The Molecular Mechanisms of

POLYCYSTIC KIDNEY DISEASE

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The Molecular Mechanisms of Polycystic Kidney Disease

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“Imagination is more important than knowledge.
Knowledge is limited, imagination encircles the world”
- Albert Einstein -

TABLE OF CONTENTS

Chapter 1	General introduction	9
Chapter 2	Cystic renal-epithelial derived Induced Pluripotent Stem Cells from Polycystic Kidney Disease patients	25
Chapter 3	Senescence is a hallmark of Polycystic Kidney Disease	57
Chapter 4	Paracrine Signaling Molecules in Cyst Fluid of Patients with Polycystic Kidney Disease	91
Chapter 5	Using CasID to screen for transcription factors that regulate PKD1 expression	115
Chapter 6	Summary, general discussion and future perspectives	145
Appendix	Nederlandse samenvatting	163
	List of abbreviations	166
	Curriculum Vitae	168
	PhD portfolio	170
	List of publications	172
	Dankwoord	174

Chapter 1

General introduction

The normal kidney versus the polycystic kidney

The kidney is a complicated organ that enables the removal of waste products from the blood and regulates the balance of body fluid and electrolyte levels. Our kidneys contain between 200.000 and 1.000.000 filtrating units – called nephrons – that filter approximately 180 liters of blood and processes this into 1-4 L urine each day. A nephron is composed of a glomerulus where the blood is filtered, and a system of tubules that determine the ultimate composition of the urine. Microscopically, the tubule is an elongated tube consisting of an epithelial monolayer with a narrow lumen. During embryonic development and kidney regeneration signaling cues ensure the accurate regulation of the diameter and directional growth of the tubule. Occasionally, this process does not take place properly, leading to epithelial cells budding off from the tubular wall. These protrusions can eventually detach from the tubulus and form a cyst, which translates as 'a balloon' or 'fluid filled sac'. Cysts can occur in any tubular segment and whilst the diameter of a normal tubule is approximately 40 µm, these cysts can grow to over 10 cm in diameter [1]. In the general population, solitary kidney cysts – also referred to as simple cysts – are quite common, with an increasing prevalence during aging [2]. In most cases simple cysts are asymptomatic. This is in contrast to kidneys that have accumulated numerous amounts of (large) cysts, a condition referred to as polycystic kidney disease (PKD). Whilst a normal kidney is the size of a fist and weighs approximately 500 grams, polycystic kidneys can grow to over 50 cm and can weigh over ten kilograms [3]. In PKD, cyst formation induces local inflammation, fibrosis and ousts the surrounding healthy kidney tissue, eventually leading to kidney failure. The first report of PKD is in the Polish king Stefan Bathory (1533-1591), where the autopsy report described his kidneys as 'large like those of bull, with an uneven and bumpy surface' [4]. Although cases of polycystic kidneys were noted by pathologists, it wasn't until late 19th century that PKD was recognized as a clinical entity with its characteristic symptoms of bilateral tumors in the flanks, hypertension, proteinuria and hematuria. The actual term 'Polycystic Kidney Disease' was first introduced by Felix Lejars in his thesis published in 1888 [4].

Acquired and hereditary Polycystic Kidney Disease

Polycystic Kidney Disease is a heterogeneous group of diseases, with a variety of acquired and congenital causes. In acquired polycystic kidney disease, cyst formation in genetically normal kidneys can be triggered by ischemia, a partial nephrectomy or nephrotoxic drugs [5–8]. In addition, patients with chronic kidney disease, especially when receiving dialysis, frequently develop acquired cystic kidney disease [9–11]. Genetic forms of PKD are caused by mutations in a myriad of genes; over 100 genes have been described that can induce a polycystic kidney phenotype [12]. Within the genetic forms

of PKD, there is a wide spectrum of the severity of the phenotype, with the age of onset of symptoms ranging from *in utero*, juvenile to late adulthood. In addition, the phenotype can be restricted to the kidney, can occur in both kidney and liver, or can be part of a broader syndrome.

Autosomal Dominant Polycystic Kidney Disease (ADPKD)

This thesis will focus on Autosomal Dominant Polycystic Kidney Disease (ADPKD), which is the most common form of PKD. The estimated prevalence is between 1:400 and 1:2,700 individuals [13]. It is estimated to affect over 10 million people worldwide, thereby being the fourth most common cause of kidney failure [13–17]. ADPDK is caused by a heterozygous germline mutation in *PKD1 (MIM: 601313)* or *PKD2 (MIM: 173910)* in ~80% and ~15% of the patients respectively [13,18–20]. In rare cases, an ADPKD-like phenotype can be caused by mutations in other genes, such as *GANAB*, *DNAJB11*, *SEC63, PKHD1*, *COL4A1* [13]. ADPKD is inherited in an autosomal dominant pattern, with a 50% risk of passing the disease on to a child. While most patients have a positive family history, approximately 10% of the ADPKD cases are caused by a *de novo* mutation. In the early phase of the disease, patients is often asymptomatic. When disease progresses, patients can suffer from hypertension, abdominal pain, urine-concentrating defects (resulting in polyuria and nocturia), nephrolithiasis, recurrent urinary tract and cyst infections, proteinuria, haematuria and a decline in renal function (for review see [12,13,21]). Although there is a large variability in disease course, ADPKD patients typically develop end-stage renal disease in their fifth decade, after which dialysis or a kidney transplantation is required [15,22]. Cyst formation is not restricted to the kidneys, but can also occur in the liver (~80%), seminal vesicles (~40%), arachnoid membrane (~8-12%), spleen (~2.6%) and pancreas (~1.8%) [21]. In addition, other extra-renal manifestations have been described, such as intracranial aneurysms, cardiac valve malformations and cardiomyopathy [21,23]. ADPKD can be diagnosed using abdominal imaging by conventional ultrasound, CT or MRI scan [24–26] or by genetic testing for mutations in *PKD1* or *PKD2* [22,27,28]. Importantly, however, sequencing analysis of *PKD1* can be quite complex, because of its large gene size and the fact that the first 33 exons share up to a 98% homology with six *PKD1*-pseudogenes [29]. In addition, there is a high allelic heterogeneity for *PKD1* and *PKD2* with more than 1,900 different reported mutations, whereas a clear mutational hotspot is lacking [22,30]. Treatment of ADPKD patients currently consists of monitoring kidney function, blood pressure control, life style advise and symptomatic treatment of pain and cyst infections (for review see [12,13,31,32]). For patients with rapidly progressing ADPKD, Tolvaptan has recently been approved in several countries. Tolvaptan is a vasopressin receptor 2 antagonist which reduces intracellular cAMP levels, and has been shown to slow down the rate of cyst growth and renal function decline [33,34].

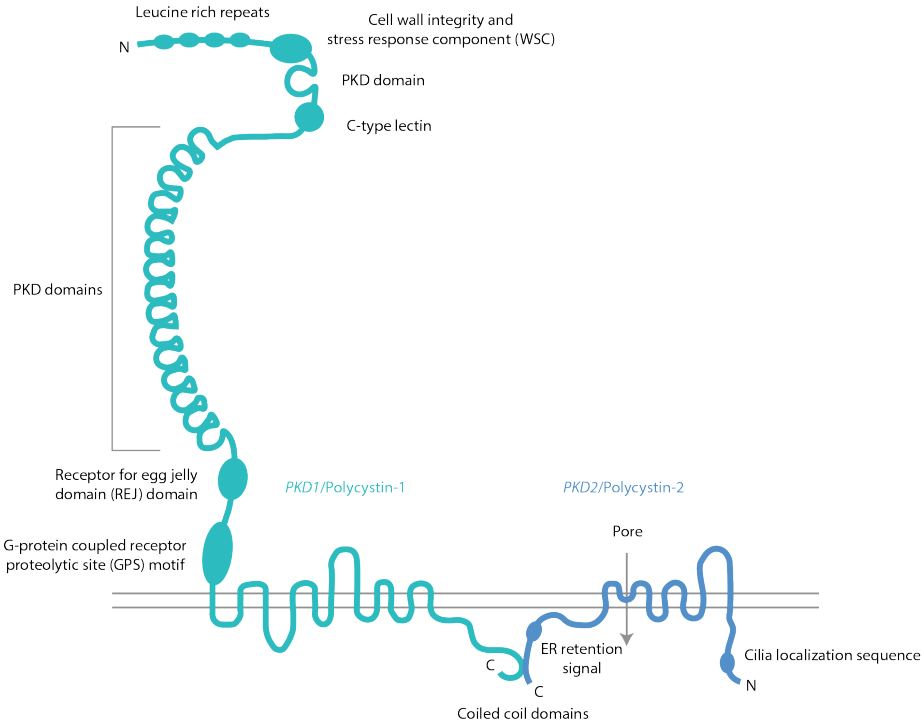
Trials regarding other therapeutics (such as somatostatin analogues, mTOR inhibitors, statins, tyrosine kinase inhibitors and Metformin) did not show a significant reduction in kidney function decline or are currently ongoing [12,13,35,36].

The polycystins

PKD1 is an evolutionary well conserved gene, with orthologs present in mammals and non-mammalian species such as fugu, zebrafish and *C. elegans* [37–40]. In humans, *PKD1* is located on chromosome 16p13.3, spanning a large genomic region of 52 Kb and consisting of 46 exons encoding a 14 kb transcript [18,41]. It is expressed in a variety of tissues and cell types, including renal tubular epithelial cells. Expression of *PKD1* has been found to be high during kidney development, in contrast to the adult kidney were expression levels are low [42,43]. However, when renal injury is induced, *PKD1* transcription levels are increased [44]. Although several *PKD1* transcription factors have been identified [45,46], the regulation of *PKD1* expression levels remains to be elucidated.

PKD1 encodes for polycystin-1, a 450 kDa transmembrane protein, with many similarities to the adhesion G-protein coupled receptor (GPCR) family [18,47]. It has a large extra-cellular N-terminal segment, 11 transmembrane spanning regions and a small intracellular C-terminus. Several domains have been identified in polycystin-1, many of which are involved in protein-protein, protein-matrix and protein-carbohydrate interactions (**Figure 1**) [13,41]. In the endoplasmic reticulum and the early Golgi system glycosylation and quality control of polycystin-1 takes place by *GANAB* (alpha subunit of glucosidase II), an endoplasmic reticulum enzyme which was recently found to be mutated in a small subset of ADPKD patients [48]. In addition, polycystin-1 is cleaved at the GPS domain, resulting in an intracellular C-terminal tail of polycystin-1, and a N-terminal fragment that remains non-covalently bound to the membrane region [49]. These post-translational modification steps are essential for polycystin-1 function and trafficking [50]. In the cell, polycystin-1 is located in the plasma membrane, at the membrane junctions (tight junctions, adherens junctions, desmosomes and focal adhesions), but also in mitochondria, the basal body and the primary cilium [13,51,52]. Cleavage of polycystin-1 at the C-terminal tail results in a small ~35 kDa and a ~15 kDa fragment that translocates into the nucleus [53,54].

Polycystin-1 interacts with polycystin-2, encoded by *PKD2* (**Figure 1**) [55]. Polycystin-2 is a non-selective cation channel member of the transient receptor potential (TRP) family, which conducts Ca^{2+}, but has also been reported to be permeable for Na^{+} and K^{+} ions [19,56]. Together they form a complex, most likely a heterotetramer, consisting of one polycystin-1 and three polycystin-2 molecules [57]. This polycystin-complex functions as a ligand gated ion-channel [58].



Reference: adjusted from Bergmann et al. Nature Reviews Disease Primers, 2018

Figure 1. The polycystins

PKD-signaling

What exactly is sensed by the polycystin complex, how the PKD signal is relayed throughout the cell and what the downstream effectors are is still poorly understood. Various cellular functions have been implied to be regulated by the polycystins. Cell-cell and cell-matrix interactions, orientated cell division, proliferation and apoptosis, cell migration, maintenance of differentiation status, autophagy, inflammation, oxidative stress and cell metabolism have all been described to be altered in ADPDK (for review see [13,59]). In any case, the primary cilium, which is an antenna-like organelle at the luminal side of the epithelial cell (for review see [60–62]), seems to play a crucial role in PKD-signalling [62]. A myriad of signalling pathways has been linked (either directly or indirectly) to the polycystins. Calcium/cAMP signalling was found to be a central effector pathway in PKD and impaired polycystin-signaling leads to reduced intracellular calcium levels. As a consequence intracellular cAMP levels are increased, which is thought to drive cystogenesis (for review see [63]). There is compelling evidence

that polycystin-1 and the Wnt-signaling pathway are intertwined. Interestingly, mutations in several components of the Wnt pathway cause a polycystic kidney and liver phenotype (e.g. beta-catenin, Apc, LGR4, LRP5, Wnt, Inversin) [20,64–71]. Also, several Wnt-pathway proteins such as beta-catenin and dishevelled (*DVL*) were found to be direct interaction partners of polycystin-1 [58,72]. Most importantly, Wnt molecules were recently identified as a ligand of polycystin-1 and binding of Wnt to polycystin-1 results in activation the polycystin-complex and calcium influx into the cell [58]. In addition, polycystin-1 itself is a target gene of the Wnt-pathway, through binding of TCF2 and beta-catenin at the *PKD1* promoter, thereby regulating its expression [73]. Moreover, polycystin-1 was found to be an inhibitor of canonical Wnt-signaling [74]. Finally, Wnt inhibitors can ameliorate cystogenesis in a PKD zebrafish and mouse model [75,76]. Hence, it is clear that polycystin signalling and the Wnt pathway are intertwined and it has been suggested that polycystin-1 is important for the balance between canonical and non-canonical Wnt signaling. Nonetheless, many other pathways are found to be altered in ADPKD, such as EGFR, VEGF, mTOR, PI3K-AKT, NF-κB, AMPK, Hippo, c-Myc, JAK-STAT, calcineurin/NFAT, amongst others [13,77–82]. Thus, further studies to unravel the PKD-pathway are needed to gain more insight in cystogenesis and for the development of novel targeted treatments for PKD.

The molecular mechanism of cystogenesis

Cystogenesis is a focal process. Several hypothetical models have been suggested to explain why only approximately 1% of the renal epithelium forms a cyst, while all epithelial cells contain the heterozygous germline mutation. Initially, a *two hit model* was suggested [83–86]. In this model, in additional to the germ line mutation, somatic mutation of the other allele of the same gene is required to initiate cystogenesis. This hypothesis was supported by DNA mutation analysis of cyst lining epithelial cells. This showed unique somatic mutations in *PKD1* in most cysts [84,86,87,85,88]. All types of mutations were found, varying from small inactivating mutations to large deletions that resulted in loss of heterozygosity. The second hit hypothesis subsequently evolved to the *gene dosage model*. This occurred after the discovery that both a functional polycystin-1 level lower than 10% and overexpression of *Pkd1* could trigger cystogenesis, illustrating that *PKD1* acts dose-dependently [89–91]. An additional level of complexity was added to the second hit hypothesis in the form of the *transheterozygous model*. The transheterozygous model proposes that the second hit could reside within a gene other than the one affected in the germ line. Indeed, patients carrying a heterozygous *PKD1* germ line mutation, were found to have somatic mutations in *PKD2* in the cystic epithelial cells and *vice versa* [92,93]. Intriguingly, genome wide somatic mutations were also detected in cyst cells [88,94]. These mutations might have occurred in genes which

affected polycystin signaling, thereby triggering cystogenesis. Or, are merely bystander mutations as a consequence of local damage followed by clonal expansion.

The hypothesis that cystogenesis is triggered by a somatic genetic hit cannot fully explain the focal aspect of cystogenesis. Patients who are trans-heterozygous for an incompletely penetrant *PKD1* allele and a pathogenic *PKD1* allele have been described [95]. In addition, some patients with the recessive form of PKD (ARPKD) are compound heterozygous for *PKHD1* mutations. In these cases, both alleles are affected in all cells, hence 'two hits' are already present while cyst formation is still focal. Importantly, there is evidence that *PKD1* is haploinsufficient, as a study using *Pkd1*$^{+/-}$ mice showed that heterozygous *Pkd1* mutant kidneys are sensitive to renal injury and acquire increased renal damage and microcysts [96]. Finally, in acquired polycystic kidney disease, genetically normal kidneys also exhibit cysts in a focal fashion. Taken together, these findings suggest that focal signalling cues must play an important role in triggering cystogenesis.

Interestingly, a 'third hit' was found to be needed for triggering cystogenesis, in addition to a germline mutation (first hit), a somatic mutation (second hit). In adult mice, the inducible deletion of *Pkd1* – resulting in *Pkd1* null cells – only leads to the formation of a few cysts. This is in contrast to the situation where *Pkd1* is deleted during kidney development or renal injury repair, in which the deletion causes a rapidly developing severe cystic phenotype [96–99]. This implies that renal epithelial cells need to be in a proliferating state in order to form cysts. This topic will be addressed in more detail in the discussion section of this thesis.

Modeling ADPKD in human cell lines

Various animal models have been used to investigate cystic kidney diseases such as pigs, cats, zebrafish, *C. elegans*, *Drosophila* and even mussels [100–107]. Most of the studies however have been performed in murine models, which have contributed tremendously to our understanding of cystogenesis (for review see [108]). Nonetheless, human *in vitro* cell line models are a valuable addition to animal models, since fundamental inter-species differences may exist. Furthermore, pharmacological and genetic manipulation is faster, and effects of such interventions can be readily observed and measured. Establishment of a suitable cell line model for ADPKD can be particularly challenging because of several aspects that need to be taken into consideration. First, cyst formation has been implied to be preceded by a somatic mutation in the kidney. Thus, a cell line must preferably originate from the renal epithelial cells that have acquired this second hit. In addition, since cystogenesis is a three-dimensional event, a three

dimensional culture system may better recapitulate the pathophysiology than a flat, two dimensional culture system [109]. Induced pluripotent stem cells and adult stem cell organoids are two *in vitro* systems that could meet these two criteria.

Induced pluripotent cells

Induced pluripotent cells (iPSCs) were first described in 2006 by the group of Yamanaka [110]. They showed that inducing expression of four pluripotency transcription factors, OCT4, KLF4, SOX2 and cMYC, in somatic cells leads to reprogramming and dedifferentiation back to embryonic-like stem cells [110]. The discovery of iPSC technology allowed for establishing pluripotent cells from various human tissues, encompassing patient specific mutations and thereby providing an ideal *in vitro* system for human disease modeling and drug discovery [111]. iPSCs can be cultured indefinitely and recent advances in gene editing – with the discovery of CRISPR/Cas9 – has made genetic manipulation of iPSC feasible. Most importantly, protocols for *in vitro* differentiation of iPSCs into various cell types (including kidney cells) have been established, some of which are three-dimensional culture based [111]. In the last decade iPSC technology has been proven to be a powerful tool to model human diseases (for review see [111]).

Adult stem cell kidney organoids

The discovery of adult stem cell markers and the establishment of culture conditions in which these adult stem cells could be maintained indefinitely, has led to the development of adult stem cell (ASC) organoid culture technology [112,113]. Seeding of ASCs isolated from somatic tissue in a 3D matrix with defined tissue-specific growth medium, results in outgrowth of self-organizing structure called organoids. These organoids recapitulate the architecture and different cell types of the tissue to which they are fated. ASC organoids were originally discovered using intestinal tissue, but soon after were established using other tissue types as well, including the kidney [114]. ASC organoid cultures are genetically stable and can, in principle, be cultured indefinitely. Patient derived or genetically modified organoids provide a powerful system for disease modeling, drug testing and hold great promise to be used for treatment in the future [115–118].

Scope of this thesis

The fact that ADPDK affects a huge number of people worldwide and many of these patients develop renal failure, makes that this disease has a high clinical and economic burden. In general, ADPKD is a slowly progressing disease which can be diagnosed relatively early, providing a long time window in which cyst growth could potentially be slowed. Although some progress has been made in the identification of potential

therapies to delay the progression of ADPKD, currently there is no effective treatment available that prevents end stage renal disease. To improve treatment options for ADPKD patients, a better understanding of the pathogenesis of cystic disease is essential. Clarifying the molecular mechanism of cyst formation could lead to the identification of novel molecular targets which could subsequently be used for the development of specific targeted therapies. Even after the discovery of the causative gene(s) over two decades, the exact molecular mechanism of cystogenesis remains to be elucidated.

We approached this complex issue by focusing on three key questions:

1. What is the function of PKD1/polycystin-1 at the cellular level?
2. What triggers cyst formation?
3. How is *PKD1* expression regulated?

In **Chapter 2** and **Chapter 3** we discuss the development of two novel *in vitro* ADPKD cell line models, based on iPSCs and on ASC kidney organoids. Both provide a unique cell line platform, which can be used to study the function of *PKD1.* **Chapter 4** assesses the role of paracrine signaling molecules, present in cyst fluid, on inducing cyst formation in wild type renal epithelial cells, aiming to gain insight in how cystogenesis is triggered. Finally, in **Chapter 5** we aimed to gain more understanding of the upstream events in PKD-signaling, by focusing on *PKD1* promoter regulation. This chapter describes an unbiased screen for the identification of *PKD1* transcription factors. Finally, chapter 6 provides a general discussion of our findings and future perspectives.

REFERENCES

1. Grantham, J. J. Polycystic kidney disease: a predominance of giant nephrons. *Am. J. Physiol. Physiol.* **244,** F3–F10 (1983).
2. Denic, A., Glassock, R. J. & Rule, A. D. Structural and Functional Changes With the Aging Kidney. *Adv. Chronic Kidney Dis.* **23,** 19–28 (2016).
3. Esker, B. & Rogotti, Paulo (University of Padua, I. Autosomal Dominant Polycystic Kidney Disease; Images in Clinical Medicine. *N. Engl. J. Med.* 363;1 (2010).
4. Torres, V. E. & Watson, M. L. Nephrology Dialysis Transplantation Polycystic kidney disease : antiquity to the 20th century. 2690–2696 (1998).
5. Dobyan DC, Hill D, Lewis T, B. R. Cyst formation in rat kidney induced by cis-platinum administration. *Lab Invest* **45,** 260–8 (1981).
6. Le Corre, S. *et al.* Cystic gene dosage influences kidney lesions after nephron reduction. *Nephron* **129,** 42–51 (2015).
7. Judit Kovác, Mónika Zilahy, Tamás Bányász, S. G. Evaluation of apoptosis and cell proliferation in experimentally induced renal cysts. *Urol. Res.* **26,** 411–416 (1998).
8. Kurbegovic, A. & Trudel, M. Acute kidney injury induces hallmarks of polycystic kidney disease. *Am. J. Physiol. Renal Physiol.* ajprenal.00167.2016 (2016). doi:10.1152/ajprenal.00167.2016
9. Grantham, J. J. Acquired Cystic Kidney Disease. *jKidney Int.* **40,** 143–152 (1991).
10. Matson, A and Cohen, E. Acquired Cystic Kidney Disease: Occurrence, Prevalence, and Renal Cancers. Medicine (Baltimore).
11. Ishikawa, I. Uremic Acquired Cystic Disease of the Kidney. *Urology* **XXVI,** 101–108 (1985).
12. Lanktree, M. B. & Chapman, A. B. New treatment paradigms for ADPKD: Moving towards precision medicine. *Nat. Rev. Nephrol.* **13,** 750–768 (2017).
13. Carsten Bergmann, Lisa M. Guay- Woodford, Peter C. Harris, S. H. & Torres, D. J. M. P. and V. E. Polycystic kidney disease. *Nat. Rev. | Dis. Prim.* (2018). doi:10.1007/978-3-319-19674-9_19
14. Scott Reule, Donal J. Sexton, Craig A. Solid, Shu-Cheng Chen, Allan J. Collins, and R. N. F. End-Stage Renal Disease From Autosomal Dominant Polycystic Kidney Disease in the United States, 2001-2010. 592–599 (2014). doi:10.1097/MOP.0000000000000311.Drug
15. Spithoven, E. M. *et al.* Renal replacement therapy for autosomal dominant polycystic kidney disease (ADPKD) in Europe: Prevalence and survival - An analysis of data from the ERA-EDTA Registry. *Nephrol. Dial. Transplant.* **29,** iv15-iv25 (2014).
16. Chapman, A. B. *et al.* Autosomal Dominant Polycystic Kidney Disease (ADPKD): Executive Summary from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. **88,** 17–27 (2016).
17. Ong, A. C. M., Devuyst, O., Knebelmann, B. & Walz, G. Autosomal dominant polycystic kidney disease: The changing face of clinical management. *Lancet* **385,** 1993–2002 (2015).
18. Aksentijevich, I. *et al.* The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. The European Polycystic Kidney Disease Consortium. *Cell* **77,** 881–94 (1994).
19. Mochizuki, T. *et al.* PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* **272,** 1339–1342 (1996).
20. Porath, B. *et al.* Mutations in GANAB, Encoding the Glucosidase IIα Subunit, Cause Autosomal-Dominant Polycystic Kidney and Liver Disease. *Am. J. Hum. Genet.* **98,** 1193–1207 (2016).
21. Simms, R. J. Autosomal dominant polycystic kidney disease. **679,** 1–10 (2016).

22. Audrézet, M. P. *et al.* Autosomal dominant polycystic kidney disease: Comprehensive mutation analysis of PKD1 and PKD2 in 700 unrelated patients. *Hum. Mutat.* **33,** 1239–1250 (2012).

23. Jere Paavola, Simon Schliffke, Sandro Rossetti, Ivana Y.-T. Kuo, Shiaulou Yuan, Zhaoxia Sun, Peter C. Harris, Vicente E. Torres, and B. E. E. Polycystin-2 mutations lead to impaired calcium cycling in the heart and predispose to dilated cardiomyopathy. *J Mol Cell Cardiol* 199–208 (2013). doi:10.1016/j.jamcollsurg.2011.02.017.Cost-Effective

24. Pei, Y. *et al.* Unified Criteria for Ultrasonographic Diagnosis of ADPKD. *J. Am. Soc. Nephrol.* **20,** 205–212 (2009).

25. Rule, D. et al. Characteristics of Renal Cystic and Solid Lesions Based on Contrast-Enhanced Computed Tomography of Potential Kidney Donors. *Am J Kidney Dis* **59,** 611–618 (2012).

26. York Pei, Young-Hwan Hwang, John Conklin, Jamie L. Sundsbak, C. M. H., Winnie Chan, Kairong Wang, Ning He, Anand Rattansingh, Mostafa Atri, P. C. & Harris, and M. A. H. Imaging-Based Diagnosis of Autosomal Dominant Polycystic Kidney Disease. *J. Am. Soc. Nephrol.* **26,** 746–753 (2014).

27. Trujillano, D. *et al.* Diagnosis of autosomal dominant polycystic kidney disease using efficient PKD1 and PKD2 targeted next-generation sequencing. *Mol. Genet. Genomic Med.* 412–421 (2014). doi:10.1002/mgg3.82

28. Mallawaarachchi, A. C. *et al.* Whole-genome sequencing overcomes pseudogene homology to diagnose autosomal dominant polycystic kidney disease. *Eur. J. Hum. Genet.* **24,** 1584–1590 (2016).

29. Sandro Rossetti, Lana Strmecki, Vicki Gamble, Sarah Burton, Vicky Sneddon, B. P., Sushmita Roy, Aysin Bakkaloglu, Radovan Komel, C. G. W. & and Peter C. Harris. Mutation Analysis of the Entire PKD1 Gene: Genetic and Diagnostic Implications. *Am. J. Hum. Genet.* **68,** 46–63 (2001).

30. Autosomal Dominant Polycystic Kidney Disease Mutation Database: PKDB. *http://pkdb.mayo.edu/* at <http://pkdb.mayo.edu/>

31. Chapman, A. B. *et al.* Autosomal-dominant polycystic kidney disease (ADPKD): executive summary from a Kidney Disease : Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int.* **88,** 17–27 (2015).

32. Harris, T. *et al.* European ADPKD Forum multidisciplinary position statement on autosomal dominant polycystic kidney disease care. *Nephrol. Dial. Transplant.* **33,** 563–573 (2017).

33. Torres, V. *et al.* Tolvaptan in Patients with Autosomal Dominant Polycystic Kidney Disease. *N Engl J Med.* **367,** 2407–2418 (2012).

34. Torres, V. E. *et al.* Tolvaptan in Later-Stage Autosomal Dominant Polycystic Kidney Disease. *N. Engl. J. Med.* NEJMoa1710030 (2017). doi:10.1056/NEJMoa1710030

35. Caroli, A. *et al.* Effect of longacting somatostatin analogue on kidney and cyst growth in autosomal dominant polycystic kidney disease (ALADIN): a randomised, placebo-controlled, multicentre trial. *Lancet* **382,** 1485–1495 (2013).

36. Meijer, E. *et al.* Effect of lanreotide on kidney function in patients with autosomal dominant polycystic kidney disease the DIPAK 1 randomized clinical trial. *JAMA - J. Am. Med. Assoc.* **320,** 2010–2019 (2018).

37. Swanson, A. J., Banerjee, S., Campbell, P. C., England, S. J. & Lewis, K. E. Identification and Expression Analysis of the Complete Family of Zebrafish pkd Genes. *Front. Cell Dev. Biol.* **5,** (2017).

38. Sandford, R. *et al.* Comparative analysis of the polycystic kidney disease 1 (PKD1) gene reveals an integral membrane glycoprotein with multiple evolutionary conserved domains. *Hum. Mol. Genet.* **6,** 1483–1489 (1997).

39. Edrees, B. M. *et al.* Functional alterations due to amino acid changes and evolutionary comparative analysis of ARPKD and ADPKD genes. *Genomics Data* **10,** 127–134 (2016).

40. Barr, M. M. & Sternberg, P. W. A polycystic kidney-disease gene homologue required for male mating behaviour in C. elegans. *Nature* **401,** 386–389 (1999).

41. Hughes, J. *et al.* The polycystic kidney disease 1 (PKD1) gene encodes a novel protein with multiple cell recognition domains. *Nat. Genet.* **10,** 151–160 (1995).

42. Geng, L. *et al.* Distribution and developmentally regulated expression of murine polycystin. *Am. J. Physiol. - Ren. Physiol.* **272,** (1997).

43. Chauvet, V. *et al.* Expression of PKD1 and PKD2 transcripts and proteins in human embryo and during normal kidney development. *Am. J. Pathol.* **160,** 973–983 (2002).

44. Le Corre, S. *et al.* Cystic gene dosage influences kidney lesions after nephron reduction. *Nephron* **129,** 42–51 (2015).

45. Puri, S. *et al.* Ets factors regulate the polycystic kidney disease-1 promoter. *Biochem. Biophys. Res. Commun.* **342,** 1005–1013 (2006).

46. Islam, M. R. *et al.* MAP/ERK Kinase Kinase 1 (MEKK1) mediates transcriptional repression by interacting with Polycystic Kidney Disease-1 (PKD1) promoter-bound p53 tumor suppressor protein. *J. Biol. Chem.* **285,** 38818–38831 (2010).

47. Zhang, B., Tran, U. & Wessely, O. Polycystin 1 loss of function is directly linked to an imbalance in G-protein signaling in the kidney. *Development* **145,** dev158931 (2018).

48. Porath, B. *et al.* Mutations in GANAB, Encoding the Glucosidase IIα Subunit, Cause Autosomal-Dominant Polycystic Kidney and Liver Disease. *Am. J. Hum. Genet.* **98,** 1193–1207 (2016).

49. Yu, S. *et al.* Essential role of cleavage of Polycystin-1 at G protein-coupled receptor proteolytic site for kidney tubular structure. *Proc. Natl. Acad. Sci. U. S. A.* **104,** 18688–93 (2007).

50. Kurbegovic, A. *et al.* Novel functional complexity of polycystin-1 by GPS cleavage in vivo: role in polycystic kidney disease. *Mol. Cell. Biol.* **34,** 3341–3353 (2014).

51. Ong, A. C. M. & Harris, P. C. Molecular pathogenesis of ADPKD: The polycystin complex gets complex. *Kidney Int.* **67,** 1234–1247 (2005).

52. Padovano, V., Podrini, C., Boletta, A. & Caplan, M. J. Metabolism and mitochondria in polycystic kidney disease research and therapy. *Nat. Rev. Nephrol.* **14,** 678–687 (2018).

53. Chauvet, V. *et al.* Mechanical stimuli induce cleavage and nuclear translocation of the polycystin-1 C terminus. *J. Clin. Invest.* **114,** 1433–1443 (2004).

54. Seng Hui Low, Shivakumar Vasanth, Claire H. Larson, Sambuddho Mukherjee, Nikunj Sharma, Michael T. Kinter, Michelle E. Kane, Tomoko Obara, and T. W. Polycystin-1, STAT6, and P100 Function in a Pathway that Transduces Ciliary Mechanosensation and Is Activated in Polycystic Kidney Disease. *Dev. Cell* 57–69 (2006). doi:10.1016/j.devcel.2005.12.005

55. Qian, F. *et al.* PKD1 interacts with PKD2 through a probable coiled-coil domain. *Nat. Genet.* **16,** 179–183 (1997).

56. DeCaen, P. G. *et al.* Polycystin-2 is an essential ion channel subunit in the primary cilium of the renal collecting duct epithelium. *Elife* **7,** 1–30 (2018).

57. Su, Q. *et al.* Structure of the human PKD1-PKD2 complex. *Science (80-.).* **361,** (2018).

58. Kim, S. *et al.* The polycystin complex mediates Wnt/Ca2+ signalling. *Nat. Cell Biol.* **1,** (2016).

59. Ong, M.-Y. C. and A. C. M. Targeting new cellular disease pathways in autosomal dominant polycystic kidney disease. *Nephrol. Dial. Transplant.* (2017). doi:10.1093/ndt/gfx071

60. Hildebrandt, F., Benzing, T. & Katsanis, N. Ciliopathies STRUCTURE AND FUNCTION OF THE CILIUM-CENTROSOME COMPLEX. *N Engl J Med.* **364,** 1533–1543 (2011).

61. Mukhopadhyay, S., Christensen, S. T., Mykytyn, K., Anvarian, Z. & Pedersen, L. B. Cellular signalling by primary cilia in development, organ function and disease. *Nat. Rev. Nephrol.* (2019). doi:10.1038/s41581-019-0116-9

62. Avasthi, P., Maser, R. L. & Tran, P. V. *Primary Cilia in Cystic Kidney Disease. In: Miller R. (eds) Kidney Development and Disease. Results and Problems in Cell Differentiation, vol 60. Springer, Cham*. **60,** (2017).

63. Calvet, J. P. *Polycystic Kidney Disease. Chapter 8, The Role of Calcium and Cyclic AMP in PKD.* (Codon Publications, 2015). doi:10.15586/codon.pkd.2015

64. Saadi-Kheddouci, S. *et al.* Early development of polycystic kidney disease in transgenic mice expressing an activated mutant of the β-catenin gene. *Oncogene* **20,** 5972–5981 (2001).

65. Qian, C.-N. *et al.* Cystic Renal Neoplasia Following Conditional Inactivation of Apc in Mouse Renal Tubular Epithelium. *J. Biol. Chem.* **280,** 3938–3945 (2004).

66. Dang, Y. *et al.* Gpr48 deficiency induces polycystic kidney lesions and renal fibrosis in mice by activating Wnt signal pathway. *PLoS One* **9,** (2014).

67. Venselaar, H. *et al.* Whole-exome sequencing reveals LRP5 mutations and canonical Wnt signaling associated with hepatic cystogenesis. *Proc. Natl. Acad. Sci.* **111,** 5343–5348 (2014).

68. Karner, C. M. *et al.* Wnt9b signaling regulates planar cell polarity and kidney tubule morphogenesis. *Nat. Genet.* **41,** 793–799 (2009).

69. Xiao, A. *et al.* A Possible Zebrafish Model of Polycystic Kidney Disease: Knockdown of wnt5a Causes Cysts in Zebrafish Kidneys. *J. Vis. Exp.* **2,** 2–7 (2014).

70. Nagy, I. I. *et al.* Impairment of Wnt11 function leads to kidney tubular abnormalities and secondary glomerular cystogenesis. *BMC Dev. Biol.* **16,** 1–14 (2016).

71. Phillips, C. L. et al. Renal Cysts of inv/inv Mice Resemble Early Infantile Nephronophthisis. *J. Am. Soc. Nephrol.* **15,** 1744–1755 (2004).

72. Huan, Y. & Van Adelsberg, J. Polycystin-1, the PKD1 gene product, is in a complex containing E- cadherin and the catenins. *J. Clin. Invest.* **104,** 1459–1468 (1999).

73. Rodova, M., Islam, M. R., Maser, R. L. & Calvet, J. P. The polycystic kidney disease-1 promoter is a target of the beta-catenin/T-cell factor pathway. *J. Biol. Chem.* **277,** 29577–29583 (2002).

74. Lal, M. *et al.* Polycystin-1 C-terminal tail associates with β-catenin and inhibits canonical Wnt signaling. *Hum. Mol. Genet.* **17,** 3105–3117 (2008).

75. Schueler, M. *et al.* DCDC2 mutations cause a renal-hepatic ciliopathy by disrupting Wnt signaling. *Am. J. Hum. Genet.* **96,** 81–92 (2015).

76. Xu, Y. *et al.* Canonical Wnt inhibitors ameliorate cystogenesis in a mouse ortholog of human ADPKD. *JCI Insight* **3,** 1–15 (2018).

77. Harskamp, L. R., Gansevoort, R. T., Van Goor, H. & Meijer, E. The epidermal growth factor receptor pathway in chronic kidney diseases. *Nat. Rev. Nephrol.* **12,** 496–506 (2016).

78. Weimbs, T., Olsan, E. E. & Talbot, J. J. Regulation of STATs by polycystin-1 and their role in polycystic kidney disease. *Jak-Stat* **2,** e23650 (2013).

79. Chapin, H. C. & Caplan, M. J. The cell biology of polycystic kidney disease. **191,** 701–710 (2010).

80. Happé, H. *et al.* Altered Hippo signalling in polycystic kidney disease. *J. Pathol.* **224,** 133–142 (2011).

81. Ong, A. C. M. & Harris, P. C. A polycystin-centric view of cyst formation and disease : the polycystins revisited. *Kidney Int.* **88,** 699–710 (2015).

82. Kim, H. J. & Edelstein, C. L. Mammalian target of rapamycin inhibition in polycystic kidney disease: From bench to bedside. *Kidney Res. Clin. Pract.* **31,** 132–138 (2012).

83. Qian, F., Watnick, T. J., Onuchic, L. F. & Germino, G. G. The molecular basis of focal cyst formation in human autosomal dominant polycystic kidney disease type I. *Cell* **87,** 979–987 (1996).

84. Brasier, J. L. & Henske, E. P. Loss of the polycystic kidney disease (PKD1) region of chromosome 16p13 in renal cyst cells supports a loss-of-function model for cyst pathogenesis. *J. Clin. Invest.* **99,** 194–199 (1997).

85. Koptides, M. Germinal and somatic mutations in the PKD2 gene of renal cysts in autosomal dominant polycystic kidney disease. *Hum. Mol. Genet.* **8,** 509–513 (1999).

86. Badenas, C. *et al.* Loss of heterozygosity in renal and hepatic epithelial cystic cells from ADPKD1 patients. *Eur. J. Hum. Genet.* **8,** 487–92 (2000).

87. Pei, Y. *et al.* Somatic PKD2 mutations in individual kidney and liver cysts support a 'two-hit' model of cystogenesis in type 2 autosomal dominant polycystic kidney disease. *J. Am. Soc. Nephrol.* **10,** 1524–1529 (1999).

88. Tan, A. Y. *et al.* Somatic Mutations in Renal Cyst Epithelium in Autosomal Dominant Polycystic Kidney Disease. *J. Am. Soc. Nephrol.* ASN.2017080878 (2018). doi:10.1681/ASN.2017080878

89. Lantinga-van Leeuwen, I. S. *et al.* Lowering of Pkd1 expression is sufficient to cause polycystic kidney disease. *Hum. Mol. Genet.* **13,** 3069–3077 (2004).

90. Thivierge, C. *et al.* Overexpression of PKD1 Causes Polycystic Kidney Disease Overexpression of PKD1 Causes Polycystic Kidney Disease. **26,** 1538–1548 (2006).

91. Kurbegovic, A. *et al.* Pkd1 transgenic mice: Adult model of polycystic kidney disease with extrarenal and renal phenotypes. *Hum. Mol. Genet.* **19,** 1174–1189 (2010).

92. Koptides, M., Mean, R., Demetriou, K., Pierides, A. & Deltas, C. C. Genetic evidence for a trans-heterozygous model for cystogenesis in autosomal dominant polycystic kidney disease. *Hum. Mol. Genet.* **9,** 447–452 (2000).

93. Watnick, T. *et al.* Mutations of PKD1 in ADPKD2 cysts suggest a pathogenic effect of trans-heterozygous mutations. *Nat. Genet.* **25,** 143–4 (2000).

94. Gogusev, J. Molecular Cytogenetic Aberrations in Autosomal Dominant Polycystic Kidney Disease Tissue. *J. Am. Soc. Nephrol.* **14,** 359–366 (2003).

95. Vujic, M. *et al.* Incompletely Penetrant PKD1 Alleles Mimic the Renal Manifestations of ARPKD. *J. Am. Soc. Nephrol.* **21,** 1097–1102 (2010).

96. Bastos, A. P. *et al.* Pkd1 haploinsufficiency increases renal damage and induces microcyst formation following ischemia/reperfusion. *J. Am. Soc. Nephrol.* **20,** 2389–2402 (2009).

97. Happé, H. *et al.* Toxic tubular injury in kidneys from Pkd1-deletion mice accelerates cystogenesis accompanied by dysregulated planar cell polarity and canonical Wnt signaling pathways. *Hum. Mol. Genet.* **18,** 2532–2542 (2009).

98. Lantinga-van leeuwen, I. S. *et al.* Kidney-specific inactivation of the Pkd1 gene induces rapid cyst formation in developing kidneys and a slow onset of disease in adult mice. *Hum. Mol. Genet.* **16,** 3188–3196 (2007).

99. Piontek, K., Menezes, L. F., Garcia-Gonzalez, M. a, Huso, D. L. & Germino, G. G. A critical developmental switch defines the kinetics of kidney cyst formation after loss of Pkd1. *Nat Med* **13,** 1490–1495 (2007).

100. He, J. *et al.* Construction of a transgenic pig model overexpressing polycystic kidney disease 2 (PKD2) gene. *Transgenic Res.* **22,** 861–867 (2013).

101. Koch, J., Ersbøll, A. K., Pedersen, H. D., Pedersen, K. M. & Haggstrom, J. Increased Mean Arterial Pressure and Aldosterone-to-Renin Ratio in Persian Cats with Polycystic Kidney Disease. *J. Vet. Intern. Med.* **17,** 21–27 (2009).

102. Obara, T. *et al.* Polycystin-2 Immunolocalization and Function in Zebrafish. *J Am Soc Nephrol* 2706–2718 (2006). doi:10.1158/0008-5472.CAN-10-4002.BONE

103. Müller, R. U., Zank, S., Fabretti, F. & Benzing, T. Caenorhabditis elegans, a model organism for kidney research: From cilia to mechanosensation and longevity. *Curr. Opin. Nephrol. Hypertens.* **20,** 400–408 (2011).

104. Ward, C. J. & Sharma, M. Polycystic Kidney Disease: Lessons Learned from Caenorhabditis elegans Mating Behavior. *Curr. Biol.* **25,** R1168–R1170 (2015).

105. Köttgen, M. *et al.* Drosophila sperm swim backwards in the female reproductive tract and are activated via TRPP2 ion channels. *PLoS One* **6,** 1–8 (2011).

106. Sunila, I. Cystic kidneys in copper exposed mussels. *Dis. Aquat. Organ.* **6,** 63–66 (2007).

107. O'Hagan, R., Wang, J. & Barr, M. M. Mating behavior, male sensory cilia, and polycystins in caenorhabditis elegans. *Semin. Cell Dev. Biol.* **33,** 25–33 (2014).

108. Happé, H. & Peters, D. J. M. Translational research in ADPKD: lessons from animal models. *Nat. Rev. Nephrol.* **10,** 587–601 (2014).

109. Dixon, E. E. & Woodward, O. M. Three-dimensional in vitro models answer the right questions in ADPKD cystogenesis. *Am. J. Physiol. Physiol.* **315,** F332–F335 (2018).

110. Takahashi, K. & Yamanaka, S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell* **126,** 663–676 (2006).

111. Shi, Y., Inoue, H., Wu, J. C. & Yamanaka, S. Induced pluripotent stem cell technology: a decade of progress. *Nat. Rev. Drug Discov.* **16,** 115–130 (2016).

112. Barker, N. *et al.* Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* **449,** 1003–1008 (2007).

113. Sato, T. *et al.* Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* **459,** 262–5 (2009).

114. Schutgens F, Rookmaaker MB, Margaritis T, Rios A, Ammerlaan C, Jansen J, Gijzen L, Vormann M, Vonk A, Viveen M, Yengej FY, Derakhshan S, de Winter-de Groot KM, Artegiani B, van Boxtel R, Cuppen E, Hendrickx APA, van den Heuvel-Eibrink MM, Heitzer E, Lanz, C. H. Tubuloids derived from human adult kidney and urine for personalized disease modeling. *Nat. Biotechnol.* 303–313 (2019).

115. Rookmaaker, M. B., Schutgens, F., Verhaar, M. C. & Clevers, H. Development and application of human adult stem or progenitor cell organoids. *Nat. Rev. Nephrol.* 1–9 (2015). doi:10.1038/nrneph.2015.118

116. Clevers, H. Modeling Development and Disease with Organoids. *Cell* **165,** 1586–1597 (2016).

117. Dutta, D., Heo, I. & Clevers, H. Disease Modeling in Stem Cell-Derived 3D Organoid Systems. *Trends Mol. Med.* 1–18 (2017). doi:10.1016/j.molmed.2017.02.007

118. Sato, T. & Clevers, H. SnapShot: Growing Organoids from Stem Cells. *Cell* **161,** 1700–1700.e1 (2015).

Chapter 4

Identifying Cystogenic Paracrine Signaling Molecules in Cyst Fluid of Patients with Polycystic Kidney Disease

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ABSTRACT

In autosomal dominant polycystic kidney disease (ADPKD) paracrine signaling molecules in cyst fluid can induce proliferation and cystogenesis of neighboring renal epithelial cells. However, the identity of this "cyst inducing factor" is still unknown. The aim of this study was to identify paracrine signaling proteins in cyst fluid using an 3D *in vitro* cystogenesis assay. We collected cyst fluid from 15 ADPKD patients who underwent kidney or liver resection (55 cysts from 13 nephrectomies, 5 cysts from 2 liver resections). For each sample, the ability to induce proliferation and cyst formation was tested using the cystogenesis assay (RPTEC/TERT1 cells in Matrigel with cyst fluid added for 14 days). Kidney cyst fluid induced proliferation and cyst growth of renal epithelial cells in a dose-dependent fashion. Liver cyst fluid also induced cystogenesis. Using size exclusion chromatography, 56 cyst fluid fractions were obtained of which only the fractions between 30-100 kDa showed cystogenic potential. Mass spectrometry analysis of samples that tested positive or negative in the assay identified 43 candidate cystogenic proteins. GO analysis showed an enrichment for proteins classified as enzymes, immunity proteins, receptors and signaling proteins. A number of these proteins have previously been implicated in ADPKD, including Secreted Frizzeld-Related Protein 4, S100A8, osteopontin, and CRELD1. In conclusion, both kidney and liver cyst fluids contain paracrine signaling molecules that drive cyst formation. Using size exclusion chromatography and mass spectrometry, we procured a candidate list for future studies. Ultimately, cystogenic paracrine signaling molecules may be targeted to abrogate cystogenesis in ADPKD.

INTRODUCTION

Polycystic Kidney Disease (PKD) is a heterogeneous group of diseases in which patients progressively develop fluid filled cysts in both kidneys, leading to destruction of kidney tissue. The most common form of PKD is Autosomal Dominant Polycystic Kidney Disease (ADPKD). ADPKD is caused by a heterozygous germ line mutation in *PKD1, PKD2* or *GANAB* [1, 24, 32]. Additional somatic mutations in the kidney affecting the remaining healthy allele have been proposed to be the initiating event for cyst formation, and this concept is referred to as the 'two-hit' model [4, 34, 41–43].

A 'third hit' was also proposed to be essential for the formation of cysts, namely *proliferation* [38, 39]. Deletion of *Pkd1* in mouse kidney shortly after birth results in rapid and massive cyst formation, whereas deletion of the gene in adult mice leads to a slower and less profound onset of cyst formation. This developmental switch in which the kidney is susceptible for loss of *Pkd1,* occurs around postnatal day 13 in mice and corresponds with a global decrease in cell proliferation in the kidney [19, 28, 31]. Moreover, in adult conditional *Pkd1* knock-out mice, cyst formation is significantly accelerated when proliferation is induced through kidney injury [12, 28, 39]. Thus, proliferation signals, which are present throughout development and during injury response, appear to be essential for cystogenesis [12, 19, 28,39].

There is also evidence that cysts can induce proliferation resulting in the formation of novel cysts. In adult conditional *Pkd1* knock out mice, the proliferation index in the kidney is high during the injury phase, normalizes when the injury is repaired, and increases again shortly after cysts are formed [12]. A second observation in conditional *Pkd1* knock out mice was that cysts are not randomly distributed throughout the kidney, but instead are localized in clusters. This suggests that the initial cyst triggers formation of novel cysts in the vicinity, a phenomenon that has been coined the "cystic snowball effect" [20]. This cystic 71 snowball effect could be explained by paracrine signaling from the cystic epithelial cells themselves or from infiltrating immune cells. Here, we hypothesize that one or more cystogenic molecules are present in cyst fluid. Indeed, previous studies showed that cyst fluid has a proliferative effect when added to wild type renal epithelial cells. Moreover, when cyst fluid was added to renal epithelial cells in 3D cultures, this resulted in cystogenesis [45, 46].

At present the identity of the paracrine signaling molecules that drive proliferation and cyst formation is unknown. Cyst fluid is a complex biofluid that consists of a variety of proteins and other biologically active substances. Analysis of cyst fluid revealed that plasma proteins like albumin, immunoglobulins, complement, and apolipoproteins are abundantly present. In addition, cyst fluid contains many growth factors, cytokines and hormones, like VEGF, EGF, TGF-α, vasopressin, IL-6, IL-8, and MCP-1 [17, 18, 21, 25]. Interestingly, previous studies showed that a small lipophilic molecule is present in cyst fluid, which induces transepithelial fluid secretion and to some extent proliferation of kidney epithelial cells *in vitro* [2, 11, 33]. However, because this small lipid did not account for the complete proliferative effect of cyst fluid, it was concluded that other cystogens must be present in the cyst fluid [45, 46]. Therefore, in the present study, our aim is to identify proteins in cyst fluid that induce cystogenesis of renal epithelial cells using a 3D cystogenesis assay, size fractionation and mass spectrometry.

METHODS

Cyst fluid sample collection

Collection of human cyst fluid was approved by the Medical Ethics Committee of the Erasmus Medical Center (MEC2013-188). Kidney and liver explants were obtained from patients diagnosed with ADPKD who were scheduled for elective nephrectomy or hepatectomy. The ADPKD diagnosis was based on established diagnostic criteria (30), including positive family history and ultrasound criteria. Cyst fluid was isolated from cysts of various sizes by needle aspiration. Aspirates with a turbid aspect due to bleeding or infection were discarded. Samples were immediately placed on ice, passed through a 0.22 µm filter and stored at -80˚C until further processing. Total protein concentration was quantified per cyst fluid sample using a BCA Protein Assay Kit (No. 23225, Thermo Fisher Scientific, Waltham, USA) according to the manufacturer's instructions.

Isolation of extracellular vesicles

Isolation of extracellular vesicles (EVs) was performed using ultracentrifugation as described previously (37). Briefly, a volume of 10 ml from a mix of cyst fluids was centrifuged (17,000 g for 15 min at 4˚C) to remove cell debris and high density particles. Supernatant was transferred to polycarbonate tubes and EVs were isolated using ultracentrifugation (200,000 g for 2 hours at 4˚C). Supernatant (non EV fraction) was removed and the pellet (EV fraction) was dissolved in 3 ml PBS (~1/3 of the original volume), and both fractions were stored at -80˚C.

Serum protein depletion

Blue Sepharose beads (Affi-Gel Blue Gel, 1537301, BioRad, Hercules, USA) were used for serum protein depletion of cyst fluid. First, 10 ml bead-solution was prewashed 3 times with distilled water and 3 times with Dulbecco's Phosphate Buffered Saline (D8537, Sigma-Aldrich, St. Louis, USA). Subsequently, 1 ml cyst fluid was added 114 to the beads, incubated at room temperature for 5 minutes and transferred onto a glass gravity flow column, after which flow through was collected. Subsequently, beads were washed 3 times with 10 ml PBS and 4 times with 10 ml elution buffer (20 mM phosphate buffer, pH 7.4 + 2 M NaCl). The eluate was buffer exchanged to double the original volume by centrifugation (4,000 g, 40 min, room temperature) using a 15 kDa MWCO UltraFilter (Amicon, Merck, Darmstadt, Germany), and reconstituted in PBS. Protein content of each fraction and original untreated sample was visualized on 4–20% Mini-PROTEAN® TGX™ Precast Protein Gels (4561096, BioRad, Hercules, USA) with a Coomassie G-250 stain SimplyBlue™ SafeStain (LC6060, Thermo Fisher Scientific, Waltham, USA)

Cell culture

RPTEC/hTERT1 cells were obtained from the American Type Culture Collection (ATCC), and cultured in DMEM:Ham'sF12 (1:1) media (31330, Gibco, Paisley, UK), supplemented with 100 U/ml penicillin streptomycin, 100x Insulin-Transferrin-Selenium (ITS, 41400045, Thermo Fisher Scientific, Waltham, USA), 5 pM triiodo-L-thyronine (T2877, Sigma-Aldrich, St. Louis, USA), 3.5 µg/ml ascorbic acid (Sigma), 10 ng/ml Recombinant human EGF AF-100-15 (Peprotech, London, UK), 25 ng/ml Prostaglandin E1 (CS0210, Sigma-Aldrich, St. Louis, USA), 25 ng/ml hydrocortisone (H4001, Sigma-Aldrich, St. Louis, USA), and 100 µg/ml G418 (11811023, Thermo Fisher Scientific, Waltham, USA). Medium was refreshed 2-3 times a week and cells were passaged by trypsinization when near confluence.

Three-dimensional in vitro cystogenesis assay

For the cystogenesis assay RPTEC/hTERT1 cells were trypsinized with Trypsin/EDTA and dissociated to a single-cell suspension. For each well 10,000 cells were pelleted by centrifugation. Supernatant was discarded, the pellet was placed on ice and resuspended in 25 µl cold Matrigel (356231, Corning, New York, USA). A drop of 25 µl Matrigel/cell-suspension was plated in each well of a pre-heated 48 wells culture plate (677180, Greiner Bio-one). Plates were incubated for 15 minutes at 37 °C to ensure solidification of the Matrigel. RPTEC/hTERT1-medium was added to the wells, with or without 10% (fractionated) cyst fluid. Medium was refreshed every other day and cyst formation was analyzed at day 14 using a IX-70S8F2 Olympus microscope.

Size exclusion chromatography

For size fractionation, a total of 4 kidney cyst fluid samples were used from the same individual (patient K5) to avoid interpatient variation. Of these 4 kidney cyst fluid samples, 2 tested positive (KCF5a+ and KCF5b+) and 2 tested negative (KCF5c- and KCF5d-) in the cystogenesis assay. Cyst fluid was cleared from cell debris using centrifugation (4,000 g for 5 min at 4˚C). Next, 5 ml cyst fluid was loaded onto a superdex 200 column 16/600 (28989335, GE Healthcare) pre-equilibrated with PBS using an AKTA FPLC (GE Healthcare) to separate components by size. This yielded 56 fractions per sample, ranging from large to small sized proteins. Column effluent was separated and collected into fractions of 2 ml and stored at -80˚C. All fractions were individually tested in the cystogenesis assay. Markerrun was done with gelfiltration standards from Biorad (151-1901) in PBS.

Proteomic analysis

Proteins were trypsinized and the resulting tryptic peptides were subjected to offline orthogonal high pH and reverse phase fractionation. Peptides were solubilized in 0.1 % TFA and loaded onto a 20 mg PLRP-S cartridge made in-house. The cartridge was washed once with 1 ml 0.1 % TFA and three times with 1 ml milliQ water. Peptides were eluted step-wise from the column with 5%, 10%, 15% and 50% acetonitrile / 10 mM ammonium formate (pH 10). The 6 fractions were dried by vacuum centrifugation and each fraction was reconstituted with 2 % ACN / 0.2 % FA for nanoLC-MS/MS analysis. Mass spectra were acquired on an Orbitrap Lumos Tribrid mass spectrometer (Thermo Fisher Scientific) coupled to an EASY-nLC 1200 system (Thermo Fisher Scientific). 162 Peptides were separated on an in-house packed 75 µm inner diameter column containing 50 cm CSH130 resin (3.5 µm, 130 Å, Waters) with a gradient of 2–20% (ACN, 0.1% FA) over 150 min at 300 nL/min. The column was kept at 50 °C in an NanoLC oven - MPI design (MS Wil GmbH). For all experiments, the instrument was operated in the data-dependent acquisition mode. MS1 spectra were collected at a resolution of 120,000, with an automated gain control target of 2E5 and a max injection time of 50 ms. The most intense ions were selected for MS/MS, top speed method 3 seconds cycle time. Precursors were filtered according to charge state (2–169 7z), and monoisotopic peak assignment. Previously interrogated precursors were dynamically excluded for 70 s. Peptide precursors were isolated with a quadrupole mass filter set to a width of 0.7 Th. Raw data files were analyzed using MaxQuant software (version 1.5.6.0). MS/MS spectra were searched against the Uniprot database (taxonomy: human, version 2017_01). The specificity was set to trypsin, and a maximum of 2 missed cleavages was allowed. Cysteine carbamidomethylation was set as a fixed modification whereas methionine oxidation and N-terminal protein acetylation were set as variable modifications. A false discovery rates for peptides and proteins were both set to 0.01. Known contaminants and reverse hits were removed from the modification specific peptide list.

RESULTS

Kidney and liver cyst fluid induce cystogenesis of human renal epithelial cells in vitro

Cyst fluid samples from 13 ADPKD kidneys and 2 ADPKD liver explants were collected (**Table 1**). Each individual sample was tested for its capacity to induce cyst formation in 3D kidney epithelial *in vitro* cultures and used for further characterization and identification of the cystogen (**Figure 1A**). We found that human renal epithelial cells (RPTEC/hTERT1) exposed to medium supplemented with kidney cyst fluid gradually formed spheres with a lumen in the center, in contrast to PBS or untreated cells (**Figure 1B**). Addition of liver cyst fluid samples resulted in growth of the same cyst-like structures, although the effect was less pronounced (**Figure 1C**). A serial dilution of cyst fluid supplemented to the medium, demonstrated that the paracrine molecules act in a dose dependent fashion (**Figure 1D**). We also observed a clear difference in cystogenic potential between cyst fluid samples derived from different cysts, even if obtained from the same ADPKD-kidney explant, ranging from ‘no’, ‘low’, ‘medium‘ to ‘high’ cystogenic effect (**Table 1** and **Figure 1E**). These effects could not be explained by differences in protein concentration (median concentrations respectively 32282, 36801, and 33911 µg/µl, $P > 0.05$).

Explant	Gender	Age	Indication for nephrectomy/ hepatectomy	eGFR (ml/min/ 1.73m²)	Weight explant (grams)	Size explant (centimeters)	Total cysts	No	Low	Medium	High
KCF1	F	69	Space kidney transplant	17	2370	30 x 12 x 9	8	-	3	3	2
KCF2	M	66	Infection	33	2477	28 x 13 x 12	6	-	3	3	-
KCF3	F	55	Space kidney transplant	6	1382	21 x 13 x 11	2	-	-	2	-
KCF4	M	44	Haemorrhagic cyst	13	3878	26 x 16 x 10	3	-	2	1	-
KCF5	M	63	Infection	76	2660 (L) 2360 (R)	30 x 17 x 12 (L) 28x 15 x 10 (R)	9	3	4	-	2
KCF6	F	46	Space kidney transplant	12	1622 (L) 1605 (R)	27 x 13 x 6 (L) 24 x12 x 10 (R)	1	-	1	-	-
KCF7	F	56	Infection and space kidney transplant	4	3348	30 x 16 x 8	4	-	2	2	-
KCF8	M	67	Space kidney transplant	7	1316	23 x 11 x 8	5	-	3	1	1
KCF9	M	55	Haemorrhagic cyst	24	4160	32 x 19 x 13	2	-	1	1	-
KCF10	M	47	Infection	19	1081	24 x 12 x 5	1	-	-	1	-
KCF11	M	60	Infection	51	2123	29 x 16 x 9	4	-	2	2	-
KCF12	M	67	Space kidney transplant	7	922	28 x 14 x 12	5	-	4	1	-
KCF13	F	73	Space kidney transplant	6	1900	24 x 17 x 8	5	-	1	4	-
LCF14	F	48	Liver transplant	N/A	4485	Not available	2	-	1	1	-
LCF15	F	58	Liver transplant	N/A	4437	Not available	3	-	2	1	-

Table 1. Patient characteristics

Abbreviations: eGFR, estimated glomerular filtration rate; KCF, kidney cyst fluid; LCF, liver cyst fluid; L, left; R, right; Total cysts, total cysts analyzed.

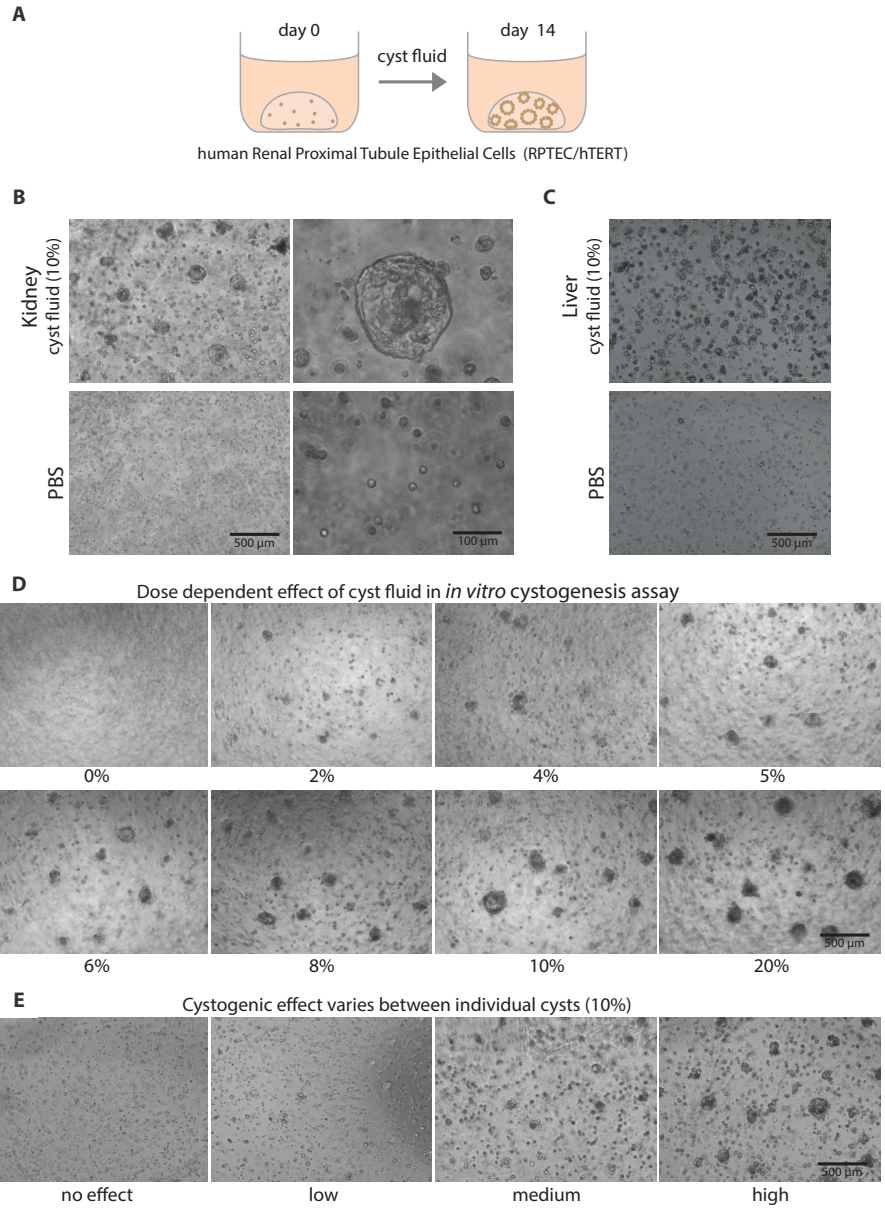
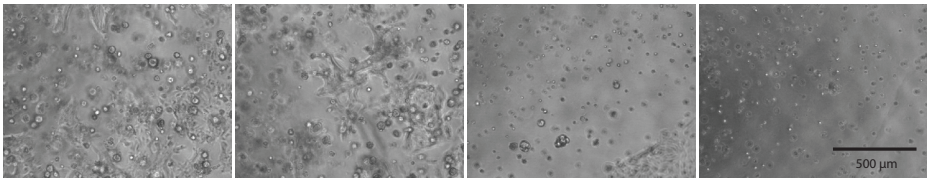


Figure 1. In vitro cystogenesis assay (**A**) Schematic representation of cystogenesis assay. (**B**) Brightfield pictures at day 14, RPTEC/hTERT cultured in Matrigel drops, upper panels: medium is supplemented with 10% kidney cyst fluid, in lower panels medium and PBS only, Right panels: close-up. (**C**) Medium supplemented with 10% liver cyst fluid. (**D**) Serial dilution of cyst fluid supplemented to the medium (final concentration 0 - 20%) shows a dose dependent cystogenic effect of the cyst fluid. (**E**) Fluids derived from different cysts of the same patient have different cystogenic capacity patient (scored at day 14).

Extracellular vesicles are not enriched for paracrine signal molecules

Extracellular vesicles (EVs) have recently emerged as mediators of cell to cell communication.(40) To test if paracrine signaling molecules are present in EVs, EVs were isolated from the cyst fluid and tested for their cystogenic capacity in our 3D *in vitro* cystogenesis assay. Using nanoparticle tracking analysis, we confirmed the presence of vesicles. While the supernatant fraction did induce cystogenesis, we observed little effect in the EV fraction (**Figure 2**). This suggests that the paracrine signaling molecules are not present in cyst fluid EVs, but more likely is solubilized in the cyst fluid itself.



whole cyst fluid | supernatant (non-EV fraction) | pellet 200.000g (EV fraction) | PBS

Figure 2. Extracellular vesicles (EVs) isolated from cyst fluid using ultracentrifugation were tested in the in vitro cystogenesis assay Proliferation and cyst-like structures were observed in the samples exposed to non-ultracentrifuged cyst fluid and the supernatant of the ultracentrifuged fluid, whereas only a modest effect is seen upon exposure to the EVs containing pellet fraction. PBS was used as negative control.

The paracrine signaling 203 molecules are bound by sepharose beads

The high abundancy of albumin and other serum-derived proteins in cyst fluid can reduce detection sensitivity of proteomic analysis. Therefore, we aimed to deplete albumin and other serum proteins from the cyst fluid using blue sepharose beads, which non-selectively bind albumin. Captured proteins were eluted from the beads using a high salt buffer and subsequently reconstituted in PBS (**Figure 3A**). In our cystogenesis assay, we tested the bound fraction (eluate), the unbound fraction (flow-through), the original sample (untreated) and a negative control (PBS). Cystogenesis was observed in the samples treated with the bound fraction but absent in the unbound fraction, indicating that the cystogenic factors are bound directly or indirectly by blue sepharose beads (**Figure 3B**).

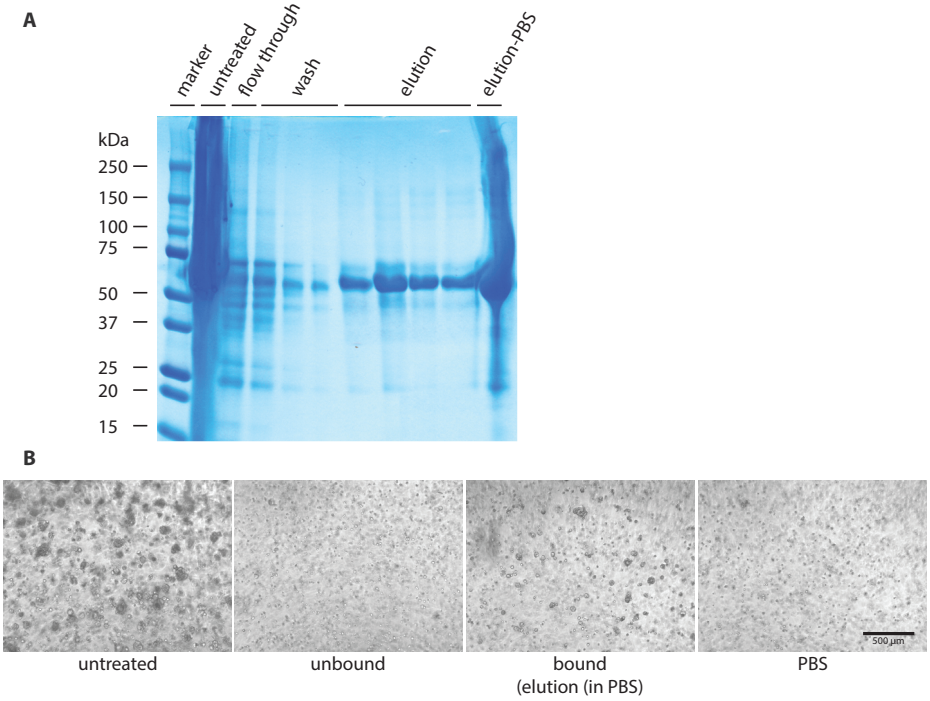


Figure 3. Serum protein depletion of cyst fluid with blue sepharose beads (A) Coomassie stained polyacrylamide gel; original sample (untreated), unbound fraction (flow through), wash and elution fractions, and the eluate reconstituted in PBS (bound fraction). **(B)** Original cyst fluid samples and fractions after serum protein depletion tested in cystogenesis assay.

Fractionation of cyst fluid identifies 30-100 kDA cystogenic fractions

In order to identify the paracrine cystogens present in cyst fluid, we separated cyst fluid using size exclusion chromatography (SEC). To limit interpatient variability, we used four cyst fluid samples from patient 5 (KCF5 in **Table 1**), including two with "no" effect in the cystogenesis assay, and two with a "high" effect. With SEC, 56 fractions were obtained per sample, which were individually tested in the cystogenesis assay (**Figure 4A-B**). Interestingly, for the samples that tested positive in our cystogenesis assay (KCF5a+ and KCF5b+) we found that fractions #23-28, corresponding to a size of ~30-100 kDa, retained the cystogenic effect, whereas this effect was absent when adding the same fractions obtained from samples which were tested negative (KCF5c- and KCF5d-) to our assay (**Figure 4B**).

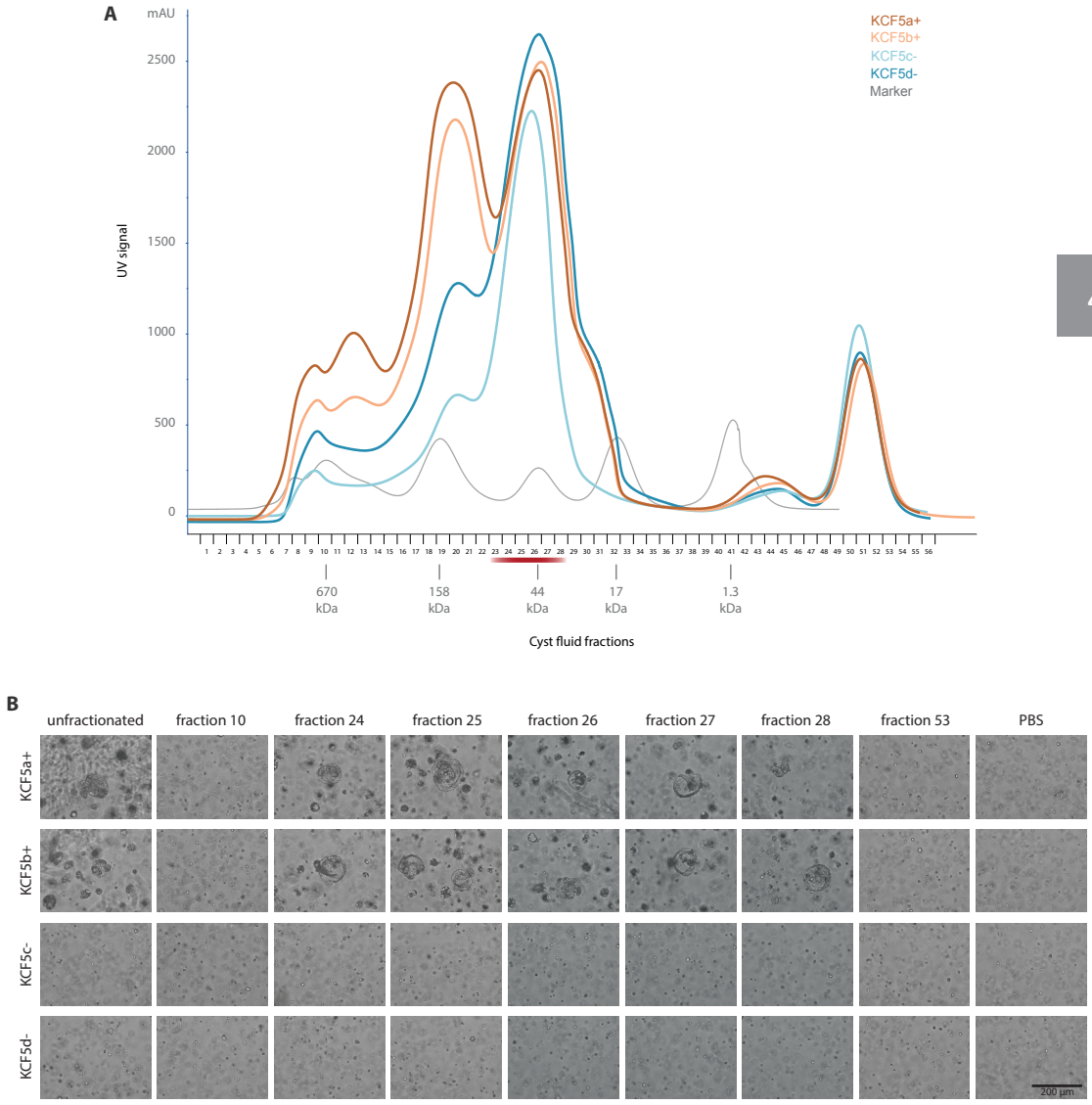


Figure 4. Size Exclusion Chromatography (SEC) (**A**) Size Exclusion Chromatography (SEC) of 4 kidney cyst fluid samples, The x-axis shows the fractions numbers collected with an isocratic elution. The y-axis represents UV signal intensity at 280 nm. Markerrun was done with gelfiltration standards, sizes indicated below the x-axe. (**B**) Cystogenesis assay testing 2 "cystogenic" (KCF5a+ and KCF5b+), 2 "non-cystogenic" (KCF5c- and KCF5d-) kidney cyst fluid samples that were fractionated by size exclusion chromatography. Representative pictures of fractions #10, 24, 25, 26, 27, 28, 53 are shown for each of the 4 cyst fluid samples.

Mass spectrometry identifies candidate paracrine signaling proteins

We analyzed fraction #25 by mass spectrometry, because this fraction showed the strongest cystogenic effect (**Figure 4**). Mass spectrometry analysis of the four #25 fractions (KCF5a+, KCF5b+, KCF5c- and KCF5d-) revealed a total of 2822 unique proteins, many of which have been previously described to be present in cyst fluid (**Supplementary Material**). Three hundred and thirty proteins were present in both the 'kidney positive' samples and 628 proteins in both the 'kidney negative' samples (**Figure 5A**). A comparison between the 'kidney positive' and 'kidney negative' group revealed 43 candidate cystogens (**Figure 5B** and **Table 2**). The candidate list was enriched for proteins predicted to be secreted extracellularly (**Figure 5C**). Moreover, GO analysis showed that the candidate list consists of different types of proteins, including enzymes, immunity proteins, receptors, and signaling proteins (**Figure 5C**). We also performed GO analysis on 1441 proteins without cystogenic potential (data not shown). Compared with the "positive" proteins, the cellular distribution of the "negative" proteins was more often "cell junction" and less often "extracellular region". The protein classes were relatively comparable with the exception that many "negative" proteins were classified as "nucleic acid binding proteins".

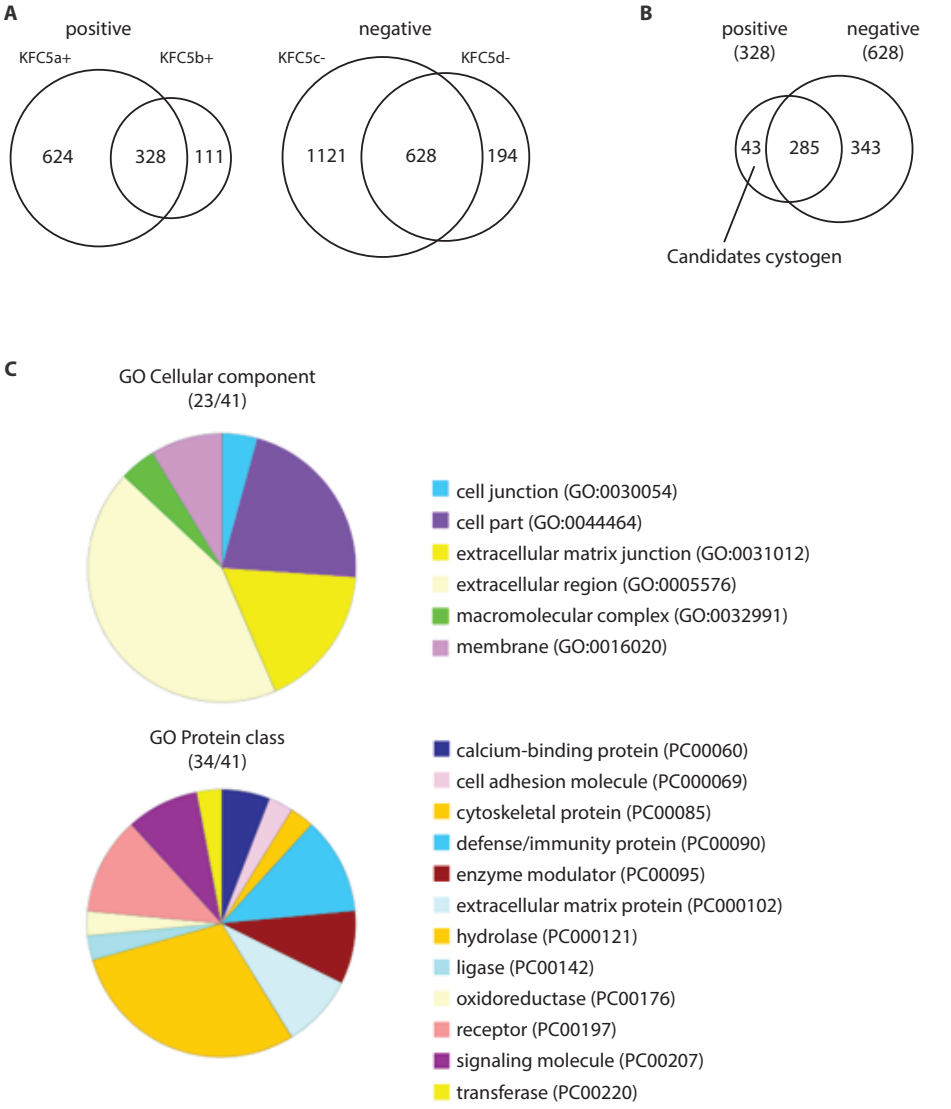


Figure 5. Proteomic analysis of cyst fluid fraction #25 (**A**) Venn diagram showing protein content overlap between the two cystogenic samples (KCF5a+ and KCF5b+) and the two non-cystogenic samples (KCF5c- and KCF5d-). (**B**) Comparative analysis identified 43 proteins that were present in both cystogenic samples (KCF5a/b+) but not in the non-cystogenic samples. (**C**) Panther GO analysis recognized 41/43 proteins, pie chart showing cellular components (23 hits out of 41 proteins) and protein classes (34 hits out of 41 proteins).

DISCUSSION

Here, we established an *in vitro* cystogenesis assay enabling us to study the cystogenic effect of cyst fluid on normal renal epithelial cells and subsequent identification of candidate cystogens. We found that the cystogens are present in kidney- and liver-derived cyst fluid. We showed that individual cysts from the same kidney or liver exerted a different cystogenic response possibly due to differences in concentration of cystogens. In addition, we found that the cystogen is not enriched in extracellular vesicles (EVs), that it is enriched in a blue sepharose binding fraction and in ~30-100 kDa Size Exclusion Chromatography fractions. Using proteomic analysis we procured a list of 43 candidate cystogen proteins.

What drives cystogenesis is currently unknown, but there is evidence that paracrine signaling initiates novel cyst formation of neighboring epithelial cells (26). It is of interest that the paracrine signaling molecules also exert their cystogenic effect on wild-type epithelial cells. We demonstrate that addition of cyst fluid to wild type epithelial cells *in vitro* results in cytogenesis, as also shown in previous studies.(45, 46) Moreover, *in vivo* wild-type cells can also form cysts, as in *Pkd1*$^{-/-}$:*Pkd1*$^{+/+}$ chimeric mice some cysts were largely formed by *Pkd1*$^{+/+}$ cells (26). Strikingly, kidney injury caused by nephrotoxic drugs, ischemia or partial nephrectomy, leads to tubular dilatation and cyst formation in mice that have otherwise normal kidneys (7, 8, 14, 16). Finally, patients with chronic kidney disease without a PKD mutation frequently develop acquired cystic kidney disease (10, 13, 23). It is possible that the paracrine molecules play a role in renal cyst formation, not only in ADPKD patients but also in cystic development in other conditions such as acquired cystic kidney disease. Previous studies indicated that the cystogen is conserved between species, because cyst fluid isolated from PKD-mice and from humans induced cystogenesis when added to Madin-Darby canine kidney cells (45, 46). Moreover, as observed previously (46), our results indicate that the concentration of the cystogen varies between individual cysts from the same kidney or liver. The reason for this is unclear, b 268 ut we speculate that this could be caused by differences between the tubular origin of the cyst, or local variation of the immune response, or the presence of pathogens.

Our results reveal four novel characteristics of the cystogen. *First*, the cystogen is not a kidney-specific molecule, since liver cyst fluid also tested positive in the assay. This suggests that the same signaling events may contribute to cyst formation in the kidney as well as in the liver. *Second*, the molecules are not excreted into the cyst fluid as part of EVs, since EVs isolated from the cyst fluid showed less cystogenic

potential than whole cyst fluid. *Third*, we observed that the cystogen is bound directly or indirectly by blue sepharose beads, which are known to bind albumin, interferon, lipoproteins, coagulation factors, and several enzymes. *Finally*, size fractionation of cyst fluid demonstrated that the cystogenic molecule or molecules are present in the fractions corresponding to a molecular weight of ~30-100 kDa. Notably, previous studies identified a small lipid (< 3.5 kDa) that has a secretory and proliferative effect on epithelial cells *in vitro.*(2, 11, 33) In this study, we did not detect cystogenic effects of any of the other fractions outside the ~30-100 kDa region. However, this does not exclude the possibility that the cystogen is a small protein, present mainly as a multimer, or in complex with larger proteins, for example bound by albumin.

Using proteomic analysis we identified 43 candidate cystogens. Interestingly, four of these candidates are proteins that are known to play a role in cystogenesis. *First,* Secreted Frizzled-Related Protein 4 (sFRP4) is a 55 kDa protein expressed in a variety of tissues including kidney and liver (29). It is a highly glycosylated soluble protein that functions as a Wnt-signaling modulator through direct interaction with Wnts (29, 47). Wnt-signaling plays an important role in kidney development, kidney injury response and is implicated to be involved in the pathogenesis of ADPKD (3, 9, 15, 44). sFRP4 expression was found to be increased in mouse kidney cyst models and human ADPKD 292 kidneys (38). Interestingly, ectopic expression of sFRP4 in zebrafish promoted cystogenesis in the pronephros (35). In addition, elevated sFRP4 serum levels in ADPKD patients correlate with rapid disease progression (47). *Second,* S100A8 is a calcium-binding protein that forms a heterodimeric complex with S100A9, called calprotectin, and is a ligand of RAGE (receptor for advanced glycation end products). S100A8 is involved in the innate immune response and induces proliferation and cell survival through NF-κβ and ERK signaling (27). Moreover, calprotectin and its receptor RAGE, were found to be overexpressed in cystic regions in PKD2 overexpressing mice and ADPKD human kidney (27). *Third,* osteopontin (SPP1) is a secreted cytokine and part of the integrin-binding ligand family. It is an important modulator of the immune response and also plays a key role in cancer progression (6). Normally, its expression in the kidney is transiently upregulated after kidney injury. However, in a rat model of polycystic kidney disease, osteopontin expression levels were persistently elevated (8). *Fourth,* Cysteine Rich with EGF Like Domains 1 (CRELD1) is a member of the family of epidermal growth factor-related proteins. These proteins are conserved between species and widely expressed during development and adulthood, including in the kidney and liver (36). CRELD1 can induce intracellular calcium release, NFATC1 dephosphorylation and nuclear translocation resulting in expression of NFATC1 target genes (22). Calcium-calcineurin-NFAT signaling has been implicated in ADPKD (5).

Our study has a number of limitations. First, it is not certain that the candidate cystogen is a protein. However, metabolites are usually very small (< 1.5 kDa) and we did not see cystogenic potential of these molecular weight fractions, although we cannot exclude complex formation of the paracrine factor with larger proteins. Second, many signaling proteins are biologically active but present in low concentrations and can therefore be missed with mass spectrometry. In addition, our results lead to several new questions that remain to be answered by future studies. 315 Is there more than one cystogen? Which cells produce the cystogen? What causes the variation in cystogen concentration between cysts?

In conclusion, we aimed to identify the paracrine signal molecules that drive cyst formation. We identified 43 candidate proteins present in cyst fluid which can be used for further testing of their cystogenic capacity *in vitro* and *in vivo.* By identifying paracrine signaling molecules that drive cystogenesis we expect to gain more insight in the molecular mechanism of cyst formation. Moreover, inhibition of these cystogens might slow down disease progression in patients with polycystic kidney disease.

REFERENCES

1. **Consortium, T. E. P. K. D**. The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. The European Polycystic Kidney Disease Consortium. *Cell* 77: 881–94, 1994.
2. **Belibi FA**, **Reif G**, **Wallace DP**, **Yamaguchi T**, **Olsen L**, **Li H**, **Helmkamp GM**, **Grantham JJ**. Cyclic AMP promotes growth and secretion in human polycystic kidney epithelial cells. *Kidney Int* 66: 964–973, 2004.
3. **Benzing T**, **Simons M**, **Walz G**. Wnt Signaling in Polycystic Kidney Disease. *J Am Soc Nephrol* 18: 1389–1398, 2007.
4. **Brasier JL**, **Henske EP**. Loss of the polycystic kidney disease (PKD1) region of chromosome 16p13 in renal cyst cells supports a loss-of-function model for cyst pathogenesis. *J Clin Invest* 99: 194–199, 1997.
5. **Calvet JP**. Polycystic Kidney Disease. Chapter 8, The Role of Calcium and Cyclic AMP in PKD. Codon Publications, 2015.
6. **Castello LM**, **Raineri D**, **Salmi L**, **Clemente N**, **Vaschetto R**, **Quaglia M**, **Garzaro M**, **Gentilli S**, **Navalesi P**, **Cantaluppi V**, **Dianzani U**, **Aspesi A**, **Chiocchetti A**. Osteopontin at the Crossroads of Inflammation and Tumor Progression. *Mediators Inflamm* 2017, 2017.
7. **Le Corre S**, **Viau A**, **Burtin M**, **El-Karoui K**, **Cnops Y**, **Terryn S**, **Debaix H**, **B??rissi S**, **Gubler MC**, **Devuyst O**, **Terzi F**. Cystic gene dosage influences kidney lesions after nephron reduction. *Nephron* 129: 42–51, 2015.
8. **Dobyan DC, Hill D, Lewis T BR**. Cyst formation in rat kidney induced by cis-platinum administration. *Lab Invest* 45: 260–8, 1981.
9. **Gewin LS**. Renal Tubule Repair : Is Wnt / β -Catenin a Friend or Foe ? *Genes, 2018*.
10. **Grantham JJ**. Acquired Cystic Kidney Disease [Online]. *jKidney Int* 40: 143–152, 1991.
11. **Grantham JJ**, **Ye M**, **Davidow C**, **Sharma M**. Evidence for a Potent Lipid Secretagogue Fluids of Patients With Autosomal Dominant Polycystic Kidney Disease. *J Am Soc Nephrol* 6, 1995.
12. **Happé H**, **Leonhard WN**, **van der Wal A**, **van de Water B**, **Lantinga-van Leeuwen IS**, **Breuning MH**, **de Heer E**, **Peters DJM**. Toxic tubular injury in kidneys from Pkd1-deletion mice accelerates cystogenesis accompanied by dysregulated planar cell polarity and canonical Wnt signaling pathways. *Hum Mol Genet* 18: 2532–2542, 2009.
13. **Ishikawa I**. Uremic Acquired Cystic Disease of the Kidney. *Urology* XXVI: 101–108, 1985.
14. **Judit Kovác, Mónika Zilahy, Tamás Bányász SG**. Evaluation of apoptosis and cell proliferation in experimentally induced renal cysts. *Urological Research* 26: 411–416, 1998.
15. **Kim S**, **Nie H**, **Nesin V**, **Tran U**, **Outeda P**, **Bai C-X**, **Keeling J**, **Maskey D**, **Watnick T**, **Wessely O**, **Tsiokas L**. The polycystin complex mediates Wnt/Ca2+ signalling. *Nat Cell Biol* 1, 2016.
16. **Kurbegovic A**, **Trudel M**. Acute kidney injury induces hallmarks of polycystic kidney disease. *Am J Physiol Renal Physiol* :00167.2016, 2016.
17. **Lai X**, **Bacallao RL**, **Blazer-yost BL**, **Hong D**, **Stephen B**, **Witzmann FA**. Characterization of the renal cyst fluid proteome in autosomal dominant polycystic kidney disease (ADPKD) patients. *Proteomics Clin Appl* .2: 1140–1152, 2008.
18. **Lai X**, **Blazer-Yost BL**, **Gattone VH**, **Muchatuta MN**, **Witzmann F a.** Protein composition of liver cyst fluid from the BALB/c-cpk/+ mouse model of autosomal recessive polycystic kidney disease. *Proteomics* 9: 3775–3782, 2009.
19. **Lantinga-van leeuwen IS**, **Leonhard WN**, **van der Wal A**, **Breuning MH**, **de Heer E**, **Peters DJM**. Kidney-specific inactivation of the Pkd1 gene induces rapid cyst formation in developing kidneys and a slow onset of disease in adult mice. *Hum Mol Genet* 16: 3188–3196, 2007.

20. **Leonhard WN**, **Zandbergen M**, **Veraar K**, **van den Berg S**, **van der Weerd L**, **Breuning M**, **de Heer E**, **Peters DJ**. Scattered Deletion of PKD1 in Kidneys Causes a Cystic Snowball Effect and Recapitulates Polycystic Kidney Disease. *J Am Soc Nephrol* 26: 1322–1333, 2015.
21. **Mason SB**, **Lai X**, **Bacallao RL**, **Blazer-yost BL**, **Vincent H**, **Ii G**, **Wang KC**, **Witzmann F a**. The biomarker enriched proteome of autosomal dominant polycystic kidney disease. *Proteomics Clin Appli* 3: 1247–1250, 2009.
22. **Mass E**, **Wachten D**, **Aschenbrenner AC**, **Voelzmann A**, **Hoch M**. Murine Creld1 controls cardiac development through activation of calcineurin/NFATc1 signaling. *Dev Cell* 28: 711–726, 2014.
23. **Matson, A and Cohen E**. Acquired Cystic Kidney Disease: Occurrence, Prevalence, and Renal Cancers. *Medicine (Baltimore)*. Jul;69(4):217-26, 1990.
24. **Mochizuki T**, **Wu G**, **Hayashi T**, **Xenophontos SL**, **Veldhuisen B**, **Saris JJ**, **Reynolds DM**, **Cai Y**, **Gabow P a**, **Pierides a**, **Kimberling WJ**, **Breuning MH**, **Deltas CC**, **Peters DJ**, **Somlo S**. PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* 272: 1339–1342, 1996.
25. **Nichols MT**, **Gidey E**, **Matzakos T**, **Dahl R**, **Stiegmann G**, **Shah RJ**, **Grantham JJ**, **Fitz JG**, **Doctor RB**. Secretion of cytokines and growth factors into autosomal dominant polycystic kidney disease liver cyst fluid. *Hepatology* 40: 836–846, 2004.
26. **Nishio S**, **Hatano M**, **Nagata M**, **Horie S**, **Koike T**, **Tokuhisa T**, **Mochizuki T**. Pkd1 regulates immortalized proliferation of renal tubular epithelial cells through p53 induction and JNK activation. *J Clin Invest* 115: 910–918, 2005.
27. **Park EY**, **Seo MJ**, **Park JH**. Effects of Specific Genes Activating RAGE on Polycystic Kidney Disease. *Am J Nephrol* 32: 169–178, 2010.
28. **Patel V**, **Li L**, **Cobo-stark P**, **Shao X**, **Somlo S**, **Lin F**, **Igarashi P**. Acute kidney injury and aberrant planar cell polarity induce cyst formation in mice lacking renal cilia. *Hum Mol Genet* 17: 1578–1590, 2008.
29. **Pawar NM**, **Rao P**. Secreted frizzled related protein 4 (sFRP4) update : A brief review. *Cell Signal* 45: 63–70, 2018.
30. **Pei Y**, **Obaji J**, **Dupuis A**, **Paterson AD**, **Magistroni R**, **Dicks E**, **Parfrey P**, **Cramer B**, **Coto E**, **Torra R**, **San Millan JL**, **Gibson R**, **Breuning M**, **Peters D**, **Ravine D**. Unified Criteria for Ultrasonographic Diagnosis of ADPKD. *J Am Soc Nephrol* 20: 205–212, 2009.
31. **Piontek K**, **Menezes LF**, **Garcia-Gonzalez M a**, **Huso DL**, **Germino GG**. A critical developmental switch defines the kinetics of kidney cyst formation after loss of Pkd1. *Nat Med* 13: 1490–1495, 2007.
32. **Porath B**, **Gainullin VG**, **Cornec-Le Gall E**, **Dillinger EK**, **Heyer CM**, **Hopp K**, **Edwards ME**, **Madsen CD**, **Mauritz SR**, **Banks CJ**, **Baheti S**, **Reddy B**, **Herrero JI**, **Bañales JM**, **Hogan MC**, **Tasic V**, **Watnick TJ**, **Chapman AB**, **Vigneau C**, **Lavainne F**, **Audrézet M-P**, **Ferec C**, **Le Meur Y**, **Torres VE**, **Harris PC**. Mutations in GANAB, Encoding the Glucosidase IIα Subunit, Cause Autosomal-Dominant Polycystic Kidney and Liver Disease. *Am J Hum Genet* 98: 1193–1207, 2016.
33. **Putnam WC**, **Swenson SM**, **Reif G a**, **Wallace DP**, **Helmkamp GM**, **Grantham JJ**. Identification of a Forskolin-Like Molecule in Human Renal Cysts. *J Am Soc Nephrol* 18: 934–943, 2007.
34. **Qian F**, **Watnick TJ**, **Onuchic LF**, **Germino GG**. The molecular basis of focal cyst formation in human autosomal dominant polycystic kidney disease type I. *Cell* 87: 979–987, 1996.
35. **Romaker D**, **Puetz M**, **Teschner S**, **Donauer J**, **Geyer M**, **Gerke P**, **Rumberger B**, **Dworniczak B**, **Pennekamp P**, **Buchholz B**, **Neumann HPH**, **Kumar R**, **Gloy J**, **Eckardt K-U**, **Walz G**. Increased Expression of Secreted Frizzled-Related Protein 4 in Polycystic Kidneys. *J Am Soc Nephrol* 20: 48–56, 2009.
36. **Rupp PA**, **Fouad GT**, **Egelston CA**, **Reifsteck CA**, **Olson SB**, **Knosp WM**, **Glanville RW**, **Thornburg KL**, **Robinson SW**, **Maslen CL**. Identification, genomic organization and mRNA expression of CRELD1, the founding member of a unique family of matricellular proteins. *Gene* 293: 47–57, 2002.

37. **Salih M**, **Demmers J a**, **Bezstarosti K**, **Leonhard WN**, **Losekoot M**, **van Kooten C**, **Gansevoort RT**, **Peters DJM**, **Zietse R**, **Hoorn EJ**, **DIPAK Consortium**. Proteomics of Urinary Vesicles Links Plakins and Complement to Polycystic Kidney Disease. *J. Am. Soc. Nephrol.* (2016). doi: 10.1681/ASN.2015090994.

38. **Takakura A**, **Contrino L**, **Beck AW**, **Zhou J**. Pkd1 inactivation induced in adulthood produces focal cystic disease. *J Am Soc Nephrol* 19: 2351–63, 2008.

39. **Takakura A**, **Contrino L**, **Zhou X**, **Bonventre J V.**, **Sun Y**, **Humphreys BD**, **Zhou J**. Renal injury is a third hit promoting rapid development of adult polycystic kidney disease. *Hum Mol Genet* 18: 2523–2531, 2009.

40. **Tkach M**, **Théry C**. Communication by Extracellular Vesicles: Where We Are and Where We Need to Go. *Cell* 164: 1226–1232, 2016.

41. **Torra R**, **Badenas C**, **San Millán JL**, **Pérez-Oller L**, **Estivill X**, **Darnell A**. A loss-of-function model for cystogenesis in human autosomal dominant polycystic kidney disease type 2. *Am J Hum Genet* 65: 345–352, 1999.

42. **Watnick TJ**, **Torres VE**, **Gandolph M a**, **Qian F**, **Onuchic LF**, **Klinger KW**, **Landes G**, **Germino GG**. Somatic mutation in individual liver cysts supports a two-hit model of cystogenesis in autosomal dominant polycystic kidney disease. *Mol Cell* 2: 247–251, 1998.

43. **Wu G**, **D'Agati V**, **Cai Y**, **Markowitz G**, **Park JH**, **Reynolds DM**, **Maeda Y**, **Le TC**, **Hou H**, **Kucherlapati R**, **Edelmann W**, **Somlo S**. Somatic inactivation of Pkd2 results in polycystic kidney disease. *Cell* 93: 177–188, 1998.

44. **Wuebken A**, **Schmidt-ott KM**. WNT/B-catenin signaling in polycystic kidney disease. *Kidney Int* 80: 135–138, 2011.

45. **Yamaguchi T**, **Nagao S**, **Takahashi H**, **Ye M**, **Grantham JJ**. Cyst fluid from a murine model of polycystic kidney disease stimulates fluid secretion, cyclic adenosine monophosphate accumulation, and cell proliferation by Madin-Darby canine kidney cells in vitro. *AmJKidney Dis* 25: 471–477, 1995.

46. **Ye M**, **Grant M**, **Sharma M**, **Elzinga L**, **Swan S**, **Torres VE**, **Phillips A**, **Kornhaus J**, **Yamaguchi T**, **Grantham JJ**. Cyst Fluid From Human Autosomal Dominant Polysystic Kidneys Promotes Cyst Formation and Expansion by Renal Epithelial Cells in Vitro. *J. Am. Soc. Nephrol.* 3: 984–994, 1992.

47. **Zschiedrich S**, **Budde K**, **Nürnberger J**, **Wanner C**, **Sommerer C**, **Kunzendorf U**, **Banas B**, **Hoerl WH**, **Obermüller N**, **Arns W**, **Pavenstädt H**, **Gaedeke J**, **Lindner TH**, **Faerber L**, **Wimmer P**, **Stork R**, **Eckardt K-U**, **Walz G**. Secreted frizzled-related protein 4 predicts progression of autosomal dominant polycystic kidney disease. *Nephrol Dial Transplant* , 2015.

Protein name	Gene name(s)	LFQ KCF5a+	LFQ KCF5b+	Log2(LFQ) KCF5a+	Log2(LFQ) KCF5b+
Immunoglobulin heavy variable 6-1	IGHV6-1	3,453,200,000	1,379,100,000	31.7	30.4
Protein S100-A8	S100A8	4,313,400,000	3,186,400,000	32.0	31.6
Prothymosin alpha	PTMA	267,070,000	33,858,000	28.0	25.0
Immunoglobulin variable lambda 8-61	IGLV8-61	1,011,400,000	833,630,000	29.9	29.6
Immunoglobulin heavy variable 2-70	IGHV2-70	563,440,000	103,800,000	29.1	26.6
Tyrosine-protein phosphatase non-receptor type substrate 1	SIRPA, SIRPB1	193,360,000	578,980,000	27.5	29.1
Osteopontin	SPP1	335,070,000	1,273,400,000	28.3	30.2
Immunoglobulin Kappa Variable 1-27	IGKV1-27	358,680,000	153,020,000	28.4	27.2
Immunoglobulin heavy variable V-III region BRO	IGHV-III BRO	96,968,000	97,488,000	26.5	26.5
Intercellular adhesion molecule 1	ICAM1	52,343,000	426,790,000	25.6	28.7
Immunoglobulin variable lambda 3-9	IGLV3-9	176,710,000	174,650,000	27.4	27.4
Carboxypeptidase B2	CPB2	975,260,000	466,650,000	29.9	28.8
Immunoglobulin J chain	IGJ, JCHAIN	205,610,000	265,700,000	27.6	28.0
Brain acid soluble protein 1	BASP1	901,140,000	141,050,000	29.7	27.1
Procollagen C-endopeptidase enhancer 1	PCOLCE	299,380,000	212,780,000	28.2	27.7
Keratin, type II cytoskeletal 7	KRT7	184,490,000	499,890,000	27.5	28.9
V-set and immunoglobulin domain-containing protein 4	VSIG4	492,860,000	174,920,000	28.9	27.4
Coagulation factor IX; Coagulation factor IXa light chain	F9	288,950,000	172,230,000	28.1	27.4
Aldo-keto reductase family 1 member C3	AKR1C3	1,463,200,000	166,490,000	30.4	27.3
Heat shock protein beta-1	HSPB1	202,980,000	75,436,000	27.6	26.2
Collagen alpha-2(I) chain	COL1A2	804,870,000	663,690,000	29.6	29.3
Secreted frizzled-related protein 4	SFRP4	72,975,000	82,214,000	26.1	26.3

Intercellular adhesion molecule 2	ICAM2	111,930,000	156,540,000	26.7	27.2
Vitamin K-dependent protein C	PROC	70,192,000	82,165,000	26.1	26.3
Coagulation factor X	F10	312,640,000	241,040,000	28.2	27.8
Complement C1r subcomponent	C1R	41,027,000	284,630,000	25.3	28.1
Keratin, type I cytoskeletal 19	KRT19	80,545,000	257,850,000	26.3	27.9
Keratin, type I cytoskeletal 17	KRT17	153,430,000	95,412,000	27.2	26.5
Sialic acid-binding Ig-like lectin 14 and 5	SIGLEC14 and 5	386,090,000	110,340,000	28.5	26.7
Hepatocyte growth factor-like protein	MST1	160,920,000	155,560,000	27.3	27.2
Poliovirus receptor	PVR	49,520,000	73,626,000	25.6	26.1
Carbamoyl-phosphate synthase [ammonia], mitochondrial	CPS1	1,211,500,000	95,050,000	30.2	26.5
Cholesteryl ester transfer protein	CETP	141,430,000	119,860,000	27.1	26.8
Osteoclast-associated immunoglobulin-like receptor	OSCAR	58,152,000	61,387,000	25.8	25.9
Carboxypeptidase N subunit 2	CPN2	43,414,000	32,552,000	25.4	25.0
Basement membrane-specific heparan sulfate proteoglycan core protein	HSPG2	33,212,000	625,720,000	25.0	29.2
Receptor-type tyrosine-protein phosphatase gamma	PTPRG	81,722,000	116,740,000	26.3	26.8
Cysteine-rich with EGF-like domain protein 1	CRELD1	49,800,000	86,371,000	25.6	26.4
ADAM DEC1	ADAMDEC1	106,950,000	38,356,000	26.7	25.2
Keratin, type I cytoskeletal 16	KRT16	75,643,000	54,882,000	26.2	25.7
Collagen alpha-2(V) chain	COL5A2	49,691,000	58,404,000	25.6	25.8
Apolipoprotein B-100;Apolipoprotein B-48	APOB	376,420,000	69,536,000	28.5	26.1
Plectin	PLEC	526,910,000	12,831,000	29.0	23.6

Table 2. Cystogen candidate list

Abbreviations: LFQ, label-free quantification.

Chapter 6

Summary, general discussion and future perspectives

SUMMARY

ADPDK is a disease with a high clinical burden and currently effective therapies that prevent end-stage renal failure are lacking. The aim of this thesis was to advance our knowledge on the molecular mechanisms of cystogenesis, to facilitate the development of new therapeutic strategies for ADPKD patients.

Chapter 2 and 3 describe the development of two novel human ADPKD cell line models – based on either induced pluripotent stem cells (iPSCs) or adult stem cell (ASC) kidney organoids – which can be used to study polycystin-1 function. In **chapter 2,** we established ADPKD patient-specific iPSCs. Tubular Epithelial Cell lines (TECs) were derived from nephrectomies of ADPKD patients and of healthy control kidney tissue, and expression of kidney and epithelial markers was confirmed. ADPKD mutant and wild type TECs were transduced to initiate iPSC formation. ADPKD iPSCs were characterized with respect to their karyotype, expression of stem cell markers and pluripotency potential. Somatic mutations in the tubular epithelium have been implied to be initiating events of cystogenesis. We are the first to report ADPKD iPSCs derived from cystic epithelium, providing a model in which these somatic mutations can be studied. Mutation analysis of the ADPDK-iPSCs identified heterozygous germ line mutations in *PKD1*, but no additional somatic hit in this gene. We did, however, find other genome wide somatic mutations and future studies will have to show whether these mutations contributed to cyst initiation. Furthermore, we showed that transcriptomes of ADPDK-iPSCs were largely similar to wild type iPSCs. However, we showed that kidney derived iPS cells maintain a kidney and disease specific DNA methylation memory. This epigenetic kidney memory might favor directed differentiation of these cells into wild type and diseased renal cells *in vitro*.

In **chapter 3**, adult kidney stem cells were used to establish an ADPKD model system. ASCs were isolated from ADPKD nephrectomies and healthy kidney tissue, and cultured in a 3D-matrigel culture platform. Similar to the ADPKD- iPSCs, ADPKD-ASC kidney organoids harbored a heterozygous *PKD1* germ line mutation and various genome wide somatic mutations. Interestingly, we found that ADPKD-ASC organoids exhibit a cellular senescent gene expression profile. In addition, we showed that increased numbers of senescent cells are present *in vivo* in ADPKD mouse and human kidneys. Moreover, we showed that several proteins are secreted into cyst fluid and belong to the Senescence Associated Secretory Phenotype (SASPs). Furthermore, cyst fluid can induce increased proliferation of renal epithelial cells *in vitro*, suggesting that SASPs might be a trigger for cystogenesis. Thus, using this novel ADPKD-ASC kidney

organoid model, we identified senescence as a hallmark of PKD. Linking senescence to cystogenesis provides important new insights in the pathophysiology of hereditary and acquired polycystic kidney disease.

In **Chapter 4** we aimed to identify paracrine signaling molecules in cyst fluid that can induce cystogenesis. We showed that cyst fluid – collected from ADPKD kidneys and livers – induce cystogenesis when added to renal epithelial cells (RPTEC/hTERT1) *in vitro.* Using size fractionating of the cyst fluid molecules, we found that only the fractions between 30-100 kDa showed cystogenic potential. In addition, we observed that cyst fluid samples from different individual cysts exhibited differences in strength of the cystogenic effect. Comparing the proteome from a fraction of cyst fluid sample with a high cystogenic potential to a sample without cystogenic effect, yielded a list of 43 candidate cystogenic proteins. We found enrichment of proteins classified as enzymes, immunity proteins, receptors and signaling proteins. Moreover, some of these proteins such as Secreted Frizzled-Related Protein 4, S100A8, osteopontin, and CRELD1, have previously been implicated in the pathogenesis of ADPKD. These candidates can be used for further studies to test if targeting of these molecules abrogates cystogenesis *in vitro* or in mouse models and perhaps ultimately in ADPKD patients.

Finally, we aimed to gain more insight in *PKD1* gene expression regulation. In **Chapter 5** we present an unbiased screen to identify transcription factors regulating *PKD1* expression, using the novel technique CasID. In this technique a promiscuous biotin ligase (BirA*) is fused to a catalytically dead Cas9 (dCas9). By co-expressing a single guide RNA in the cell, the BirA-dCas9 fusion protein is targeted to a specific genomic locus and enables biotinylation of proteins in the proximity of the locus of interest. Next, biotinylated proteins are identified using a high-affinity streptavidin pull down and mass spectrometry analysis. We have exploited this technique to discover novel *PKD1* promoter binding proteins, by using a single guide RNA just upstream of the *PKD1* transcription start site. Our screen yielded several candidate *PKD1* transcription factors that have been linked to PKD previously such as MAX, CUX1, ATF2 and ZEB2. Although the involvement of the identified candidate *PDK1* transcription factors has not yet been confirmed, our results suggest CasID is a useful technique to identify such regulators of gene expression.

In **Chapter 6** we discuss our findings in light of the current knowledge concerning the pathophysiology of cyst formation. In addition, future directions are proposed to further enhance our understanding of the molecular mechanism of cystogenesis and development of future therapies for ADPKD patients.

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

In this thesis we have studied the molecular mechanism of ADPKD by focusing on three central questions. First, we aimed to better understand what the function of *PKD1*/ polycystin-1 is at the cellular level. The second question focused on what triggers cyst formation. Third, we aimed to improve the current understanding of polycystin-1-regulation. Here, I will focus on these three central questions, the lessons learned from our work and novel questions that have arisen during the course of the work.

Modeling ADPKD in human cell lines

Following the discovery of *PKD1* as the first ADPKD gene, this gene has been a focal point for studies on cystic kidney diseases. Mouse and other animal models, patient derived tissue and cell lines, biochemical studies and computational analysis have all been employed to unravel the function of *PKD1* and its gene product polycystin-1 at the cellular level. However, despite extensive research it remains incompletely understood why *PKD1*/ polycystin-1 dysfunction leads to cystogenesis. Using human renal epithelial cell lines as a model system for ADPKD, several aspects need to be taken into consideration. First, the choice between primary cells versus immortalized cell lines is important. Primary cells have a finite life span thus can be difficult to maintain in culture over a longer time period and will be insufficient when large cell numbers are needed. Primary cells that are isolated at different time points can lead to variation between experiments. Moreover, obtaining sufficient numbers of patient and healthy control kidney tissue for cell line isolation can be challenging. Immortalized cell lines on the other hand provide an unlimited supply of cells. However, normal cellular functions can be altered in these cells, due to the immortalization process. Second, when using renal epithelial cells *in vitro*, verification of renal and epithelial marker expression is essential, as exposure to culture medium can influence expression profiles and cell identity. Third, cyst formation is a three-dimensional event, therefore a 3D culture system is preferred over a 2D monolayer. Finally, somatic mutations in the renal epithelium have been implicated in the pathogenesis of cyst formation. When using cyst derived cell lines, these somatic mutations will be present in the model system. In this thesis, we took two different approaches for generating an *in vitro* ADPKD model; one based on iPSCs and one based on adult stem cell kidney organoids described in **chapters 2 and 3**.

Second hit

Several fibroblast derived ADPKD-iPSC models have been published (2, 23, 29, 30, 41, 63, 71). However, we are the first to establish iPSCs from cystic renal epithelial cells. In addition, ADPKD-ASC kidney organoids have not been described yet. Both our iPSC and ASC kidney organoid cell lines have been derived from cystic epithelium. Thus, somatic mutations that occurred in the cystic renal epithelium have been captured in the model. Genetic second hits have been implied to be the initiating event in cystogenesis and various studies provide evidence that, in addition to the patients germ line mutation, somatic mutations in *PKD1* can be detected in a significant number of individual cysts (68). Surprisingly, in the cystic tubular renal epithelial cells (TECs) – which we used as starting material for the iPSCs – we did not detect somatic mutations in *PKD1*. Similarly, in cyst derived ADPKD organoids we did not detect a second hit in *PKD1.* There are several possible explanations for this. First, somatic *PKD1* mutations could have been missed due to technical reasons. However, this option is not very likely because three different techniques (WES, LR-PCR, MLPA) were used to specifically search for somatic hits in *PKD1*. Second, it is possible that a second hit in *PKD1* initiated cystogenesis, but that this mutation was not present in all epithelial cells lining the cysts. In other words; the cyst is not monoclonal. Subsequently, due to an *in vitro* growth selection in favor of *PKD1*+/- cells, cells with a second hit in *PKD1* were selected against in culture. Or, finally, cyst growth was not initiated by a second hit in *PKD1*, but other factors were responsible for triggering cystogenesis such as somatic mutations in non-PKD genes, or focal signaling events. Intriguingly, we did observe various somatic mutations that were unique for each individual cyst, an observation that has been reported previously by other groups as well (25, 68). Many of these somatic mutations were found in cilia-related genes or genes linked to oncogenesis, suggesting that these genes might have played a causal role in initiating cystogenesis (68). On the other hand these somatic mutations can also merely reflect DNA damage as a consequence of the local deleterious effects of inflammation, proliferation and mechanical stress due to cyst growth. Evidence that cystogenesis is not triggered by a second hit in *Pkd1* comes from a previous study showing that *Pkd1+/-* mice develop cysts shortly after induction of kidney injury, showing that *Pkd1* heterozygosity results in haploinsufficiency (4). In addition, ARPKD patients are compound heterozygous for *PKHD1* mutation, thus already have a 'second hit' but also exhibit focal cyst formation. Finally, injury also triggers cyst formation in genetically normal kidneys. Taken together, these observations suggest that the focal aspect of cystogenesis is not necessarily caused by a second genetic hit, but could also be triggered by local signaling cues. In **chapter 4** we describe that indeed paracrine signaling molecules are present in cyst fluid which can trigger cystogenesis.

Function of *PKD1*/polycystin-1

Interestingly, we observed that ADPKD versus control iPSCs were quite similar with respect to morphology, growth rate, stem cell marker expression, pluripotency, and RNA expression. This suggests that stem cell function is not evidently affected by a heterozygous *PKD1* mutation, which is in line with the fact that a heterozygous *Pkd1*/*PKD1* mutation does not affect embryonic development. Therefore, ADPKD iPSCs should preferably be differentiated into renal epithelial cells before studying *PKD1*/polycystin-1 function. In ADPKD ASC kidney organoids we did detect a disease specific phenotype pointing towards a role for *PKD1*/polycystin-1 in senescence during injury repair. Whilst patient derived and control ASC kidney organoids are morphologically similar, ADPKD ACS organoids exhibited a cellular senescent transcriptome profile. This is marked by downregulation of cell cycle genes and LaminB1 and upregulation of SASPs expression. Of note, we observed that ADPDK TECs have an increased nuclear size, another hallmark of cellular senescence. The observation that also *in vivo* cystic epithelial cells have larger nuclei has been described previously (1, 8, 77), and validated by our studies. In addition, we found an increased number of senescent cells in ADPKD patient tissue. Absence of p21(*CDKN1A*) upregulation and high levels of p16(*CDKN2A)* suggest that this senescence is controlled via p16 signaling. Furthermore, we found that SASPs are present in cyst fluid and that cyst fluid can induce increased cell proliferation of renal epithelial cells *in vitro*. Thus, our human ADPKD-ASCs kidney organoid model has led to the discovery that cellular senescence is a hallmark of cystogenesis. We will further elaborate on the role of cellular senescence in cystogenesis and the function of polycystin-1 herein in the paragraph 'Cystogenesis is triggered by renal injury' and 'Senescence in renal injury repair at the molecular level'.

iPSC versus ASC kidney organoids

With the ADPKD iPSCs and ASCs we generated two novel culture systems suitable for ADPKD disease modeling. Both systems have several features in common; iPSCs and ASCs can both be expanded indefinitely *in vitro*, differentiated into kidney organoids, grown in a three dimensional platform, and used for gene editing. There are, however, some important differences between these two types of stem cell cultures. For example, ACS kidney organoids spontaneously form epithelial tubuloids due to the culture conditions that mimic the stem cell niche (64), while iPSCs have to be induced into nephron-like structures. Over the last couple of years, advances in differentiation protocols have further enhanced the possibility of generating *in vitro* kidney organoid structures from iPSCs (6, 9, 22, 51, 67). However, generating kidney structures *in vivo* remains challenging and optimization of the current protocols is required to increase reproducibility (57). Moreover, although these *in vitro* kidney organoid

structures encompass glomeruli, tubuli, collecting ducts, stroma and vasculature, they do not fully recapitulate the complexity of the adult kidney *in vivo*. Nonetheless, future studies should focus on utilizing the progress that has been made in *in vitro* kidney development strategies for differentiation of our ADPKD iPSC cells. This will be specifically useful to study the function of *PKD1* during kidney 'development' and homeostasis. In addition, since iPSCs are pluripotent, our ADPKD iPSCs can be used to study *PKD1*/polycystin-1 function in other tissue types affected in ADPKD patients, like chondrocytes, cardiomyocytes and cholangiocytes (19, 32, 45). Also for ASC kidney organoids, further optimization of kidney organoid culture conditions is required and additional analysis regarding renal cell markers and functionality of the kidney organoids are needed. Another focus for future studies should include the development of *PKD1* heterozygous and homozygous CRISPR/Cas9 knock out ASC kidney organoids. This will be helpful to answer the key question whether a senescence phenotype is present in these artificially gene edited ASC kidney organoids that have not been exposed to a cyst-microenvironment. In conclusion, both ADPKD *in vitro* models can be used for further analysis of the molecular mechanisms of cystogenesis and the function of *PKD1*/polycystin-1. A crucial step for enabling large drug screens in either iPSC or ACS ADPKD kidney organoids will be to find a practical readout for *PKD1*/polycystin-1 function in these cells.

Cystogenesis is triggered by renal injury

In wild type kidneys, various forms of renal injury, such as nephrotoxic drugs, ischemia or partial nephrectomy, chronic kidney disease in general and kidney aging, can induce cyst formation, which can lead to acquired polycystic kidney disease (16, 18, 21, 26, 31, 34, 39, 47). Also in ADPKD, injury is a trigger for cyst initiation. Heterozygous *Pkd1* and *Pkd2* mice and *Pkd1* inducible knock out mice – where deletion results in *Pkd1* null cells – do not develop a polycystic kidney disease phenotype until injury is induced in these mice, resulting in massive cystogenesis (4, 27, 59). Renal injury induces a complex repair response process in the kidney. An important feature of the physiological injury – or wound healing – response is cellular senescence (17, 33, 53). Cellular senescence is a response to various types of cell stress, which results in a proliferative arrest of the cell and secretion of senescence associated secretory phenotype (SASP) factors that modulate the surrounding tissue (for review see 12, 41, 46). After injury, senescent cells promote tissue renewal by recruiting immune cells through SASPs. In turn, these immune cells ensure clearance of senescent cells after which tissue regeneration is completed, coined 'acute' senescence (53). Persistent damage and aging can lead to impairment of this physiological injury response; senescent cells accumulate resulting in persistent inflammation and fibrosis (53). Thus, whilst senescent cells exert a

beneficial effect during the early phase of acute kidney injury, prolonged 'chronic' senescence has a detrimental effect in repetitive and chronic kidney injury (74). Indeed, accumulation of senescent cells has been reported in kidneys from experimental mouse models and human renal diseases (33, 74). Moreover, depletion of accumulated senescent cells has been proven to reduce age related kidney damage and maintain renal function (46). Shortly after kidney injury *Pkd1* expression is upregulated (16), suggesting that it plays an important role in renal injury repair. Indeed, in ADPKD the renal injury response is impaired. *Pkd1+/-* or *Pkd2+/-* mice exhibit increased cortical damage, apoptosis and immune cell infiltration and tubular dilatations shortly after injury, and fibrosis and microcysts after several weeks (4, 59). In **chapter 3** we demonstrate that senescence is a hallmark of ADPKD. We describe that ADPKD ASC kidney organoids express senescent phenotype related genes. Moreover, we detected an increase in senescent cell numbers in the epithelium as well as stroma cells in mouse and human ADPKD renal tissue. Damage in ADPKD kidneys is often attributed to the local deleterious effect of extensive cyst growth. Intriguingly, however, in non-cystic *Pkd2+/-* mice increased cell proliferation, immune cell invasion and fibrosis was found to precede cyst formation (59). This implies that impaired wound healing is a direct consequence of dysfunction of the polycystin complex . Thus, our results suggest that the sequence of senescence – clearance – and regeneration is impaired in ADPKD leading to accumulation of senescent cells and accelerated kidney aging.

Senescence in renal injury repair at the molecular level

At the molecular level, cellular senescence is controlled through either p53-p21 or p16 signalling (11, 48, 53). Shortly after acute injury there is a tremendous upregulation of p53 and p21 in the tubular epithelium (49, 55, 66, 69). Massive damage leads to loss of tubular epithelial cells after which remnant quiescent progenitor cells enter a proliferative state and repopulate the tubular epithelial monolayer (3). Upregulation of p21 and p53 exerts a renal protective effect by inducing a transient cell cycle arrest and providing time for the damaged epithelium to repair cellular and DNA-damage, avoiding malignant transformation or cell death (3, 49, 50, 55, 66, 69). Both p53-/- and p21 conditional knock out mice are sensitive to renal injury with a more rapid decline in renal function and histological changes including tubular dilatation (55, 66). In ADPKD, p21 upregulation during kidney injury repair is abrogated. This was observed for *Pkd1+/-* or *Pkd2+/-* mice in which p21 expression remains low after injury, due to defective JAK/STAT signalling (4, 5, 59). Accordingly, we could not detect any differences in p21 expression levels between ADPKD and control organoids and human tissues (**chapter 3**), an observation that has been reported for human tissue previously (56). Since polycystin-1 depletion impairs p21 and p53 upregulation and results in

increased proliferation, polycystin-1 has been suggested to act as a regulator of the G1 checkpoint, controlling entry into the S phase and preventing replication of damaged DNA. Interestingly, we found DNA repair enriched in our GO analysis.

An explanation for the inability to induce p21 expression in ADPKD could be elevated C-MYC levels, which is a p21 repressor. We detected high *C-MYC* mRNA expression in our ADPKD iPSCs as compared to control iPSC (data not shown) and increased C-MYC levels has been reported previously for ADPKD (8, 43). In addition, mice harbouring constitutively active c-myc expression develop polycystic kidney disease (73). Taken together, we hypothesize that shortly after injury, polycystin-1 might aid in inducing acute senescence by causing a cell cycle pause through the p53-p21 pathway, enabling proper injury repair of the tubuli. In ADPKD, this beneficial acute senescence response is impaired and leads to accelerated accumulation of chronic senescence – through p16 signalling – resulting in fibrosis, inflammation and cystogenesis. Future studies should be performed to test whether inhibition of chronic senescence in ADPKD kidneys can prevent or reduce cystogenesis. For this, either a genetic or pharmacological approach can be used, such as a crossover between an ADPKD mouse model and $p16^{ink4a}$ knockout mice knock out mice, or the use of senolytics in ADPKD mice.

Senescence might trigger cystogenesis via SASPS

We propose that senescence itself can trigger cystogenesis. Senescent cells secrete SASPs, a variety of growth factors, pro-inflammatory and matrix proteins (13, 53). SASPs can induce senescence as well as proliferation of neighboring cells in a paracrine manner (13, 14, 62). Previously it was found that local signalling cues might induce cystogenesis in epithelial cells that are in close proximity of existing cysts, a phenomenon coined the cystic snowball effect (42). In **chapter 4** we hypothesized that paracrine signalling molecules responsible for this cystic snowball effect are secreted into cyst fluid. We showed that cyst fluid indeed induces cystogenesis of wild type renal epithelial cells in an *in vitro* assay. Size fractionation and comparative analysis of cyst fluid with high versus low cystogenic capacity yielded a list of candidate cystogenic proteins. Interestingly, many candidate proteins are involved in injury repair such as immunity proteins, growth factors, adhesion molecules and matrix proteins. We and others found that various SASPs are present in cyst fluid, some of which are part of the cystogen candidate list in **chapter 4**. Moreover in **chapter 3**, we showed that – compared to control organoids – ADPKD ASC organoids show higher expression of several SASPs, including CCN1 (*Cyr61*) an important regulator of senescence during injury repair (35, 37). Thus, our results suggest that accumulated senescent cells in ADPKD kidneys secrete SASPs which can induce cystogenesis in the surrounding tissue.

Further evidence that senescence is a trigger for cystogenesis comes from previous studies showing that drug-induced senescence – for example by cisplatinum – triggers cyst formation (20, 21). In addition, experimental mouse models harbouring mutations that cause either oncogene, DNA damage or oxidative stress induced senescence also exhibit a polycystic phenotype (61, 70, 76, 77). Moreover, in humans, aging is associated with increased prevalence of kidney cysts (18). Senescence as a trigger for cystogenesis can also explain intra-familial variability in disease severity. Whilst these patients harbor the exact same *PKD1* mutation, exposure to renal injury inducing factors will differ between these family members and thereby influence cystic load in the kidney.

PKD1/polycystin-1 signaling pathway

To comprehend how polycystin-1 regulates injury repair exactly, it is essential to unravel the polycystin-1 signal transduction cascade and understand how *PKD1* transcription is regulated. *PKD1*/polycystin-1 acts in a dose dependent fashion; low transcription levels and over-expression have been proven to result in a cystic phenotype (38, 40, 60, 72). Also, during kidney development injury repair *PKD1* expression needs to be upregulated (10, 15, 24). Thus, proper control of *PKD1* expression and functional polycystin-1 levels are crucial to prevent cystogenesis. Only a few transcription factors that regulate the *PKD1* promoter are known, such as p53. Further identification of *PKD1* transcription factors is needed to gain insight into *PKD1* regulation. In **chapter 5** we describe the use of a novel technique, CasID, to screen for proteins enriched at the *PKD1* promoter. We found several transcriptions factors that have been linked to *PKD1* signaling or even have been described to cause a polycystic phenotype such as CUX1, ATF-2 and MAX. Interestingly, these candidates also play a role in kidney injury and cellular senescence. ATF2 is a DNA damage response protein and a transcription factor that regulates genes involved in cellular stress, DNA damage, cell proliferation and inflammation (44, 75). It is involved in regulation of tubular morphology during kidney development (12) and upregulated in response to kidney injury (52). Moreover, ATF2 is constitutively activated in ADPKD (54). CUX1 drives proliferation through p21 repression and c-MYC activation (7, 58). CUX1 is involved in kidney development and overexpressed in the cyst epithelium (28, 65). Overexpression of Cux1 in mice results in a cyst kidney phenotype (7). MAX is part of a transcription factor complex with c-MYC which can induce senescence and is also upregulated in ADPKD (43). Thus, these candidates might link acute kidney injury and senescence to PKD1 expression regulation. Further validation of these PKD promoter associated candidate proteins is needed in order to verify binding at the *PKD1* promoter, which can be done for example by repeating the pull down experiment, perform ChiP-seq /ChiP-PCR on the candidate

proteins and a pull down using a biotinylated *PKD1* promoter construct in combination with an epitope tagged candidate protein. Next, whether these candidates activate or repress *PKD1* transcription can be tested using *PKD1* promoter expression assays or by generating knock out cells for each candidate and measure PKD1 expression levels. Moreover, CasID can be used to screen for transcription factors binding at various regions of the *PKD1* proximal, distal promoter and the enhancer element that is present downstream of the transcription start site. Finally, BioID is an ideal technique for identifying interaction partners of membrane proteins. This technique is therefore very useful to unravel the polycystin-signaling pathway. Improving our understanding of the PKD signaling cascade and how *PKD1*/polycystin-1 levels are controlled will be helpful in designing therapeutic strategies in the near future.

Concluding remarks

Acute senescence is essential for proper kidney repair. We propose a model where a *PKD1* mutation in ADPKD patients or (repetitive) injury in wild type kidneys leads to impaired kidney repair. This results in accumulation of senescent cells which secrete SASPs. These SASPs cause fibrosis, persistent inflammation, and we propose that these SASPs also trigger cystogenesis (**Figure 1**). Future studies should focus on clarifying the role of polycystin-1 in renal injury repair. For this, combining findings from the ADPKD field with knowledge on cellular senescence and wound healing is essential. Second, we need to further identify which paracrine (SASP) molecule(s) induce(s) cystogenesis. Third, we should investigate if stringent prevention of acute kidney injury in ADPKD patients can slow down disease progression (e.g. by avoiding nephrotoxic drugs, prevention of contrast nephropathy and prerenal injury). Finally, ablation of chronic senescent cells with senolytics could be a promising therapeutic strategy to slow down cyst growth in ADPKD experimental mouse models and, if successful, in patients.

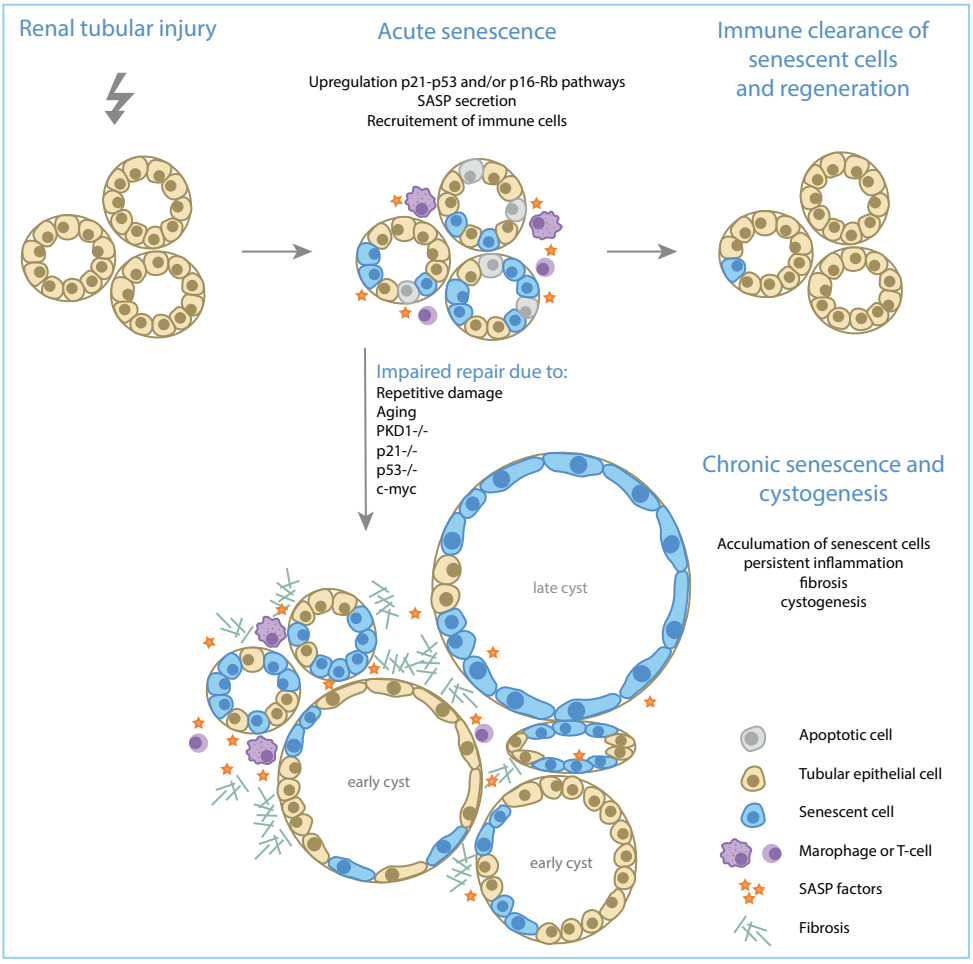


Figure 1. Senescence in cystogenesis
Schematic representation of hypothetical model on senescence in cystogenesis

REFERENCES

1. **AbouAlaiwi WA**, **Ratnam S**, **Booth RL**, **Shah J V.**, **Nauli SM**. Endothelial cells from humans and mice with polycystic kidney disease are characterized by polyploidy and chromosome segregation defects through survivin down-regulation. *Hum Mol Genet* 20: 354–367, 2011.
2. **Ameku T**, **Taura D**, **Sone M**, **Numata T**, **Nakamura M**, **Shiota F**, **Toyoda T**, **Matsui S**, **Araoka T**, **Yasuno T**, **Mae S-I**, **Kobayashi H**, **Kondo N**, **Kitaoka F**, **Amano N**, **Arai S**, **Ichisaka T**, **Matsuura N**, **Inoue S**, **Yamamoto T**, **Takahashi K**, **Asaka I**, **Yamada Y**, **Ubara Y**, **Muso E**, **Fukatsu A**, **Watanabe A**, **Sato Y**, **Nakahata T**, **Mori Y**, **Koizumi A**, **Nakao K**, **Yamanaka S**, **Osafune K**. Identification of MMP1 as a novel risk factor for intracranial aneurysms in ADPKD using iPSC models. *Sci Rep* 6: 30013, 2016.
3. **Andrade L**, **Rodrigues CE**, **Gomes SA**, **Noronha IL**. Acute Kidney Injury as a Condition of Renal Senescence. 27: 739–753, 2018.
4. **Bastos AP**, **Piontek K**, **Silva AM**, **Martini D**, **Menezes LF**, **Fonseca JM**, **Fonseca II**, **Germino GG**, **Onuchic LF**. Pkd1 haploinsufficiency increases renal damage and induces microcyst formation following ischemia/reperfusion. *J Am Soc Nephrol* 20: 2389–2402, 2009.
5. **Bhunia AK**, **Piontek K**, **Boletta A**, **Liu L**, **Qian F**, **Xu PN**, **Germino FJ**, **Germino GG**. PKD1 induces p21waf1 and regulation of the cell cycle via direct activation of the JAK-STAT signaling pathway in a process requiring PKD2. *Cell* 109: 157–168, 2002.
6. **Bonventre J V**. Kidney organoids—a new tool for kidney therapeutic development. *Kidney Int* 94: 1040–1042, 2018.
7. **Cadieux C**, **Harada R**, **Paquet M**, **Côté O**, **Trudel M**, **Nepveu A**, **Bouchard M**. Polycystic kidneys caused by sustained expression of Cux1 isoform p75. *J Biol Chem* 283: 13817–13824, 2008.
8. **Camila Parrot, Almira Kurbegovic, Guanhan Yao, Martin Couillard OC and MT**. C-MYC is a regulator of the PKD1 gene and PC1-induced pathogenesis. *Hum. Mol. Genet.* .
9. **Cecilia Boreström, Anna Jonebring, Jing Guo, Henrik Palmgren, Linda Cederblad, Anna Forslöw, Anna Svensson, Magnus Söderberg, Anna Reznichenko, Jenny Nyström, Jaakko Patrakka, Ryan Hicks, Marcello Maresca, Barbara Valastro, Anna CollénCecilia Boreström, A AC**. A CRISP(e)R view on kidney organoids allows generation of an induced pluripotent stem cell – derived kidney model for drug discovery. *Kidney Int.* (2018). doi: 10.1016/j.kint.2018.05.003.
10. **Chauvet V**, **Qian F**, **Boute N**, **Cai Y**, **Phakdeekitacharoen B**, **Onuchic LF**, **Attié-Bitach T**, **Guicharnaud L**, **Devuyst O**, **Germino GG**, **Gubler M-C**. Expression of PKD1 and PKD2 transcripts and proteins in human embryo and during normal kidney development. *Am J Pathol* 160: 973–983, 2002.
11. **Childs BG**, **Durik M**, **Baker DJ**, **Deursen JM Van**. Cellular senescence in aging and age-related disease : from mechanisms to therapy. *Nat Genet* 21: 1424–1435, 2015.
12. **Cizelsky W**, **Tata A**, **Kuhl M**, **Kuhl SJ**. The Wnt/JNK signaling target gene alcam is required for embryonic kidney development. *Development* 141: 2064–2074, 2014.
13. **Coppé J-P**, **Desprez P-Y**, **Krtolica A**, **Campisi J**. The Senescence-Associated Secretory Phenotype: The Dark Side of Tumor Suppression. *Annu Rev Pathol Mech Dis* 5: 99–118, 2010.
14. **Coppé J-P**, **Patil CK**, **Rodier F**, **Sun Y**, **Muñoz DP**, **Goldstein J**, **Nelson PS**, **Desprez P-Y**, **Campisi J**. Senescence-Associated Secretory Phenotypes Reveal Cell-Nonautonomous Functions of Oncogenic RAS and the p53 Tumor Suppressor. *PLoS Biol* 6: e301, 2008.
15. **Le Corre S**, **Viau A**, **Burtin M**, **El-Karoui K**, **Cnops Y**, **Terryn S**, **Debaix H**, **B??rissi S**, **Gubler MC**, **Devuyst O**, **Terzi F**. Cystic gene dosage influences kidney lesions after nephron reduction. *Nephron* 129: 42–51, 2015.
16. **Le Corre S**, **Viau A**, **Burtin M**, **El-Karoui K**, **Cnops Y**, **Terryn S**, **Debaix H**, **Bérissi S**, **Gubler MC**, **Devuyst O**, **Terzi F**. Cystic gene dosage influences kidney lesions after nephron reduction. *Nephron* 129: 42–51, 2015.

17. **Demaria M**, **Ohtani N**, **Youssef SA**, **Rodier F**, **Toussaint W**, **Mitchell JR**, **Hoeijmakers JHJ**, **Bruin A De**, **Hara E**, **Laberge R**, **Vijg J**, **Steeg H Van**, **Dolle MET**. Article An Essential Role for Senescent Cells in Optimal Wound Healing through Secretion of PDGF-AA. (2014). doi: 10.1016/j.devcel.2014.11.012.

18. **Denic A**, **Glassock RJ**, **Rule AD**. Structural and Functional Changes With the Aging Kidney. *Adv Chronic Kidney Dis* 23: 19–28, 2016.

19. **Development RB**, **Taz TC**, **Merrick D**, **Mistry K**, **Wu J**, **Gresko N**, **Baggs JE**, **Hogenesch JB**, **Sun Z**, **Caplan MJ**, **Physiology M**, **Biology C**, **Haven N**, **Biology P**, **Children C**, **Author C**, **Caplan MJ**. *Polycystin-1 regulates bone development through an interaction with the transcriptional coactivator TAZ*. 2019.

20. **Dobyan DC**. Long-term consequences of cis-platinum-induced renal injury: A structural and functional study. *Anat Rec* 212: 239–245, 1985.

21. **Dobyan DC, Hill D, Lewis T BR**. Cyst formation in rat kidney induced by cis-platinum administration. *Lab Invest* 45: 260–8, 1981.

22. **Freedman BS**, **Brooks CR**, **Lam AQ**, **Fu H**, **Morizane R**, **Agrawal V**, **Saad AF**, **Li MK**, **Hughes MR**, **Werff R Vander**, **Peters DT**, **Lu J**, **Baccei A**, **Siedlecki AM**, **Valerius MT**, **Musunuru K**, **McNagny KM**, **Steinman TI**, **Zhou J**, **Lerou PH**, **Bonventre J V.** Modelling kidney disease with CRISPR-mutant kidney organoids derived from human pluripotent epiblast spheroids. *Nat Commun* 6: 8715, 2015.

23. **Freedman BS**, **Lam AQ**, **Sundsbak JL**, **Iatrino R**, **Su X**, **Koon SJ**, **Wu M**, **Daheron L**, **Harris PC**, **Zhou J**, **Bonventre J V**. Reduced Ciliary Polycystin-2 in Induced Pluripotent Stem Cells from Polycystic Kidney Disease Patients with PKD1 Mutations. *J Am Soc Nephrol* 24: 1571–1586, 2013.

24. **Geng L**, **Segal Y**, **Pavlova A**, **Barros EJG**, **Löhning C**, **Lu W**, **Nigam SK**, **Frischauf A-M**, **Reeders ST**, **Zhou J**. Distribution and developmentally regulated expression of murine polycystin. *Am J Physiol - Ren Physiol* 272, 1997.

25. **Gogusev J**. Molecular Cytogenetic Aberrations in Autosomal Dominant Polycystic Kidney Disease Tissue. *J Am Soc Nephrol* 14: 359–366, 2003.

26. **Grantham JJ**. Acquired Cystic Kidney Disease [Online]. *jKidney Int* 40: 143–152, 1991. http://emedicine.medscape.com/article/246888-overview#a5.

27. **Happé H**, **Leonhard WN**, **van der Wal A**, **van de Water B**, **Lantinga-van Leeuwen IS**, **Breuning MH**, **de Heer E**, **Peters DJM**. Toxic tubular injury in kidneys from Pkd1-deletion mice accelerates cystogenesis accompanied by dysregulated planar cell polarity and canonical Wnt signaling pathways. *Hum Mol Genet* 18: 2532–2542, 2009.

28. **Vanden Heuvel GB**, **Bodmer R**, **Mcconnell KR**, **Nagami GT**, **Igarashi P**. Expression of a cut-related homeobox gene in developing and polycystic mouse kidney. *Kidney Int* 50: 453–461, 1996.

29. **Ho M**, **Huang C**, **Lee J**, **Hsu S**, **Cheng Y**, **Hsu Y**, **Hwang D**, **Lu H**, **Chen H**, **Hsieh PCH**. Generation of an induced pluripotent stem cell line , IBMS-iPSC-014-05 , from a female autosomal dominant polycystic kidney disease patient carrying a common mutation of R803X in PKD2. *Stem Cell Res* 25: 38–41, 2017.

30. **Huang C**, **Ho M**, **Lee J**, **Hwang D**, **Ko H**, **Cheng Y**, **Hsu Y**, **Lu H**, **Chen H**, **Hsieh PCH**. Generation of induced pluripotent stem cells derived from an autosomal dominant polycystic kidney disease patient with a p . Ser1457fs mutation in PKD1. *Stem Cell Res* 24: 139–143, 2017.

31. **Ishikawa I**. Uremic Acquired Cystic Disease of the Kidney. *Urology* XXVI: 101–108, 1985.

32. **Jere Paavola, Simon Schliffke, Sandro Rossetti, Ivana Y.-T. Kuo, Shiaulou Yuan, Zhaoxia Sun, Peter C. Harris, Vicente E. Torres and BEE**. Polycystin-2 mutations lead to impaired calcium cycling in the heart and predispose to dilated cardiomyopathy. *J Mol Cell Cardiol* (2013). doi: 10.1016/j.jamcollsurg.2011.02.017.Cost-Effective.

33. **Jin H**, **Campisi J**, **Attanasio M**, **Jin H**, **Zhang Y**, **Ding Q**, **Wang SS**, **Rastogi P**, **Dai D**, **Lu D**, **Purvis M**, **Cao C**, **Wang A**, **Liu D**, **Ren C**, **Attanasio M**. Epithelial innate immunity mediates tubular cell senescence after kidney injury Find the latest version : Epithelial innate immunity mediates tubular cell senescence after kidney injury. 4, 2019.

34. **Judit Kovác, Mónika Zilahy, Tamás Bányász SG**. Evaluation of apoptosis and cell proliferation in experimentally induced renal cysts. *Urol Res* 26: 411–416, 1998.

35. **Jun J**, **Lau LF**. The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. *Nat Cell Biol* 12: 1249, 2010.

36. **Kim H**, **Bae Y**, **Jeong W**, **Ahn C**, **Kang S**. Depletion of PKD1 by an antisense oligodeoxynucleotide induces premature G1/S-phase transition. *Eur J Hum Genet* 12: 433–440, 2004.

37. **Kim K**, **Chen C**, **Monzon RI**, **Lau LF**. Matricellular Protein CCN1 Promotes Regression of Liver Fibrosis through Induction of Cellular Senescence in Hepatic Myofibroblasts. 33: 2078–2090, 2013.

38. **Kurbegovic A**, **Côté O**, **Couillard M**, **Ward CJ**, **Harris PC**, **Trudel M**. Pkd1 transgenic mice: Adult model of polycystic kidney disease with extrarenal and renal phenotypes. *Hum Mol Genet* 19: 1174–1189, 2010.

39. **Kurbegovic A**, **Trudel M**. Acute kidney injury induces hallmarks of polycystic kidney disease. *Am J Physiol Renal Physiol* : ajprenal.00167.2016, 2016.

40. **Lantinga-van Leeuwen IS**, **Dauwerse JG**, **Baelde HJ**, **Leonhard WN**, **van de Wal A**, **Ward CJ**, **Verbeek S**, **DeRuiter MC**, **Breuning MH**, **de Heer E**, **Peters DJM**. Lowering of Pkd1 expression is sufficient to cause polycystic kidney disease. *Hum Mol Genet* 13: 3069–3077, 2004.

41. **Lee J**, **Ho M**, **Huang C**, **Wen C**, **Cheng Y**, **Hsu Y**, **Hwang D**, **Lu H**, **Chen H**, **Hsieh PCH**. Induced pluripotent stem cells derived from an autosomal dominant polycystic kidney disease patient carrying a PKD1 Q533X mutation. *Stem Cell Res* 25: 83–87, 2017.

42. **Leonhard WN**, **Zandbergen M**, **Veraar K**, **van den Berg S**, **van der Weerd L**, **Breuning M**, **de Heer E**, **Peters DJ**. Scattered Deletion of PKD1 in Kidneys Causes a Cystic Snowball Effect and Recapitulates Polycystic Kidney Disease. *J Am Soc Nephrol* 26: 1322–1333, 2015.

43. **Li X**, **Trudel M**. *Polycystic Kidney Disease*. [date unknown].

44. **Lopez-Bergami P**, **Lau E**, **Ronai Z**. Emerging roles of ATF2 and the dynamic AP1 network in cancer. *Nat Rev Cancer* 10: 65–76, 2010.

45. **Lu W**. Comparison of Pkd1-targeted mutants reveals that loss of polycystin-1 causes cystogenesis and bone defects. *Hum Mol Genet* 10: 2385–2396, 2002.

46. **Marjolein P. Baar, Renata M.C. Brandt, Diana A. Putavet, Julian D.D. Klein, Kasper W.J. Derks, Benjamin R. M. Bourgeois, Sarah Stryeck, Yvonne Rijksen, Hester van Willigenburg, Danny A. Feijtel, Ingrid van der Pluijm, Jeroen Essers, Wiggert A. van Cappell and PLJ de K**. Targeted Apoptosis of Senescent Cells Restores Tissue Homeostasis in Response to Chemotoxicity and Aging Marjolein. *Cell* 169: 132–147, 2018.

47. **Matson, A and Cohen E**. Acquired Cystic Kidney Disease: Occurrence, Prevalence, and Renal Cancers. Medicine (Baltimore)., [date unknown].

48. **Mchugh D**, **Gil J**. Senescence and aging : Causes , consequences , and therapeutic avenues. *J Cell Biol* 217: 65–77, 2018.

49. **Megyesi J**, **Andrade L**, **Vieira JM**, **Safirstein RL**, **Price PM**. Positive effect of the induction of p21WAF1/CIP1 on the course of ischemic acute renal failure. *Kidney Int* 60: 2164–2172, 2001.

50. **Megyesi J**, **Andrade L**, **Vieira JM**, **Safirstein RL**, **Price PM**, **Andrade L**, **Vieira JM**, **Safirstein RL**, **Price PM**. Coordination of the cell cycle is an important determinant of the syndrome of acute renal failure. *Am J Physiol Ren. Physiol*.

51. **Morizane R**, **Lam AQ**, **Freedman BS**, **Kishi S**, **Valerius MT**, **Bonventre J V**. Nephron organoids derived from human pluripotent stem cells model kidney development and injury. *Nat Biotechnol* 33: 1193–1200, 2015.

52. **Morooka H**, **Bonventre J V.**, **Pombo CM**, **Kyriakis JM**, **Force T**. Ischemia and reperfusion enhance ATF-2 and c-Jun binding to cAMP response elements and to an AP-1 binding site from the c-jun promoter. *J Biol Chem* 270: 30084–30092, 1995.

53. **Muñoz-Espín D**, **Serrano M**. Cellular senescence: From physiology to pathology. *Nat Rev Mol Cell Biol* 15: 482–496, 2014.

54. **Ngoc Hang Le, Annemieke van der Wal, Paola van der Bent, Irma S. Lantinga-van Leeuwen, Martijn H. Breuning, Hans van Dam, Emile de Heer and DJMP**. Increased Activity of Activator Protein-1 Transcription Factor Components ATF2, c-Jun, and c-Fos in Human and Mouse Autosomal Dominant Polycystic Kidney Disease. *J Am Soc Nephrol* 16: 2724–2731, 2005.

55. **Nishioka S**, **Nakano D**, **Kitada K**, **Sofue T**, **Ohsaki H**, **Moriwaki K**, **Hara T**, **Ohmori K**, **Kohno M**, **Nishiyama A**. The cyclin-dependent kinase inhibitor p21 is essential for the beneficial effects of renal ischemic preconditioning on renal ischemia / reperfusion injury in mice. *Kidney Int* 85: 871–879, 2013.

56. **Park J**, **Schutzer WE**, **Lindsley JN**, **Bagby SP**, **Oyama TT**, **Anderson S**, **Weiss RH**. p21 is decreased in polycystic kidney disease and leads to increased epithelial cell cycle progression : roscovitine augments p21 levels. 11: 1–11, 2007.

57. **Phipson B**, **Er PX**, **Combes AN**, **Forbes TA**, **Howden SE**, **Zappia L**, **Yen H**, **Lawlor KT**, **Hale LJ**, **Sun J**, **Wolvetang E**, **Takasato M**, **Oshlack A**, **Little MH**. Evaluation of variability in human kidney organoids. *Nat. Methods* (2018). doi: 10.1038/s41592-018-0253-2.

58. **Porath B**, **Livingston S**, **Andres EL**, **Petrie AM**, **Wright JC**, **Woo AE**, **Carlton CG**, **Baybutt R**, **Vanden Heuvel GB**. Cux1 promotes cell proliferation and polycystic kidney disease progression in an ADPKD mouse model. *Am J Physiol - Ren Physiol* 313: F1050–F1059, 2017.

59. **Prasad S**, **McDaid JP**, **Tam FWK**, **Haylor JL**, **Ong ACM**. Pkd2 dosage influences cellular repair responses following ischemia-reperfusion injury. *Am J Pathol* 175: 1493–1503, 2009.

60. **Pritchard L**, **Sloane-Stanley JA**, **Sharpe JA**, **Aspinwall R**, **Lu W**, **Buckle V**, **Strmecki L**, **Walker D**, **Ward CJ**, **Alpers CE**, **Zhou J**, **Wood WG**, **Harris PC**. A human PKD1 transgene generates functional polycystin-1 in mice and is associated with a cystic phenotype. *Hum Mol Genet* 9: 2617–2627, 2000.

61. **Rankin EB**, **Tomaszewski JE**, **Haase VH**. Renal cyst development in mice with conditional inactivation of the von Hippel-Lindau tumor suppressor. *Cancer Res* 66: 2576–2583, 2006.

62. **Ritschka B**, **Storer M**, **Mas A**, **Heinzmann F**, **Ortells MC**, **Morton JP**, **Sansom OJ**, **Zender L**, **Keyes WM**. The senescence-associated secretory phenotype induces cellular plasticity and tissue regeneration. *Genes Dev* 31: 172–183, 2017.

63. **Sampaziotis F**, **Cardoso de Brito M**, **Madrigal P**, **Bertero A**, **Saeb-Parsy K**, **Soares FAC**, **Schrumpf E**, **Melum E**, **Karlsen TH**, **Bradley JA**, **Gelson WTH**, **Davies S**, **Baker A**, **Kaser A**, **Alexander GJ**, **Hannan NRF**, **Vallier L**. Cholangiocytes derived from human induced pluripotent stem cells for disease modeling and drug validation. *Nat Biotechnol* 33: 845–852, 2015.

64. **Schutgens F, Rookmaaker MB, Margaritis T, Rios A, Ammerlaan C, Jansen J, Gijzen L, Vormann M, Vonk A, Viveen M, Yengej FY, Derakhshan S, de Winter-de Groot KM, Artegiani B, van Boxtel R, Cuppen E, Hendrickx APA, van den Heuvel-Eibrink MM, Heitzer E, Lanz CH**. Tubuloids derived from human adult kidney and urine for personalized disease modeling. *Nat Biotechnol* : 303–313, 2019.

65. **Sharma M**, **Brantley JG**, **Alcalay NI**, **Zhou J**, **Heystek E**, **Maser RL**, **Vanden Heuvel GB**. Differential expression of Cux-1 and p21 in polycystic kidneys from Pkd1 null and cpk mice. *Kidney Int* 67: 432–442, 2005.

66. **Sutton TA**, **Hato T**, **Mai E**, **Yoshimoto M**, **Kuehl S**, **Anderson M**, **Mang H**, **Plotkin Z**, **Chan RJ**, **Dagher PC**. p53 Is Renoprotective after Ischemic Kidney Injury by Reducing In fl ammation. *JASN* (2013). doi: 10.1681/ASN.2012050469.

67. **Takasato M**, **Er PX**, **Chiu HS**, **Maier B**, **Baillie GJ**, **Ferguson C**, **Parton RG**, **Wolvetang EJ**, **Roost MS**, **Chuva de Sousa Lopes SM**, **Little MH**. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis. *Nature* 526: 564–568, 2015.

68. **Tan AY**, **Zhang T**, **Michaeel A**, **Blumenfeld J**, **Liu G**, **Zhang W**, **Zhang Z**, **Zhu Y**, **Rennert L**, **Martin C**, **Xiang J**, **Salvatore SP**, **Robinson BD**, **Kapur S**, **Donahue S**, **Bobb WO**, **Rennert H**. Somatic Mutations in Renal Cyst Epithelium in Autosomal Dominant Polycystic Kidney Disease. *J. Am. Soc. Nephrol.* (2018). doi: 10.1681/ASN.2017080878.

69. **Tang C**, **Ma Z**, **Zhu J**, **Liu Z**, **Liu Y**, **Liu Y**, **Cai J**, **Dong Z**. P53 in kidney injury and repair : Mechanism and therapeutic potentials. *Pharmacol. Ther.* (2018). doi: 10.1016/j.pharmthera.2018.10.013.

70. **Teixeira A**, **Edery P**, **Cochat P**. Cowden disease and multicystic dysplastic kidney : increased risk of renal cancer ? *Clin Kidney J* 5: 453–455, 2012.

71. **Thatava T**, **Armstrong AS**, **De Lamo JG**, **Edukulla R**, **Khan YK**, **Sakuma T**, **Ohmine S**, **Sundsbak JL**, **Harris PC**, **Kudva YC**, **Ikeda Y**. Successful disease-specific induced pluripotent stem cell generation from patients with kidney transplantation. *Stem Cell Res Ther* 2: 48, 2011.

72. **Thivierge C**, **Kurbegovic A**, **Couillard M**, **Guillaume R**, **Coté O**, **Trudel M**. Overexpression of PKD1 Causes Polycystic Kidney Disease Overexpression of PKD1 Causes Polycystic Kidney Disease. 26: 1538–1548, 2006.

73. **Trudel M**, **Lanoix J**, **Barisoni L**, **Blouin MJ**, **Desforges M**, **L'Italien C**, **D'Agati V**. C-myc-induced apoptosis in polycystic kidney disease is Bcl-2 and p53 independent. *J Exp Med* 186: 1873–84, 1997.

74. **Valentijn FA**, **Falke LL**, **Nguyen TQ**, **Goldschmeding R**. Cellular senescence in the aging and diseased kidney. .

75. **Yu T**, **Li YJ**, **Bian AH**, **Zuo H Bin**, **Zhu TW**, **Ji SX**, **Kong F**, **Yin DQ**, **Wang CB**, **Wang ZF**, **Wang HQ**, **Yang Y**, **Yoo BC**, **Cho JY**. The Regulatory Role of Activating Transcription Factor 2 in Inflammation. *Mediators Inflamm* 2014: 1–10, 2014.

76. **Zheng L**, **Cardaci S**, **Jerby L**, **Mackenzie ED**, **Sciacovelli M**, **Johnson TI**, **Gaude E**, **King A**, **Leach JDG**, **Edrada-ebel R**, **Hedley A**, **Morrice NA**, **Kalna G**, **Blyth K**, **Ruppin E**, **Frezza C**, **Gottlieb E**. Fumarate induces redox-dependent senescence by modifying glutathione metabolism. *Nat Commun* 6: 1–12, 2015.

77. **Zhou W**, **Otto E a**, **Cluckey A**, **Airik R**, **Hurd TW**, **Chaki M**, **Diaz K**, **Lach FP**, **Bennett GR**, **Gee HY**, **Ghosh AK**, **Natarajan S**, **Thongthip S**, **Veturi U**, **Allen SJ**, **Janssen S**, **Ramaswami G**, **Dixon J**, **Burkhalter F**, **Spoendlin M**, **Moch H**, **Mihatsch MJ**, **Verine J**, **Reade R**, **Soliman H**, **Godin M**, **Kiss D**, **Monga G**, **Mazzucco G**, **Amann K**, **Artunc F**, **Newland RC**, **Wiech T**, **Zschiedrich S**, **Huber TB**, **Friedl A**, **Slaats GG**, **Joles J a**, **Goldschmeding R**, **Washburn J**, **Giles RH**, **Levy S**, **Smogorzewska A**, **Hildebrandt F**. FAN1 mutations cause karyomegalic interstitial nephritis, linking chronic kidney failure to defective DNA damage repair. *Nat Genet* 44: 910–5, 2012.

NEDERLANDSE SAMENVATTING

ADPKD – ofwel cystenieren – is een ziekte waarbij er zeer veel vochtblazen (cysten) ontstaan in de nieren, waardoor het gezonde nierweefsel verdrongen wordt. Een groot deel van de patiënten ontwikkelt hierdoor nierfalen en moet dialyse of een niertransplantatie ondergaan. Op dit moment is er nog geen effectieve behandeling beschikbaar die het ontstaan van nieuwe cysten en cystegroei tegengaat. Bij het grootste deel van de ADPKD patiënten wordt de ziekte veroorzaakt door een mutatie in het *PKD1* gen dat codeert voor het polycystine-1 eiwit. Echter, wat de functie van *PKD1*/ polycystine-1 in de cel is en waarom disregulatie hiervan leidt tot cyste vorming is nog onbekend. Het doel van deze thesis was om meer inzicht te krijgen in het moleculaire mechanisme van cyste vorming wat essentieel is voor het ontwikkelen van nieuwe gerichte behandelingen tegen ADPKD. We hebben dit onderzocht door ons te richten op drie belangrijke vragen: ten eerste, wat is de functie van *PKD1*/polycystine-1 in de cel? Ten tweede, zijn er eiwitten aanwezig in cyste vloeistof die aan kunnen zetten tot groei van nieuwe cysten? Als laatste, welke transcriptie factoren reguleren het aan- en uitzetten van het *PKD1* gen?

Hoofdstuk 2 en 3 beschrijven de ontwikkeling van twee cellijnmodellen die gebruikt kunnen worden om de functie van polycystine-1 te bestuderen. Hierbij hebben wij gebruik gemaakt van geïnduceerde pluripotente stamcellen (iPSCs) of van nierorganoïden ontwikkeld uit volwassen stamcellen (ASC). In **Hoofdstuk 2** zijn iPS cellen genereerd om te dienen als ADPKD cellijnmodel. Hiervoor hebben wij tubulus epitheel cellen (TEC) geïsoleerd uit ADPKD nieren en gezond nierweefsel. Na verificatie van expressie van nier- en epitheelmarkers door de TECs, hebben we deze gebruikt voor het genereren van iPSCs. De iPSCs werden gevalideerd met betrekking tot hun karyotype, expressie van stamcelmarkers en de pluripotente differentiatie capaciteit. We zijn de eerste die ADPKD iPSCs hebben gemaakt vanuit cyste-epitheel. Andere groepen hadden al eerder iPSCs gemaakt van fibroblasten van ADPKD patiënten. De iPSCs gemaakt van cyste epitheel zijn van belang omdat somatische mutaties in de nierepitheelcellen mogelijk een rol spelen in het in gang zetten van cystegroei. In ons iPSC ADPKD model kunnen deze somatische mutaties bestudeerd worden. Mutatieanalyse van ADPKD-iPSCs liet zien dat de TEC en iPS cellen een heterozygote kiembaanmutatie in het *PKD1* gen bevatten, maar geen additionele somatische mutaties in *PKD1*. Wel vonden we somatische mutaties in diverse andere genen. Het is echter nog onbekend of deze somatische mutaties bijgedragen hebben aan de cystegroei. Daarnaast zagen we dat het RNA expressieprofiel van ADPKD iPSCs en wild type controles grotendeels overeenkwamen. Tenslotte laten we zien dat de iPCS die

gemaakt zijn vanuit niercellen een nier- en ziekte-specifiek DNA-methylatieprofiel hebben. Dit methylatieprofiel kan mogelijk voordelig zijn voor het genereren van gekweekt nierweefsel in het laboratorium, aangezien het als een soort geheugen voor de cel kan dienen over diens herkomst.

Hoofdstuk 3 beschrijft de ontwikkeling van een ADPKD cellijnmodel waarbij gebruik is gemaakt van nierorganoïden gegenereerd uit volwassen stamcellen (ASC adult stem cells). ASC geïsoleerd uit cystenieren en gezond nierweefsel werden gekweekt in 3D matrigeldruppels. Net als bij de ADPKD iPSCs, vonden wij in de ASC nierorganoïden een heterozygote kiembaanmutatie in het *PKD1* gen en additionele somatische mutaties in diverse genen maar niet in *PKD1*. Een interessante bevinding was dat ADPKD ASC nierorganoïden een genexpressie profiel hadden passend bij celveroudering, ofwel senescence. We hebben aangetoond dat er meer verouderde cellen aanwezig zijn in cystenieren ten opzichte van gezond nierweefsel. Daarnaast hebben we gevonden dat er verschillende eiwitten in cystevloeistof uitgescheiden worden waarvan bekend is dat ze worden uitgescheiden door verouderde cellen (SASPs, senescence associated secretory phenotype factors). Verder vonden we dat wanneer cystevloeistof wordt toegevoegd aan nierepitheel cellen in kweek, dit resulteert in toename de snelheid van celdeling, hetgeen suggereert dat SASPs mogelijk bijdragen aan cystegroei. Kortom, met dit nieuwe ASC nierorganoïden ADPKD model, hebben wij gevonden dat veroudering een kenmerk is ADPKD nieren. Door het verband te leggen tussen het ontstaan van cysten en veroudering van de nier hebben wij nieuwe inzichten gekregen in de pathofysiologie van erfelijke en verworven polycysteuze nierziekten.

In **hoofdstuk 4** hebben we getracht om eiwitten in cystevloeistof te identificeren die cystegroei kunnen veroorzaken door middel van paracriene signalen. cystevloeistof, van zowel ADPKD nieren als van ADPKD levers, induceert cystegroei wanneer het wordt toegevoegd aan nierepitheelcellen (RPTEC/hTERT1) in kweek. Door de eiwitten in cystevloeistof te fractioneren op basis van grootte en de activiteit van de verschillende fracties te testen, hebben we gevonden dat het cyste inducerende eiwit (of eiwitten) tussen de 30 en 100 kDa groot is. Verder hebben we gevonden dat de sterkte van het cystegroei inducerende effect van cystevloeistof uit verschillende cysten van dezelfde nier varieert. Een vergelijking van het proteoom van fracties van cystevloeistof met een sterk en zonder cyste-inducerend effect resulteerde in identificatie van 43 kandidaat-eiwitten die mogelijk cystegroei kunnen veroorzaken. Veel van deze kandidaten zijn enzymen, afweereiwitten, receptoren en signaaleiwitten. Van enkele van de kandidaat-eiwitten, zoals Secreted Frizzled-Related Protein 4, S100A8, osteopontin en CRELD1, is eerder beschreven dat zij een rol spelen in de pathogenese van ADPKD.

Deze kandidaten kunnen gebruikt worden voor verder onderzoek naar de rol van deze eiwitten in cystegroei in cellijn- of muismodellen en mogelijk uiteindelijk in ADPKD patiënten.

Tenslotte hebben meer inzicht proberen te krijgen in hoe de regulatie van *PKD1* genexpressie plaats vindt. In **hoofdstuk 5** hebben we met een nieuwe techniek (CasID), een screen gedaan om nieuwe transcriptie factoren te identificeren de *PKD1* expressie regelen. Deze techniek maakt gebruik van een niet-selectieve biotine ligase (BirA*) die gefuseerd is met een katalytisch inactief Cas9 eiwit (dCas9). Co-expressie in de cel met een single guide RNA zorgt ervoor dat het BirA-dCas9 fusie-eiwit heel gericht een specifieke plek in het DNA bindt. Eiwitten die zich daar bevinden worden door het BirA-dCas9 voorzien van een biotine label. Deze gebiotinyleerde eiwitten kunnen daarna geïdentificeerd worden door middel van een streptavidine pull-down en massaspectrometrie. Wij hebben deze CasID techniek ingezet om nieuwe eiwitten te identificeren die de *PKD1* promoter binden, door een single guide RNA te gebruiken die aangrijpt net voor de transcriptie start. Onze screen heeft enkele kandidaat transcriptie factoren opgeleverd, die in de literatuur eerder in verband zijn gebracht met ADPKD, zoals MAX, CUX1, ATF2 en ZEB2. Hoewel deze nieuwe *PKD1* transcriptie factoren nog gevalideerd moeten worden, laten onze resultaten zien dat CasID een geschikte techniek is voor het identificeren van nieuwe eiwitten die expressie van een specifiek gen reguleren.

In **hoofdstuk 6** bespreken we onze bevindingen in het licht van de huidige kennis omtrent de pathofysiologie van cyste vorming. Daarnaast worden toekomstige onderzoeksrichtingen besproken die kunnen bijdragen aan meer inzicht in het moleculaire mechanisme van cystogenese en het ontwikkelen van nieuwe therapieën voor ADPKD patiënten.

LIST OF ABBREVIATIONS

2D	Two-dimensional
3D	Three-dimensional
ACKD	Acquired cystic kidney disease
ADPKD	Autosomal dominant polycystic kidney disease
ARPKD	Autosomal recessive polycystic kidney disease
ASC	Adult stem cell
BioID	Proximity-dependent biotin identification
BirA*	Biotin ligase with an R118G mutation
Ca^{2+}	Calcium
CasID	proximity-dependent BirA*-dCas9 identification
cDNA	Complementary DNA
ChIP	Chromatin immunoprecipitation
dCas9	Catalytically dead Cas9
DNA	Deoxyriboducleic acid
EB	Embryoid bodies
ER	Endoplasmic reticulum
FACS	Fluorescence-activated cell sorting
FPC	Fibrocystin
hES cells	Human embryonic stem cell
IF	Immunofluorescence
Indel	Insertion or deletion mutation
iPSC	Induced pluripotent stem cell
LR-PCR	Long-range polymerase chain reaction
MeD-seq	Methylated DNA sequencing
MLPA	Multiplex Ligation-dependent Probe Amplification
mRNA	Messenger RNA
PKD	Polycystic kidney disease
PKD1	Polycystic kidney disease-1 gene
PKD2	Polycystic kidney disease-2 gene
RNA	Ribonucleic acid

RNA-seq	RNA sequencing
RPTEC/hTERT1	Immortalized human renal proximal tubular epithelial cell line
SASP	Senescence-associated secretory phenotype
sgRNA	Single guide RNA
SNP	Single nucleotide polymorphism
TEC	Tubular epithelial cells
WES	Whole exome sequencing
WT	Wild type

CURRICULUM VITAE

Annegien Kenter is geboren in 1982 te Aerdenhout. Na het afronden van de HAVO op het Lyceum Sancta Maria te Haarlem heeft zij Atheneum op het Nova College te Haarlem gedaan. In afwachting van toelating tot de geneeskunde studie heeft zij een jaar fysiotherapie en een jaar bewegingswetenschappen gestudeerd en tevens twee jaar in de verpleging gewerkt.

In 2004 werd zij toegelaten tot de studie geneeskunde aan de Erasmus Universiteit Rotterdam. Voor haar co-schappen is zij afgereisd naar de spoedeisende hulp van het Academisch Ziekenhuis Paramaribo te Suriname en voor tropengeneeskunde naar het Ndala Hospital te Tanzania. Over dit laatste co-schap heeft zij enkele columns voor het tijdschrift Medisch Contact geschreven. Tijdens haar studie was zij actief in diverse commissies van de Medische Faculteits Vereniging Rotterdam (MFVR) en daarnaast is zij tien jaar actief geweest als zeilinstructeur, begeleider, examinator en bestuurslid van Vinea Zeil- en Surfschool in Friesland. In 2012 behaalde zij haar artsexamen en werkte één jaar als ANIOS interne in het Maasstadziekenhuis en de Daniel den Hoed kliniek te Rotterdam.

Naast de studie geneeskunde voltooide zij een Master of Science Molecular Medicine aan de Erasmus Universiteit Rotterdam. Haar afstudeerthesis 'Skewing of X Chromosome Inactivation' voerde zij uit op het laboratorium van Prof. dr. J. H. Gribnau. Hierna schreef zij een promotie-onderzoeksvoorstel en subsidieaanvraag die werd gehonoreerd met een Kolff arts-onderzoeker beurs van de Nierstichting en een beurs van het Graduate Programme Erasmus MC - Medical Genetics van de Nederlandse Organisatie voor Wetenschappelijk Onderzoek (NWO). In 2013 startte zij met haar promotie onderzoek naar het moleculaire mechanisme van cystenieren aan de Erasmus Universiteit Rotterdam bij de afdelingen Ontwikkelingsbiologie, Interne geneeskunde – Nefrologie en Transplantatie en Celbiologie onder leiding van Prof. dr. J. H. Gribnau, Prof. dr. R. Zietse, Prof. dr. E. J. Hoorn en dr. G. Jansen. Tevens maakte ze onderdeel uit van het eerste vrouwentalentenprogramma van het Erasmus MC: Jonge Vrouwen in de Academie. Daarnaast werd zij geselecteerd voor het Kidney Stars programma van de American Society of Nephrology (ASN) Kidney week in San Diego, Amerika.

Sinds 2018 is zij werkzaam bij de interne geneeskunde in het Reinier de Graaf Gasthuis te Delft. In 2019 is zij onder supervisie van dr. H. Boom en A.A.M. Zandbergen gestart met de opleiding tot internist.

PHD PORTFOLIO

PhD candidate:	Annegien Kenter
PhD period:	2013 – 2019
Departments:	Developmental Biology, Cell biology, Internal Medicine-Nephrology
Research school:	Medisch-Genetisch Centrum Zuidwest (MGC)
Promotoren:	Prof. dr. Joost H. Gribnau
	Prof. dr. Robert Zietse
	Prof. dr. Ewout J. Hoorn
Copromotor:	Dr. Gert Jansen

General academic and research skills	**Year**
Introduction to confocal microscopy (OIC, EMC)	2013
Biochemistry and Biophysics, MGC	2013
Genetics, MGC	2014
Cell and Developmental Biology, MGC	2014
Biomedical Research Techniques, MolMed	2014
Veilig Werken in laboratoria, MGC	2015
Biomedical English Writing and Communication, EMC	2015
Ensembl: Browsing the genome, MolMed	2015
Course on 'R' programming, MolMed	2016
Photoshop, illustrator, Indesign	2016
Biostatistical Methods I, NIHES	2016

Courses, seminars and workshops	**Year**
Cell Biology and Developmental biology department seminars and journal club, EMC	2013-2017
Regionale Klinische avond Interne Geneeskunde, Rotterdam	2013-2017
Dutch Kidney Foundation Winterschool, Driebergen	2014
Renal fluid and electrolytes from bench to bedside (WGIKD), Nijmegen	2014
New Frontier in Cilia Medicine, Nijmegen, the Netherlands	2015
Cilia meeting EMBO, Amsterdam	2016
Medical Business Masterclass, Stichting Medical Business, Amsterdam	2017
Talentenprogramma Erasmus MC: 'Jonge Vrouwen in de Academie'	2017

International conferences	Year
FASEB PKD, from molecular mechanism to therapy, Lucca, Italy (poster)	2014
ASN Kidney week and early program 'building a kidney; from stem cell to function', Philadelphia USA	2014
Harvard Center for PKD Research, PKD group meeting Harvard, Boston, USA (presentation)	2015
Kidney: Development Disease and Regeneration Nephrotools, Liverpool, UK	2015
ASN Kidney week and early program 'Polycystic Kidney Disease', San Diego, USA	2015
ASN Kidney week, Chicago, USA	2016
FASEB PKD, Big Sky Montana, USA (poster)	2017

Teaching activities and supervising students	Year
V. Xavier, (Master of Science in Cell and Stem Cell Biology, Université Pierre et Marie Curie, Paris, France) Master's Thesis: "Unravelling Polycystic Kidney Disease"	2016
J. Okai, (Life science, Hogeschool Rotterdam) 2nd year internship	2016-2017
M. Hosseinzadeh (Life science, Hogeschool Rotterdam) Final thesis	2016-2017
Medical students: 'VO: Vroege embryo's, klonen en transgenese'	2017

Grands	Year
Kolff Physician Researcher Grant, Dutch Kidney Foundation	2012
Graduate Programme Erasmus MC - Medical Genetics, NWO	2013
ASN kidney week, Kidney STARS program and travel grant	2015
MRACE (co-author): 'Kidney Regeneration by use of induced Pluripotent Stem Cells'	2015
Trustfonds travel grant, Erasmus University	2017

LIST OF PUBLICATIONS

Iris Jonkers, Tahsin Stefan Barakat, Eskeatnaf Mulugeta Achame, Kim Monkhorst, **Annegien Kenter**, Eveline Rentmeester, Frank Grosveld, J. Anton Grootegoed, and Joost Gribnau.RNF12 Is an X-Encoded Dose-Dependent Activator of X Chromosome Inactivation. *Cell.* 2009 nov 25;139(5):865-7

Tahsin Stefan Barakat, Nilhan Gunhanlar, Cristina Gontan Pardo, Eskeatnaf Mulugeta Achame, Mehrnaz Ghazvini, Ruben Boers, **Annegien Kenter**, Eveline Rentmeester, J. Anton Grootegoed and Joost Gribnau. RNF12 activates Xist and is essential for X chromosome inactivation. *PLoS Genetics.* 2011 jan 27;7(1)

Annegien Kenter, Sari E. van Rossum-Fikkert, Mahdi Salih, Paul C.M.S. Verhagen, Geert J.L.H. van Leenders, Jeroen A.A. Demmers, Gert Jansen, Joost H. Gribnau, Robert Zietse, Ewout J. Hoorn. Identifying Cystogenic Paracrine Signaling Molecules in Cyst Fluid of Patients with Polycystic Kidney Disease. *AJP Renal Physiology* 7 Nov 2018

Annegien Kenter, Eveline Rentmeester, Job van Riet, Ruben Boers, Joachim Boers, Mehrnaz Ghazvini, Vanessa J. Xavier, Geert J.L.H. van Leenders, Paul C.M.S. Verhagen, Marjan. E. van Til, Monique Losekoot, Dorien J.M. Peters, Wilfred F.J. van IJcken, Harmen J.G. van de Werken, Robert Zietse, Ewout J. Hoorn, Gert Jansen, Joost H. Gribnau. Cystic renal-epithelial derived Induced Pluripotent Stem Cells from Polycystic Kidney Disease patients. *Submitted for publication*

Annegien Kenter, Frans Schutgens, Hester van Willigenburg, Eveline Rentmeester, Job van Riet, Harmen J.G. van de Werken, M.J. Hoogduijn, Geert J.L.H. van Leenders, Paul C.M.S. Verhagen, Wilfred F.J. van IJcken, R.W.F. de Bruin, Peter L.J. de Keizer, Marjan C. Verhaar, Hans Clevers, Gert Jansen, Robert Zietse, Maarten B. Rookmaaker, Dorien J.M. Peters, Ewout J. Hoorn, Joost H. Gribnau. Senescence is a hallmark of Polycystic Kidney Disease. *Submitted for publication*

Annegien Kenter, Sarra Merzouk, Jeremy Okai, Mitra Hosseinzadeh, Robbert-jan T.S. Palstra, Jeroen A.A. Demmers, Ewout J. Hoorn, Robert Zietse, Joost H. Gribnau, Gert Jansen. Using CasID to screen for transcription factors that regulate PKD1 expression. *Manuscript in preparation*

Luiz Gustavo Dufner de Almeida, Santoesha Nanhoe, Andrea Zonta, Mitra Hosseinzadeh, Regina Kom-Gortat, Peter Elfferich, Gerben Schaaf, **Annegien Kenter**, Daniel Kümmel, Nicola Migone, Sue Povey, Rosemary Ekong and Mark Nellist. Comparison of the functional and structural characteristics of rare TSC2 variants with clinical and genetic findings. *Submitted for publication*