1

Introduction
Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common form of inherited kidney disease. The disease has a prevalence of 3 to 4 per 10,000 in the general population\(^1\) and is characterized by bilateral progressive renal cyst formation and growth throughout life.

**Pathophysiology of ADPKD**

ADPKD is caused by a mutation in the *PKD1* gene (85% of cases) or in the *PKD2* gene (15% of cases). These genes encode for the proteins Polycystin-1 and Polycystin-2 respectively\(^3\). These proteins form the so-called polycystin complex that is localized at the basis of the primary cilium of renal epithelial cells, which acts as a mechanosensor that detects flow in the renal tubules. When this sensor is stimulated, calcium influx occurs from pre-urine into the cytoplasm of renal tubular epithelial cells and from intracellular stores. High intracellular calcium inhibits the enzyme adenylyl cyclase (AC), which is localized at the basolateral side of renal tubular epithelial cells, and that stimulates the conversion of adenosine triphosphate (ATP) into cyclic adenosinemonophosphate (cAMP). In normal physiology, cAMP is involved via a cascade of down-stream intracellular processes with regulation of cell growth and chloride driven fluid transport. In ADPKD, the polycystin complex is dysfunctional because of mutations in *PKD1* or *PKD2* and consequently, calcium cannot enter the cells, nor can calcium be released from intracellular stores. Low intracellular calcium leads to high activity of AC and high intracellular cAMP levels. In turn, this results in high intracellular cAMP levels, which lead to aberrant renal tubular epithelial cell proliferation and chloride driven fluid excretion in the kidney\(^4\). These are the two key components of the process of cyst formation and growth in ADPKD (Figure 1).

**Variable disease course in ADPKD**

Eventually, ADPKD can lead to development of end stage kidney disease (ESKD). However, only 70% of patients reach ESKD and the age at which patient reach ESKD shows large interindividual variability. This is partly explained by the locus and type of mutation, since patients with a *PKD1* mutation, especially truncating mutations, generally progress faster to ESKD compared to patients with a *PKD2* mutation\(^5\). Figure 2 represents this variable disease course in the families of our Groningen ADPKD patient cohort per *PKD* mutation. This figure shows that even between family members that share the same mutation, a large interindividual variability exists in the age at which family members with ADPKD reach ESKD.
Introduction

**Figure 1.** Schematic representation of the pathophysiological progresses that drive cyst formation and growth in renal tubular epithelial cells of the collecting duct in ADPKD and the mechanism of action of vasopressin V2 receptor antagonists and somatostatin analogues (modified from Zittema et al.). In ADPKD the polycystin complex (formed by the proteins PC1 and PC2 on the apical membrane) is dysfunctional which leads to diminished calcium influx or diminished release of calcium from intracellular stores. Low intracellular calcium levels in turn stimulate activation of adenylate cyclase (AC), which converts adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP). cAMP is an important player in several pathways that could possibly lead to cell proliferation and cell growth, for instance, via activation of the Ras/B-Raf/MEK/ERK-pathway. Furthermore, cAMP activates apical positioned chloride channels (CFTR-channels) leading to fluid secretion, which together results in cyst formation. cAMP production can be inhibited by blocking the vasopressin V2 receptor (V2R), which is coupled to G stimulatory (Gs) proteins that can activate AC. Activation of the somatostatin receptor (SSTR) can inhibit cAMP production in a direct and indirect way. AC can directly be inhibited by the receptor coupled G inhibitory (Gi) proteins. Activation of these Gi proteins can also activate calcium channels and stimulate intracellular release of calcium via phospholipase C (PLC) which can restore intracellular calcium stores. This leads indirectly to inhibition of cAMP production. Orange and grey lines indicate that the pathway is either activated or inactivated. Abbreviations are: ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; CFTR, cystic fibrosis transmembrane conductance regulator; ER, endoplasmic reticulum; Gi, G inhibitory; Gs, G stimulatory; mTOR, mammalian target of rapamycin; PC, polycystin; PKA, protein kinase A; PLC, phospholipase C; SSTR, somatostatin receptor; TSC, tuberous sclerosis; V2R, vasopressin V2 receptor.

Remarkably, this figure does not confirm differences between *PKD1* truncating and non-truncating mutations, which may be explained by referral bias, since the UMCG is a tertiary referral center, and especially subjects with rapidly progressive disease are referred for consultation of inclusion in trials. However, this figure does give the impression that patients with a *PKD2* mutation develop ESKD at an older age than subjects with a *PKD1* mutation.
Factors associated with disease progression

Given the variability in age at which ESKD is reached within families, other factors than type of gene mutation are likely involved in determining the rate of disease progression. Intrafamilial variability may, for instance, be caused by other genes that can modify disease progression\(^6\). Furthermore, several studies have reported that males in general show a faster rate of disease progression compared to females, suggesting that sex hormones may be of importance\(^7,8\). Finally, environmental factors that may cause a more rapid rate of disease progression are a low birth weight, high caffeine intake, smoking, low water intake and high protein intake\(^9-14\).

Currently used predictors of disease progression

Since the disease course of ADPKD is highly variable, it is difficult to predict the rate of disease progression in an individual patient. Obviously, the ability to predict the rate of
disease progression in patients with ADPKD would help patients and caregivers alike in treatment related decisions. Patients with a higher rate of disease progression will probably benefit the most from therapy, since in these patients the benefit to risk ratio of treatment is expected to be better, especially when treatment is started early\textsuperscript{15}.

Currently, there are several variables used to predict the rate of disease progression in ADPKD. ADPKD is in general a slowly progressive kidney disease. As ESKD is the endpoint to be prevented, it makes sense to use glomerular filtration rate (GFR), indexed for age, as a predictor for the rate of disease progression. However, GFR indexed for age may be less sensitive in early stages of this disease, as kidney function remains relatively stable in the near-normal range for prolonged periods of time before it starts to decline. It is hypothesized that in early stage ADPKD, GFR remains in the normal range due to compensatory hyperfiltration of remnant nephrons, while cysts are progressively formed and nephrons are lost\textsuperscript{16} (Figure 3). Therefore, much attention has focused on total kidney volume (TKV) as a predictor of disease progression, because an increase in TKV starts already at a very young age. The CRISP (Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease) study was one of the largest and most important studies to confirm that TKV can indeed be used as a surrogate for disease progression in ADPKD. Thereafter several other studies have corroborated this finding\textsuperscript{17}.

As mentioned above, the rate of disease progression is also partly explained by ADPKD genotype, and genotype is therefore also commonly used to predict disease progression\textsuperscript{5}. However, genotype and TKV are often not available in routine clinical care, because their assessment is laborious and expensive. Furthermore, at an individual patient level their predictive power for the rate of disease progression is limited. Therefore cheap and easy to measure risk markers need to be developed that alone, or in combination with conventional risk markers, can predict the rate of disease progression in ADPKD.
Figure 3. Representation of the natural disease course of ADPKD.

**Promising new risk markers**

**Urinary damage and inflammation markers**
Measurement of urinary damage and inflammation markers are of interest, especially because these markers are relatively inexpensive and easy to measure. Many of these markers have been shown to be associated with disease severity and disease progression in non-ADPKD chronic kidney disease\(^{18-30}\) and some are even approved by the FDA as an official biomarker for kidney damage\(^{31}\). In ADPKD, cross-sectional studies already showed that urinary damage markers are associated with disease severity, assessed as GFR and TKV\(^{32, 33}\). However, little attention has been focused on the possible ability of these markers to predict disease progression in ADPKD\(^{34-37}\). In Table 1 various urinary damage and inflammation markers are shown. This table displays the performance of the assays, and whether previous studies have shown associations of these markers cross-sectionally with disease severity and longitudinally with disease progression in patients with ADPKD.
Table 1. Urinary inflammation and tubular damage markers representing different segments of the nephron and their association with disease severity and disease progression in patients with ADPKD.

<table>
<thead>
<tr>
<th>Assay performance</th>
<th>Cross-sectional</th>
<th>Longitudinal</th>
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<tbody>
<tr>
<td></td>
<td>Intra-CV (%)</td>
<td>Inter-CV (%)</td>
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<tr>
<td>General</td>
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<td></td>
</tr>
<tr>
<td>- Albumin</td>
<td>2.2</td>
<td>2.6</td>
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<tr>
<td>Glomerular</td>
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<tr>
<td>- IgG</td>
<td>8.4</td>
<td>16.4</td>
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<tr>
<td>Proximal tubular</td>
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<td></td>
</tr>
<tr>
<td>- β2MG</td>
<td>6.3</td>
<td>8.0</td>
</tr>
<tr>
<td>- KIM-1</td>
<td>7.4</td>
<td>14.5</td>
</tr>
<tr>
<td>- NAG</td>
<td>3.1</td>
<td>13.7</td>
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<tr>
<td>Distal tubular</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- H-FABP</td>
<td>9.3</td>
<td>17.6</td>
</tr>
<tr>
<td>Inflammatory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- MCP-1</td>
<td>8.3</td>
<td>12.7</td>
</tr>
<tr>
<td>- MIF</td>
<td>5.2</td>
<td>9.2</td>
</tr>
<tr>
<td>- NGAL</td>
<td>6.8</td>
<td>19.6</td>
</tr>
</tbody>
</table>

Abbreviations are: IgG, immunoglobulin G; β2MG, β₂-microglobulin; KIM-1, kidney injury molecule 1; NAG, N-acetyl-β-D-glucosaminidase; HFABP, heart-type fatty acid binding protein; MCP-1, monocyte chemotactic protein 1; MIF, macrophage migration inhibitory factor; NGAL, neutrophil gelatinase-associated lipocalin; GFR, glomerular filtration rate; TKV, total kidney volume; NA, not applicable.

Hyperfiltration: the earliest marker of disease severity and possible future disease progression?

As it is hypothesized that kidney function stays stable in the early stages of the disease by compensatory hyperfiltration of remnant nephrons, the extent to which a patient is hyperfiltrating may be an early marker of disease severity and predictor of future disease progression. Unfortunately, renal hyperfiltration cannot be directly measured in humans, and therefore surrogate measures are used. Hyperfiltration is sometimes defined as an unstimulated increased kidney function, but this does not seem applicable for patients with ADPKD since it is speculated that patients hyperfilter in ADPKD to compensate for GFR loss. In these cases, glomerular hyperfiltration is sometimes defined as an increased filtration fraction, determined by GFR divided by the effective kidney plasma flow. Indeed increased filtration fraction indicates that...
the kidney is attempting to maintain kidney function to a certain level despite lower effective kidney plasma flow. However, measurement of effective kidney plasma flow by infusion of exogenous tracers such as para-aminohippuric acid (PAH) may be inaccurate, and lead to overestimation of filtration fraction, especially when tubular function is compromised as in ADPKD\(^4\). Glomerular hyperfiltration is therefore more commonly defined as the loss of kidney function reserve capacity, i.e. the impairment of the kidney to increase GFR in response to stimuli such as dopamine or amino acids\(^4\), \(^4\). If patients with ADPKD hyperfilter in early stages of the disease, a loss of kidney function reserve capacity is expected to occur prior to a decline in GFR.

**Endogenous substances involved in ADPKD disease progression**

One of the pivotal detrimental factors in the pathophysiology of ADPKD are elevated levels of cAMP in renal tubular cells. These levels will increase further by stimulation of the vasopressin V2 receptor by vasopressin (Figure 1). Thus, high levels of vasopressin are expected to be associated with a worse disease course and might therefore serve as a risk marker for future rapid disease progression\(^4\). Indeed, studies have shown that copeptin, a surrogate marker for vasopressin, is associated with a faster rate of kidney function decline in patients with ADPKD\(^4\) and blocking the vasopressin V2 receptor, attenuates ADPKD disease progression\(^4\), \(^4\). Another hormone that may interfere in this pathway is somatostatin. Somatostatin is a hormone that is involved in many cell processes, and that can induce a broad spectrum of biological effects. Importantly, somatostatin has the ability to directly and indirectly inhibit tubular cAMP production\(^4\), \(^5\)-\(^7\) (Figure 1). Therefore, it may be that patients with low levels of endogenous somatostatin have a worse disease course compared to patients with high levels. In line, the administration of somatostatin analogues are of interest as a possible therapeutic option in ADPKD.

**Somatostatin analogues in ADPKD**

Somatostatin analogues indeed have the ability to inhibit cAMP production by the inhibition of AC activity, which resembles the working mechanism of tolvaptan. Nine distinct membrane-bound AC isoforms (AC1-9) and one soluble AC (sAC) have been identified. Except for AC8, all of these isoforms are expressed in the kidney. Specific AC isoforms can exert unique effects in various cell types of the kidney, potentially relevant for channel activation and thus cystogenesis\(^5\). It is currently unknown if there are specific AC isoforms associated with the vasopressin V2 or somatostatin receptor. It may therefore well be that both receptors interact with the same AC isoform and that there is a pharmacological interaction between somatostatin analogues and
tolvaptan. Interestingly, some studies have suggested involvement of somatostatin in renal water handling\textsuperscript{52-55}, which suggests that there indeed may be an interaction between the somatostatin and vasopressin pathways.

**AIMS OF THE THESIS**

The general aim of this thesis is to study if current risk markers that predict disease progression in ADPKD can be improved, and to search for new markers that may predict disease progression beyond the currently used risk markers.

**OUTLINE OF THE THESIS**

The assessment of TKV by the gold standard manual tracing method is very laborious and therefore not generally applicable in routine clinical care. In Chapter 2 we investigated if TKV could be assessed by easy and less laborious estimation methods instead. Historically, gadolinium enhanced T1 weighted images were used for the measurement of TKV because of the short scanning time, low variations in image quality and high contrast of the renal structures against the surrounding tissues\textsuperscript{56}. Gadolinium, however, is currently not routinely used in patients with impaired kidney function, because exposure to gadolinium has been found to be associated with a higher incidence of nephrogenic systemic fibrosis\textsuperscript{57}. When not using gadolinium contrast, T2 weighted images might be preferred over T1 weighted images for the measurement of the TKV, because this technique shows high kidney tissue-contrast and hyperintense renal cysts, that may help to better delineate the kidney boundaries against background tissue\textsuperscript{58}. Moreover, the single-shot T2 weighted techniques have evolved over the last years with for instance shorter examination time and fewer motion artifacts. This makes T2 weighted imaging potentially preferable over T1 weighted imaging for TKV measurement. In Chapter 3 we therefore compared the performance of using T2 and T1 weighted MR images for measurement of TKV and growth in TKV in patients with ADPKD.

Since ADPKD is a tubular disease with an inflammatory component, measurement of urinary tubular damage and inflammation markers is of interest to predict the rate of disease progression, especially because these markers are relatively inexpensive and easy to measure. In Chapter 4 we therefore investigated if urinary markers were
associated with ADPKD disease progression. In chapter 5 we investigated whether the markers that were identified in chapter 4 were associated with disease progression in an independent cohort of ADPKD patients.

Although it is never formally been tested, it is assumed that patients with ADPKD hyperfilter prior to a decline in GFR. If this can be confirmed, then the extent to which a patient is hyperfiltrating may possibly be the earliest marker of disease severity and predictor of disease progression. In chapter 6 we are the first to formally investigate if patients hyperfilter prior to a decline in GFR by using a generally accepted definition of hyperfiltration in chronic kidney disease, i.e. loss of kidney function reserve capacity.

Elevated cAMP levels are one of the pivotal detrimental factors in the pathophysiology of ADPKD. As somatostatin has the ability to inhibit intracellular cAMP production, we hypothesized that endogenous somatostatin levels may be associated with ADPKD disease progression. In chapter 7 we therefore investigated if endogenous plasma somatostatin has potential to serve as a prognostic biomarker. Extending this line of reasoning, administration of somatostatin, in the form of somatostatin analogues, is a possible therapeutic option in ADPKD. In chapter 8 a review is given of the complex physiology of somatostatin, in particular in renal physiology and its potential therapeutic role in ADPKD. In addition, the results of studies with somatostatin analogues in ADPKD are discussed. As somatostatin analogues can inhibit cAMP production in a similar way as tolvaptan, through inhibition of AC activity, there may be an interaction between both pathways. In line, some studies have suggested that somatostatin is involved in renal water handling. In chapter 9 we investigated therefore if there are differences in diuresis and free water clearance in ADPKD patients using the somatostatin analogue lanreotide compared to patients using standard care. Furthermore, we investigated if differences were dependent on patient characteristics.

In 2016, tolvaptan, the first disease modifying drug has become available to treat patients with ADPKD. Initially, patients with signs of rapid disease progression and a relatively preserved kidney function were eligible for treatment. Now clinical experience has accumulated and with the results of an additional recent clinical trial with tolvaptan, an update was needed of the recommendations for the use of tolvaptan in ADPKD. In chapter 10 we therefore propose novel recommendations how to select patients with rapidly progressive disease for treatment with tolvaptan and demonstrate how these recommendations work in clinical practice.
REFERENCES


