidney and cultured podocytes together with the analysis of kidney biopsies from ZDF rats and DN patients, we demonstrated that in diabetes angiotensin II plays a relevant role in perpetuating podocyte dysfunction after the initial cell injury induced by glomerular hypertension. A specific intracellular pathway was identified, represented by activation of Notch1 and Snail signaling in podocytes that resulted in down-regulation of nephrin expression, the integrity of which is crucial for the glomerular filtration barrier. Both in ZDF rats and DN patients, ACE inhibitor therapy that reduced proteinuria restored nephrin and Snail expression in podocytes. The translational relevance of this study rests on the strong similarity of changes in the Notch1/Snail/nephrin axis induced by angiotensin II in diabetic rats and in diabetic patients, thereby providing robust insights into novel molecular mechanisms underlying kidney disease progression in DN.

Mediators

Molecules and substances accumulate in chronic kidney failure and may negatively impact on the regenerative capacity of circulating bone marrow derived stem cells by turning the microenvironment in the vasculature and internal organs from pro-regenerative into anti-regenerative. Consequently, circulating mesenchymal stem cells (MSC) as vascular progenitor cells might contribute to vascular calcification, the major risk factor for disability and death in CKD patients, neointima formation, and tissue fibrosis rather than to repair. Aim of this activity was to characterize the biologic effects of single uremic retention solutes (URS) on adverse differentiation processes of MSC and to put forward novel treatment strategies based on the biochemical and physical characteristics of the most important URS.

In our systematic approach, bone marrow derived MSCs were separately treated with 64 individual URS at uremic concentrations in osteoblastic induction medium and screened for biologic effects. There was only minor influence on fibroblast and vascular smooth muscle (VSMC) differentiation. Therefore we focused on adverse osteoblastic differentiation in subsequent studies. Pro-inflammatory cytokines (IL-1 β , TNF- α) followed by three other middle-sized molecules (FGF-2, FGF-23, PTH) were the strongest inducers of a pro-calcifying osteoblastic MSC phenotype in a dose dependent manner. Co-incubation of MSCs exposed to serum from uremic patients with specific blockers of IL-1, TNF- α , and the FGFs either alone or in combination effectively obviated osteoblastic transformation. In an additional translational approach, the osteoblastic potential of serum obtained upon high cut-off (HCO) dialyzer treatment was compared to that obtained upon conventional dialysis. Unselective removal of middle-sized molecules with innovative HCO dialyzers achieved similar favourable effects attenuating osteoblastic differentiation and calcium deposition as in the blocking experiments.

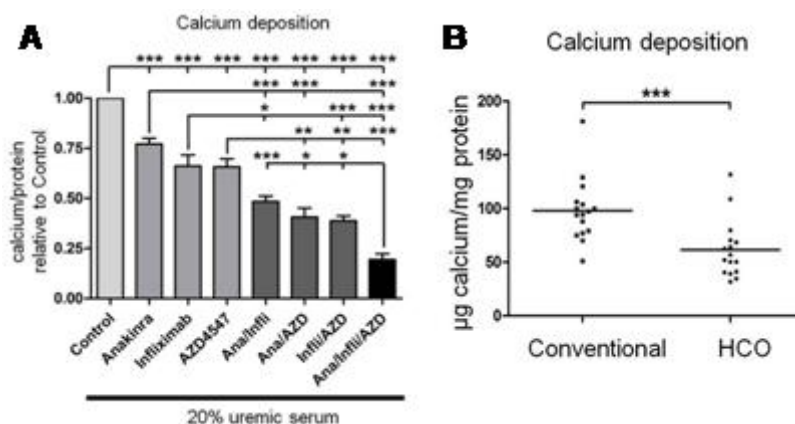


Figure: **A:** MSCs were exposed to serum from dialysis patients in osteoblast induction medium (OM). Inhibitors of IL-1 (Anakinra, Ana), TNF- α (Infliximab, Infli), and fibroblast growth factors (AZD4547, AZD) were added. Deposited calcium normalized to sample protein content was quantified. **B:** MSCs were incubated with osteoblast induction medium containing serum from dialysis patients treated either with conventional high-flux dialyzers (Conventional) or from the same patients after a 3 weeks course of dialysis with high cut-off membranes (HCO). Quantification of deposited calcium normalized to protein content is shown.

Our findings emphasize the importance of middle-sized molecules and pro-inflammatory cytokines as key mediators of uremic calcifying MSC phenotype. Specific pharmacologic interventions may be instrumental for reduction of the unsolved problem of vascular calcification in chronic kidney diseases.

Mesenchymal progenitors and mTOR

We further aimed to understand mechanisms of maladaptive phenotypic modulation and dysfunction of human bone marrow derived mesenchymal progenitor cells (MSC) in the context of chronic kidney disease that might result in loss of endogenous regeneration potential and rapid progress of kidney diseases and related pathologies. We investigated the involvement of the mTOR candidate pathway, a cellular integrator of metabolic changes, growth factor and cytokine inputs, and energy sensor, in pro-inflammatory and pro-fibrotic processes as well as in osteoblast differentiation of MSCs.

The normotensive DOCA/salt mouse model of mineralocorticoid excess in CKD was used to study mTOR signalling in systemic adaptation processes during chronic progredient kidney damage in a sex specific manner. Male and female uninephrectomized mice were subject either to control treatment or DOCA/salt treatment with or without intervention with the pharmacologic mTOR modulator rapamycin (rapa). Female mice were protected from development of left ventricular cardiac hypertrophy in response to DOCA/salt in comparison to male mice. This was accompanied by high intrinsic activity of mTOR complex 2 (mTORC2), an upstream mediator of anti-apoptotic pathways. However, rapa treatment disrupted female protection by blocking mTORC1 and mTORC2 signalling. Male rapa treated mice had blocked mTORC1 signaling but reciprocally activated mTORC2 resulting in decreased hypertrophy and an adaptive cardiac phenotype. Thus, maintenance of both, mTORC1 and mTORC2 signaling, appears to be essential in adaptive cardiac remodelling in CKD. Our findings support a rationale for sex-specific therapeutic strategies to address left ventricular hypertrophy in the reno-cardial syndrome.

In our in-vitro studies on the influence of uremic retention solutes on MSC biology, we identified the mTOR pathway as an important regulator of osteoblastic differentiation in MSCs. The mTOR modulator rapa effectively inhibited calcification as well as osteoblastic differentiation of MSCs in terms of pro-arteriosclerotic transformation. In parallel, we documented reduced mTORC1 signaling and enhanced mTORC2 activity. To delineate the relative importance of reduced mTORC1 and increased mTORC2 signal transduction, we established knock down of the mTORC2 defining protein Rictor in MSCs via lentiviral gene transfer. These studies revealed that the protective rapa effect was dependent on intact mTORC2 signaling.

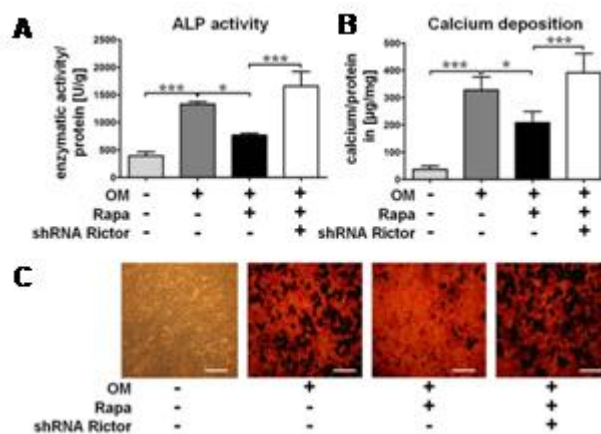


Figure: **A:** Alkaline phosphatase activity in MSC lysates normalized to total protein content was measured at day 7. **B:** Quantification of deposited calcium normalized to total protein content. **C:** Staining of calcium deposits with alizarin red S. Original magnification x200, scale bar = 100 µm. Bars represent the mean±SEM, n=6. *P<0.05, ***P<0.001.

The beneficial signalling pattern induced by rapa with inhibited mTORC1 and activated mTORC2 resulted in initiation of protective cell fate programs such as anti-senescence, pro-autophagy, and anti-apoptosis. Since apoptotic bodies function as a nidus for calcification we propose that enhancement of mTORC2 activity in addition to direct inhibition of osteoblastic differentiation by Rapa reduces calcified extracellular matrix produced by MSCs. Our findings reveal mTORC1 activation and mTORC2 deregulation as early events linked to cell fate programs operative during osteoblastic MSC transformation. Therapeutic modulation of mTOR signaling may induce alternative cell fate sequences and confer protection from accelerated arteriosclerosis in metabolic diseases.

RAAS and Vitamin D

Although being the current state-of-the-art renoprotective therapy, blockade of the renin-angiotensin-aldosterone-system (RAAS), is not able to fully stop renal disease progression in most patients. Recent data include data on renoprotective effects of vitamin D, presence of interaction between RAAS-activity and the vitamin D-FGF23-klotho pathway, and impact of genetic variability in the vitamin D pathway on albuminuria. We aimed to investigate the impact of combined intervention with a vitamin D receptor activator (VDRA) and RAAS inhibitor, combined with dietary sodium restriction, on outcome of therapeutic intervention in vivo, in a rodent model of proteinuria-driven chronic kidney disease.

Our main finding was that the VDRA paricalcitol has an additional renoprotective effect on top of ACE inhibition and dietary sodium restriction – the current cornerstone of renoprotective therapy. During low sodium diet, both paricalcitol and the ACE inhibitor lisinopril, as well as their combination reduced proteinuria compared with vehicle. In contrast, none of the treatments significantly affected proteinuria during high sodium diet. Paricalcitol also reduced interstitial pre-fibrotic changes, inflammation, and interstitial fibrosis during ACE inhibition under a low, but not high sodium diet.

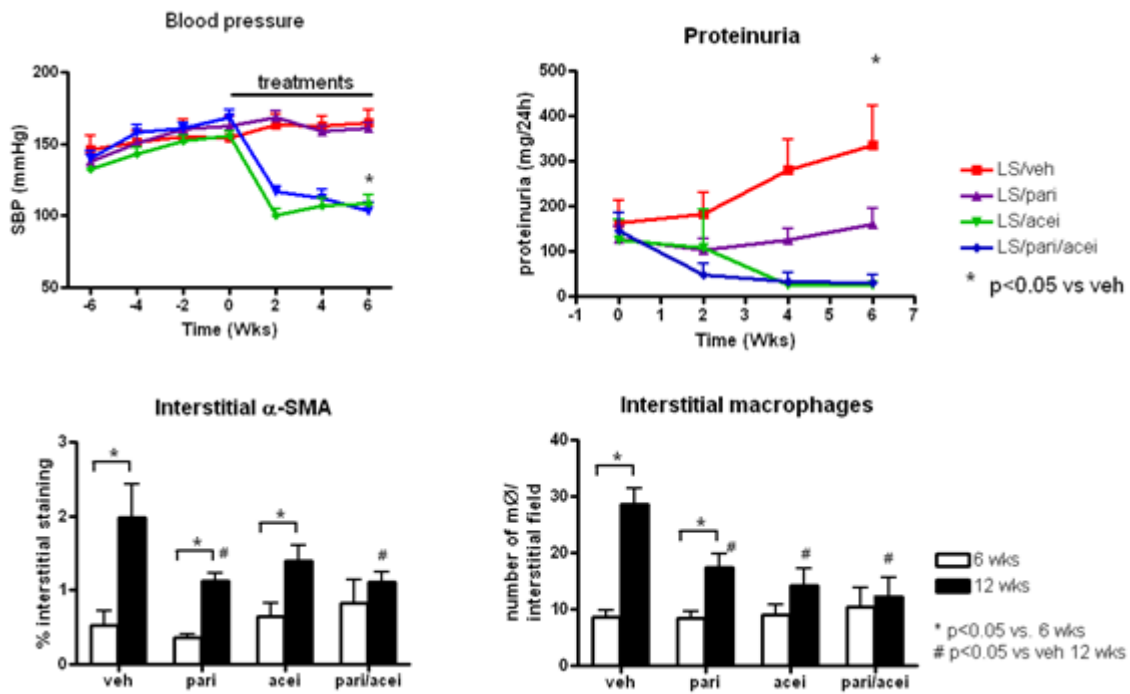


Figure: Overview of the effects of vehicle (red), paricalcitol (purple), ACEi (green), or ACEi+paricalcitol (blue), all during dietary sodium restriction, on the course of blood pressure, proteinuria, the pre-fibrotic marker alpha-SMA and interstitial macrophage accumulation in the rat adriamycin model. Note that proteinuria and blood pressure are significantly lowered by ACEi+low sodium, but not alpha-SMA expression, which remains progressive under ACEi+LS, the standard therapy in CKD. Addition of paricalcitol to ACEi+LS however prevented progression of the pre-fibrotic marker alpha-SMA and interstitial inflammation.

We further studied the potential role of vitamin D binding protein (VDBP), the major transporter molecule for vitamin D in the circulation, as a urinary biomarker of tubulointerstitial fibrosis and inflammation. Our data from animal and human studies suggest that VDBP is a biomarker of tubulo-interstitial inflammatory and fibrotic damage, also in analyses adjusted for albuminuria, and is reduced in response to renoprotective therapy.

The HGF context

Renal scarring is a common feature of chronic nephropathies. Growth factors such as transforming growth factor- β (TGF- β) and connective tissue growth factor (CTGF) are involved in the progression of renal damage. There are very few therapies that induce renal repair in chronic nephropathies. HGF is a mesenchyme-derived cytokine with anti-fibrotic and regenerative properties in some experimental models of CKD. We demonstrated hepatocyte growth factor (HGF) gene therapy was able to induce regression of glomerulosclerosis in diabetic

nephropathy through local reparative mechanisms. We on this basis tested whether bone marrow-derived cells are also involved in this HGF-induced reparative process. To address this hypothesis we created chimeric db/db mice as a model of diabetes that produce enhanced green fluorescent protein (EGFP) in bone marrow cells. We performed treatment with HGF gene therapy either alone or in combination with granulocyte-colony stimulating factor, in order to induce mobilisation of haematopoietic stem cells in these diabetic and chimeric animals. We have demonstrated that HGF gene therapy enhances the number of bone marrow-derived cells (BMDCs) into the diabetic kidney by enhancing SDF-1 in the renal tissue, and that these BMDCs infiltrating the diabetic kidney are mainly macrophages located surrounding glomeruli.

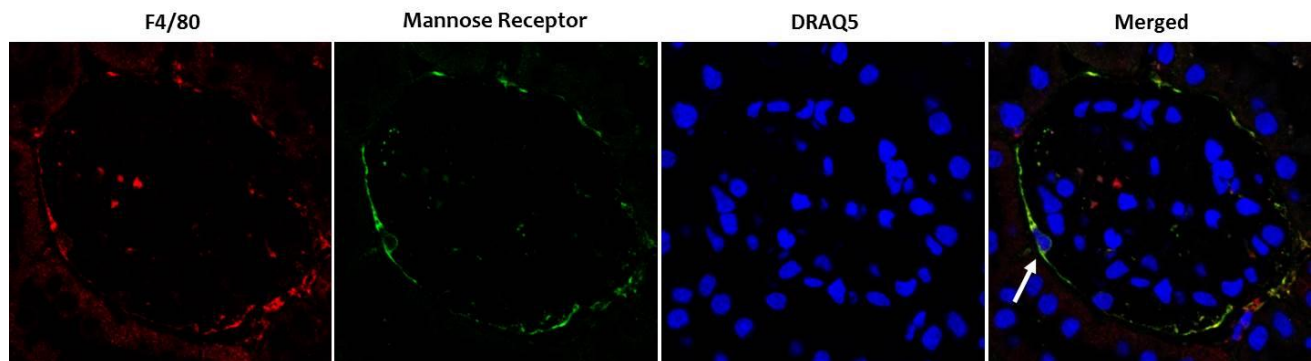


Figure: HGF gene therapy enhances the proportion of M2 macrophages in the db/db chimeric diabetic kidney.

Moreover, HGF gene therapy is able to reduce pro-inflammatory cytokines and to enhance anti-inflammatory cytokines, promoting an anti-inflammatory environment in the diabetic kidney. Consequently, HGF-treated db/db mice presented a higher proportion of M2 macrophages in the diabetic kidney than non-treated animals. Finally, HGF gene therapy is related to a possible fusion between parietal epithelial cells (PECs) from the Bowman's capsule and BMDCs, probably macrophages. These highly specialized cells (PECs), which cover the glomerular capillary tuft, are crucial for podocyte repair after injury. Accordingly, the number of podocytes was well preserved in HGF-treated mice. Therefore, these data suggest that HGF gene therapy attracts bone marrow-derived cells, M2 macrophages, around the renal capsule, and these can fuse with PECs and probably help repair. As other authors suggest, results showed in this study based on HGF gene therapy in db/db chimeric mice contribute to the idea that M2 macrophages exert beneficial effects in a mouse model of CKD. In fact, in order to analyze the efficacy of macrophage cell infusion in a mouse model of kidney injury, initial bone marrow-derived M2 macrophage cell therapy experiments have been performed in a UUO mouse model.

Thus, M2 macrophage cell infusion emerges as a new possible therapy to enhance renal regenerative potential in diabetic nephropathy and other chronic nephropathies.

The FGF23 context

Abnormalities in mineral metabolism parameters in renal failure are key pathogenic factors on cardiovascular disease and progression of renal failure. These parameters include phosphate, calcium, PTH, calcitriol or FGF23. SysKid focused on the relationship between FGF23 and klotho receptors in CKD and the role in the progression of vascular calcification.

We have shown that in the context of uremia a reduction of FGF23 contributes to increase vascular calcification and cardiac damage. These results are associated with a significant increase in serum phosphate levels.

We further investigated the regulation of the FGF23 receptor in normal and uremic rats. Our results demonstrate that in uremic rats a high phosphate excretion leads to a down-regulation of renal klotho expression. This was observed even when circulating FGF23 was neutralized by administration of anti-FGF23 antibodies. It was also observed that high levels of FGF23 (continuous administration) induce up-regulation of renal FGFR1. In addition we checked that high doses of calcitriol do not increase renal klotho expression.

Further analysis focused on the effects of FGF23 on cell proliferation of vascular smooth muscle cells (VSMC). Our results showed that high levels of FGF23 increase proliferation of VSMC promoting the expression of cyclin D1 and PCNA. Moreover FGF23 activates cell migration and a characteristic change from contractile to synthetic VSMC; contractile VSMC were spindle and filamentous whereas synthetic VSMC had

a cobblestone structures with more organelles in order to produce more proteins and secrete extracellular matrix. Finally we observed that high concentrations of FGF23 induced the expression of specific genes of synthetic VSMC. All these changes were also associated with an increase of calcification in presence of phosphate. Therefore in VSMC the increased levels of FGF23 could promote phosphate-induced calcification through the imbalance from a contractile phenotype to synthetic VSMCs.

Additional in vivo and in vitro studies documented the mechanisms of FGF23 regulation. We have demonstrated that stimulation of FGF23 by high phosphate diet is limited if there is a situation of calcium deficiency. This decrease in FGF23 in response to calcium deficiency could be a response to avoid a subsequent reduction in calcitriol, which could exacerbate hypocalcemia.

In relation to vascular calcification, we have shown that obese rats with renal failure are predisposed to vascular calcification. This is mediated by an increase of oxidative stress since antioxidant therapy with vitamin E prevents oxidative stress and reduces vascular calcification.

In vitro we have demonstrated that high phosphate induces the activation of Wnt/b-catenin pathway in VSMC and mesenchymal stem cells promoting osteogenic transdifferentiation of these cells. Similarly we have shown that the addition of moderately high concentrations of Magnesium inhibit calcification of VSMC through a down-regulation of this pro-calcificant pathway. In relation with magnesium another study revealed that magnesium inhibits PTH production of primary culture of parathyroid glands contributing with it to decrease vascular calcification.

Integration perspective – model systems

Syskid had the opportunity of comparative and integrative analysis of in vitro, in vivo and human data on CKD, providing essential information for the translational drug development process. For the ZDF rat model as well as for the RPTEC/TERT in vitro model mRNA and miRNA profiling was executed, offering a data matrix for comparing with 48 Omics profiles consolidated on the human disease side.

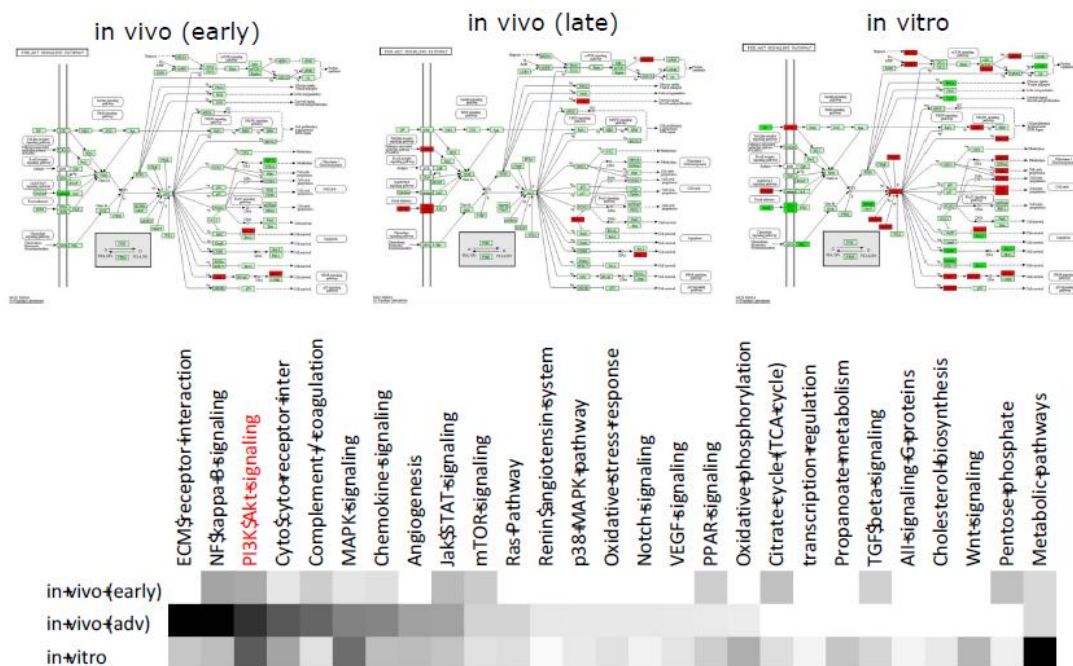


Figure: top: mapping of transcript profiling of in vivo model data (ZDF rats) and in vitro model (RPTEC/TERT1) on a pathway identified as relevant on the basis of human profiling data (PI3AKT signaling). bottom: cross-pathway comparison of model systems.

With this data assignment an optimized evaluation of targets as well as drugs itself on the level of model systems has become feasible, e.g. regarding evaluation of ACE inhibitor activity in the light of FGF23 and Klotho.

Integration perspective – targets/drugs

With the SysKid work on molecular mechanisms afflicted with early stage of DN, as derived by a combination of biomarker validation, generation of molecular process and pathway maps, and assignment of biomarkers to molecular processes a novel strategy for evaluating drug effect became feasible. Utilizing technology for computing molecular models was applied also for identifying drug mechanism of action molecular models, and algorithmic interference of disease pathophysiology models and drug effect models allowed identifying impact of drug activity on specific disease mechanisms beyond the mere drug target.

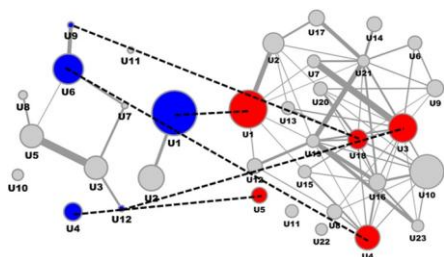


Figure A: drug mechanism of action interference, ACE inhibitors; ACE MoA model on the left, DN molecular model on the right.

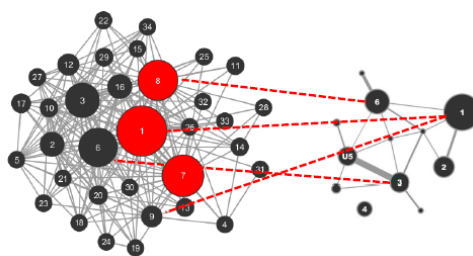


Figure B: drug mechanism of action interference, Erlotinib: DN molecular model on the left, Erlotinib MoA model on the right.

SysKid – “Systems Biology towards novel chronic kidney disease diagnosis and treatment” proposed and delivered on an integrative perspective: Large scale molecular data on human and animal models combined with detailed molecular characterization of selected molecular processes brought forward a network of molecular mechanisms afflicted with DN. Assignment of a large spectrum of biomarkers together with validation results allowed pinpointing specific mechanisms relevant in progressive disease. Interference with drug mechanism of action finally allowed proposing precision medicine for given medication, and opened up repurposing opportunities for identifying alternative medication.

1.4 Potential impact

1.4.1 Contributions to State-of-the-Art

On the level of SysKid objectives we added to State-of-the-Art in all aims and objectives as outlined in our work programme. Knowledge became available to the scientific community via publications in peer reviewed journals as well as via contributions to scientific conferences.

Objective 1: *Integrate existing, and extend clinically well-defined **sample cohorts** of patients with chronic kidney disease.*

SysKid had access to a number of existing, large databases and biobanks for discovery as well as validation of findings on biomarkers and risk factors, and also extended on such retrospective cohorts via establishing prospective collections.

This data and sample environment on the one hand allowed setting up well planned discovery cohorts, and equally important enabled validation of discovery results in large and clinically relevant cohorts involving thousands of samples. In general, discovery findings only become relevant after executing appropriate validation, and this procedure was enabled in the SysKid consortium structure via combining analytical capabilities (experimental as well as computational) with clinical teams experienced in data validation in a clinical setting.

Objective 2: *Establish and unify a **broad ‘omics’ repository** characterizing CKD.*

SysKid built on significant Omics profile background available with the team, combined this background with public domain profiling data and further integrated with foreground data spanning genetics, transcripts, proteins and metabolites.

Both, human as well as model Omics (in vitro as well as in vivo) was integrated in a dedicated SysKid data infrastructure, allowing to execute comparative analysis between disease models and human situation, contrasting genetic background with environmental factors, and linking molecular mechanistic aspects of the disease with biomarker candidates.

Objective 3: *Decipher processes, molecular pathways and associated CKD biomarkers utilizing a **Systems Biology approach**.*

The broad Omics repository built in the project allowed SysKid implementation of three computational approaches for coming up with CKD-associated molecular mechanisms (kidney disease interactome) following different algorithmic procedures/different levels of granularity. Further, pathways and molecular process segments were retrieved from selected components of the data space.

In total close to 400 biomarker candidates were evaluated during the different studies of SysKid, culminating in a full assignment of biomarkers to underlying molecular mechanisms. Execution of biomarker validation studies on top allowed us to annotate such mechanisms regarding involvement in progressive disease, hence serving as prime targets for novel therapy approaches.

Objective 4: *Use **cell cultures** and **animal models** to deepen our understanding of identified processes associated with early CKD.*

SysKid established and utilized a range of model systems covering cell lines (in vitro) as well as animal models (mouse, rat). These model systems were on the one hand side utilized for testing hypotheses on biomarkers and specific molecular mechanisms deemed relevant in the human situation. On the other hand, selected model systems underwent Omics profiling. This in turn allowed comparative analysis of model systems regarding fit to human disease signatures.

Executing hypothesis-driven analysis on target molecular mechanisms of relevance together with explorative profiling of models on the transcript as well as protein level added to practical utilization of CKD model systems specifically for testing novel targets and drug candidates.

Objective 5: *Delineate novel **therapeutic strategies** and **pre-clinical evaluation** for prevention and slowing of progression of chronic renal disease.*

SysKid identified molecular risk factors afflicted with progressive disease with respect to both, renal function decline as well as cardiovascular complications. Such factors were contributed from analysis of human data as well as retrieved out of analysis of model systems. For selected components evaluation work was implemented up to the animal model level.

As additional approach SysKid could leverage on the molecular mechanistic map retrieved for DN via applying technologies for computing molecular models also for drug effect. Proposals became available on optimized use of given medication as well as on alternative drugs (repurposing).

Objective 6: *Clinical **validation of identified biomarkers** for generating **early stage diagnosis** and **prognosis IVD kits***

Biomarker validation work involved single biomarkers, marker panels as well as multi-marker-based classifiers. Biomarkers included in validation work span from genetic background and regulatory components of transcriptional control up to the effector level (proteins and metabolites), and involved tissue, blood and urine as sample matrix.

In project runtime a urinary proteomics classifier (CKD273) advanced significantly in multiple validation work, at present seeing application in a clinical interventional study in early stage DN (PRIORITY trial), and further being presented to regulatory agencies.

Objective 7: Delineate a novel **risk score** for the development of chronic kidney disease

SysKid brought forward a risk calculator and scoring schemes.

One such scoring scheme was implemented in a Web-based risk calculator for general use. SysKid work on risk factors and epidemiology further faced significant disparity on appropriate use of statistical methods, in this light developing and providing 10 guideline publications.

Objective 8: *Deepen our understanding of the **epidemiological aspects** of early CKD with particular focus on **consequences for healthcare policies***

SysKid specifically addressed CKD prevalence on a European level. Approaches included first time consolidation of available studies from several European countries, prediction of the development of CKD prevalence on the basis of given registries, further following a prospective data collection effort in five European countries.

SysKid executed studies regarding the status of implementing guidelines in CKD, clearly identifying the need for improving screening regarding development and progression of CKD, as well as clear beneficial effects of multi-objective therapy (better control of BP, use of RAS blocking treatment, and improved cholesterol and diabetes treatment).

With respect to preventive measures SysKid analyzed the impact of modifiable nutritional and life style factors on CKD. Respective publications received decent media coverage adding to raising awareness on such modifiable factors on the level of the general public.

Having met the objectives as defined at project start the SysKid collaborative effort managed adding to state-of-the-art in the project aims:

Aim 1: Identify persons at risk of developing chronic kidney disease utilizing epidemiology as well as molecular tools.

Aim 2: Understand the molecular processes triggering early stage chronic kidney disease and identify associated biomarkers.

Aim 3: Develop novel diagnostic and therapeutic strategies to control progression of chronic kidney disease.

Aim 4: Perform pre-clinical verification of novel therapy approaches and perform clinical testing of novel diagnostics.

Along the way of implementing SysKid aims and objectives we further added to conceptual work on the upcoming theme of personalization and precision medicine.

Conceptual expansion: Precision Medicine

SysKid stated in the abstract of the work programme: “We will focus on early stage CKD with diabetes mellitus and hypertension as the most prevalent causative conditions, and hypothesize that, although being diverse in aetiology, underlying molecular pathophysiology may be similar”.

SysKid results clearly show a complex pathophysiology coming with CKD in the background of diabetes, according to our map on molecular mechanisms seeing multiple molecular processes in an intricate interplay. A number of these processes play a role in each individual patient, although with presumably varying effective contribution to progressive disease. In accordance, the concept of personalization of disease pathophysiology comes in, seeing discussions on precision medicine aspects also coming into the area of chronic kidney disease.

Yet another indicator for supporting relevance of personalization is the improved performance of marker panels when compared to single markers. This finding may on the one hand side be explained by biochemical variability of “one and the same” when it comes to involvement and relevance of molecular disease mechanisms driving onset and progression of disease, but on the other hand may also indicate the presence of different “subgroups” of patients.

In such case, the concepts of stratification and personalization come in, in SysKid taking up momentum over project runtime.

As recently outlined by Klonoff in the context of managing diabetes (J Diabetes Sci Technol 2015) precision medicine aims at tailoring medication with respect to a specific clinical presentation. Precision medicine may be seen as successor of personalized medicine, where the latter to some extent got scrutinized in the realm of developing and providing specific medication for a specific individual, in such definition not amenable in the framework of evidence based medicine. Precision medicine comes much closer to stratified medicine, where therapies are tailored at specific patient populations selected according to clinical parameters or disease biomarkers (Trusheim et al., Net Rev Drug Discov 2007).

Hence, precision medicine rests on phenotype profiling allowing selecting patients responsive for a specific treatment, where the group of such patients resembles a stratum for such therapy. This concept is certainly not new, and has been applied in clinical trials focusing on halting DN progression as well by e.g. defining cutoff values of albuminuria and eGFR as inclusion criteria. However, with respect to treatment benefit such criteria have not proven being comprehensive for identifying non-responders or identifying patients prone to side effects. This bears the issue to what extent risk factors for disease development and progression identified on a population basis, or functional markers as eGFR (as such not educating on the specific molecular context underlying loss of filtration) can be used in the context of precision medicine for managing diabetes complications. On top, precision with respect to drug use demands a beneficial effect of a drug's molecular mechanism of action in the light of a specific molecular pathophysiology of an individual patient; hence, the entire concept needs to rest on molecular mechanisms. Risk factors determined on the clinical level as well as indicators of organ function may serve as proxy for such specific molecular match, however, specificity for beneficial interference appears to hold room for improvement.

Following this line of argument the following strategy may be pursued, as exemplified in integrative work of SysKid:

- i) Derive a molecular mechanistic map of the clinical phenotype via integration of multi-level Omics profiling data
- ii) Identify biomarker candidates allowing to serve as proxy for the molecular mechanism map
- iii) Test the mechanism biomarkers in the clinical context of relevance (in our case onset/progression of disease).

- iv) From the set of biomarkers being progression-associated gain knowledge on respective underlying molecular mechanisms being progression-associated.
- v) From drug mechanism of action profiling identify drug effects seeing interference with such progression-associated mechanisms

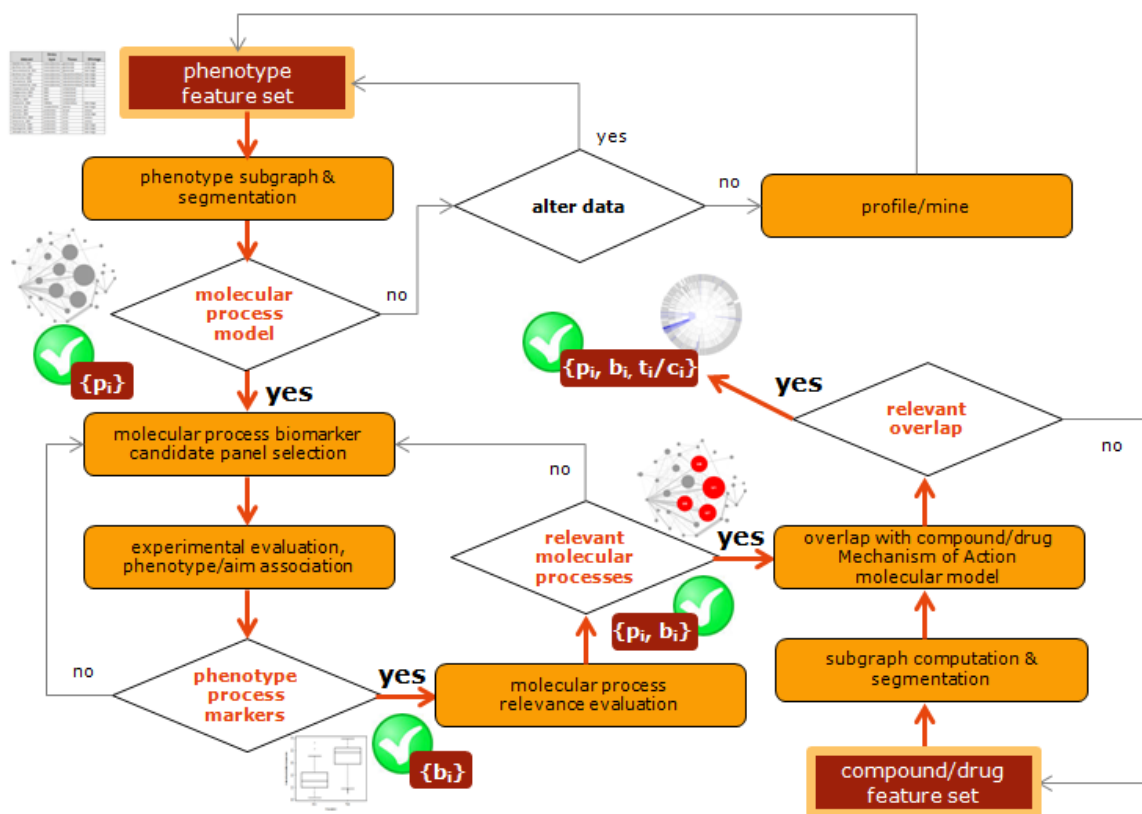


Figure: a concept on realizing precision medicine in chronic kidney disease, being end: end executed in SysKid.

At first, a detailed molecular map of DN needs to be retrieved allowing for delineating a molecular pathway representation of DN pathophysiology. Second, biomarker candidates are to be determined serving as proxy for such individual pathways, followed by testing such candidate markers with respect to association with disease progression. Having such biomarker panel in hand provides enhanced molecular phenotyping on a patient-specific level via associating activation status of molecular pathways to individual patients. As drugs address mechanisms (i.e. molecular pathways) such profiling in turn allows categorization of patient strata for which specific targets hence drugs indicate beneficial interference.

According to such concept the following tuple needs to be identified: $\{p_i, b_i, t_i/c_i\}$

p_i resembles the set of molecular processes being associated with progressive disease, and the respective set of biomarkers b_i allows to determine the status of such process. Determining b_i in a patient presenting with the phenotype allows improving molecular profiling, i.e. what is the “status” of the relevant molecular process set for the specific patient. Holding then knowledge on a set of drugs regarding their detailed molecular mechanism of action provides the option of selecting such drug seeing beneficial “overlap” with the disease mechanisms (t_i/c_i) active at the individual patient level.

1.4.2 Socio-economic impact

In the landmark interventional studies of advanced CKD in patients with diabetes such as IDNT and RENAAL, a reduction of the relative risk for doubling of creatinine or ESRD could be achieved in the range of 16 to 30% but still half of the subjects could not be saved from these events over a period of roughly four years (Lewis et al., N Engl J Med 2001; Brenner et al., N Engl J Med 2001).

A recent study by Andr sd ttir et al. showed that intensified RAS blockade, and improved cholesterol and diabetes treatment results in an almost 50% reduction in mortality, clearly demonstrating improved prognosis in both type 1 and type 2 diabetic subjects with diabetic nephropathy applying current multifactorial treatment (Andr sd ttir et al., Diabetes Care 2014).

One of the most recent phase III trials in the field was evaluating Bardoxolone Methyl addressing inflammation and oxidative stress, however, the trial had to be stopped due to severe adverse events (de Zeeuw et al., N Engl J Med 2013).

This recent example on developing novel treatment for complex pathophysiologies as DN reveals a fundamental issue in drug R&D productivity, expressed by an overall cycle time of 13 years and capitalized costs of about 1.7 billion USD for bringing a new molecular entity to the patient (Paul et al., Net Rev Drug Discov 2010).



Figure: Drug development steps for bringing one new molecular entity to the market. p(TS) probability of technical success; WIP work in progress (adapted from Paul et al., Nat Rev Drug Discov 9, 203-214, 2010).

R&D productivity is a composite of efficiency and effectiveness expressed as a pharmaceutical value equation setting the number of individual drug candidates in a development pipeline, their transition probabilities of technical success (p(TS)) along preclinical and clinical development steps and value gained in relation to cycle time and costs. Technological advances add to decreasing cycle time and costs, however, the key item remains being attrition, defined as $1 - p(TS)$, and drug target selection is considered as the central determinant.

In this R&D model at least 24 drug target candidates are needed in the target-to-hit development stage for finally reaching a drug launch. This large number is mostly determined by attrition in advanced clinical testing, going down to 34% for p(TS) in phase II clinical testing. A straight forward yet not feasible strategy is to simply increase the number of candidates included at early stage hit and lead screening, as this according to Little's law significantly impacts cycle time under rate-limiting development resources. Still, getting an increased number of development candidates into phase I clinical testing may allow improved segregation of development candidates finally showing an increased transition probability in later stage clinical testing. Such approach may be cost effective via shifting investment from later stage clinical testing to earlier stages.

In the context of DN a broad spectrum of targets addressing diverse mechanisms are presently undergoing preclinical evaluation. Mining scientific literature deposited in NCBI Pubmed e.g. provides 104 protein coding genes being at least on a discovery level discussed as target in DN (Heinzel et al., Front Cell Dev Biol 2014). From this plethora of candidates a key question needs to be addressed: fit-for-purpose of a drug and its target mechanism in the pathophysiological background of DN

SysKid established broad molecular profiling of DN on human and in vitro/in vivo model level, validated biomarker candidates and classifiers for improving phenotype profiling on risk for onset and progression of disease on the background of risk factors, and finally on a conceptual as well as implementation level demonstrated bridging phenotype profiling and target/drug effect via biomarkers. As a result, SysKid provided significant contributions promising to increasing p(TS) for bringing novel medication to patients with chronic kidney disease.

Just for comparison, increasing the p(TS) at early stage of DN drug development by 1%, and at clinical stage by 0.1% already significantly exceeds the investment done in SysKid. Interdisciplinary efforts bringing together a broad base of technologies allow addressing the entire translational and preclinical spectrum, and combined with clinical institutions bridges into relevant validation on the level of human disease. From a R&D productivity perspective such collaborative approaches prove highly cost efficient.

1.4.3 Scientific impact

SysKid brought forward more than 170 peer reviewed publications tackling biomarker, healthcare as well as molecular mechanism and target/drug aspects focusing on chronic kidney disease. Another about 30 publications are in the status “submitted” promising further dissemination of SysKid results throughout 2015. On top, a set of the papers covered fundamentals in data analysis and statistics for offering guidelines to the scientific community in the specific field.

The majority of publications included at least two members of the SysKid consortium documenting the collaborative work executed. Further to note is the balance of publications regarding the areas of biomarkers, clinical statistics and epidemiology, and molecular mechanisms/targets/drugs.

SysKid further provided > 330 contributions to scientific conferences, including main events in the specific field as the ERA-EDTA and the ASN. SysKid results managed of being covered by a dedicated session at the ERA-EDTA conference 2015 (London), entitled “*Prognosis and therapy of diabetic nephropathy: one size does not fit them all*”.

Prognosis and therapy of diabetic nephropathy: one size does not fit them all	Chairs: Gert Mayer, Rainer Oberbauer
The epidemiology of CKD in Europe: implications for healthcare policies	Kitty Jager
Clinical utility of risk scores/biomarkers to predict progression and outcome in diabetic nephropathy	Peter Rossing
Linking biomarkers to pathophysiology and treatment response: a systems biology approach	Bernd Mayer
Enrichment strategies in clinical trials - the road to personalized medicine in nephrology?	Dick de Zeeuw

Further, the SysKid team could contribute to a special issue of the journal Nephrology, Dialysis and Transplantation entirely dedicated to research results coming out of the SysKid project (publication planned for Q3/2015).

Out of SysKid two major initiatives were triggered, the PROVALID study and the CKD burden consortium. PROVALID expanded the prospective clinical data and biobanking initiative in SysKid (covering 800 CKD patients in four European Countries) to 4,000 patients in five European countries. External funding could be secured for this major initiative, allowing continuation of the study well beyond SysKid runtime. The CKD burden consortium involves partners throughout Europe (also outside of the SysKid team) for enabling a comparative perspective on CKD prevalence.

Beyond the core theme of SysKid, team members linked with major other activities in the field. Selected examples from the scientific side include:

- EuroKUP (European Kidney Urinary Proteomics, EU COST initiative, www.eurokup.org)
- Cooperation efforts for joining forces on prospective studies in the realm of PROVALID (German Chronic Kidney Disease study, the German DIACORE study, and the EU sponsored PREDICTIVE consortium)
- Exchange with the EUTox consortium (working in the field of uremic retention solutes)
- Cooperation with the German NT-CVD consortium
- Cooperation with the ADVANCE team (The Georg Institute for Global Health in Australia)
- Active contribution of SysKid consortium members to other FP7 programs of relevance in the field: EU-MASCARA (Markers for subclinical cardiovascular risk assessment), the PRIORITY study (Proteomic prediction and renin angiotensin aldosterone system Inhibition prevention of early diabetic nephropathy In type 2 diabetic patients with normoalbuminuria), the iMODE-CKD program (Clinical and system –omics for the identification of the Molecular DEterminants of established Chronic Kidney Disease, Marie Curie Action Initial Training Network), and the CKD-BIO initiative (multi-centric observational study, aiming at identifying omics-based biomarkers for prognosis of CKD progression centered around histopathology from biopsies)

Selected examples from the public side include:

- The Italian National Health Institute (ISS) (work on prevalence of CKD)
- Collaboration with the Main Association of Austrian Social Security Institutions (HVB) and the Austrian Agency for Health and Food Safety (AGES)
- Liaising with the European Kidney Health Alliance (EKHA)

Equally important, SysKid significantly contributed to educating young researchers by introducing to a truly interdisciplinary research approach in the light of Systems Biology. The consortium triggered active contribution of young fellows as part of the annual consortium meetings (poster sessions with best poster awards), and enabled execution and completion of >10 PhD theses.

1.4.4 Main dissemination activities and exploitation of results

Dissemination activities

SysKid throughout the entire project period provided monthly dissemination of project results. Channels included the project web site, as well as newsletters offered on the Web site and on top distributed via email to > 600 registered professionals in the field.



Figure A: SysKid web platform.



Figure B: SysKid monthly newsletters.

At start of the second project phase we additionally entered twitter and Facebook for adding to visibility of the project.



Figure A: SysKid on twitter, @ syskid.eu.



Figure B: SysKid on Facebook.

The European Fit-for-Health program as well as the Commission Conferences on research and innovation provided video coverage of SysKid:



Figure A: fit-for-health video covering SysKid on youtube (<https://www.youtube.com/watch?v=lq3KQTY4Zs>).



Figure B: SysKid @ the Research and Innovation Conference, Brussels 2010
http://webcast.ec.europa.eu/eutv/portal/res/_v_fl_300_de/player/index_player.html?id=9420&pid=9401&userlocale=de.

Throughout the project press work was done for bringing SysKid to the general public, examples include:

News Release

February 23, 2010

SysKid
Collaborative Project
Systems Biology towards Novel Chronic
Kidney Disease Diagnosis and Treatment

Europe tackles Chronic Kidney Disease with Systems Biology

25 Research groups from 15 countries will study a severe complication of diabetes and hypertension

(Vienna) SysKid, a large-scale integrating European research project, aims at understanding chronic kidney disease in the context of diabetes and hypertension. SysKid – which stands for »Systems Biology towards Novel Chronic Kidney Disease Diagnosis and Treatment« – will pave the way for progress in prevention, new diagnostic strategies and treatment options for declining kidney function, which affects millions of patients suffering from diabetes and hypertension. The project is driven by a European Union grant of EUR 11,8 million from Framework Programme 7 (FP 7) and a total project volume of close to EUR 16 million. SysKid's first general assembly will be held March 9-11, 2010 at the Medical University of Vienna and the project will be introduced at the Medical University of Innsbruck on March 12, 2010.

Tückische Nierenschwäche

Chronische Nierenschäden sind im Vormarsch und tückisch, weil sie lange symptomlos und unentdeckt bleiben. Die EU fördert das großangelegte Forschungsprojekt SysKid, das von einem Linzer Primar mit-initiiert wurde. Es soll neue therapeutische Ansätze liefern.



Mediziner, Statistiker, Molekularbiologen, Epidemiologen etc. arbeiten für SysKid. Initiatoren hoffen auf bessere Früherkennung (v.l.): Oberbauer, Landa, B. Mayer, G. Mayer. Bild: ges

Diet Choices May Help Diabetics Stave Off Kidney Disease

Fruit, protein and moderate alcohol intake tied to lower risk of chronic disease in study

MONDAY, Aug. 12, 2013 (HealthDay News) -- A healthy diet and moderate alcohol consumption may help people with type 2 diabetes reduce their risk of chronic kidney disease or slow its progression, a new study indicates.

Researchers looked at more than 6,200 diabetes patients, and found that nearly 32 percent of them developed chronic kidney disease and about 8 percent died during 5.5 years of follow-up.

Patients with the healthiest diets had a lower risk of kidney disease and of dying than those with the least healthy diets. Patients who ate more than three servings of fruit per week were less likely to develop chronic kidney disease than those who ate less fruit.

The study was published online Aug. 12 in the journal *JAMA Internal Medicine*.



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Systems Biology in Nephrology: SysKid Researchers at the European Renal Congress Reveal a Picture of Complex and Dynamic Networks



Exploitation of results

The SysKid consortium resembled a relevant mix of academic/translational R&D, clinical research, industry partners and SMEs (small and medium sized enterprises). Intellectual property became available throughout the consortium, resulting in exploitation opportunities beyond scientific dissemination.

IP leading to patents filed:

- EP 12156890.1 “*Symmetric dimethylarginine (SMDA) modifies high density lipoprotein (HDL) to induce endothelial dysfunction*” filed 24.02.2012
- “*Biomarkers for improved diagnosis and prognosis of chronic kidney disease*” Submission number: 500081760, PCT application number: PCT/NL2013/050191

IP leading to new products:

- The high-dimensional classifier developed in SysKid, CKD273, was submitted to be evaluated for routine application to the “Gemeinsamer Bundesausschuss” in Germany, as well as to the FDA and the European Medicines Agency (EMA)
- The routines for computing molecular models on phenotypes and drug effect were included in the software platform e.valuation being actively offered to biotech/pharma R&D
- A global mean normalization module developed for executing SysKid analysis was included in the QBASE software being actively offered to biotech/pharma R&D

IP leading to new technologies:

- One of the developed statistical software products, the SAS macro PSHREG, has been made available online in May 2012 and is since then highly demanded by registered users all over the world. It received a recommendation by Paul Allison, who is a well-known author of numerous data analysis textbooks and course instructor. The SAS macro PSHREG is freely available (under a GNU-GPL-2 license) at <http://cemsis.meduniwien.ac.at/en/kb/science-research/software/statistical-software/pshreg/>
- The R package shrink developed in SysKid is freely available to the scientific community (under a GNU-GPL-2 license) at <http://cran.r-project.org/web/packages/shrink>
- Technology elements developed in SysKid were incorporated into the popular Genecards platform (www.genecards.org) making the technologies available to the general scientific community

1.5 Participants and relevant contact details

Participant list:

No.	Team Leader	Beneficiary name	Short Name	Country
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26	Mariano Rodriguez	Universidad de Cordoba	UCO	Spain
23/27	Josep Grinyo	Institut Catala de la Salut	ICS-HUB	Spain
28	Joachim Jankowski	Universitätsklinikum Aachen	UKA	Germany

Project web site: www.syskid.eu

