CHAPTER 7
Summary and future perspectives
Chapter 7

SUMMARY

The overall premise of this thesis is that earlier detection of diabetic kidney disease (DKD) in patients with type 2 diabetes and subsequent intervention for increased risk of DKD may ultimately help alleviate the burden of end-stage renal disease (ESRD). One strategy to accomplish this may be the use of novel biomarker panels to predict progression of renal disease. Furthermore, this thesis examined the use novel biomarker panels for predicting response to therapy and monitoring the effect of therapeutic intervention.

Type 2 diabetes is a multifactorial disease involving different pathophysiological molecular processes with a heterogeneous histopathological structure [1]. We hypothesized that a combination of biomarkers that capture different pathogenic processes of renal damage may provide a more realistic picture of a patient’s actual pathophysiological status. This thesis therefore focused on multiple biomarkers within a panel instead of a single biomarker. Recent advances in laboratory and throughput technologies have helped generate an expansive inventory of potential biomarker panels for renal disease in type 2 diabetes [2].

Novel biomarker panels can be used not only for risk prediction at early disease stages but also importantly for drug response prediction and monitoring therapeutic effect. Many patients with type 2 diabetes treated with guideline recommended therapy still face a high risk of renal disease progression and variability in response to therapy. Therefore, the use of novel biomarker panels in the context of drug response prediction or monitoring therapy is extremely important to reduce variability drug response, minimize side effects, or off-target effects, which is observed with many established and novel drugs. Using novel biomarkers to improve on current practices to improve risk stratification, help increase our understanding of renal disease pathophysiology, and provide insight into novel therapeutic targets can ultimately help reduce the burden renal disease in type 2 diabetes.

**Novel biomarker panels as predictors of renal disease**

In Part 1, this thesis investigated the predictive ability of novel biomarker panels for the progression of renal disease in patients with type 2 diabetes. Novel biomarkers may provide deeper understanding into the pathophysiology of DKD. Furthermore, identification of progression-associated molecular pathways via biomarkers as proxy may also help to identify novel therapeutic targets. Given the complexity of the multiple pathophysiological processes involved in progression of renal disease in type 2 diabetes together with the intra-individual variability of biomarkers, it is questionable if a single biomarker may possess useful diagnostic and prognostic power. Alternatively, a panel of clearly defined biomarkers may provide a more robust and reproducible tool as it tolerates changes in single biomarkers without jeopardizing their diagnostic ability. A combination of biomarkers that capture different pathways of renal damage may provide a more realistic picture of a patient’s actual pathophysiological status and hence may yield better assessment of disease prognosis performance.

Chapter 2 presented an observational cohort study of 82 patients with type 2 diabetes followed for 4 years. A panel of 13 novel biomarkers was associated with accelerated renal function decline beyond established risk markers. This study identified a novel panel of biomarkers representing different pathways of renal damage, including inflammation, fibrosis, angiogenesis, and endothelial function. This combined panel improved prediction of accelerated renal function decline in patients with type 2 diabetes on top of established risk markers. There was however, no external validation of this biomarker panel, and the small sample size limit the study results. Therefore, these results need to be validated in a large, prospective cohort to validate and assess this novel biomarker panel's applicability in a broad type 2 diabetes population.

The measurement of peptides and metabolites, known as proteomics and metabolomics, have emerged as strong tools in biomarker discovery [3]. Chapter 3 assessed the predictive ability of plasma proteomics classifiers to improve risk prediction of transition in albuminuria stage in patients with hypertension or type 2 diabetes. The developed plasma proteomics classifiers were able to predict transition in stage of albuminuria in hypertensive patients and transition from micro- to macroalbuminuria in patients suffering from type 2 diabetes. This was independent of established the renal risk markers urinary albumin excretion (UAE) and estimated glomerular filtration rate (eGFR), as well as use of renin-angiotensin-aldosterone system (RAAS) intervention. The plasma peptides identified in hypertension and type 2 diabetes were linked to pathways associated with established mechanisms of renal disease, including fibrosis, inflammation, angiogenesis, and mineral metabolism. These results support the growing evidence of using peptidomic platforms as a strategy for risk stratification of renal disease.

Metabolomics is another potential tool to discover novel biomarkers for renal disease. Chapter 4 investigated the predictive ability of urine and plasma metabolites for the development of diabetic nephropathy in patients with type 2 diabetes. In this discovery study, plasma metabolites histidine and butenolycarnatine and urine metabolites hexoses, glutamine, and tyrosine were able to predict progression from micro- to macroalbuminuria on top of established renal risk markers in type 2 diabetes. These metabolites did not predict albuminuria progression in patients with hypertension, suggesting a type 2 diabetes specific metabolite profile. In this study, the urinary metabolome profile performed better than the plasma profile, and addition of the plasma metabolites to the urinary metabolites did not improve outcome prediction. This may point at urinary metabolomics as a better clinical approach to identify people with type 2 diabetes at risk of progressive renal disease (also due to practical advantages of collecting urine compared to blood samples). However, the results from the plasma metabolomics may be useful to identify underlying mechanisms and pathways of disease and should not be neglected. Again, external validation of these metabolites is still needed to give firm conclusions for these metabolites’ predictive ability in a broader population.

Chapter 2

Chapter 3

Chapter 4
Novel biomarker panels for predicting response to therapy
First choice, guideline recommended therapy for treatment of hypertension and albuminuria for patients with type 2 diabetes are either angiotensin converting enzyme inhibitors (ACEI) or angiotensin II receptor blockers (ARB) [4]. However, many patients still do not respond optimally to ACEI or ARBs [5-9]. This may result in progressive renal function loss. One approach to reduce this variation in response to therapy is to use novel biomarkers to predict response to therapy. Chapter 5 discovered and externally validated a serum metabolite classifier that significantly improves prediction of albuminuria response to ARBs in patients with diabetes mellitus on top of traditional clinical risk markers. Metabolites included in the classifier were related to oxidative stress, inflammation, and fibrosis pathways. Specifically, increased nitric oxide synthase 3 (NOS3) activity appears to be a factor in predicting the albuminuria lowering response to ARBs. These findings suggest the use of serum metabolomics as a tool to help optimize treatment of antiproteinuric ARB intervention. Metabolites included in the classifier were assigned to stress/inflammation pathways and downstream consequences of fibrosis and extra cellular matrix remodeling. Furthermore, NOS3 appears to be a specific factor relevant in ARB response. These results indicate that for assessing drug response, both disease progression status and specific drug molecular effects need to be taken into account. The results of this metabolomics study support the growing evidence of using omics tools as a strategy to improve treatment of renal disease in diabetes mellitus. The complementary use of omics platforms can help bring personalized medicine one step closer to implementation in clinical practice.

Novel biomarker panels for monitoring the effect of therapy
Novel biomarker panels can be used to monitor the response to treatment. Chapter 6 investigated the effect of atrasentan on the previously identified metabolomics signature of diabetic kidney disease [10]. This study first demonstrated that concentrations of urinary metabolites from this panel were significantly correlated with eGFR levels in patients with type 2 diabetes and nephropathy. We then assessed the beneficial impact of atrasentan on this urinary metabolite panel. Treatment with atrasentan 1.25 mg/d for 12 weeks stabilized the levels of the metabolites while they declined with placebo treatment, implying that treatment with atrasentan may prevent their reduction as compared to placebo. Individual changes in the metabolites after 12 week treatment positively correlated with changes in eGFR. Lower metabolite concentrations have been previously shown to reflect reduced mitochondrial content and renal function [10], therefore, it is plausible that treatment with atrasentan may stabilize several aspects of mitochondrial function. Long-term hard outcome trials are required to assess whether these short-term beneficial effects portend in improvement in overall renal function. Results of further studies may help provide insight into the mechanisms through which atrasentan exerts renoprotective effects and may yield novel biomarkers to monitor response to therapy in patients with type 2 diabetes and DKD.

FUTURE PERSPECTIVES
Before a biomarker or panel of biomarkers can be used in clinical practice, it needs to be extensively validated in large studies to assess accuracy, reproducibility, sensitivity, and specificity. The translation of a biomarker or a combination of biomarkers from discovery to clinical practice is a process full of pitfalls and limitations. In 2009, Hlatky et al. proposed a framework for the development of biomarkers in collaboration with the American Heart Association [11], as listed in Box 1. Unfortunately, many novel biomarker panel studies for renal disease in type 2 diabetes stop at the end of the third phase [12], and do not proceed to external validation or assessment of clinical utility. More awareness and investments need to be made to perform studies in the clinical utility and clinical outcome phases in order to start implementing novel biomarker panels in clinical practice. Better study designs that can test the biomarkers’ practical value to translate the predictive power of a biomarker panel into decisions for clinical practice would help address non-acceptance of novel biomarkers by professional communities. Furthermore, collaborations between academia and industry may be a strategy to share expertise from different areas, to promote effective dissemination of results, and to support implementation of these findings in clinical practice.


1. Proof of concept
   - Do novel marker levels differ between subjects with and without outcome?
2. Prospective validation
   - Does the novel marker predict development of future outcomes in a prospective cohort or nested case-cohort/case-cohort study?
3. Incremental value
   - Does the novel marker add predictive information to established, standard risk markers?
4. Clinical utility
   - Does the novel risk marker change predicted risk sufficiently to change recommended therapy?
5. Clinical outcomes
   - Does use of the novel risk marker improve clinical outcomes, especially when tested in a randomized clinical trial?
6. Cost-effectiveness
   - Does use of the marker improve clinical outcomes sufficiently to justify the additional costs of testing and treatment?

Much of this thesis consists of research performed with the SysKid program (Systems Biology towards Novel Chronic Kidney Disease Diagnosis and Treatment, www.syskid.eu). SysKid was a large-scale European research project involving both academia and industry partners. SysKid aimed at improving characterization of the molecular mechanisms underlying diabetic kidney disease at the interference of the molecular impact of individual drugs in order to tailor optimal therapy to individual patients. Results and continuing
work stemming from SysKid have deepened our understanding of chronic kidney disease (CKD) in terms of prevention, new diagnostic strategies, and treatment options for renal disease in diabetes and hypertension. Furthermore, there is an ongoing validation study in a large, type 2 diabetes population that will assess the validity of the novel biomarker panels described in Chapter 2-4 of this thesis. Though this validation study will not be able to provide further knowledge toward clinical utility or cost-effectiveness, there is the opportunity to evaluate the predictive ability of these novel biomarkers panels for hard renal outcomes. Furthermore, this validation study provides the opportunity to compare the biomarkers at early stage, mid stage, and late stage DKD.

Use of biomarker panels for risk stratification in clinical practice is one strategy to guide treatment decisions and target interventions for patients at highest risk for progressive renal disease. Within SysKid, validation of a novel urinary peptide panel known as the CKD273 score was performed [13]. This CE-MS based urinary proteomics classifier was developed in a cross-sectional study in CKD patient groups with varying underlying etiologies of disease [14]. The clinical utility of the CKD273 score is currently being tested in the Proteomic Prediction and Renin Angiotensin Aldosterone System Inhibition Prevention Of Early Diabetic nephropathy In Type 2 Diabetic Patients With Normoalbuminuria (PRIORITY) trial (Clinical Trials Identifier NCT02040441) [15]. The PRIORITY trial will assess the clinical utility of the CKD273 score for the purpose of guiding treatment of patients with type 2 diabetes at high risk for renal disease progression. The PRIORITY trial uses the CKD273 score to identify patients at high risk of renal disease while still in early disease stages. The primary objective of the PRIORITY trial is to confirm whether urinary proteomics can predict development of microalbuminuria. The PRIORITY study will also assess whether high-risk patients identified with the CKD273 score will benefit from spironolactone therapy.

Current treatment of type 2 diabetes relies on optimal glycemic, lipid, and blood pressure control. Past randomized control studies has shown that targeting HbA1c, lipid management, and blood pressure control delays the progression of DKD [16-20]. However, even in these successful studies, the residual renal risk is still quite high, implying that the current treatment regime is insufficient to prevent progression to ESRD in a substantial proportion of patients. Given the large heterogeneity in pathophysiology of DKD and the substantial variability in response to renoprotective drugs, treatment strategies need to go in a new direction. Using novel biomarker panels to identify patients who would respond well to treatment before therapy has even been started, may help decrease variability in treatment response. An ideal scenario to implement use of novel biomarkers in clinical practice would be to identify individuals at risk for progressive disease while at the same time identify those who would respond well to treatment. However there is currently a paucity of studies that evaluate whether novel biomarkers predict drug response. Chapter 5 of this thesis is just the start in utilizing novel biomarkers to predict response to therapy, and future studies are needed to push this field forward.

Studies that assess whether treatment induced changes in novel biomarkers are associated with renal risk are lacking. This will be an area of interest for the future as it may help us to identify novel biomarkers that can be used to monitor drug efficacy, generate information about the molecular mechanisms through which drugs exert their effects, and provide insight into novel drug targets. It may be possible that a patient benefits from a drug without changing the urinary albumin levels and thus other biomarkers are needed to monitor drug efficacy or safety. Chapter 6 of this thesis provides some evidence towards proof of concept for use of a novel biomarker panel to monitor the effect of drug therapy, though long-term hard outcome trials are still required to confirm these findings and assess whether the short-term changes in these metabolites portend an improvement in long-term renal function.

In order to go beyond the status quo, novel approaches to treat patients with renal disease are necessary to improve disease outcomes. As advancing laboratory techniques become more and more realistic in clinical practice, the use of omics techniques as a tool to reduce the burden of renal disease complications represents a real strategy to improve disease diagnosis, prognosis, and treatment. Rich omics datasets have improved molecular phenotyping and characterization of the underlying molecular mechanisms of DKD on the level of individual patients. These datasets should also be used in the future to assess mechanisms of drug response and discover biomarkers that predict drug response. Collectively the omics methodologies, molecular process mapping, and from there developing novel biomarkers would contribute to the identification of the optimal therapeutic approach to provide the greatest benefit with minimal side effects or off-target effects (Figure 1). This may help improve on the current “trial-and-error” approach to choosing drugs for treatment of DKD in patients with type 2 diabetes and bring personalized medicine one step closer to clinical practice.

Figure 1. Creating a systems medicine approach to study drug response variability by adding post-genomic information to the genotype information, thereby connecting the complex genotype with the phenotype.
REFERENCES


