Summary, discussion and future perspectives
**Chapter 11**

**SUMMARY**

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary kidney disease with a prevalence of 3 to 4 patients per 10,000 of the general population\(^1\). ADPKD leads to end-stage renal disease with median ages of onset of 58.1 and 79.7 years, for the \(PKD1\) and \(PKD2\) mutation, respectively\(^2\). The vasopressin pathway is known to be activated in ADPKD\(^3\)\(^-\)\(^5\), and in recent years the first ADPKD-specific treatment has become available in the form of a vasopressin V2 receptor antagonist, tolvaptan. In the TEMPO 3:4 trial, tolvaptan reduced annual TKV growth from 5.5% to 2.8% and decreased the estimated glomerular filtration rate (GFR) slope from \(-3.70\) to \(-2.72\) \(\text{mL/min/1.73m}^2\)\(^6\). Since registration of this drug optimization of treatment has become of interest, as aquaretic side-effects lead to down-titration and even treatment discontinuation in considerable number of patients that used tolvaptan\(^6\). In addition, predicting which patients will have rapid disease progression has become increasingly important, since it is likely that subjects with more rapid disease progression will benefit more from drug treatment.

The aim of the first part of this thesis was to investigate whether the prognosis of ADPKD could be improved via lowering vasopressin concentration by lifestyle modification, and whether tolvaptan treatment could be optimized.

The second part of this thesis focuses on improvement of total kidney and liver volumes (TKV and TLV) assessment. TKV is an officially recognized early biomarker to assess disease severity and progression in ADPKD that can identify rapidly progressive patients in an early phase of the disease, even before kidney function starts to decline.

**Part I. Treating ADPKD by influencing the vasopressin pathway**

*Chapter 2* provides a summary of the current evidence of the pathophysiological relation of vasopressin with PKD, and gives a comprehensive overview of potential treatment options. These options consist of either blocking the effect of vasopressin using the kidney specific vasopressin V2 receptor antagonist tolvaptan or lowering circulating vasopressin concentration by changing lifestyle, fluid intake in particular.

In the following chapters it was investigated which lifestyle factors are associated with vasopressin – assessed using its surrogate marker copeptin – in a large general population cohort and a cohort of ADPKD patients, respectively. As described in *Chapter 3*, in the general population cohort the final stepwise backward regression model revealed associations with higher copeptin concentration for higher systolic blood pressure, current smoking, higher alcohol use, higher urea excretion, lower potassium excretion, use of glucose lowering drugs, higher BMI and higher plasma glucose. Higher copeptin concentration for higher systolic blood pressure, current smoking, higher alcohol use, higher...
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urea excretion, lower potassium excretion, use of glucose lowering drugs, higher BMI and higher plasma glucose. Higher copeptin concentration was especially strongly associated with lower 24-hour urine volume and higher sodium excretion, used as markers for fluid and salt intake, respectively.

In Chapter 4, a cohort of ADPKD patients was analyzed. The final stepwise backward regression model revealed associations with higher copeptin concentration for higher systolic blood pressure, lower 24-hour urine volume and higher sodium excretion, like previously found in the large general population cohort. None of the other lifestyle factors reached significance using a multivariable stepwise approach in which the associations were adjusted for sex, age, measured GFR and TKV. After adjustment for various not modifiable factors, vasopressin concentration was, in a multivariable stepwise backward linear regression analyzed, mainly associated with the factors that directly influence plasma osmolality, being fluid and salt intake. Other lifestyle factors that are known to influence vasopressin concentration when analyzed univariably, like smoking and alcohol consumption, did not reach statistical significance, indicating that these lifestyle factors do not influence vasopressin concentration as much as sodium and fluid intake do.

Chapter 5 presents a posthoc analysis of the TEMPO 3:4 trial, a large randomized controlled trial that showed the renoprotective efficacy of tolvaptan in patients with ADPKD. We aimed to see whether plasma copeptin levels were associated with disease progression. In addition, we studied whether pre-treatment copeptin concentration and treatment induced change in copeptin concentration were associated with tolvaptan treatment efficacy. In line with previous findings, baseline copeptin predicted kidney growth and eGFR decline in placebo treated subjects with ADPKD, independent of sex, age and baseline estimated GFR. In tolvaptan treated subjects copeptin increased after three weeks of treatment. In subjects with higher pre-treatment copeptin, a larger tolvaptan treatment effect was noted with respect to kidney growth rate and a similar trend was found for estimated GFR decline. Tolvaptan treated subjects with a larger percent increase in copeptin from pre-treatment to week 3 had better treatment efficacy, with less kidney growth and estimated GFR decline after three years. Copeptin thus is a promising biomarker to predict outcome and tolvaptan treatment efficacy in ADPKD.

Development of new treatment modalities as well as optimization of tolvaptan treatment are the scope of Chapters 6 and 7. Chapter 6 is an interventional murine study, in cooperation with Mayo Clinic (Rochester, MN, USA) that investigated the effects of sodium intake on disease progression as this is one of the main stimuli for vasopressin release. It furthermore studied the effect of sodium on vasopressin V2 receptor antagonism efficacy as well as its association with aquaretic side-effects. Treatment with a
vasopressin V2 receptor antagonist did not ameliorate disease progression in the rapid and slowly progressive murine Pkd1-model that were studied. Studying whether a low sodium diet improved vasopressin V2 receptor antagonism was thus not possible. It also suggests that the models that were used are not fit to investigate whether changing sodium intake will influence disease progression, because this intervention is suggested to be mediated by changes in the AVP axis. Notwithstanding, in 1 of the 2 models there was a suggestion that a low sodium diet did reduce the aquaretic side-effects of treatment with a vasopressin V2 receptor antagonist. Aquaresis was reduced on a low versus high sodium diet during vasopressin V2 receptor antagonist treatment by 23.2% in the rapidly and 20.6% in the slowly progressive model of Pkd1. This indicates that lowering sodium intake might attenuate the aquaretic side-effects of tolvaptan in patients with ADPKD.

Interestingly, sodium restriction has long been known to limit urine production in nephrogenic diabetes insipidus (NDI), a disease where the vasopressin V2 receptor is dysfunctional. The pathophysiology of NDI is similar to the pharmacological effect of tolvaptan, where the vasopressin V2 receptor is blocked. Chapter 7 describes a patient with ADPKD that used tolvaptan and started hydrochlorothiazide (HCT) treatment. HCT has been an established treatment for polyuria in NDI for over 50 years and is known to lower urine output by up to 50% within 2-4 days\(^7\). After starting HCT, no side-effects were reported, nor were any electrolyte abnormalities or signs of dehydration noted. However, 24-hour urine volume lowered from 4.9 liter on tolvaptan alone to 2.9 liter while concomitantly using tolvaptan and HCT. Copeptin concentrations rose from 22.7 to 29.7 pmol/L, and estimated GFR slopes changed from -1.35 to -3.97 mL/min/1.73m\(^2\) per year. Our case suggests that prescription of HCT during tolvaptan use may lead to less polyuria. Increased vasopressin is known to be associated with disease progression in ADPKD and could hypothetically lead to accelerated renal function decline. This is also suggested by our findings. This potential side effect of HCT warrants a careful approach towards co-prescription of this drug with tolvaptan.

Part II. Assessing ADPKD severity by MR imaging
In the early phase of ADPKD, renal function (glomerular filtration rate) is not notably affected, while TKV already increases. TKV is strongly associated with rate of disease progression and the age at which affected patients reach end-stage renal disease. TKV is therefore recognized by the American Food and Drug Administration and European Medicines Agency as an official biomarker in ADPKD. Now treatment options are emerging, it becomes of interest to select patients that will benefit most from these treatments, i.e. patients with rapid disease progression. TKV could be a very useful tool in order to identify these patients in an early phase of the disease. However, assessment
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is laborious and requires specific expertise, limiting its applicability as biomarker for this purpose.

In Chapter 8 we compared the performance of two different MRI sequences to assess TKV using the gold standard of manual tracing in ADPKD patients. Historically T1 weighted images (T1) were used, but the methodology of T2 weighted imaging (T2) has evolved, making T2 potentially preferable over T1 for TKV measurement. Here, we found that the measurement of TKV and its growth rate perform similarly when using T2 compared to T1 weighted images. T2 performed even better on secondary outcome parameters, because assessment of TKV using this type of imaging has slightly lower intra-and interreader variability. In addition, the series obtained with T2 imaging are more often of sufficient quality to be used for volume measurement.

Chapter 9 and 10 study alternative methodologies to assess TKV that are more feasible to use in clinical care. These methods were compared to the gold standard of manual tracing the kidneys. Ideally, a method is developed that performs as accurate as manual tracing, making it a reliable alternative to use in patient care, and in patient trials. In Chapter 9, two recently developed estimation methods were compared to manually tracing the kidneys, the ellipsoid and mid-slice method. Both methods resulted in similar kidney volumes compared to manually traced TKV. Both detected similar changes in TKV over time compared to manually traced TKV. They, however, had a lower reproducibility compared to the gold standard method. To assess the feasibility of estimation methods for clinical care, it was analyzed whether the estimated TKVs affected the Mayo Clinic risk classification category. This risk classification is used to assess whether a patient is likely to have a rapid disease progression and is used in clinical care to select patients for (drug) treatment. Using the estimated TKV, the risk category remained the same for the majority of patients, making estimation of TKV feasible for screening disease progression of ADPKD patients in clinical care. Since the ellipsoid method requires less time and expertise, we suggest this method may be preferable over the mid-slice estimation method in clinical care.

Chapter 10, for which the work was performed in the Mayo Clinic (Rochester, MN, USA) where we validated an artificial deep neural network for fully automated method to assess TKV and introduced this method to assess total liver volume (TLV) as well. This method provides a reliable alternative for manual tracing TKV and TLV in patients with polycystic kidney and/or liver disease. The precision and accuracy of the automated methodology correlated with the inter-reader variability of manually traced TKV, as well as TKV growth. The fully automated liver segmentations had significantly different, but numerically similar, liver volumes compared to manual tracing. When analyzing the sub
set of subjects with moderate and severe polycystic liver disease, our data showed that the automated method performed equally well compared to manually traced liver volumes. Automated TLV measurement therefore seems a reliable alternative for manual TLV measurement in polycystic liver disease. Automated measurements performed equally compared to manual measurements with respect to reproducibility and precision. Automated measurements may therefore be used as an alternative for kidney and liver assessment in randomized controlled trials where volumetry is used as endpoint. Benefits of using automatically traced measurements are less time per measurement, but also the avoidance of inter-reader variability (as the automated program will always measure the exact same volume for the same MRI) and no need for experienced readers/radiologists to perform the measurements, leading to less costs.

FUTURE PERSPECTIVES

Part I. Treating ADPKD by influencing the vasopressin pathway

Even though the vasopressin pathway is clearly involved in the pathophysiology of ADPKD and pharmacologic blockade of the vasopressin V2 receptor is proven to be beneficial, it remains unclear whether interference in the vasopressin pathway via vasopressin lowering lifestyle interventions are similarly beneficial in the management of ADPKD. We confirmed that fluid as well as osmolar intake, sodium in particular, are associated with copeptin concentration both in the general population as well as in ADPKD. Recently, a short-term dietary intervention study in ADPKD confirmed that a combined increased fluid and lower osmolar dietary regimen lowers copeptin in patients. However, long-term follow-up is needed to see whether this effect on plasma vasopressin concentration can be maintained, and whether the effect on plasma vasopressin concentration is sufficiently strong to result in less disease progression. Moreover, the individual effects of increased fluid and reduced osmolar (sodium and protein) diets remain to be studied, to see what the individual contribution is of these dietary interventions.

Although there may not yet be formal evidence to prove the efficacy of these interventions to influence disease progression in ADPKD, indirect evidence is emerging. For instance, in a recent post-hoc analysis of the HALT-PKD trials dietary sodium showed to be associated with the rate of TKV increase, but not with the rate of estimated GFR decline in ADPKD patients with an estimated GFR over 60 mL/min/1.73m². In an observational study with patients with lower renal function (estimated GFR 25-60 mL/min/1.73m²) sodium excretion was associated with reaching a composite endpoint of 50% reduction of estimated GFR, ESRD or death, and with a faster rate of estimated GFR decline. The effects of hydration on disease progression in ADPKD have been studied for many years, with contradictory results. Promising results were obtained by Nagao et al. and Hopp et
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al., who showed in rats with PKD that enhanced water intake led to amelioration of disease progression, accompanied by lower urinary vasopressin excretion\textsuperscript{12,13}. In contrast, a not-randomized study with 34 ADPKD patients who followed either a normal or increased water intake diet during one year did not confirm the beneficial effects of increased water intake. Even though 24-hour urine volumes were almost doubled from 1.4 to 2.6 liter per day and plasma copeptin and vasopressin concentrations were lower on the high water intake, there was a non-significant trend towards a faster disease progression on high water intake. Both estimated GFR decline and TKV growth tended to be higher after one year of increased water intake\textsuperscript{14}. Not finding a beneficial effect in this study might have been due to the study design (not-randomized, as patients could choose the treatment group of their preference), the limited number of patients included, or differences in salt intake. Therefore, there still is a need to study the effects of an increased water intake, as well as sodium and protein intake, on disease progression in ADPKD in a randomized controlled setting. Currently, two randomized controlled trials are being performed to analyze the effects of enhanced hydration on disease progression in chronic kidney disease in general (Clinicaltrial.gov Identifier: NCT01766687), as well as in 180 ADPKD patients, of which half will be prescribed a fluid intake to reduce urine osmolality to $\leq 270 \text{mOsm/kg}$\textsuperscript{15}.

As tolvaptan is marketed as first drug to ameliorate the rate of disease progression in ADPKD, a new era of optimizing treatment with tolvaptan via either affecting its side-effects or increasing its efficacy has begun. As the pathophysiology of NDI, where the vasopressin V2 receptor is dysfunctional, is similar to the pharmacological effect of tolvaptan, it would be of interest to study whether treatment modalities that lower urine volume in NDI could also ameliorate the aquaretic side-effects of tolvaptan. In our case study we indeed found that hydrochlorothiazide, known to lower urine volume in NDI, lowered urine volume in our ADPKD patient concomitantly treated with tolvaptan. Further studies in patients with ADPKD should illuminate the potential of hydrochlorothiazide in ameliorating the rate of disease progression, as well as its potential in lowering aquaretic side-effects and synergistic treatment effect when concomitantly used with tolvaptan. Furthermore, other commonly used therapeutic interventions to lower polyuria in NDI could be the topic of investigation in future studies that aim to lower the aquaretic side-effects of tolvaptan. For instance via lowering the osmolar intake. Another potentially interesting treatment modality could be the addition of metformin to tolvaptan treatment. Metformin has shown to lower urine production in a rat model of NDI, in which tolvaptan was used to induce NDI\textsuperscript{16}. Furthermore, it has been suggested that metformin could be renoprotective in PKD via a vasopressin independent pathway\textsuperscript{17}. Currently two phase 2 studies are investigating the effect of metformin treatment on disease progression in ADPKD (Clinicaltrial.gov Identifiers: NCT02656017 and NCT02903511). As metformin acts
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via a vasopressin independent pathway, concomitant use of metformin and tolvaptan could synergistically affect disease progression in PKD via cyclic AMP downregulation\textsuperscript{17}. Secondly, statins have previously shown to lower aquaretic side-effects through induction of accumulation of AQP2, as has previously been shown in a rat model of diabetes insipidus\textsuperscript{18}. They may also have synergistic treatment effects with concomitant tolvaptan use, as they lower cyclic AMP vasopressin independently through downregulation of G\textsubscript{s} protein\textsuperscript{19}.

Besides treatment modalities that have been proven to be effective in NDI, there might be other options to ameliorate tolvaptans aquaretic side-effects, that potentially have a synergist effect on disease progression when concomitantly used with tolvaptan in ADPKD. Hopp et al. found an additive efficacy in ameliorating kidney growth of concomitant tolvaptan and pasireotide, a long-acting somatostatin analogs acting on somatostatin receptor (SSTR), use compared to either treatment alone in Pkd1-mice. Furthermore, less aquaresis was observed when adding SSTR to the tolvaptan treatment\textsuperscript{20}. Lastly, tetracycline antibiotics demeclocycline and doxycycline might have synergistic treatment effects with tolvaptan. Demeclocycline decreases adenylate cyclase 5/6 expression and consequently cAMP generation as well as AQP2 expression\textsuperscript{21}. In animal studies doxycycline inhibited disease progression in rats with PKD\textsuperscript{22}. However, the nephrotoxicity of these drugs when used at high doses will probably limit their potential for treatment in ADPKD. Evaluating the effects of one of the above mentioned treatment modalities on disease progression and aquaretic side-effects in ADPKD patients that concomitantly use tolvaptan, could be the scope of future studies.

Part II. Assessing ADPKD severity by MR imaging

With the acknowledgement of TKV as biomarker for disease progression in ADPKD, there is an unmet need to optimize TKV measurement in order to make its measurement more feasible and applicable in clinical care. With our studies, we showed that T2 and T1 weighted MR images can both be used for manual tracing of TKV. We showed that measured TKV is superior over estimated TKV. In addition, we found that a fully automated deep learning method to measure TKV was as accurate as manual tracing, both cross-sectionally, as well as with respect to its capability to measure change in kidney volume over time. We trained the method for measurement of liver volume as well and unfortunately found significant differences in cross-sectional measurement, as well as growth analysis for small liver volumes. As liver volume measurement is mostly relevant in case of polycystic liver disease (PLD), future studies to optimize the deep learning program for patients with PLD, with or without ADPKD, should be performed. Furthermore, it could be studied whether T1 weighted images are better for measurement of liver volumes. To further confirm the potential of the automated method compared to
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manually traced TKV and TLV, future studies could compare both methods as endpoint in randomized controlled trials to see whether automated TKV measurement is a reliable alternative in the assessment of kidney and liver volumes and growth in clinical trials. This could be done as post hoc investigations of studies that found a significant benefit of treatment. Furthermore, to fully use its potential, it would be interesting to implement the program in clinical care for quick and reliable assessment of kidney and liver volume in patients with ADPKD to see how rapidly their disease is progressing and whether they qualify for drug treatment to ameliorate kidney and/or liver growth.

In the rapidly changing field of ADPKD research, the studies described in this thesis add to a better understanding of the detrimental effects of the vasopressin pathway, optimization of tolvaptan treatment and improvement of renal and liver imaging. This understanding will help to better select those patients with ADPKD that will benefit most from these optimized treatment options.
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REFERENCES


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