Renal genetics in transition

On the clinical relevancy of genetic testing in renal disease

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in renal disease

Niergenetica in transitie
Over de klinische relevantie van genetisch testen bij nierziekten

(met een samenvatting in het Nederlands)

Proefschrift

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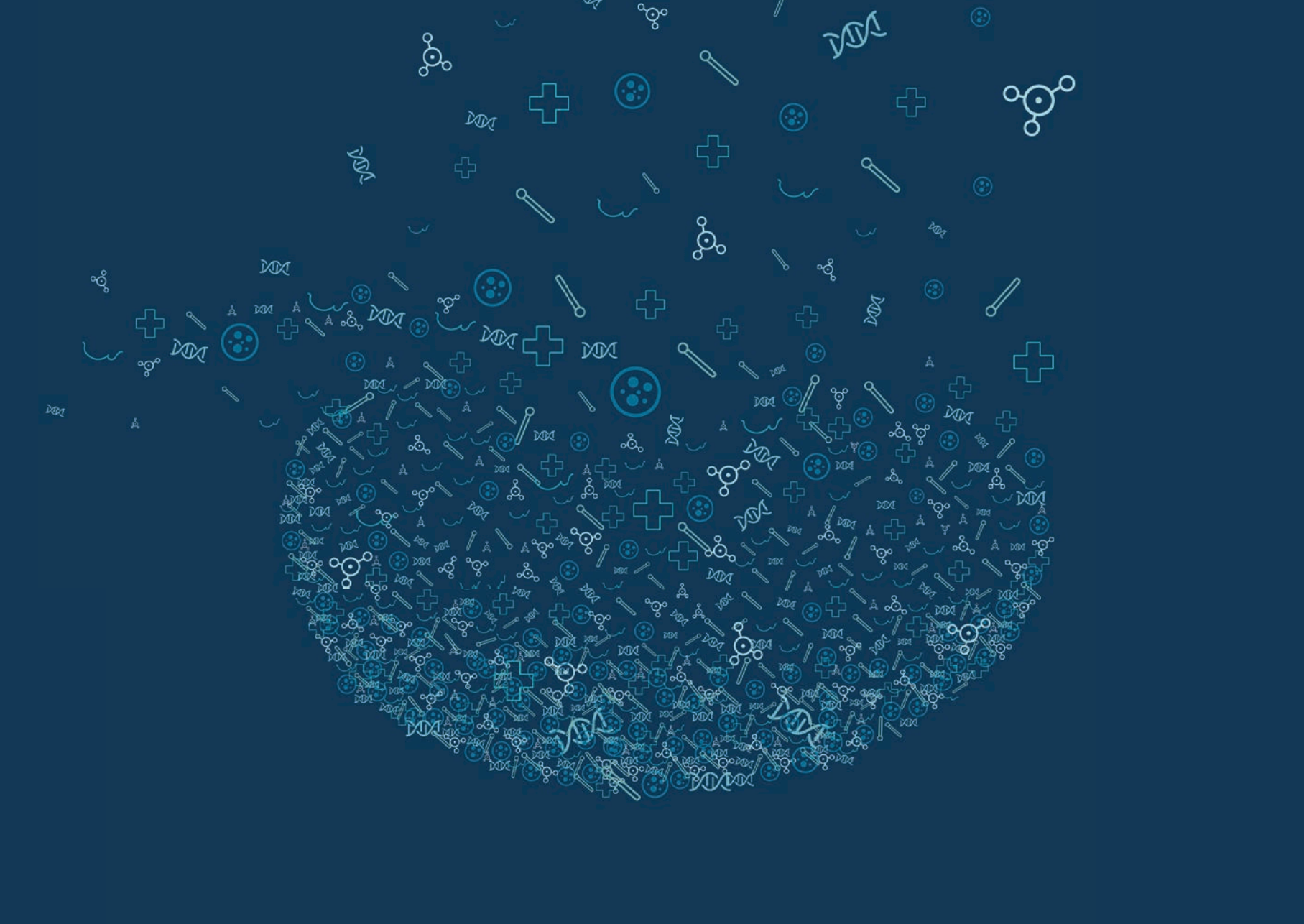

Introduction: Monogenic causes for kidney failure

Rozemarijn Snoek, Albertien M. van Eerde, Nine V.A.M. Knoers

Adapted and expanded from:
Importance of reliable variant calling and clear phenotyping when reporting on gene panel testing in renal disease

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In chronic kidney disease (CKD) the kidneys lose their ability to filter, reabsorb and secrete water, electrolytes and other small molecules from the blood into the urine, and keep larger molecules such as proteins in.[1] The prevalence of CKD is estimated to be 10-13%, and continually increasing.[2–4] Disease burden is high in CKD, as it is associated with increased risks of cardiovascular morbidity, premature mortality and decreased quality of life.[2–4]

CKD is defined by decreased glomerular filtration rate (GFR) or the amount of protein lost in the urine (proteinuria), for at least 3 months.[5–8] GFR and degree of proteinuria are also used to determine the severity of renal disease, from mild disease in stage 1 to end-stage renal disease (ESRD) in stage 5.[5,9–11] In ESRD the kidneys are failing, requiring renal replacement therapy in the form of dialysis or a renal transplant.[5,9–11]

1.1 Underlying causes of CKD

The criteria to stage CKD are based on laboratory findings, and do not depend on nor characterize the underlying cause for the renal disease.[5–8] Nevertheless, identifying the underlying cause is essential, because it gives information regarding the prognosis and can influence treatment decisions (addressed in detail below). To identify the underlying cause, the tools routinely used are blood and urine investigations, renal ultrasounds or computed tomography, and invasive renal biopsies.[5–8,12–14]

However, accurately determining the underlying cause in a specific patient can prove difficult. This is because CKD can be caused by many different disorders that often have indistinguishable clinical features. The type of diseases that cause CKD range from highly prevalent diseases such as diabetes and hypertension, to less prevalent conditions like IgA nephropathy and finally to very rare disorders, for example Fabry disease.[1,15,16]

1.2 Monogenic renal diseases

Interestingly, the very rare causes of CKD are often monogenic kidney diseases (MGKD). MGKD are the result of mutations in genes encoding proteins essential for renal structure or function.[17] Also called Mendelian diseases, after their discoverer Gregor Mendel (1822-1884), monogenic diseases are caused by defects in a single gene.[18,19] The defect can be in one autosomal allele (autosomal dominant disease), two autosomal alleles (autosomal recessive disease), or it can be in a gene on the X or Y chromosome (sex linked disease).[18,19]

Though each individual disease is rare, together MGKD are estimated to account for 10-15% of the overall prevalence of ESRD in adults and 70% in children.[20–24] These are likely underestimations, since many studies only include a specific population or exclude patients with an already established genetic diagnosis.[25] For instance, in adult familial disease diagnostic yields of 38% have been reported, while a study that excluded patients with a genetic diagnosis still reported a yield of 10%.[26,27]

MGKD can affect the kidney in different ways, from aberrant gross organ development to a faulty subcellular structure.[17,21,28] The knowledge of genotype-phenotype relationships in MGKD is continually evolving, expanding the types of diseases a specific gene can be associated with.[28,29] MGKD are a heterogeneous group of disorders, with the phenotype characteristics varying between diseases and even within specific disorders.[17,21,28] Thus, clear phenotyping is key in identifying a possible MGKD patient. The main phenotypical characteristics that increase the likelihood of MGKD are familial disease, unusually severe disease, extra-renal features and disease presenting at a young age.[17,21,26,28]

Identification of cases with possible MGKD is essential, in order to provide adequate genetic testing.[30] As **Chapter 2** shows, there is a significant under- and misdiagnosis of MGKD, leading to a call for a "genetics first" approach in ESRD patients.

1.3 Diagnosis of MGKD with next-generation sequencing (NGS)

The possibilities of accurately diagnosing MGKD have increased since the advent of NGS.[31,32] This genetic testing technique enables simultaneous sequencing of the complete genome (whole genome sequencing), all ~21,000 human genes (whole exome sequencing), or a smaller subset of genes associated with a particular phenotype (disease-associated multigene panels).[31–33] Therefore NGS is flexible in its application, especially since cost and turnaround time are steadily decreasing.[34]

NGS creates a bulk of genomic data, thus the sequencing data need to be adequately annotated and filtered for variant calling.[35] Estimating the pathogenicity of variants is performed based on the population frequency of a variant, the in silico prediction of the variant's effect on a.o. the protein structure, if the variant could explain the phenotype, and if the variant segregates with the disease in the family (Figure 1.1).[35–38] If a variant is then deemed (likely) pathogenic, results can be translated back to the individual patient.[30]

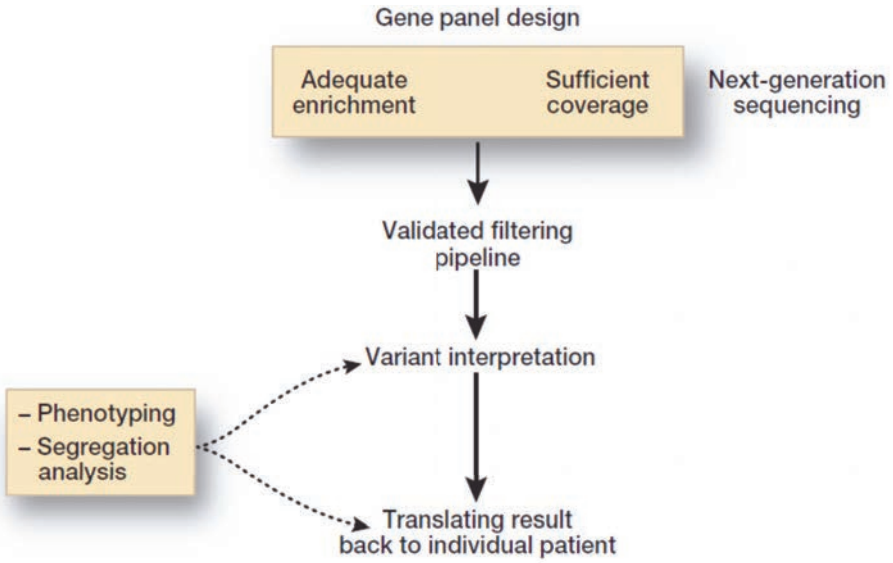


Figure 1.1 | The process of gene panel testing for renal disease: gene panel design with the use of next-generation sequencing techniques, variant calling, and interpretation, as well as the influence of phenotyping and segregation analysis in variant interpretation and translating the result back to the individual patient.

Definite calling of pathogenicity is impossible however, if a variant is detected in a gene that has not previously been associated with the patient's phenotype.[19,39] In those cases, *in vitro* or *in vivo* functional studies can be performed to determine the causal relationship between mutations in a certain gene and a particular phenotype.[40–47] For example, by showing that cells or a model organism in which the gene is knocked out show a phenotype similar to the patient.[40–47] With the development of the CRISPR-Cas9 technique, the functional studies field has taken flight, as the technique allows for high-throughput testing.[41,42,48]

1.4 The major genetic causes for CKD

Genetic causes of CKD are more prevalent in the young-onset CKD population, for ~20% of all renal diseases that progress to ESRD before age 25 are estimated to be genetic.[21] Therefore, MGKD is a large contributor to disease burden in the young ESRD population. Notably, three disease groups by themselves cause ~65% of young-onset ESRD, namely congenital anomalies of the kidney and urinary tract (CAKUT), steroid-resistant nephrotic syndrome (SNRS) and renal ciliopathies.[21]

CAKUT, the collective term for all congenital structural anomalies of the kidney and urinary tract, is estimated to cause 49% of all young-onset CKD.[21] Due to a complex and yet unsettled disease etiology, it can be difficult to find an underlying genetic cause in CAKUT. Recent studies show a diagnostic yield of genetic testing of 14% in familial CAKUT.[27,49] As CAKUT is congenital, it can present in utero and be identified by fetal ultrasound.[50–52] However, disease severity and renal function outcome is variable in CAKUT, within CAKUT subtypes and even within families.[53,54] This makes prognostication challenging. To develop a non-invasive way to predict outcome in fetuses with CAKUT, **Chapter 4** focusses on discerning the fetal renal development in congenital solitary functioning kidneys (a subtype of CAKUT) by assessing the number of renal papillae with fetal ultrasound.

Steroid-resistant nephrotic syndrome (SRNS) is the second main cause of young-onset CKD, with a prevalence of ~10%.[21] Nephrotic syndrome is caused by high molecular weight protein loss that can arise due to damage to the podocytes ('footcells'), renal cells which with their 'feet' create a slit diaphragm providing ultrafiltration in the glomerulus.[55] If the nephrotic syndrome does not respond to corticosteroid treatment, it is an indicator of genetic disease.[56–58] SRNS can be congenital, which greatly increases the likelihood of a genetic disease.[59] **Chapter 5** describes the functional experiments to prove the causality of mutations in the novel genes *YRDC* and *GON7* in Galloway-Mowat syndrome, in which SRNS is a major symptom. Another phenotype that is characterized by podocyte damage and presents with nephrotic syndrome or isolated proteinuria, is focal segmental glomerulosclerosis (FSGS).[60,61] **Chapter 3** illustrates the importance of genetic testing in FSGS, by showcasing the challenges and clinical implications of genetic diagnostics in three cases of young adult-onset FSGS.

Renal ciliopathies represent ~5% of all young-onset CKD causes.[21] Ciliopathies are the result of mutations in genes essential to the primary cilium, an organelle protruding from almost every mammalian cell.[62–64] Though its precise function in the kidney has not been elucidated yet, some hypothesize the cilium provides a flow sensor in the tubules.[62–65] Ciliopathies have a broad phenotype. For example the disease nephronophthisis presents with small kidneys and ESRD around age 13, while the most prevalent renal ciliopathy (autosomal dominant polycystic kidney disease, ADPKD) entails large cystic kidneys and onset of ESRD around age 60.[64,66,67] A further expansion of the ciliopathy phenotypical spectrum is offered in **Chapter 6** where we show, through the genotyping of >5600 renal transplant recipients, that the classic pediatric-onset ciliopathy NPHP1-nephronophthisis in fact also causes 1 in 200 cases of adult-onset ESRD.

1.5 Implications of MGKD diagnosis

Genetic testing can offer information on the cause of the disease, but can also give direction to therapy (e.g. coenzyme-Q10 supplementation in SNRS caused by mutations in the co-Q10 pathway) and guide decisions regarding renal transplantation from a related donor.[58,68,69] In a significant number of cases genetic testing can even be a low-invasive first mode of diagnostics and obviate the need for an invasive renal biopsy.[70] In addition, family members may be at risk of having the same disorder and can thus be counselled their individual risks and the possibility of presymptomatic (genetic) testing. This offers presymptomatic carriers the opportunity to receive treatment in early CKD stages halting rapid disease progression (e.g. angiotensin-converting enzyme inhibitors in *COL4A3-5*-related disease) and severity of secondary symptoms such as hypertension.[17,71–74]

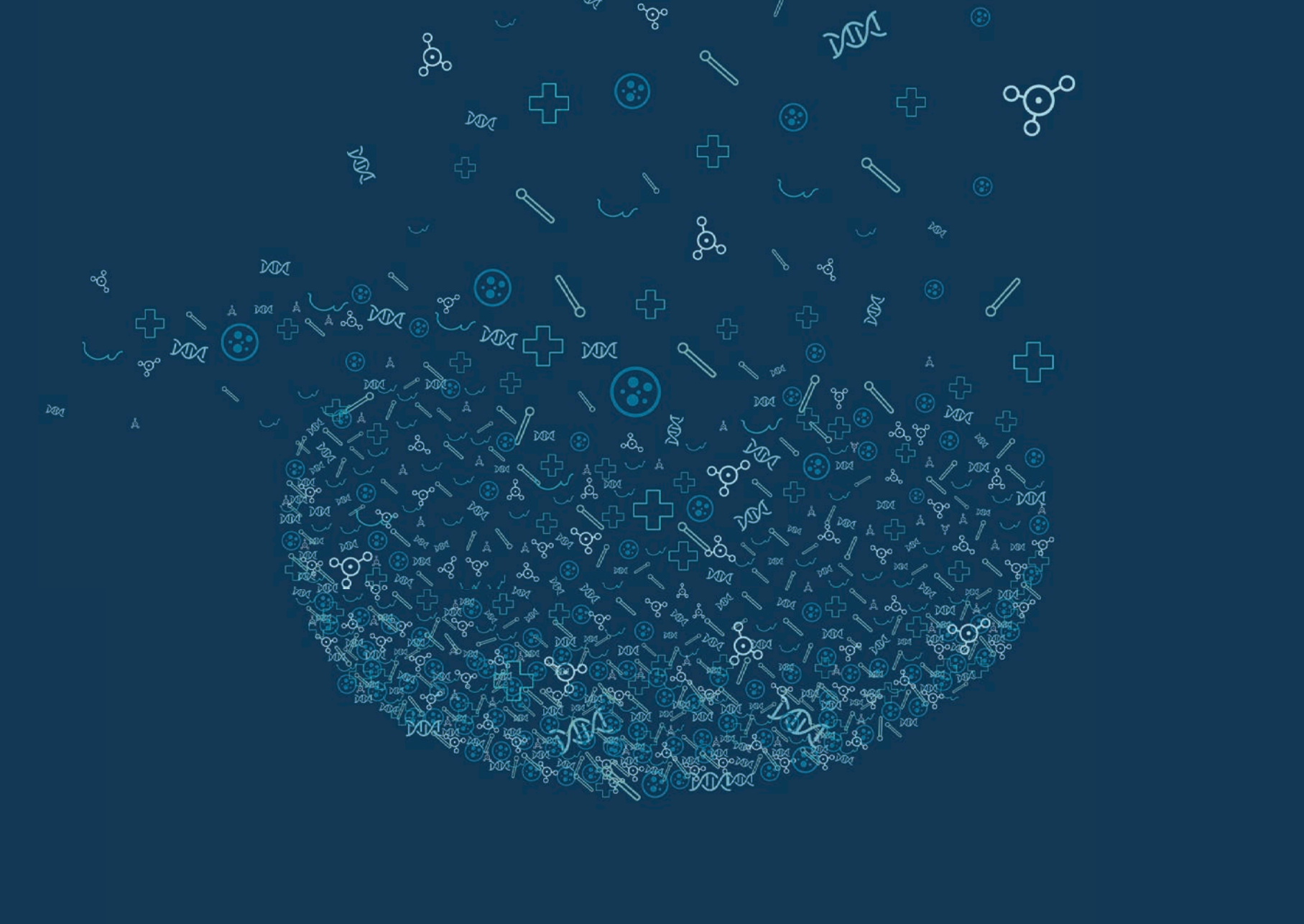
Unique to the young CKD population is the impact a MGKD diagnosis can have on family planning. Depending on the disease, a MGKD patient has up to 50% chance of passing the disease on to their off spring.[18,19] Depending on disease severity, patient preference and local availability, MGKD couples have many options regarding family planning. These include testing whether a fetus carries the same MGKD with prenatal diagnostics (PND).[28,75–79] In specific cases, patients can also opt for performing pre-implantation genetic testing (PGT, 'embryoselection') to prevent passing on the disease to future off spring.[28,80,81] **Chapter 9** showcases the unique and extensive Dutch experience with PGT for MGKD.

Pregnancy in CKD is associated with an increased risk of maternal and fetal pregnancy complications such as pre-eclampsia, prematurity and low birthweight.[82–89] Furthermore, pregnancy also has a negative impact on the renal outcome, meaning that pregnancy will likely worsen the renal function and consequently the patient may go into ESRD earlier.[83,84,90] **Chapter 8** presents a case series of female patients with *COL4A3-5*-related disease (Alport syndrome), taking stock of the maternal and fetal outcomes in these women.[73,74,91] **Chapter 7** offers perspective on good clinical practice when counselling young CKD patients who want to become pregnant, by providing ways to deal with these dilemmas and to adequately secure decision-making in daily practice.

1.6 Renal genetics in transition

In **Chapter 10** the studies presented in this thesis are further discussed, offering context and an outlook into future applications and impact of genetic testing in CKD. The work for this thesis has also culminated in a national clinical practice

recommendation for nephrologists (**Appendix 1**). Since its implementation in early 2019, the recommendation has aided in recognizing, counselling and diagnosing MGKD patients in Dutch daily clinical practice. Lastly, **Appendix 2** is the result of the author's contribution to a think-tank of young scientists, who call other young researchers into action to transform the scientific landscape.



‘Genetics first’ approach improves diagnostics of ESRD patients younger than 50 years

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Submitted

Abstract

In clinical practice only chronic kidney disease (CKD) patients with a higher likelihood of genetic disease are offered genetic testing. Early genetic testing could obviate the need for invasive kidney biopsies, and allow for precise prognostication and adequate treatment. To test the viability of such a 'genetics first' approach for CKD, we performed multi-gene panel testing in a group of renal transplant recipients <50 years, irrespective of cause of transplant.

In total, 273 patients had received a first renal transplant prior to the age of 50 years. Patients had to be in care in het UMC Utrecht, have DNA available and be without clear-cut non-genetic disease. Forty-three patients had been diagnosed with a genetic disease prior to enrollment, in the remaining 70 patients we performed a whole exome sequencing based multi-gene panel analysis consisting of 379 known renal disease genes.

Genetic analysis of 113 patients yielded a genetic diagnosis in 52%, reclassifying the original clinical diagnosis in 6%. Extrapolated to the 273 patients transplanted in our center, who did not all fit the inclusion criteria, the diagnostic yield was still 22%. In six of the 14 patients with a diagnostic kidney biopsy, the genetic diagnosis was different from the histological diagnosis.

Burden of monogenic disease in transplant patients with end-stage renal disease (ESRD) of any cause prior to the age of 50 is 52%. In 43% of cases with a renal biopsy the biopsy would not have had added diagnostic value if genetic testing had been performed as a first tier diagnostic. This shows that early genetic testing can provide a non-invasive diagnostic test that impacts prognostication and treatment and can obviate the need for an invasive biopsy. We conclude that in patients who one expects to develop ESRD prior to the age of 50, genetic testing should be considered as first mode of diagnostics.

2.1 Introduction

Chronic kidney disease (CKD) has a continually increasing prevalence, with latest estimates of 10-13% in the general population.[1–4] Especially for those patients that progress to end-stage renal disease (ESRD), there are increased risks of related morbidity and mortality, which prove to be a worldwide healthcare burden.[2–4] ESRD is caused by many underlying conditions, and therefore finding the underlying cause can be challenging.[1–4] One type of underlying causes that is not picked up by routine diagnostics, and thus can be overlooked, are monogenic kidney diseases (MGKDs).

MGKDs are caused by variants in genes that encode proteins essential for renal structure, function or development.[17,21,28] Despite each specific disease being rare, together MGKDs are estimated to account for 70% of the overall CKD prevalence in children and 10% in adults.[20–24] These MGKD prevalences are rough estimates, as many studies only include a specific population or exclude patients with an already established genetic diagnosis.[25] Depending on the extent of the genetic testing and the group of patients selected for a study, diagnostic yields vary from 10-73%.[26,27,92–99]

In both clinical practice as well as in research, often only patients with an increased likelihood of MGKD undergo genetic testing.[26,27,100,101] The likelihood of MGKD is higher in patients with specific phenotypes, CKD at an age <25 years, renal disease with extra-renal symptoms, familial disease and with unusually severe disease.[17,21,28] Nevertheless, we have previously shown that MGKDs can also have a late adult-onset, even in the classically pediatric MGKD nephronophthisis.[102] The use of next generation sequencing in MGKDs has led to an expansion in our understanding of the phenotypic spectrum, both across and within current kidney disease categories.[28,29]

Genetic testing has the potential to provide adequate diagnosis of the underlying cause for CKD through a minimally invasive and increasingly cost-effective test.[34] Such a 'genetics first' approach could reduce the need for invasive diagnostic kidney biopsies, allow for more precise prognostication and adequate etiology-based treatment.[17,70–74,91] It can also impact family members, in case of living related renal transplantation, pre-symptomatic nephrological care, genetic counselling, and questions regarding family planning.[17,28]

To test the viability of a genetics first approach for CKD in daily practice, we performed genetic testing in a broad group of renal transplant recipients, including all patients with a first transplant at an age <50 years without a clear-cut non-genetic disease. We deliberately did not select for familial cases, cases with a very

young age at onset, or phenotypes with a high likelihood of an underlying genetic cause, to best match the population in the daily nephrology out-patient clinic eligible for a genetics first approach. We applied a multi-gene panel (379 genes) and analyzed its diagnostic yield. In addition, we evaluated the added value of genetic testing as a first tier diagnostic when compared to invasive kidney biopsies.

2.2 Methods

Approval for this study was granted by the University Medical Center Utrecht (UMC Utrecht) Institutional Review Board. The inclusion criteria were: (1) the patient had received one or more renal transplant(s) in our center, (2) the first transplant was at an age <50 years, and (3) residual DNA from transplant-related human leukocyte antigen (HLA) typing was available. Since DNA-based HLA typing was first performed in our center in 2005, we only included patients that were transplanted after that time. Patients who were not in care in the UMC Utrecht anymore or had a clear-cut non-genetic diagnosis (i.e. infectious disease, loss of renal function within 7 days of using a nephrotoxic drug or diabetic nephropathy) were excluded. Patients with other types of CKD, e.g. related to long-term drug use or post-traumatic disease, were not excluded as there was no way to rule out the possibility of a genetic disease in those patients. Prior to being enrolled, patients provided informed consent for sequencing using a WES-based multi-gene panel (379 genes, Supplemental Table 2.1). Information on patient and clinical disease characteristics were retrospectively obtained from the patients' electronic medical records. If a genetic diagnosis had been established in regular diagnostic care prior to enrollment of this study, only clinical data was gathered and no additional sequencing was performed.

Genomic DNA was isolated from EDTA anti-coagulated blood using the MagNA Pure Compact system (Roche Diagnostics). After initial HLA typing at time of transplant, it was stored at -80 degrees until retrieval for WES.

Exomes were enriched using the SureSelectXT Clinical Research Exome V2 (Agilent [elid S30409818], genome build GRCh37) and sequenced on a Illumina Novaseq 6000 targeting a minimal average sequence depth of >100X. The sequencing data was processed with an inhouse developed pipeline, IAP v2.7.0, based on the Genome Analysis Toolkit (GATK v3.8-1-0-gf15c1c3ef) best practices guidelines.[103,104] The read pairs were mapped with BWA-MEM v0.7.5a, marking duplicates and merging lanes using Sambamba v0.6.5 and realigning indels using GATK IndelRealigner.[105,106] Next, the GATK Haplotypecaller was used to call single nucleotide polymorphisms and indels, creating variant call formatted files.

Variants were annotated, filtered and prioritized using the Alissa Interpret Clinical Informatics Platform (Platform dataset version 17, Agilent) based on whether they were located in a gene of interest by applying the 379 multi-gene panel (Supplemental Table 2.1) and a validated filtering tree routinely used in our genome diagnostics center. Tree filtering criteria were that the variant be exonic or intronic within 20 base pairs of the exon boundary and a maximum allele frequency of 0.5% in the GnomAD database (version 3), consisting of >140,000 healthy controls.[107] At least one functional effect predictor should predict the variant to be "likely pathogenic" (PolyPhen2 HumDiv and HumVar prediction, SIFT score <0.05) and at least one of the conservation scores should predict high conservation, namely Grantham score >100, GERP++ >2 or PhyloP >2.5.[37,108–111] All analyses were finished by November 1st 2019.

Patients were evaluated according to our standard clinical practice, fitting the American College of Medical Genetics (ACMG) criteria, and all laboratory processes were performed in our ISO15189 accredited diagnostic laboratory.[39] All filtered variants were manually assessed by two research reviewers. Decisions were re-assessed by a trained clinical genetic labspecialist reviewer, discrepancies were resolved through consensus discussion with all three reviewers. Manual review involved the following stringent criteria: variants were excluded in case of synonymous variants, intronic variants with no predicted effect on splicing according to the Splice Prediction Module in Alamut Visual (Version 2.14), the variant being present in <10 reads and in case of a single variant being present in a heterozygous state in a known autosomal recessive disease gene.[112] Variants were then assessed on GnomAD allele frequency, with the cut-of dependent on the associated disease population frequency, i.e. the specific allele frequency could maximally explain 10% of all disease cases.[107] The final step of manual review involved assessment of whether the disease associated with that particular variant could fit the known patient phenotype, based on review of the medical records, the OMIM database, the ClinVar database and literature.[113,114] All variants that through manual review were deemed to be an ACMG class 4 (likely pathogenic) or class 5 (pathogenic) variant were independently validated with Sanger sequencing.[39]

Copy number variation (CNV) detection was performed using in-house modified version of ExomeDepth.[115,116] Exomedepth uses an algorithm to determine CNVs based on the read depth information of a patient compared to a matched reference set. The reference set used consisted of 145 female and 124 male control samples with similar library preparation and sequencing as our patients. Samples were matched to either the female or male reference set based on their genetically determined sex. Target regions for CNV calling were based on robust capture

regions of the Agilent SureSelect CREv2 (elid S30409818) enrichment design; robust regions were determined on 106 control samples (55 females, 51 males) and defined as regions with an average coverage between 30X and 500X, and with low variability (coefficient of variation less or equal to 20%). Only CNVs spanning the regions of the genes on the 379 multi-gene panel were assessed.

Clinical and histological patient data was compared to the genetic results. If the genetic testing revealed a diagnosis that differed from the original clinical and/or histological diagnosis or if the genetic result would have caused a change in patient management, we defined this as a 'reclassification of the original diagnosis'. As a genetic diagnosis always has an impact on available options for family planning, this impact was not regarded as 'a change in patient management'.

If a patient was diagnosed with a MGKD and a native kidney biopsy at time of diagnosis was available, this biopsy was reexamined by one blinded observer according to current diagnostic standards. Because the original immunofluorescence (IF) slides and electron microscopy (EM) pictures were not available, these findings were retrieved from the original pathology reports. Only the light microscopy glass slides were reexamined. The observer did not have access to clinical data of the patients. Control biopsies, both from the subset of the cohort without a MGKD diagnosis and biopsies from outside the study, were also reexamined to allow for blinding (data not shown). The blinded observer also scored the likelihood of MGKD based on the biopsy reexamination on a 5 point qualitative scale.

χ^2-tests and Mann-Whitney U tests were performed with SPSS for Windows (version 25, IBM, NY).

2.3 Results

A total of 273 patients received their first transplant at an age <50 years since 2005 in our center (Figure 2.1), of whom 54 were not in care in the UMC Utrecht anymore and for 24 patients the stored DNA was not of sufficient quantity or quality. 31 patients had a clear-cut non-genetic renal disease, namely 27 patients with infectious disease, two patients with nephrotoxic drug-use and onset of kidney disease within 7 days and two patients with diabetic nephropathy. We approached 164 patients for enrollment, of whom 49 declined to participate and two patients were not enrolled as previous genetic testing had led to a likely pathogenic variant in a candidate gene.

2.3.1 Diagnostic yield of genetic testing

The remaining 113 patients were included, for 43 a genetic diagnosis had been established prior to enrollment and 70 patients were sequenced according to the methods described above. Baseline characteristics for all 113 patients are shown in

Table 2.1 | Baseline characteristics of the n=113 patients described in this study, including the separate baseline characteristics in the n=43 patients who received a molecular diagnosis prior to the start of this study and the n=70 patients who were sequenced in this study. These characteristics did not differ significantly between groups.

Characteristic	Whole cohort (n=113)	Patients with molecular diagnosis prior to the start of this study (n=43)	Patients sequenced in this study (n=70)	Patients with prior molecular diagnosis versus sequenced in this study (*p*-value)
Male (%, n)	61 (n=69)	54 (n=23)	66 (n=46)	0.57*
Age at onset of renal disease (years, median [range])	23 (0-47)	22 (0-43)	24 (0-47)	0.24**
Age at ESRD (years, median [range])	36 (2-49)	37 (2-49)	36 (2-49)	0.75**

ESRD=end-stage renal disease, *=χ^2-test, **=Mann-Whitney U test

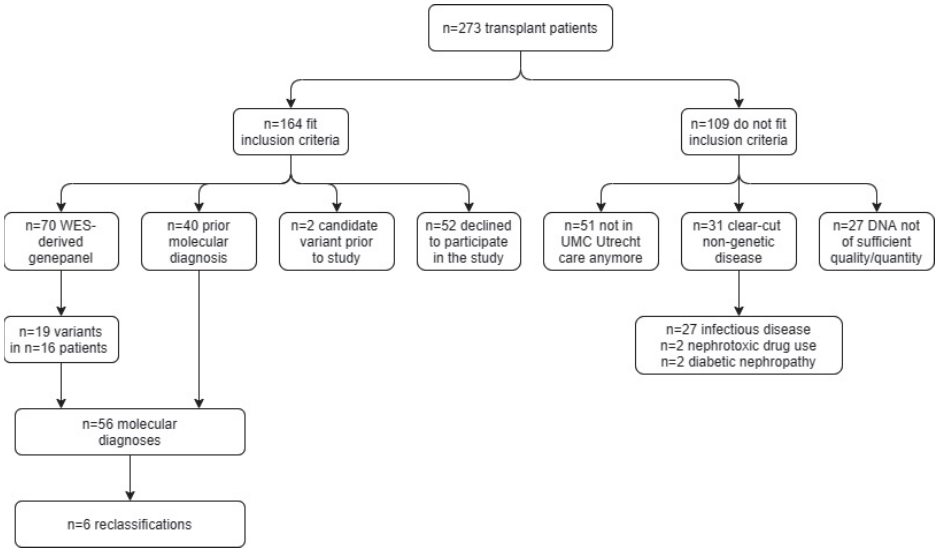


Figure 2.1 | Flowchart of patients eligible for enrollment in the study, including patients that were excluded and the reason for exclusion.

Table 2.1. Median age at ESRD was 36, with 13% of the cohort reaching ESRD as a child. There were no significant differences in the baseline characteristics between the patients with a prior genetic diagnosis compared to the patients sequenced in this study.

Sequencing to a minimal whole exome depth of 100X resulted in an average >15X coverage of the targeted bases in the gene panel of >99,5%. This yielded 1543 variants in the 70 patients that were sequenced. We identified 1405 variants as ACMG class 1 or 2, leaving 135 variants of interest for which we performed manual review of the literature. After this review, 116 were deemed a class 3 variant (VUS) due to the variant not being associated with the patient phenotype at all. Thus, 19 variants in 16 patients were classified as ACMG class 4 and 5 variants (Table 2.2 and Supplemental Table 2.2). CNV analyses in the 70 patients yielded no (likely) pathogenic deletions or duplications in the panel genes. There were no class 3-5 variants in the ACMG incidental finding genes that were part of the panel.

Together with the previously clinically tested eligible patients, the diagnostic yield was 52% (n=59 out of 113, Figure 2.1 and Table 2.2). Additionally, there were two patients with class 4-5 variants in complement factor genes, for whom we could not objectify that the variants caused the patient phenotype. In some patients the previously established genetic diagnosis had been made in another clinical center, we did not always have access to the specific genetic data (Supplemental Table 2.2). Median age at ESRD in the genetic diagnosis patients was 37 years (range 2-49), similar to the rest of the cohort (Table 2.1).

Table 2.2 | Overview of the original clinical diagnoses, molecular diagnoses and reclassifications in the n=113 patients described in this study

Original clinical and/or histological diagnosis	**Total (n, %)**	**Of which molecular diagnosis (n, %)**	**Gene(s)**	**Of which reclassifciation (n, %)**
Ciliopathy				
ADPKD	19 (17%)	19 (100%)	*PKD1* (n=19)	0 (0%)
Nephronophthisis	3 (3%)	3 (100%)	*NPHP1* (n=3)	0 (0%)
Bardet-Biedl syndrome	2 (2%)	2 (100%)	*BBS10* (n=2)	0 (0%)
Glomerular disease				
IgA nephropathy	14 (12%)	2 (14%)	*COL4A3* (n=1) *COL4A4* (n=1)	2 (100%)
FSGS	8 (7%)	5 (63%)	*COL4A3* (n=1) *COL4A5* (n=1) *INF2* (n=2) *WT1* (n=1)	3 (60%)
Congenital nephrotic syndrome	7 (6%)	6 (86%)	*NPHS1* (n=2) *NPHS2* (n=3) *WT1* (n=1)	0 (0%)

Table 2.2 Continued.

Original clinical and/or histological diagnosis	Total (n, %)	Of which molecular diagnosis (n, %)	Gene(s)	Of which reclassifciation (n, %)
Alport syndrome	5 (4%)	5 (100%)	*COL4A3* (n=4) *COL4A5* (n=1)	0 (0%)
Non-congenital nephrotic syndrome (without FSGS)	4 (4%)	1 (25%)	*COL4A3* (n=1)	0 (0%)
Membranoproliferative glomerulonephritis	3 (3%)	0 (0%)	N/A	0 (0%)
Anti-GBM nephropathy	1 (1%)	0 (0%)	N/A	0 (0%)
Congenital anomalies				
CAKUT	7 (6%)	1 (14%)	*HNF1B* (n=1)	1 (100%)
Isolated VUR	6 (5%)	0 (0%)	N/A	0 (0%)
Tumor				
Tuberous sclerosis	4 (4%)	4 (100%)	*TSC1* (n=2) *TSC2* (n=2)	0 (0%)
Renal carcinoma	3 (3%)	3 (100%)	*MET* (n=1) *VHL* (n=1) *WT1* (n=1)	0 (0%)
Vascular disease				
Hypertension	7 (6%)	0 (0%)	N/A	0 (0%)
Microscopic polyangiitis	1 (1%)	0 (0%)	N/A	0 (0%)
Tubular disease				
Cystinosis	2 (2%)	2 (100%)	*CTNS* (n=1) *CLCN5* (n=1)	1 (50%)
Dent disease	1 (1%)	1 (100%)	*CLCN5* (n=1)	0 (0%)
Other				
CKD with unknown cause	4 (4%)	1 (25%)	*PAX2* (n=1)	1 (100%)
aHUS	3 (3%)	2 (67%)	*C3* and *CD46* (n=1) *CFH* (n=1)	0 (0%)
Urethrastricture	2 (2%)	0 (0%)	N/A	0 (0%)
Nephrolithiasis	1 (1%)	0 (0%)	N/A	0 (0%)
Chronic drug use	3 (3%)	0 (0%)	N/A	0 (0%)
Post-traumatic CKD	1 (1%)	0 (0%)	N/A	0 (0%)
Cystic fibrosis	1 (1%)	1 (100%)	*CFTR* (n=1)*	0 (0%)
Syndromal condition	1 (1%)	1 (1%)	22q11.1 deletion*	0 (0%)

ADPKD=autosomal dominant polycystic kidney disease, aHUS=atypical hemolytic-uremic syndrome, CAKUT=congenital anomalies of the kidney and urinary tract, CKD=chronic kidney disease, FSGS=focal segmental glomerulosclerosis, GBM=glomerular basement membrane, N/A=not applicable, VUR=vesico-urethral reflux, *region not on multi-gene panel applied in n=70 cases sequenced in this study, diagnosis made prior to enrollment in this study

2.3.2 Added value of genetic testing

When comparing the genetic diagnosis with the original clinical and/or histological diagnosis, there was a reclassification in 6% of the total cohort (n=7/113, Table 2.2) and 12% of the 59 cases with a genetic diagnosis. We identified a class 5 variant in one patient with CKD of unknown cause, namely in *PAX2* (n=1).We found class 4 and 5 variants in the *COL4A3-5* genes (Alport syndrome) in two patients with "Primary FSGS", in two with "IgA nephropathy" and one patient with "Nephrotic syndrome, biopsy normal".[16,117] One patient with isolated renal dysplasia had a *HNF1B* variant and one patient was clinically diagnosed with cystinosis in the 1990's, but during the regular cystinosis follow-up a class 5 *CLCN5* variant was detected, meaning he in fact had Dent's disease.[118]

2.3.3 Reexamination of kidney biopsy shows added value of genetic testing

In 8 patients with a genetic diagnosis, the original kidney biopsy was available for reexamination and analysis of likelihood of MGKD (Table 2.3). This blinded reexamination resulted in a 'Very likely MGKD' diagnosis in one patient with *NPHS2* variants and one *COL4A3* patient. Two biopsies were categorized as secondary FSGS after reexamination, not likely to be MGKD-related, while patients carried *INF2* and *PAX2* variants. One biopsy showed IgA nephropathy, which could reflect the possibility of IgA nephropathy coinciding with Alport syndrome (*COL4A4* variant).[119] The biopsy of the cystic fibrosis patient was classified as diabetic nephropathy. The observer did not know the patient did not have diabetes; the nodular sclerosis of cystic fibrosis can mimic that of diabetic nephropathy.[120] For two biopsies the likelihood of MGKD could not be determined due to lack of IF and EM, but light microscopy showed no signs of hereditary disease. The updated histological diagnosis was thus different from the genetic diagnosis in 6 patients. This indicates that in 43% of the 14 patients in whom a kidney biopsy was performed at original time of diagnosis, the biopsy retrospectively would have had little diagnostic value when genetic testing had been performed as first tier diagnostic.

Table 2.3 | The original and reexamined biopsy diagnosis, including likelihood of MGKD, in 8 biopsies available for blinded reexamination. "Original diagnosis" was made at time of first presentation.

Sample number	Molecular diagnosis	Original observations light microscopy	Original observations immunofluoresence	Original observations electron miscroscopy	Original histological diagnosis	Reexamined histological diagnosis	Likelihood of MGKD based on reexamined biopsy
13	*NPHS2*	FSGS	Not available	Not available	FSGS	FSGS	Not determinable due to lack of IF and EM
28	*COL4A3*	FSGS	Not available	Not available	Primary FSGS	FSGS	Not determinable due to lack of IF and EM
39	*INF2*	FSGS	No IgG, IgM, IgA, C1q or C3 staining	Partial podocyte feet effacement. No abberant GBM. No electron-dense depositions.	Secondary FSGS	Secondary FSGS	Not likely
74	*NPHS2*	FSGS	Not available	Not available	FSGS, not all classifications for Finnish type	Diffuse mesangial sclerosis	Very likely
102	*PAX2*	FSGS	IgA and IgG negative, IgM, C3c and C1q aspecific fluoresence in the mesangium	Partial podocyte effacement, GBM normal, no electron-dense deposition	Secondary FSGS	Secondary FSGS	Not likely
106	*COL4A3*	FSGS	Not available	Thin GBM	Possibly Alport syndrome	Alport syndrome	Very likely
130	*COL4A4*	Endocapillary hypercellularity and extracapillary proliferation	IgG negative, IgA 4+, IgM 3+ with IgA trapping, C1q 1+. C3, Kappa and Lambda 4+ with similar pattern to IgA.	Podocyte feet effacement and immunocomplexes	IgA nephropathy	IgA nefropathie. Oxford-score: M1, E1, S1, T1	Not likely
190	*CFTR*	Nodular glomerulosclerosis	Linear IgG staining in the medulla, Kappa and Lambda equally fluorescent.	No glomerulus, basal membrane no fibrils	Nodular glomerulosclerosis	Diabetic nephropathy	Not likely

2.4 Discussion

We assessed genetic data of 113 patients who had received a renal transplant prior to the age of 50 due to ESRD of any cause. The diagnostic yield was 52% (n=59/113), reclassifying the original clinical and/or histological diagnosis in 6% (n=7/113).

This diagnostic yield of 52% is higher than in familial CKD (37% yield) and pediatric-onset CKD cases (34% yield).[26,92] Our higher yield might be due to the fact that we sequenced ESRD patients younger than 50 years old, which is a relatively severe phenotype. We cannot report on family history due to the retrospective nature of clinical data retrieval and family history often not being noted in the patient file. A significant portion of the patients in this cohort were diagnosed with MGKD prior to enrollment, thus in those cases there may have been a family history or extra-renal disease that prompted their physician to perform genetic testing. Nevertheless, our high diagnostic yield is remarkable as we imposed limited selection, and with a median age at ESRD of 36 years one would expect that MGKD prevalence would be lower than in familial or early-onset cases.[17,21]

Even if we assume that none of the patients who declined to participate had genetic disease, diagnostic yield would still be 36% (n=59/164; Figure 2.1). Extrapolating to the entire cohort of transplanted patients, including those with clear-cut non-genetic disease, the prevalence of MGKD is at least 22% (n=59/273; Figure 2.1). Previous studies have shown that depending on the phenotype, diagnostic yield can vary enormously, from 12% in transplant waiting list patients with 'undetermined ESRD' to 73% in suspected cystic and glomerular MGKD.[27,92,95,99,121–123] The impact of phenotype on diagnostic yield is underscored by our cohort not yielding any relevant CNVs, likely due to the low number of CAKUT patients.[49] The differences in reported diagnostic yield are impacted by the genes selected for analysis, the sequencing approach used, differences in sample size and inclusion criteria.[124]

We chose to impose minimal exclusion criteria to ensure we tested a population closest to the population present in the daily nephrology out-patient clinic where one would perform genetics first in. We only excluded patient with clear-cut non-genetic disease, since we felt it was not reasonable to approach patients with e.g. HIV-related disease to study a genetic cause of their clearly non-heritable disease.[125–127] On the other hand, patients with e.g. year-long lithium use were approached, as there was no way to rule out the possibility of a genetic disease in those patients.[128]

There was selection on age at first transplant, as it is not likely that a genetics first approach would be applied in daily practice in patients with CKD onset at an age much older than 50. This is due to the high burden of diabetes and cardiovascular disease in advanced age patients, though it is yet unclear what the prevalence of

MGKD is in these older patients and we and others have shown that in some rare cases MGKD can present up to 60 years of age.[102,129–131] Naturally, kidney transplant recipients do not reflect the entire ESRD population, though below the age of 50 the vast majority of patients with ESRD is transplanted in the Netherlands.[132] The broad selection with limited exclusions based on patient characteristics in our cohort, means that the our results can be relevant to the broader CKD population of patients developing severe or progressive CKD at a relatively young age.

The notion of MGKD underdiagnosis is further reiterated by the observation that the genetic diagnosis reclassified the original clinical and/or histological diagnosis in 6%. This is lower than the one in five patients found by others, possibly due to the high percentage of well-known phenotypes such as ADPKD and congenital nephrotic syndrome in our cohort (specifically in the patients with a genetic diagnosis prior to enrollment).[99,102,133] Reclassification can directly impact patient care, for instance in the patient who showed IgA nephropathy on kidney biopsy, while genetic testing diagnosed *COL4A4* related disease.[16,134] Had this patient been diagnosed with Alport syndrome earlier, he might have received ACE-inhibitors in an earlier stage, slowing down disease progression.[135] Conversely, patients with nephrotic syndrome might not have been prescribed corticosteroids if their physician had known the disease was genetic and therefore likely to be resistant to steroid treatment.[136] Monitoring extra-renal features is also a result of a reclassification, as was the case in our *HNF1B* patient.[137] By extension, all patients in whom a genetic diagnosis is made may profit from the possible new avenues of treatment and options regarding family planning that come with knowing one has a heritable disease.[17,28,70–74,91] Early genetic testing therefore shows a clear benefit, as it informs prognostication and facilitates management.[123]

Notably, in 43% of the 14 patients in whom a kidney biopsy had been performed at renal disease onset, in retrospect the biopsy would not have had an added value, had genetic testing been applied as a first tier diagnostic. Almost half of the biopsied patients in our cohort would unnecessarily have been subjected to the risks posed by an invasive biopsy procedure.[14] One should note that IF and EM material was not available for three cases, though only in one case of a *COL4A3* variant the availability of EM would have made diagnosing the MGKD through histology more likely.[138] Our findings highlight another potential benefit of a genetics first approach, namely that genetic testing can provide an accurate diagnosis through a minimally invasive test.

There are case-reports of patients with FSGS being diagnosed successfully with a genetics first approach, obviating the need for a kidney biopsy altogether.[70] For many patients a biopsy is not possible, e.g. due to an end-stage kidney, in

this group genetic testing is of special relevance to reach a diagnosis.[14] Obviously, a kidney biopsy can still be informative, e.g. to measure disease progression or therapy effectiveness.[14]

Next to adequate diagnostics for the kidney disease patient, a genetic diagnosis may have an impact on family members, specifically when they might be potential kidney donors.[139] A significant number of the genetic diagnoses in this study are autosomal dominant diseases, some with variable penetrance.[140] Screening pre-transplant patients for MGKD would allow one to identify the patients with heritable disease and subsequently screen potential donors, for the benefit of both the donor and recipient.[20,141]

We applied a large multi-gene WES-based panel in a portion of the patients enrolled in this study, which is a similar sequencing approach compared to other studies applying large panels.[26,92,96,97,122,123,133] We opted for a WES-based multi-gene panel as this allowed us to limit the risk of incidental findings that come with broad genetic testing, while maximizing the opportunity to find causal variants in known renal disease genes.[142,143] If no causal variant is found in the (phenotype-associated) panel, one can easily assess a broader panel or 'open up' the entire WES data to look for variants in candidate genes, as was done in two patients that were sequenced prior to the start of this study (Figure 2.1).[144] That way, only patients for whom complete WES analysis is needed, are subjected to the risk of incidental findings.[142,144] This study shows that a WES-based multi-gene panel is useful as a first tier diagnostic in progressive CKD.

This study was limited by several factors. As for test-related factors, the DNA assessed in this study was material left from the HLA-typing at time of transplantation, meaning the DNA was sometimes of poor quantity or quality.[145] Though overall coverage was sufficient in all regions of the multi-gene panel, we may have missed variants due to a limited amount of reads at a specific location.[144] We performed a WES-based multi-gene panel, therefore we could not assess variants or CNVs outside of the genes on the applied panel. Thus the diagnostic yield we report is likely an underestimation, especially in populations with a large CNV burden such as CAKUT.[49]

There were patient-related limitations as well. For the sake of DNA availability, we only enrolled transplant patients. We only included patients from one tertiary center, perhaps introducing a sample bias. It is conceivable that patients who choose to participate are more willing to undergo genetic testing due to e.g. a positive family history, creating an inclusion bias. In our extrapolations to the overall transplant population yield, these factors were not of issue, making these highly relevant for clinical practice.

In conclusion, in our cohort of patients who received a renal transplant prior to the age of 50 years due to ESRD of any cause, the proportion of genetic disease is 52%. Even when patients who were not sequenced (e.g. due to rejection of enrollment, clear-cut non-genetic disease, loss to follow-up or no availability of DNA) are considered to be all without genetic disease, the diagnostic yield is still at least 22%. This is the first study in which hardly any selection on underlying disease phenotype was made, providing a cross-section of the patients who develop ESRD prior to the age of 50 years. We found indications that in 43% of patients with a kidney biopsy, the biopsy would not have added diagnostic value if a genetic test had been applied as a first tier diagnostic. This shows the relevance of early genetic testing in providing a minimally invasive test that impacts prognostication and treatment. We conclude that in patients who one expects to develop ESRD prior to the age of 50, genetic testing should be considered as first mode of diagnostics.

Supplementary material 2

Supplemental Table 2.1 | Genes on the n=379 gene panel applied on n=70 patients without a previous molecular diagnosis

ACE	*BMP4*	*CRB2*	*FREM2*	*KANK4*	*NUP205*	*SGPL1*	*TP53RK*
ACTG2	*BMPR2*	*CSPP1*	*FXYD2*	*KCNJ1*	*NUP93*	*SIX1*	*TPRKB*
ACTN4	*BSND*	*CTNS*	*G6PC*	*KCNJ10*	*NXF5*	*SIX2*	*TRAF3IP1*
ADAMTS13	*C2CD3*	*CUBN*	*GALNT3*	*KCNJ5*	*OCRL*	*SIX5*	*TRAP1*
ADCK3	*C3*	*CUL3*	*GALT*	*KIAA0556*	*OFD1*	*SLC12A1*	*TRIM32*
ADCK4	*C5orf42*	*CYP11B1*	*GANAB*	*KIAA0586*	*OSGEP*	*SLC12A3*	*TRPC6*
AGT	*CA2*	*CYP11B2*	*GATA3*	*KIF14*	*PAX2*	*SLC16A12*	*TRPM6*
AGTR1	*CACNA1H*	*CYP17A1*	*GDNF*	*KIF7*	*PAX8*	*SLC22A12*	*TSC1*
AGXT	*CACNA1S*	*CYP24A1*	*GLA*	*KL*	*PBX1*	*SLC26A3*	*TSC2*
AHI1	*CASR*	*DACT1*	*GLI3*	*KLHL3*	*PCBD1*	*SLC2A2*	*TTC21B*
ALDOB	*CC2D2A*	*DCDC2*	*GLIS2*	*KYNU*	*PDE6D*	*SLC2A9*	*TTC8*
ALG1	*CCDC114*	*DDX59*	*GLIS3*	*LAGE3*	*PDSS1*	*SLC34A1*	*UMOD*
ALG8	*CD151*	*DGAT1*	*GNA11*	*LAMB2*	*PDSS2*	*SLC34A3*	*UPK3A*
ALMS1	*CD2AP*	*DGKE*	*GPC3*	*LCAT*	*PHEX*	*SLC36A2*	*UQCC2*
AMN	*CD46*	*DMP1*	*GPC5*	*LMNA*	*PKD1*	*SLC37A4*	*VDR*
ANKS3	*CDKN1C*	*DNAJB11*	*GREB1L*	*LMOD1*	*PKD2*	*SLC3A1*	*VHL*
ANKS6	*CEP120*	*DST*	*GRHPR*	*LMX1B*	*PKHD1*	*SLC41A1*	*VIPAS39*
ANLN	*CEP164*	*DSTYK*	*GRIP1*	*LPP*	*PLCE1*	*SLC4A1*	*VPS33B*
ANO1	*CEP290*	*DYNC2H1*	*GSN*	*LRIG2*	*PMM2*	*SLC4A4*	*WDPCP*
AP2S1	*CEP41*	*DYNC2LI1*	*GUCY2C*	*LRP2*	*PODXL*	*SLC5A2*	*WDR19*
APOA1	*CEP83*	*DZIP1L*	*HAAO*	*LRP4*	*PRDM12*	*SLC6A19*	*WDR34*
APOL1	*CFB*	*EGF*	*HNF1B*	*LYZ*	*PRKCSH*	*SLC6A20*	*WDR35*
APRT	*CFH*	*EHHADH*	*HNF4A*	*LZTFL1*	*PSAP*	*SLC7A7*	*WDR60*
AQP2	*CFHR1*	*EMP2*	*HOGA1*	*MAFB*	*PTEN*	*SLC7A9*	*WDR73*
ARHGAP24	*CFHR2*	*ENPP1*	*HOXD13*	*MAGED2*	*PTH1R*	*SLC9A3*	*WNK1*
ARHGDIA	*CFHR3*	*EPCAM*	*HPRT1*	*MAGI2*	*PTPRO*	*SLC9A3R1*	*WNK4*
ARL13B	*CFHR4*	*EVC*	*HPSE2*	*MAP7D3*	*PYGM*	*SLIT2*	*WNT4*
ARL6	*CFHR5*	*EVC2*	*HSD11B2*	*MAPKBP1*	*REN*	*SMARCAL1*	*WT1*
ARSA	*CFI*	*EYA1*	*IFT122*	*MET*	*RET*	*SOX17*	*XDH*

Supplemental Table 2.1 | Genes on the n=379 gene panel applied on n=70 patients without a previous molecular diagnosis

ATP6V0A4	*CHD1L*	*FAH*	*IFT140*	*MKKS*	*RMND1*	*SPINT2*	*XPNPEP3*
ATP6V1B1	*CHD7*	*FAHD2A*	*IFT172*	*MKS1*	*ROBO2*	*SPTLC1*	*XPO5*
ATP7B	*CHRM3*	*FAM134B*	*IFT27*	*MUC1**	*RPGRIP1*	*SPTLC2*	*YRDC*
ATXN10	*CLCN5*	*FAM20A*	*IFT43*	*MYH11*	*RPGRIP1L*	*STRA6*	*ZEB2*
AVP	*CLCNKA*	*FAM58A*	*IFT52*	*MYH9*	*RRM2B*	*STX16*	*ZIC3*
AVPR2	*CLCNKB*	*FAN1*	*IFT57*	*MYO1E*	*SALL1*	*TBC1D1*	*ZMPSTE24*
B2M	*CLDN16*	*FAT1*	*IFT80*	*MYO5B*	*SALL4*	*TBX18*	*ZNF423*
B9D1	*CLDN19*	*FBXL4*	*IFT81*	*NEK1*	*SARS2*	*TCTEX1D2*	
B9D2	*CNNM2*	*FGA*	*IKBKAP*	*NEK8*	*SCARB2*	*TCTN1*	
BBIP1	*COL4A1*	*FGF20*	*INF2*	*NEUROG3*	*SCLT1*	*TCTN2*	
BBS1	*COL4A3*	*FGF23*	*INPP5E*	*NGF*	*SCN11A*	*TCTN3*	
BBS10	*COL4A4*	*FGF8*	*INVS*	*NOTCH2*	*SCN4A*	*THBD*	
BBS12	*COL4A5*	*FGFR1*	*IQCB1*	*NPHP1*	*SCNN1A*	*TMEM104*	
BBS2	*COQ2*	*FH*	*ITGA3*	*NPHP3*	*SCNN1B*	*TMEM107*	
BBS4	*COQ4*	*FLCN*	*ITGA8*	*NPHP4*	*SCNN1G*	*TMEM138*	
BBS5	*COQ6*	*FN1*	*ITGB4*	*NPHS1*	*SDCCAG8*	*TMEM216*	
BBS7	*COQ7*	*FOXC2*	*JAG1*	*NPHS2*	*SDHB*	*TMEM231*	
BBS9	*COQ9*	*FOXF1*	*KAL1*	*NR3C1*	*SEC61A1*	*TMEM237*	
BCS1L	*COX10*	*FRAS1*	*KANK1*	*NR3C2*	*SEC61B*	*TMEM67*	
BICC1	*CPT2*	*FREM1*	*KANK2*	*NUP107*	*SEC63*	*TNXB*	

*NGS sequencing of *MUC1* does not reveal the pathogenic cytosin insertion in the *MUC1* variable number tandem repeat[146]

Supplemental Table 2.2 | The clinical information on the 59 patients with a ACMG class 4 or 5 molecular diagnosis in this cohort, including variant details and in silico predictions.

Time of sequencing	Sample number	Gender	Age at onset of renal disease (years)	Age at ESRD (years)	Original clinical diagnosis	Original renal biopsy diagnosis	Molecular diagnosis	Gene
Prior to this study	1	F	18	42	Tuberous sclerosis	Angiomyolipomas	Tuberous sclerosis	*TSC2*
Prior to this study	4	M	1	15	Wilms tumor	NP	Wilms tumor	*WT1*
Prior to this study	8	M	26	47	ADPKD	NP	ADPKD	*PKD1*
Prior to this study	10	F	26	41	Von Hippel-Lindau syndrome	Renal clear cell carcinoma	Von Hippel-Lindau syndrome	*VHL*
Prior to this study	13	M	0	7	Congenital nephrotic syndrome	FSGS	Congenital nephrotic syndrome	*NPHS2*
Prior to this study	21	M	21	21	FSGS	NP	Alport syndrome	*COL4A5*
This study	28	F	4	27	Primary FSGS	Primary FSGS	Alport syndrome	*COL4A3*
This study	29	F	0	2	Congenital nephrotic syndrome	Nephrotic syndrome, not all classifications present for diagnosis of Finnish type	Nephrotic syndrome, Finnish type	*NPHS1* *NPHS1*
Prior to this study	35	F	10	11	Nephronophthisis	NP	Nephronophthisis	*NPHP1*
Prior to this study	39	M	20	25	Secondary FSGS	Secondary FSGS	Hereditary FSGS	*INF2*
This study	42	M	38	45	ADPKD	NP	ADPKD	*PKD1*
Prior to this study	51	M	10	27	Alport syndrome	NP	Alport syndrome	*COL4A5*
This study	63	F	0	34	Bardet-Biedl syndrome	NP	Bardet-Biedl syndrome	*BBS10*
Prior to this study	65	M	42	45	ADPKD	NP	ADPKD	*PKD1*
Prior to this study	66	M	0	3	Congenital nephrotic syndrome	NP	Congenital nephrotic syndrome	*WT1*
Prior to this study	72	F	9	19	FSGS	FSGS	FSGS	*WT1*
Prior to this study	74	M	0	13	Congenital nephrotic syndrome	FSGS, not all classifications for Finnish type	Congenital nephrotic syndrome	*NPHS2*
Prior to this study	76	M	30	43	Tuberous sclerosis	NP	Tuberous sclerosis	*TSC1*
This study	80	F	0	24	Congenital nephrotic syndrome	NP	Nephrotic syndrome, Finnish type	*NPHS1* *NPHS1*
This study	82	M	0	32	Bardet-Biedl syndrome	NP	Bardet-Biedl syndrome	*BBS10* *BBS10*

Variant nomen (cDNA)	Variant nomen (protein)	Zygosity	Allele frequency in gnomAD	SIFT score	PolyPhen2 prediction	VariantTaster prediction	Reference for variant	Reclassification of original clinical and/ or histological diagnosis
Variant detected elsewhere	Variant detected elsewhere	Heterozygous	N/A	N/A	N/A	N/A	N/A	No
Variant detected elsewhere	Variant detected elsewhere	Heterozygous	N/A	N/A	N/A	N/A	N/A	No
Variant detected elsewhere	Variant detected elsewhere	Heterozygous	N/A	N/A	N/A	N/A	N/A	No
c.509T>A	p.Val170Asp	Heterozygous	Not in database	0	Probably damaging	Disease causing	Maher *et al.*, 1996[147]	No
c.413G>A	p.Arg138Gln	Homozygous	163/282690	0	Probably damaging	Disease causing	Huber *et al.*, 2003[148]	No
Fibroblast and renal biopsy diagnosis	N/A	X-linked	N/A	N/A	N/A	N/A	N/A	Yes
c.172G>A	p.Gly58Ser	Heterozygous	6/240254	0	Probably damaging	Disease causing	Morinière *et al.*, 2014[149]	Yes
c.1936C>G	p.Pro646Ala	Compound heterozygous	Not in database	0	Probably damaging	Disease causing	Novel	No
c.1760T>C	p.Leu587Pro	Compound heterozygous	0/235468	0.001	Probably damaging	Disease causing	Schoeb *et al.*, 2010[150]	
Full gene deletion	N/A	Homozygous	N/A	N/A	N/A	N/A	Full gene deletion[102]	No
c.217G>A	p.Gly73Ser	Heterozygous	Not in database	0	Probably damaging	Disease causing	Barua *et al.*, 2013[151]	No
c.7409delC	p.Pro2470Argfs*150	Heterozygous	Not in database	N/A	N/A	N/A	Novel	No
c.4889C>T	p.Phe1630*	X-linked	Not in database	N/A	N/A	N/A	Novel	No
c.271dupT	p.Cys91Leufs*5	Homozygous	17/30930	N/A	N/A	N/A	Stoetzel *et al.*, 2006[152]	No
Variant detected elsewhere	Variant detected elsewhere	Heterozygous	N/A	N/A	N/A	N/A	N/A	No
c.1339+2T>A	p.?	Heterozygous	Not in database	N/A	N/A	N/A	Novel	No
c.1432G>A	p.?	Heterozygous	Not in database	N/A	N/A	N/A	Wang *et al.*, 2012[153]	No
c.413G>A	p.Arg138Gln	Homozygous	163/282690	0	Probably damaging	Disease causing	Huber *et al.*, 2003[148]	No
Deletion final exons	N/A	Heterozygous	N/A	N/A	N/A	N/A	Kozlowski *et al.*, 2007[154]	No
c.1211T>G	p.Phe404Cys	Compound heterozygous	Not in database	0.107	Possibly damaging	Polymorphism	Novel	No
c.2491C>T	p.Arg831Cys	Compound heterozygous	7/276974	0.028	Probably damaging	Disease causing	Liu *et al.*, 2001[155]	
164T>C	p.Leu55Pro	Compound heterozygous	1/30958	0.001	Probably damaging	Disease causing	Billingsley *et al.*, 2010[156]	No
c.461delT	p.Leu154Cysfs*17	Compound heterozygous	Not in database	N/A	N/A	N/A	Novel	

Supplemental Table 2.2 | Continued.

Time of sequencing	Sample number	Gender	Age at onset of renal disease (years)	Age at ESRD (years)	Original clinical diagnosis	Original renal biopsy diagnosis	Molecular diagnosis	Gene
Prior to this study	85	M	43	40	Tuberous sclerosis	NP	Tuberous sclerosis	*TSC2*
Prior to this study	86	M	0	37	IgA nephropathy	Iga nephropathy	Alport syndrome	*COL4A3* *COL4A3*
Prior to this study	88	M	34	37	aHUS	NP	aHUS	*C3* *CD46*
Prior to this study	90	M	0	20	Cystinosis	NP	Cystinosis	*CTNS*
This study	94	M	37	40	ADPKD	NP	ADPKD	*PKD1*
Prior to this study	96	F	20	36	Tuberous sclerosis	NP	Tuberous sclerosis	*TSC1*
Prior to this study	102	M	25	25	CKD with unknown cause	Secondary FSGS	*PAX2*-related disease	*PAX2*
Prior to this study	105	F	30	46	ADPKD	NP	ADPKD	*PKD1*
Prior to this study	106	F	23	40	Alport syndrome	FSGS with thin GBM, possibly Alport syndrome	Alport syndrome	*COL4A3*
Prior to this study	108	F	6	28	Alport syndrome	NP	Alport syndrome	*COL4A3* *COL4A3*
Prior to this study	119	F	35	41	Papillary renal carcinoma	Multifocal papillary renal carcinoma	*MET*-related cancer	*MET*
Prior to this study	129	F	26	37	Nephrotic syndrome	Normal	Alport syndrome	*COL4A3*
This study	130	M	13	16	IgA nephropathy	IgA nephropathy	Alport syndrome	*COL4A4*
This study	131	F	24	24	ADPKD	NP	ADPKD	*PKD1*
Prior to this study	135	M	35	44	ADPKD	NP	ADPKD	*PKD1*
Prior to this study	147	M	29	29	Dent disease	NP	Dent disease	*CLCN5*
This study	157	F	34	35	ADPKD	NP	ADPKD	*PKD1*
Prior to this study	160	F	23	40	ADPKD	NP	ADPKD	*PKD1*
Prior to this study	161	M	28	32	FSGS	NP	FSGS	*INF2*
Prior to this study	162	M	22	45	ADPKD	NP	ADPKD	*PKD1*
Prior to this study	169	F	19	49	ADPKD	NP	ADPKD	*PKD1*
Prior to this study	177	F	15	22	Syndromal condition	NP	Syndromal condition	22q11.1 deletion
This study	178	M	35	46	ADPKD	NP	ADPKD	*PKD1*

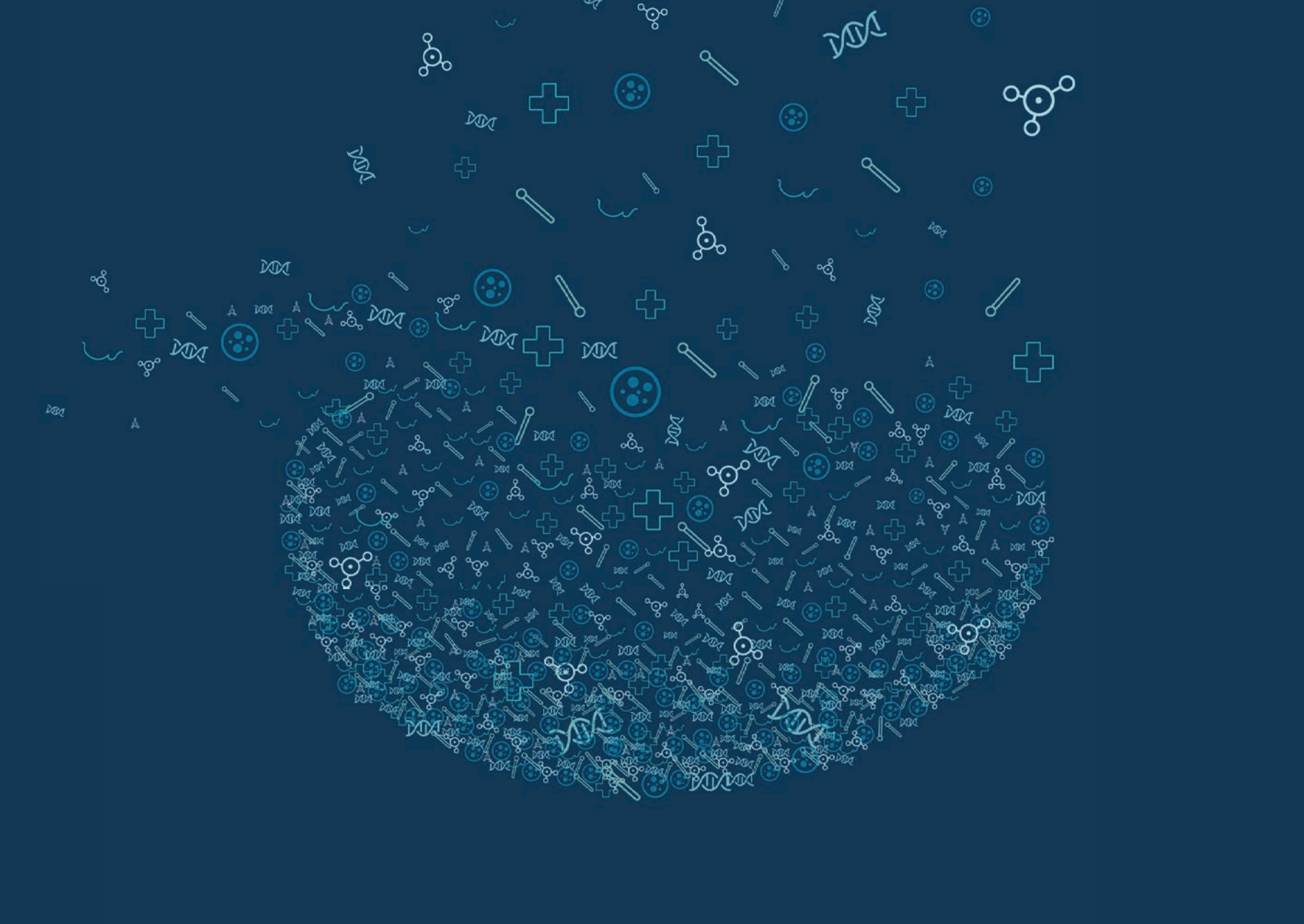
Variant nomen (cDNA)	Variant nomen (protein)	Zygosity	Allele frequency in gnomAD	SIFT score	PolyPhen2 prediction	VariantTaster prediction	Reference for variant	Reclassification of original clinical and/ or histological diagnosis
Variant detected elsewhere	Variant detected elsewhere	Heterozygous	N/A	N/A	N/A	N/A	N/A	No
c.40_63de124	p.Leu14_Leu21del	Compound heterozygous	17/121044	N/A	N/A	N/A	Longo *et al.*, 2002[157]	Yes
c.4370T>C	p.Ile1457Thr	Compound heterozygous	1/249522	0.22	Benign	Disease causing	Novel	
c.4855A>C	p.Ser1619Arg	Digenic	1/31352	0.07	Possibly damaging	Disease causing	Bu *et al.*, 2014[158]	No
c.307C>T	p.Arg103Trp	Digenic	Not in database	0.02	Possibly damaging	Disease causing	Fang *et al.*, 2008[159]	
Variant detected elsewhere	Variant detected elsewhere	Homozygous	N/A	N/A	N/A	N/A	N/A	No
c.1386-3C>G	Splice site variant	Heterozygous	Not in database	N/A	N/A	N/A	Novel	No
Variant detected elsewhere	Variant detected elsewhere	Homozygous	N/A	N/A	N/A	N/A	N/A	No
c.685C>T	p.Arg229*	Heterozygous	Not in database	N/A	N/A	N/A	Novel	Yes
c.5968_5969del	p.Arg1990fs	Heterozygous	N/A	N/A	N/A	N/A	Audrézet *et al.*, 2012[160]	No
c.725G>A	p.Gly242Glu	Heterozygous	Not in database	0	Probably damaging	Disease causing	Novel	No
c.3733G>A	p.Gly1245Ser	Compound Heterozygous	Not in database	0	Probably damaging	Disease causing	Novel	No
c.4421T>C	p.Leu1474Pro	Compound Heterozygous	748/280794	0	Probably damaging	Disease causing	Chatterjee *et al.*, 2013[161]	
c.3446T>C	p.Met1149Thr	Heterozygous	Not in database	0	Probably damaging	Disease causing	Schmidt *et al.*, 1997[162]	No
c.725G>A	p.Gly242Glu	Homozygous	Not in database	0	Probably damaging	Disease causing	Novel	Yes
c.292G>A	p.Gly98Ser	Heterozygous	1/243930	0	Probably damaging	Disease causing	Novel	Yes
c.2398dupG	p.Ala800Glyfs*17	Heterozygous	Not in database	N/A	N/A	N/A	Novel	No
Alu repeat insertion exon 11	N/A	Heterozygous	N/A	N/A	N/A	N/A	Novel	No
Variant detected elsewhere	Variant detected elsewhere	X-linked	N/A	N/A	N/A	N/A	N/A	No
c.9841G>C	p.Ala3281Pro	Heterozygous	Not in database	0.024	Probably damaging	Disease causing	Novel	No
c.8017-2_8017-1delAG	p.Gly2673fs	Heterozygous	Not in database	N/A	N/A	N/A	Rossetti *et al.*, 2001[163]	No
Variant detected elsewhere	Variant detected elsewhere	Heterozygous	N/A	N/A	N/A	N/A	N/A	No
Variant detected elsewhere	Variant detected elsewhere	Heterozygous	N/A	N/A	N/A	N/A	N/A	No
Variant detected elsewhere	Variant detected elsewhere	Heterozygous	N/A	N/A	N/A	N/A	N/A	No
22q11.1 deletion	N/A	Structural variant	N/A	N/A	N/A	N/A	McDonald-McGinn *et al.*, 1999[164]	No
c.1570G>C	p.Ala524Pro	Heterozygous	Not in database	0.015	Probably damaging	Polymorphism	Novel	No

Supplemental Table 2.2 | Continued.

Time of sequencing	Sample number	Gender	Age at onset of renal disease (years)	Age at ESRD (years)	Original clinical diagnosis	Original renal biopsy diagnosis	Molecular diagnosis	Gene
This study	186	F	0	46	Alport syndrome	NP	Alport syndrome	*COL4A5*
Prior to this study	190	F	0	47	Cystic fibrosis	Nodular glomerulosclerosis	Cystic fibrosis	*CFTR*
Prior to this study	196	M	35	39	aHUS	NP	aHUS	*CFH*
Prior to this study	201	F	37	42	Renal dysplasia	NP	*HNF1B*-related disease	*HNF1B*
This study	206	F	NR	47	ADPKD	NP	ADPKD	*PKD1*
This study	209	M	33	46	ADPKD	NP	ADPKD	*PKD1*
Prior to this study	215	F	0	2	Congenital nephrotic syndrome	NP	Nephrotic syndrome, Finnish type	*NPHS2*
Prior to this study	227	F	38	47	Alport syndrome	NP	Alport syndrome	*COL4A3*
This study	232	M	39	49	ADPKD	NP	ADPKD	*PKD1*
Prior to this study	247	F	13	13	Nephronophthisis	NP	Nephronophthisis	*NPHP1*
This study	248	F	NR	39	ADPKD	NP	ADPKD	*PKD1*
Prior to this study	251	M	22	27	Nephronophthisis	FSGS and ischemia	Nephronophthisis	*NPHP1* *NPHP1*
Prior to this study	263	F	36	40	ADPKD	NP	ADPKD	*PKD1*
Prior to this study	266	M	28	29	ADPKD	NP	ADPKD	*PKD1*
Prior to this study	268	M	4	42	Cystinosis	FSGS	Dent disease	*CLCN5*
Prior to this study	274	M	15	40	ADPKD	NP	ADPKD	*PKD1*

ADPKD=autosomal dominant polycystic kidney disease, FSGS=focal segmental glomerulosclerosis, N/A=not applicable, NP=not performed

Variant nomen (cDNA)	Variant nomen (protein)	Zygosity	Allele frequency in gnomAD	SIFT score	PolyPhen2 prediction	VariantTaster prediction	Reference for variant	Reclassification of original clinical and/ or histological diagnosis
c.991G>A	p.Gly331Ser	Heterozygous	Not in database	0	Probably damaging	Disease causing	Nozu *et al.*, 2014[165]	No
3272-26A>G	p.Phe508del	Homozygous	2027/282630	N/A	N/A	N/A	Collins, 1992[166]	No
Variant detected elsewhere	Variant detected elsewhere	Heterozygous	N/A	N/A	N/A	N/A	N/A	No
Variant detected elsewhere	Variant detected elsewhere	Heterozygous	N/A	N/A	N/A	N/A	N/A	Yes
c.9324delC	p.Ile3109Serfs*207	Heterozygous	Not in database	N/A	N/A	N/A	Borras *et al.*, 2017[167]	No
c.12010C>T	p.Gln4004*	Heterozygous	1/240348	N/A	N/A	Disease causing	Herbert *et al.*, 2013[168]	No
Variant detected elsewhere	Variant detected elsewhere	Homozygous	N/A	N/A	N/A	N/A	N/A	No
c.888+1G>A	N/A	Heterozygous	Not in database	N/A	N/A	N/A	Novel	No
c.7544G>A	p.Arg2515Gln	Heterozygous	4/272336	0.506	Benign	Polymorphism	Xu *et al.*, 2018[169]	No
Full gene deletion	N/A	Homozygous	N/A	N/A	N/A	N/A	Full gene deletion[102]	No
c.3262G>T	p.Glu1088*	Heterozygous	Not in database	N/A	N/A	Disease causing	Novel	No
c.1-?_2202+del	p.?	Compound Heterozygous	Not in database	N/A	N/A	N/A	Novel	No
c.1027G>A	p.Gly343Arg	Compound Heterozygous	31/282388	0	Probably damaging	Disease causing	Halbritter *et al.*, 2013[170]	
c.12010C>T	p.G1n4004*	Heterozygous	1/244454	N/A	N/A	Disease causing	Herbert *et al.*, 2013[168]	No
c.8162-2A>G	p.?	Heterozygous	Not in database	N/A	N/A	N/A	Novel	No
c.1909C>T	p.Arg637*	Heterozygous	Not in database	N/A	N/A	Disease causing	Takemura *et al.*, 2001[171]	Yes
c.4197G>A	p.Trp1399*	Heterozygous	Not in database	N/A	N/A	Disease causing	Novel	No



Importance of Genetic Diagnostics in Adult-Onset Focal Segmental Glomerulosclerosis

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Abstract

Focal segmental glomerulosclerosis (FSGS) is a histological pattern of podocyte and glomerulus injury. FSGS can be primary, secondary to other diseases or due to a genetic cause. Strikingly, genetic causes for adult-onset FSGS are often overlooked, likely because identifying patients with genetic forms of FSGS based on clinical presentation and histopathology is difficult. Yet diagnosing genetic FSGS does not only have implications for prognostication and therapy, but also for family and family planning.

In this case series we present three adult patients who presented with advanced renal disease with the histological picture of FSGS and proved to have a genetic cause of the disease, namely variants in INF2, COL4A4 and HNF1B respectively. We show the possibilities of identifying genetic FSGS based on clinical clues of a positive family history, early age at onset of disease and/or severe therapy-resistant disease. We discuss ways to select the method of genetic testing for individual patients. Finally, we examine how the judicious use of genetic investigations can obviate potential harmful diagnostic procedures and direct clinical decisions in patients and their relatives.

3.1 Background

With the advances in genetic testing methods, genetic analysis is an increasingly important diagnostic tool in nephrology.[30] This is also the case for genetic focal segmental glomerulosclerosis (FSGS), which is the focus of this paper.

FSGS is a histological pattern of podocyte loss and glomerular injury. It is characterized in a renal biopsy, by segmental sclerotic lesions in at least one glomerulus (observed with lightmicroscopy, LM) and effacement of the podocyte foot processes (observed with electron microscopy, EM).[172,173] The underlying causes for FSGS are heterogeneous.[117,174]

FSGS is traditionally categorized according to those underlying causes, namely primary (often involves a circulating factor causing podocyte dysfunction), secondary to a non-renal disease, and genetic FSGS.[117,175] Depending on the underlying cause patients can present with proteinuria, or nephrotic syndrome (most in primary FSGS), and end-stage renal disease (ESRD), or progress to ESRD over the course of 5-10 years.[176]

There are no clear-cut clinical or histopathological findings to distinguish genetic FSGS from other types.[177] However, there are several hallmarks of genetic disease. Namely a positive family history, early age at onset of disease (~30% of FSGS with an onset before 25 years of age is genetic), and uncharacteristically severe and/or steroid resistant disease.[21,61,177,178] Conversely, because genetic disease often presents at a young age, it is often unjustly overlooked in adult-onset FSGS patients.[61]

With the advances of genetic testing however, diagnosing genetic FSGS has become much more feasible over the past few years. Not only because over 50 genes are currently known to be involved in FSGS, but also since the costs and turn-around-time for genetic tests are continuously dropping, increasing their availability in daily clinical practice.[17,34,61,177,179,180]

The technique most frequently used for genetic testing is next-generation sequencing (NGS).[17,61,177,179,180] NGS can identify disease-causing mutations in the entire genome (whole-genome sequencing), the protein coding regions (whole-exome sequencing) or a specific set of genes of interest (targeted gene panel, TGP).[181] For instance, the TGP on FSGS in Supplemental Table 3.1 contains the classic FSGS genes *NPHS1* and *NPHS2* as well as genes recently associated with FSGS such as the *COL4A* genes (the Alport syndrome genes) and *PAX2* (involved in nephrogenesis). Selecting the right NGS test is essential, to be able to come to a diagnosis with limited risk of the incidental findings that testing many genes (e.g. whole-exome sequencing) can bring.

Despite the abovementioned challenges, considering a genetic cause in adult-onset FSGS patients is important as it can have a large impact on the patient and his/her family members. Here, we present three patients with adult-onset chronic kidney disease (CKD) who were clinically and histopathologically diagnosed with FSGS and were shown to carry a genetic cause thanks to a close collaboration between nephrologists, pathologists and clinical geneticists. We use these cases to discuss the expanding possibilities of diagnosing genetic FSGS and the clinical implications of such a diagnosis.

3.2 Case 1 – FSGS with ESRD at a young age

A 30-year old man with asymptomatic 2 gr/day proteinuria at age 20 and ESRD at age 29 (no signs of nephrotic syndrome, Table 3.1) was referred to our nephrogenetics out-patient clinic. There was no family history of renal disease. Renal biopsy at age 29, when the patient developed ESRD, showed FSGS (Figure 3.1A), with 80% globally sclerosed glomeruli and partial podocyte foot process effacement (Figure 3.1D).[182] The patient was referred because he was planned to undergo a kidney transplant from a family member.

Due to the young age of onset of proteinuria in this patient, there was a marked probability of genetic FSGS and a diagnostic TGP analysis for FSGS was performed (Supplemental Methods 3.1 and Supplemental Table 3.1). This revealed a heterozygous known pathogenic mutation in the *INF2* gene (OMIM610982, Table 3.2).[36,151,183,184] The mutation had been previously detected in FSGS patients, though one should note that no functional assessment of that specific mutation was performed.[151] Mutations in *INF2* are known to be a major cause for autosomal dominant FSGS.[185–187]

To adequately counsel family members, segregation analysis was performed in the patient's healthy parents. The father did not carry the mutation and later successfully donated a kidney to our patient. In the, otherwise healthy, mother a 20% mosaicism for the *INF2* mutation was detected in DNA from peripheral blood. The mother was referred for extensive health screening, which revealed no abnormalities. Since she had had a son with *INF2* mutation, it must therefore be present in the germline and thus possibly have been passed down to the patient's siblings. One sibling decided on testing (revealing no *INF2* mutation) and one decided to undergo periodic evaluation of renal function. The patient's young child will be counseled regarding pre-symptomatic genetic testing when it is of age. As the earliest presentation reported in literature is at seven years of age, the child will undergo proteinuria screening.[188]

Next to the implications for family members, the molecular diagnosis impacted the patient's care directly. Mutations in *INF2* can also be associated with dominant

intermediate Charcot-Marie-Tooth disease, thus the patient was neurologically evaluated, showing no abnormalities.[189] Additionally, the patient and his partner wanted to have more children. After counseling they opted to try to conceive via pre-implantation genetic diagnostics (PGD), an in-vitro fertilization procedure where an embryo *without* the *INF2* mutation is transferred into the uterus.[81] At time of this publication, this has not yet led to an ongoing pregnancy.

3.3 Case 2 – FSGS with a family history of ESRD

A 50-year old obese woman (BMI 34) of Hindustani Surinam descent (Table 3.1) presented in the referring hospital with mild CKD (eGFR=90), distinct proteinuria (1.6 gr/day, no signs of nephrotic syndrome) and erythrocyturia of 30 cells/μL.

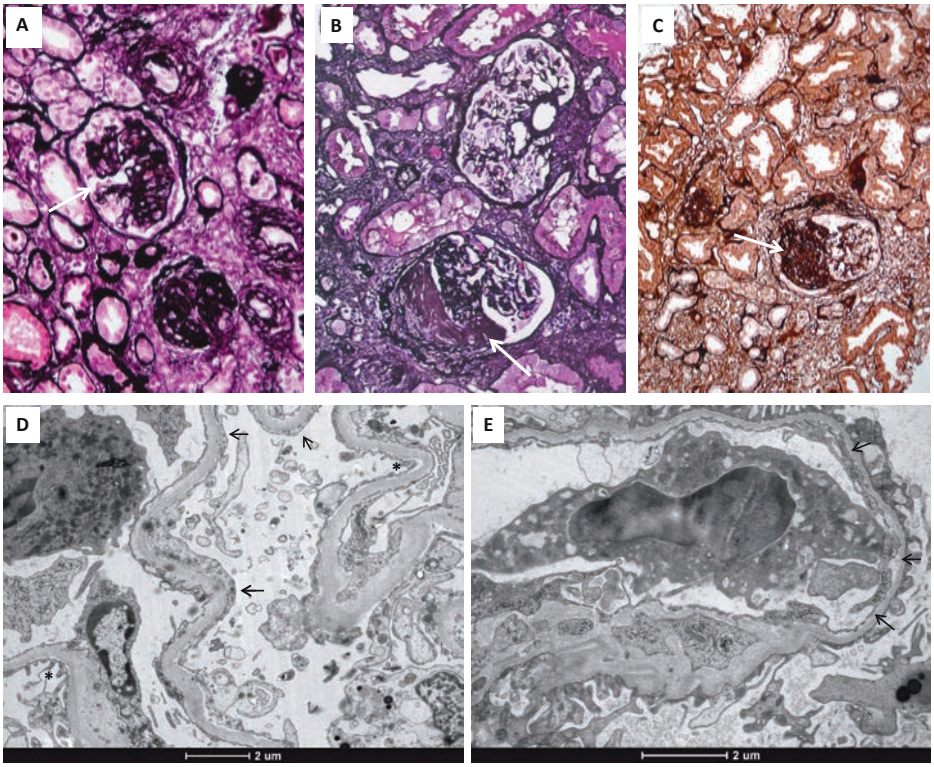


Figure 3.1 | Kidney biopsy images in the three cases. Light microscopy (Jones staining) showed glomeruli with segmental sclerosis (arrows) in case 1 (A), case 2 (B) and case 3 (C). Electron microscopy of case 1 showed partial foot process effacement, with areas of intact foot processes (asterisks) alternating with areas with foot process effacement (arrows, D). In addition to partial foot process effacement, electron microscopy of case 2 also showed a thin GBM thickness with a mean of 252 nm (arrows, E).

Table 3.1 | Age at first presentation, laboratory findings and morphological findings per case.

Casenumber	Age at first presentation	Positive family history	Clinical diagnosis	eGFR at presentation (CKD-EPI) [7]	Laboratory analysis at presentation	Renal ultrasound results	Light microscopy	Immunofluoresence microscopy	Electron microscopy	Histological classification[182]
Case 1 UMCU_NG_012_01	20 years	No	Secondary FSGS	<20 mL/min/1.73m^2	*Blood* Albumin normal Lipids normal PT & APTT normal *Urine* Protein (2 g/day)	Echodense kidneys, otherwise no abnormalities. Length 9.9 and 9.8 cm (normal). Changes likely due to CKD.	FSGS with 80% glomerulo-sclerosis	No immunoreactivity	Partial podocyte effacement	FSGS NOS
Case 2 UMCU_NG_044_01	50 years	Yes	Secondary FSGS	90 mL/min/1.73m^2	*Blood* Albumin normal Triglycerids high PT & APTT normal *Urine* Protein (1.6 g/day) 30 erythrocytes/uL	No abnormalities. Length 12.5 and 11.6 cm (normal).	FSGS with 50% glomerulo-sclerosis	Aspecific immunoreactivity for IgA and IgM	Partial podocyte effacement Thin basement membrane (mean 172 nm)	FSGS NOS
Case 3 UMCU_NG_100_01	33 years	Yes	FSGS, etiology unknown	39 mL/min/1.73m^2	*Blood* Albumin normal Triglycerids high PT & APTT normal *Urine* Protein (0.6 g/day)	No abnormalities. Length 10.2 and 10.5 cm (normal).	FSGS with 45% glomerulo-sclerosis	No immunoreactivity	No material	FSGS NOS

APTT=activated partial thromboplatin time, CKD=chronic kidney disease, cm=centimeter, eGFR=electronic Glomerular Filtration Rate, FSGS=focal segmental glomerulosclerosis, gr=gram, Ig=immunoglobulin, L=liter, m=meter, min=minute, mL=milliliter, nm=nanometer, NOS=not otherwise specified, PT=prothrombin time, SRNS=steroid resistant nephrotic syndrome, uL=microliter

Table 3.2 | Molecular diagnosis, including the performed genetic testing and information on the genetic variant, per case.

Casenumber	Genetic testing performed	HGNC approved gene name (transcript number)	OMIM number	Variant	Homozygous or heterozygous	Variant type	Reference / in silico predictions[36,151,184,203]
Case 1 UMCU_NG_012_01	FSGS	*INF2* (NM_022489.3)	610982	c.217G>A p.(Gly73Ser)	Heterozygous	Pathogenic	Barua *et al*., Kidney Int. 2013 eb;83(2):316-22 (no functional analysis of this variant) PolyPhen HumDiv score 1.000, sensitivity 0.00, specificity 1.00 Polyphen HumVar score 1.000, sensitivity 0.00, specificity 1.00 SIFT score 0.13 (tolerated) Not present in the gnomAD database
Case 2 UMCU_NG_044_01	FSGS	*COL4A4* (NM_000092.4)	12131	c.2038G>C p.(Gly680Arg)	Heterozygous	Likely pathogenic	PolyPhen HumDiv score 1.000, sensitivity 0.00, specificity 1.00 Polyphen HumVar score 1.000, sensitivity 0.00, specificity 1.00 SIFT score 0.00 (deleterious) Not present in the gnomAD database
Case 3 UMCU_NG_100_01	FSGS PAX2 Sanger sequencing Full diagnostic renal diseases ('RENome')	*HNF1B* (NM_000458.3)	189907	c.908G>A p.(Arg303His)	Heterozygous	VUS	PolyPhen HumDiv score 0.998, sensitivity 0.27, specificity 0.99 PolyPhen HumVar score 0.877, sensitivity 0.71, specificity 0.89 SIFT score 0.04 (deleterious) Not present in the gnomAD database

Arg=Arginine, del=deletion, FSGS=focal segmental glomerulosclerosis, Glu=Glutamic acid, Gly=Glycine, HGNC=HUGO Gene Nomenclature Committee, His=Histidine, OMIM=Online Mendelian Inheritance in Man®, Ser=Serine, VUS=Variant of Unknown Significance.

Her parents had ESRD, both with an age of onset around 60 years, of which the father was diagnosed as having diabetic nephropathy. In our patient, renal biopsy displayed FSGS (Figure 3.1B) with 50% globally sclerosed glomeruli, thought to be secondary to a metabolic syndrome. However, because of the erythrocyturia, the referring nephrologist wondered if *COL4A3-5* related disease (mutations in these genes are detected in patients with thin basement membrane nephropathy and classical Alport syndrome) might play a role in this patient's phenotype.

To assess this possibility, the renal biopsy was revised with EM. This showed a thin GBM with a mean thickness of 172 nm, (Figure 3.1E), which was well below the lower limit of 252 nm determined in our center for normal GBM thickness for females and also below the lower limit of 215 nm for the normal thickness for females reported in literature, further pointing towards an *COL4A3-5* related disease .[190,191] Therefore, the diagnostic TGP analysis on FSGS was performed (Supplemental Methods and Supplemental Table 3.1). This analysis includes the *COL4A* genes, since mutations in these genes have been shown to cause a histological FSGS phenotype in some cases.[190,192–196] The TGP analysis showed a heterozygous likely pathogenic mutation in the *COL4A4* gene (OMIM120131, Table 3.2), with no variants in other FSGS linked genes.[36,183,184]

COL4A4 codes for the type IV collagen alpha-4 chain, a protein essential to the GBM.[197] Heterozygous mutations in *COL4A4* have been associated with familial hematuria.[198] There are reports suggesting that specific mutations in *COL4A4* or unknown genetic modifiers might cause FSGS lesions in heterozygous carriers, while others suggest that heterozygous *COL4A3-5* mutations are the most frequent underlying cause in patients with FSGS on biopsy.[29,199–201] It is clear that the penetrance of renal disease in carriers of heterozygous *COL4A3-4* mutations is far from complete.[29,199–201] There is debate over whether this is best called autosomal dominant Alport syndrome, or e.g. *COL4A3-4* related disease.[29,199–201]

The specific mutation detected in our patient has not been described as pathogenic before. However, the variant causes the substitution of a highly conserved Glycine residue in the collagen triple-helix repeat by a more bulky amino acid (Table 3.2). Based on the fact that most known pathogenic mutations in *COL4A4* lead to similar substitutions, the mutation was classified as 'likely pathogenic'. Segregation analysis was performed and the mother (no diabetes) proved to be a carrier for the same mutation. The presence of the *COL4A4* variant in two affected family members, along with erythrocyturia and a thin GBM likely explains at least a part of our patient's *COL4A3-5* related disease phenotype. With this, it is important to note that people of Hindustani Surinam descent are known to have higher risk of metabolic syndrome, which likely also played a role in this families' renal phenotype(s).[199,202]

Genetic counseling was offered to the patient's children. Furthermore, the finding of a *COL4A4* likely pathogenic variant triggered the referring nephrologist to prescribe Lisinopril, as the patient needed anti-hypertensive medication and ACE-inhibition is also used to attenuate renal function decline in Alport syndrome.[71]

3.4 Case 3 – 'IgA-related FSGS' with a family history of ESRD

An otherwise healthy 33-year old man presented with an eGFR of 39 and proteinuria (0.6 g/day, no signs of nephrotic syndrome). The family history revealed that the mother had died with ESRD at age 50 most likely due to hypodysplastic kidneys. Renal ultrasound in the patient showed no abnormalities and normal sized kidneys (Table 3.1). In the referring hospital, renal biopsy was classified as FSGS secondary to IgA depositions. The patient wondered if he could pass on the disease to his children.

Biopsy revision at our facility showed FSGS (Figure 3.1C) with 45% of glomeruli globally sclerosed, but no immunoreactivity for IgA. There was not enough material to perform EM. Since the diagnosis of IgA nephropathy was doubtful, genetic diagnostics using the FSGS TGP analysis was performed (Supplemental Methods 3.1 and Supplemental Table 3.1). This did not lead to a molecular diagnosis. Due to the high clinical suspicion, the analysis was expanded to a larger panel of ~225 published renal genes. This revealed a heterozygous variant of unknown significance (VUS) in the *HNF1B* gene (OMIM189907, Table 3.2).[36,39,183,184]

The variant had not been observed before in patients or large healthy control populations, in silico predictions suggest a possible pathogenic effect (Table 3.2) and the variant segregated in the patient's deceased parent. Laboratory work-up in our patient for glucose, electrolyte and liver enzyme imbalances associated with *HNF1B*-related disease showed no clear abnormalities, however genotype-phenotype correlations can be unclear.[204,205] The *HNF1B* variant might thus be causal in our patient's disease and the mother's renal hypodysplasia. This is underscored by studies showing that HNF1B works as a modifier on PAX2, in which gene mutations are known to cause both isolated congenital anomalies of the kidney and urinary tract (CAKUT, such as hypodysplasia) as well as FSGS.[137,206,207] Also, mutations in *HNF1B* sometimes cause a CAKUT phenotype without abnormalities in other organs.[137,206] Hence it could be that mutations in *HNF1B* also lead to FSGS. Publication of this, to our knowledge first ever, case will hopefully stimulate further research into the *HNF1B*-FSGS relationship.

Though the patient cannot be conclusively diagnosed, the combination of the variant and the positive family history has led to all at risk family members receiving advice for periodic evaluation of renal function.

3.5 Discussion

The cases presented in this paper show that although the identification of a genetic cause for FSGS presenting at an adult age can be complex, an adequate diagnosis can have far-reaching implications. That the cases were re-diagnosed as genetic FSGS is due to the multidisciplinary approach with input from a nephrologist, pathologist, and clinical geneticist. These specialists discussed the possibility of genetic disease and the appropriate application of genetic testing for each patient individually. We discuss the items at the core of this discussion in detail below.

First it is vital to recognize that though patient characteristics can give clues on patients with high risk of a genetic disease, not all patients display those hallmarks of genetic disease.[21,61,177] Similar to the *INF2* case we presented, a family history might be absent due to germline mosaicism, or mutations that are recessive, *de novo* or incompletely penetrant.[17] Additionally, though a young age at presentation is an indication of genetic disease, our *COL4A4* patient presented at 50 years of age.[21] The notion that genetic renal disease can present later in life is underscored by our recent finding that the classic pediatric disease nephronophthisis actually can present with end stage renal disease to up to 61 years.[102]

Second, one should consider the appropriate NGS scale for each patient. In order to test a sufficient number of genes without risk of incidental findings, we apply a tiered approach, starting with analysis of TGPs that are limited to strictly FSGS-associated genes. If a limited TGP does not yield a diagnosis, one can opt to analyze a larger panel (as we did for our *HNF1B* case), or to perform whole-exome sequencing to look for variants in genes not yet associated with the patient's phenotype. To make such a step-up process even easier, we decided in 2017 to derive all TGP analyses from whole-exome sequencing data. Adequate pre- and post-test counseling (described by our group previously[29]) regarding analyses of the whole-exome data should be offered to patients, as these can reveal incidental findings.

With the continuous decrease in cost and turn-around-time of NGS the precise selection of patients and a step-up NGS method will likely become less of a question.[34] However, genetic testing should always be applied after consideration of the prognostic and therapeutic implications of finding a genetic variant for the patient and his/her family members.

For the patient, it can provide information on useful treatment strategies. Though genetic FSGS generally does not respond to corticosteroid treatment, other drugs might be beneficial, such as ACE-inhibition in *COL4A*-related disease.[56,71,208] Furthermore, a molecular diagnosis is relevant when deliberating on a renal transplantation. First, because it usually offers a favorable prognosis with respect

to recurrence in a renal graft, since chances of this are very low in genetic FSGS.[209] Second, if living related transplantation is considered, it is safest to have a genetically unaffected family member donate.[72] For this reason we tested the *INF2* patient's parent before proceeding to donation.

Family members are impacted, as they are at risk of also developing FSGS. Those at risk should be offered counseling on genetic testing and/or (pre-symptomatic) evaluation of renal function.[72] Likewise, *future* children of a genetic FSGS patient could inherit the disease. It is our experience that the knowledge that the disease is genetic, is very important for patients when contemplating how to establish their family. As we saw in our *INF2* case, the options for not passing the disease on not only include having less or no children, but also advanced techniques such as PGD, when locally available.[210]

In conclusion, the cases presented in this paper show that a genetic diagnosis in adult onset FSGS can have far reaching consequences not only for the patient but also for his/her family (planning). Identification of patients with a higher likelihood of a genetic FSGS often proves challenging, though there are several hallmarks of genetic disease. Currently we apply a tiered method to genetic testing, to limit incidental findings. In the future, a genetic-first approach could obviate invasive renal biopsies.[70] The probability of a monogenic disease and the potential impact of a genetic diagnosis should be considered in the diagnostic work-up of all adult-onset FSGS cases.

Supplementary material 3

Supplemental Methods 3.1 – Sequencing and variant selection

The genomic data presented in this paper is derived from SOLiD 5500XL system (Life Technologies) sequencing data. All sequencing and variant selection was performed at the ISO15189 accredited Genome Diagnostics section of the Department of Genetics, UMC Utrecht (The Netherlands). This laboratory has now moved from SOLiD sequencing to whole exome sequencing on an Illumina HiSeq platform, allowing for all genepanels to be derived from whole exome data (see Supplemental Table 1, available online).

SOLiD sequencing

For each patient genomic DNA was isolated from a peripheral blood sample. Subsequently, a sequencing library was prepared from the sheared genomic DNA. Each sequencing library, corresponding with a single patient, received a unique 10 nucleotide barcode allowing a cost-effective approach of ~50 samples per single enrichment procedure. Libraries were pooled and target DNA capture was performed with a custom-designed Agilent SureSelectXT assay containing the ~225 gene panel ('RENome', see Supplemental Table 1). All sequencing acquired an average depth of ~100X horizontal coverage to allow for optimal variant calling.

Variant calling and filtering

Variant calling and filtering was performed using the Cartagenia BENCHlab NGS module (V.3.1.2), with a validated filtering tree. To exclude common variants, variants were compared with our in-house database, the Exome Variant (6500 exomes), dbSNP, and GoNL (Genome of the Netherlands) databases. Non-synonymous variants, nonsense variants, essential splice site variants or coding frame-shift insertions or deletions were selected. Variants were subsequently analyzed with in silico prediction programs (a.o. Polyphen2, SIFT, GERP and Grantham scores, and multiple splice-site prediction programs) in the Alamut mutation interpretation software program (V.2.6.0) to determine the possible clinical relevance. All probable pathogenic mutations were independently validated by Sanger sequencing.

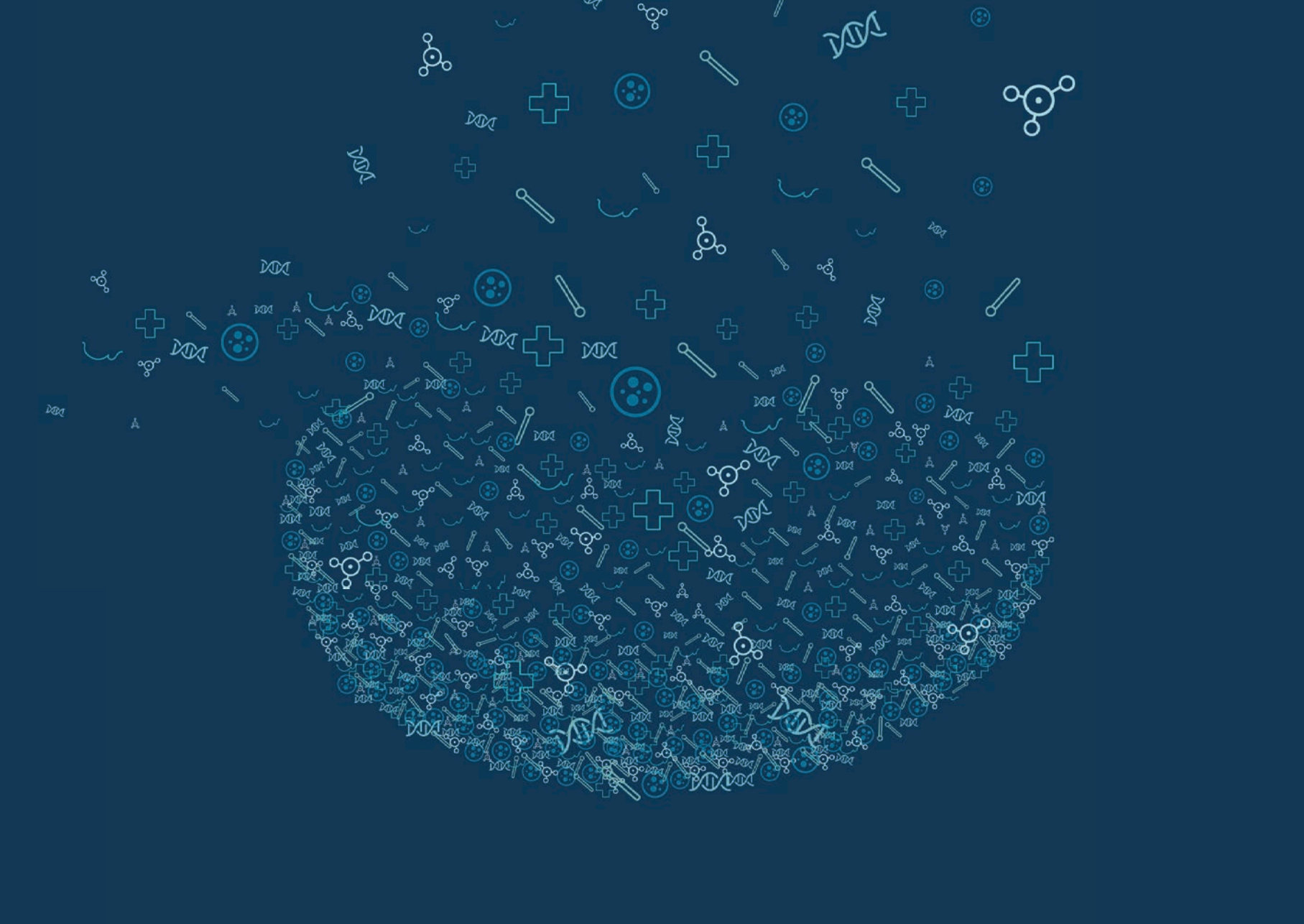
Mosaicism analysis

To determine whether a patient was mosaic for a specific variant, genomic DNA was isolated from a peripheral blood sample and a semi-quantitative Sanger analysis was applied. In this analysis the height and intensity of the Sanger sequencing peak at the genomic location of the variant of interest is compared with those in

healthy controls and family members who are proven not to be mosaic for that variant. The relative height and intensity provides an estimation of the mosaicism percentage in white blood cells. In this paper, semi-quantitative Sanger analysis was only performed in peripheral blood, not in any other tissues.

Supplemental Table 3.1

For Supplemental Table 3.1 please see the online version of this article.



Assessing nephron hyperplasia in fetal congenital solitary functioning kidneys by measuring renal papilla number

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Congenital solitary functioning kidney (CSFK) is caused by unilateral renal agenesis or multicystic dysplastic kidney, often diagnosed with fetal ultrasound (fUS) during pregnancy.[211,212] The contralateral, healthy, kidney is often enlarged.[213] In theory, CSFK leads to low nephron endowment, causing glomerular hyperfiltration, nephron hypertrophy and consequent long-term renal injury.[214] However, a study performed in our group on slaughter pig CSFKs showed nephron hyperplasia, i.e. an increase in the number of functioning nephrons, with a concurrent increase in renal papilla number (RPN).[215] We aim to investigate if hyperplasia is also present in humans CSFKs. As we could not perform invasive kidney biopsies on fetuses, we measured RPN with conventional (2D) and three dimensional (3D) fUS as a proxy. We hypothesize that RPN will increase during gestation, indicating that there might be a role for hyperplasia in CSFK.

After approval of the University Medical Center Utrecht institutional review board and informed consent from all participants, we performed a prospective case-control study. We included 60 fetuses with CSFK[211,212] and subsequently enrolled 60 gestational age, gender and ethnicity matched healthy control fetuses (no congenital anomalies, normal fetal growth). Exclusion criteria were defined as: presence of multiple anomalies and no postnatal confirmation of diagnosis.

US was performed with the Voluson E6 US system (GE Healthcare, Milwaukee, WI), with a 2–5 megahertz transabdominal 2D probe and 2–8 megahertz array transabdominal 3D probe, or a Voluson E8 US system (GE Healthcare, Milwaukee, WI), with a 2.14–6.10 megahertz transabdominal 2D probe and 3.7–9.3 megahertz array transabdominal 3D probe. In 3D fUS, the volume box was placed over the entire kidney, sweep angle set at 65 degrees at maximal quality.

To measure RPN, a papilla was defined as a hyper-echogenic focus near the renal pelvis, often with a hypo-echogenic calix connecting it towards the renal parenchyma (Supplemental Image 4.1).[216] Papillae could be well discerned at all gestational ages. All images were assessed in the sagittal plane, and twice by two independent observers blinded for gestational age and case/control status (RS and TdJ). 3D volume data were analyzed using the Tomographic US Imaging interface of the 4D view program (version 14.0, GE Healthcare, Milwaukee, WI), with slice thickness at 0.5 mm, assessing each slice separately to assure papillae were only measured once.

Presenting our results, the groups showed comparable baseline characteristics (Table 4.1). RPN (mean of all measurements) on concurrent 2D and 3D fUS was comparable in both cases (p=0.7) and controls (p=0.9), as was prenatal imaging compared to postnatal imaging (p=0.5). All cases showed compensatory kidney growth (>95th percentile).

Figure 4.1 displays the differences in RPN between both groups in both modalities. First, the difference (p<0.001) in mean RPN between groups, namely 8.1 papillae (±1.1) in CSFK and 6.1 (±1.2) in controls. Secondly, that RPN increases during gestation, with 0.14 (95%CI 0.09–0.19, p<0.001) papillae per gestational week in CSFK fetuses and 0.10 papillae (95%CI 0.06–0.14, p<0.001) in controls. This increase in RPN is not significantly different between groups (p=0.5).

When analyzing intra- and inter-observer variability, we find low intra-observer variability, namely an interclass correlation coefficient of 0.90 (95%CI 0.85–0.93). The 0.59 (95%CI 0.13–0.79) correlation indicates moderate inter-observer variability. Bland-Altman analysis shows a limit of agreement of ±1.9 papillae within observers and ±2.7 papillae between observers, with no proportional bias (respectively p=0.1 and p=0.9).

When considering our results, we show that at 24 weeks gestational age CSFK fetuses display 1.3 fold increase in RPN compared to controls. This increase is similar to the histologically proven 1.4 increase in a pig model, correlating with a 50% increase in nephron number (hyperplasia).[215] A 45% nephron number increase was also seen in a fetal ovine CSFK model.[217] This papilla-nephron correlation is underscored by our results, as we see an increase in RPN of 0.10-0.14 papillae per gestational week that coincides with the surge in nephron proliferation, starting mid-trimester and continuing up to the 36th week of gestation.[218]

Thus, as our results are in line with animal models finding a correlation between RPN and nephron number, we believe the increase in RPN in our study points towards a hyperplasia mechanism in human CSFK. Additional studies comparing fUS to histology in assessing nephron number with certainty are warranted to further research this hypothesis.[215,219]

Table 4.1 | Baseline characteristics.

Characteristics	CSFK fetuses (n=60)	Healthy fetuses (n=60)	*p*-value
Maternal age in years (mean, SD)	29.7 (4.5)	31.2 (4.6)	p=0.1†
Parity (median, IQR)	1 (0-3)	1 (0-3)	p=0.4‡
Twin pregnancies (n, %)	4 (7%)	5 (8%)	p=0.9#
CSFK due to unilateral renal agenesis (n, %)	33 (55%)	N/A	N/A
CSFK due to multicystic dysplastic kidney (n, %)	27 (45%)	N/A	N/A
Left-sided kidney (n, %)	25 (42%)	23 (38%)	p=0.1*
GA at US in weeks (median, IQR)	24.5 (15.1-33.9)	23.0 (18.4-38.6)	p=0.1‡
Only 2D US performed (n, %)	44 (73%)	44 (73%)	p=0.9*
Concurrent 2D and 3D US performed (n, %)	16 (27%)	16 (27%)	p=0.9*

CSFK=congenital solitary functioning kidney, GA=gestational age, 2D US=two dimensional fetal ultrasound, 3D US=three dimensional fetal ultrasound, N/A=not applicable, SD=standard deviation, IQR=interquartile range. Calculated with: †independent samples *t*-test, ‡Mann Whitney-U test, #Fishers' exact test and *Pearson Chi-square test

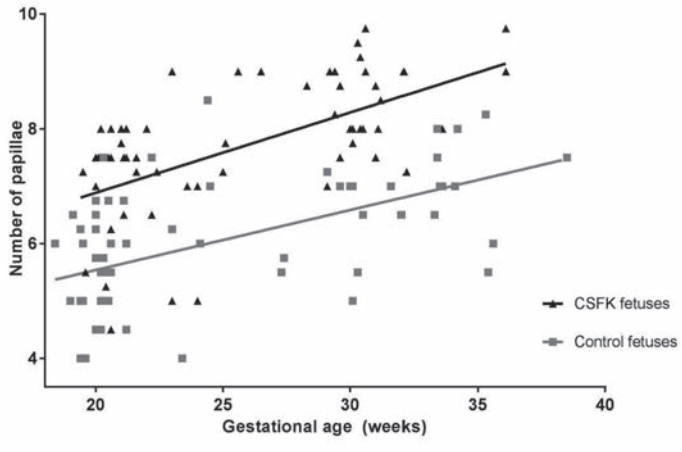
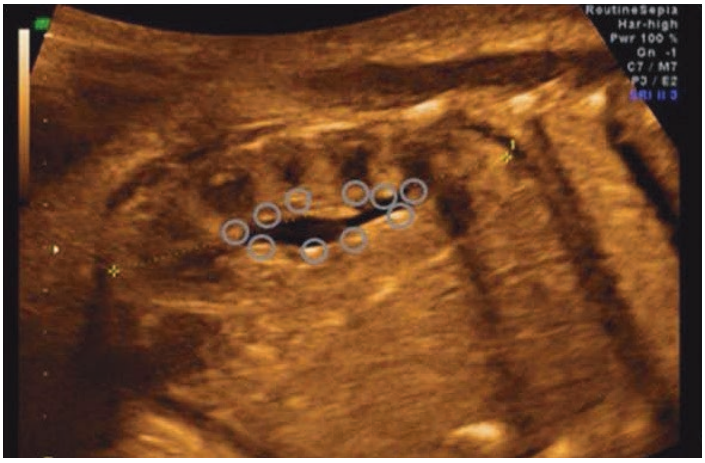


Figure 4.1 | Number of renal papillae, based on fetal ultrasound, is significantly increased (p<0.001) by 2.0 papillae in fetuses with congenital solitary functioning kidneys when compared to healthy controls. The number of papillae increases significantly (p<0.001) during gestation, with an increase of 0.14 and 0.10 papillae per gestational week in congenital solitary functioning kidney fetuses and healthy fetuses respectively.

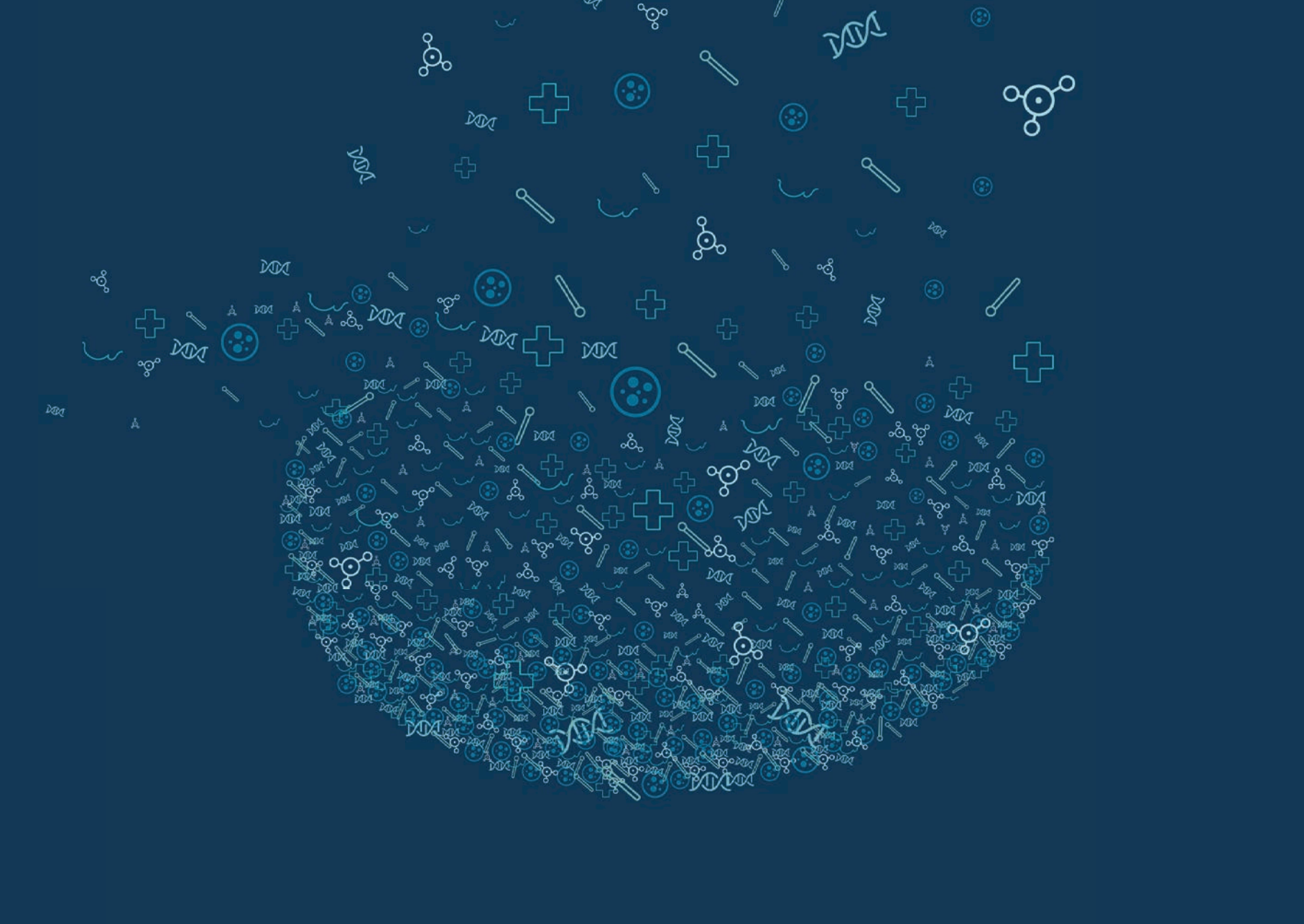
In theory, a higher nephron endowment (i.e. hyperplasia) would lead to a lower risk of developing renal injury.[220] Thus, the main limitation of this study is the lack of comparison between RPN number and postnatal renal injury outcomes, something we hope to investigate in future studies. If further research also indicates nephron hyperplasia, our reliable fUS method to assess RPN could be applied as a non-invasive measure for nephron hyperplasia.

In conclusion, we find a significantly increased RPN in fetal CSFK, suggesting compensatory hyperplasia, and not only hypertrophy, may play a role in CSFK.

Supplementary material 4



Supplemental Image 4.1 | Two-dimensional fetal ultrasound image of a left kidney (healthy fetus, no congenital solitary functioning kidney) at 30 weeks and 4 days of gestational age. The renal papillae (n=10, circled in gray) are seen as hyperechogenic foci next to the renal pelvis, at the base of the hypoechogenic renal pyramids.



5

Defects in t6A tRNA modification due to *GON7* and *YRDC* mutations lead to Galloway-Mowat syndrome

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Abstract

N^6-threonyl-carbamoylation of adenosine 37 of ANN-type tRNAs (t^6A) is a universal modification essential for translational accuracy and efficiency. The t^6A pathway uses two sequentially acting enzymes, YRDC and OSGEP, the latter being a subunit of the multiprotein KEOPS complex. We recently identified mutations in genes encoding four out of the five KEOPS subunits in children with Galloway-Mowat syndrome (GAMOS), a clinically heterogeneous autosomal recessive disease characterized by early-onset steroid-resistant nephrotic syndrome and microcephaly. Here we show that mutations in *YRDC* cause an extremely severe form of GAMOS whereas mutations in *GON7*, encoding the fifth KEOPS subunit, lead to a milder form of the disease. The crystal structure of the GON7/LAGE3/OSGEP subcomplex shows that the intrinsically disordered GON7 protein becomes partially structured upon binding to LAGE3. The structure and cellular characterization of GON7 suggest its involvement in the cellular stability and quaternary arrangement of the KEOPS complex.

5.1 Introduction

Transfer RNAs (tRNA) are subject to multiple post-transcriptional modifications that are important for the stabilization of their ternary structure and the precision of the decoding process.[221] The majority of the complex modifications are concentrated in the anticodon region of the tRNAs and are crucial for accuracy of protein synthesis. The threonylcarbamoylation of the N^6 nitrogen of the adenosine at position 37 (t^6A) of most ANN-accepting tRNAs represents one of the very few nucleotide modifications that exists in every domain of life.[222,223] The t^6A biosynthesis pathway consists of two steps: firstly, the YRDC enzyme (Sua5 in yeast) synthesizes an unsTable 5.threonylcarbamoyl-AMP intermediate (TC-AMP) and in a second step, the KEOPS protein complex transfers the TC-moiety from TC-AMP onto the tRNA substrate.[224] Enzymes that synthesize TC-AMP exist in two versions depending on the organism: a short form which only has the YrdC domain (such as human YRDC) and a long form which has an extra Sua5 domain (yeast SUA5 for instance). The eukaryotic KEOPS complex contains five subunits GON7/LAGE3/OSGEP/TP53RK/TPRKB that are arranged linearly in that order.[225–228] OSGEP is the catalytic subunit that carries out the TC-transfer reaction and its orthologues are present in virtually all sequenced genomes.[228] The other subunits are essential for the t^6A modification of tRNA, but their precise roles are as yet unknown, especially that of GON7, an intrinsically disordered protein (IDP), which was only recently identified in humans.[225,227] Fungal Gon7 was shown to be an IDP that adopts a well-defined structure covering 50% of its sequence upon complex formation with Pcc1 (LAGE3 homolog).[228] In humans, GON7 was recently shown to be also structurally disordered in absence of the other KEOPS complex subunits. GON7 was proposed to be a very remote homolog of the yeast Gon7 protein although its structure upon complex formation remains unknown.[225,227]

tRNA modifications have been demonstrated to play a role in the development of the brain and nervous system, and an increasing number of defects in these modifications are now being linked to various human neurodevelopmental disorders.[229] We recently identified autosomal recessive mutations in genes encoding four of the five subunits of human KEOPS complex, namely *LAGE3*, *OSGEP*, *TP53RK* and *TPRKB* in patients with Galloway-Mowat syndrome (GAMOS, OMIM#251300). GAMOS is a rare neuro-renal disorder characterised by the co-occurrence of steroid-resistant nephrotic syndrome (SRNS) with microcephaly and neurological impairment.[230] GAMOS is clinically heterogeneous, reflecting a genetic heterogeneity. Indeed, disease-causing mutations have been identified in eight genes to date: four in KEOPS genes and four in other unrelated genes, *WDR73*,

WDR4, *NUP133* and *NUP107*.[231–236] SRNS is typically detected in the first months of life and most often rapidly progresses to end-stage renal disease (ESRD) within a few months; however, there are rarer cases with preserved renal function in adulthood.[237] Kidney lesions range from minimal change disease (MCD), to focal segmental glomerulosclerosis (FSGS) that might be of the collapsing type, or diffuse mesangial sclerosis (DMS). Cerebral imaging findings include cerebral and cerebellar atrophy, and gyration and/or myelination defects. These anomalies are associated with neurological deficits such as psychomotor impairment, hypotonia, seizures, and more rarely sensorineural blindness and deafness. Affected children may also present with facial and/or skeletal dysmorphic features. The prognosis of GAMOS is poor, and most affected children die before six years of age.

Here we present 14 GAMOS affected individuals from seven families, with mutations in *GON7* (alias *C14orf142*) and *YRDC*, both genes encoding proteins involved in the biosynthesis of the t^6A modification. These data, together with our previous work, show that mutations in genes encoding all the proteins involved in the two chemical steps of t^6A lead to GAMOS. Furthermore, we determine the crystal structure of the GON7/LAGE3/OSGEP KEOPS subcomplex showing that GON7 becomes structured upon binding to LAGE3. The structure also explains our observations that GON7 stabilizes the remainder of the KEOPS complex and directs its quaternary organization.

5.2 Material and Methods

5.2.1 Patients and families

Written informed consent was obtained from participants or their legal guardians, and the study was approved by the Comité de Protection des Personnes "Ile-De-France II." Genomic DNA samples were isolated from peripheral blood leukocytes using standard procedures.

5.2.2 Whole exome sequencing and mutation calling

We performed whole exome sequencing using Agilent SureSelect All Exon 51 Mb V5 capture-kit on a HiSeq2500 (Illumina) sequencer (paired-end reads: 2 × 100 bases). Sequences were aligned to the human Genome Reference Consortium Human Build 37 (GRCh37) genome assembly with the Lifescope suite from Life Technologies. Variant calling was made using the Genome Analysis Toolkit pipeline. Then, variants were annotated using a pipeline designed by the Paris Descartes University Bioinformatics platform. We assumed the causal variant: i) segregates with the disease status, ii) is novel or has a minor allele frequency <1/1,000 in

gnomAD, iii) was not found in >10/2,352 projects of our in-house database. Missense variant pathogenicity was evaluated using in silico prediction tools (PolyPhen2, SIFT and Mutation Taster). Sanger sequencing was used to validate the variant identified by exome sequencing and to perform segregation analysis in the families. Sequence were analyzed with the Sequencher software (Gene Codes, Ann Arbor, MI) and positions of mutations were numbered from the A of the ATG-translation initiation codon. For Family G, WES and SNP-array were performed according to standard diagnostic procedures and WES quality criteria at the UMC Utrecht, the Netherlands. The patient-parent quartet WES with sibling-sharing analysis focused on the regions of homozygosity determined by SNP-array (parents are consanguineous in the 8th degree).

5.2.3 Plasmids, cell culture, establishment of cell lines

The following expression vectors were used in this publication: LentiORF pLEX-MCS (Open Biosystems), pESC-TRP with a c-myc tag (Agilent) and pLKO.1-TRC Cloning vector (# Plasmid 10878, Addgene). The LentiORF pLEX-MCS plasmid was modified by site-directed mutagenesis (QuickChange kit, Agilent) to insert one *Nhe*I restriction site, and either 2 copies of the Human influenza hemagglutinin (HA) tag or one copy of the V5 epitope tag allowing epitope-tagging at the N-terminal of the encoded protein. Human full-length *GON7*, *LAGE3* and *YRDC* cDNA (NM_032490.5, NM_006014.4, and NM_024640.4, respectively) were amplified by PCR from IMAGE cDNA clones (IMAGE 4796574, IMAGE 5485603, IMAGE 5211591 and IMAGE 6147134, respectively), and subcloned into the modified pLEX-MCS plasmid using either *Spe*I and *Xho*I (for *GON7* and *LAGE3*) or *Nhe*I and *Xho*I (for *YRDC*). Human YRDC cDNA was also subcloned into the *Bam*HI and *Sal*I sites of pESC-TRP. Site-directed mutagenesis (QuickChange kit, Agilent) was used to generate the mutations used in this study. An adapted cloning protocol was used to obtain the C-terminal extension found for the YRDC p.Val241Ilefs*72 mutant. For gene silencing, the shRNA sequences described in Supplementary Table 5.6 were cloned into the lentiviral pLKO.1-TRC Cloning Vector using the *Age*I and *Eco*RI restriction sites. This vector contains a cassette conferring puromycin resistance. All constructs were verified by Sanger sequencing.

The human immortalized podocyte cell line (AB8/13) provided by M. Saleem (University of Bristol, UK) was grown at 33°C with 7% CO2 in RPMI 1640 medium supplemented with 10% fetal bovine serum, insulin-transferrin-selenium, glutamine, and penicillin and streptomycin (all from Life Technologies).and human primary fibroblasts, obtained from patient skin biopsies were cultured in OPTIMEM medium supplemented with 10% fetal bovine serum, sodium pyruvate, glutamine, fungizone,

and penicillin and streptomycin (all from Life Technologies) at 37°C with 7% CO2. Obtention and culture of lymphoblastoid cell lines are detailed in Supplementary Methods. Human podocytes stably overexpressing 2HA-GON7 or V5-LAGE3, or transiently depleted for *GON7*, *LAGE3*, *OSGEP* or *YRDC* were obtained by transduction with lentiviral particles and subsequent puromycin selection (2 µg/ml). HEK293T cells (ATCC CRL-3216) were transiently transfected using Lipofectamine® 2000 (ThermoFisher Scientific).

5.2.4 Antibodies and chemical compounds

The following antibodies were used in the study: mouse anti-α-tubulin (T5168, used at 1:1000), mouse anti-actin (A5316, used at 1:1000), mouse anti-HA (12CA5, at 1/1000), rabbit anti-GON7 (HPA 051832, used at 1:500), rabbit anti-LAGE3 (HPA 036122, used at 1/500), rabbit anti-TPRKB (HPA035712, used at 1:500), rabbit anti-OSGEP (HPA 039751, used at 1/1000) and mouse anti-GAPDH (MAB374, used at 1/2000) from Sigma-Aldrich; mouse anti-V5 (MCA1360, used at 1/1000) from Bio-Rad; rabbit anti-YRDC (PA5-56366, used at 1:500) from Thermo Fisher Scientific; rabbit anti-LAGE3 (NBP2-32715, used at 1:1000) and mouse anti-OSGEP (NBP2-00823, used at 1:500) from Novus Biologicals; rabbit anti-TP53RK (AP17010b, used at 1:500) from Abgent. Secondary antibodies for immunoblotting were sheep: anti-mouse and donkey anti-rabbit HRP-conjugated antibodies (GE Healthcare UK), and IRDye 800CW Donkey anti-rabbit (926-32213) and IRDye 680RD Donkey anti-mouse (926-68072) antibodies (LI-COR). Cycloheximide (C7698), Nuclease P1 (N8630), phosphodiesterase I from snake venom (P3243) and alkaline phosphatase (P4252) were purchased from Sigma-Aldrich.

5.2.5 Yeast culture and heterologous complementation assay

Yeast cells were grown at 28°C in standard rich medium YEPD (1% yeast extract, 2% peptone, 2% glucose) or minimal supplemented media (0.67% YNB, 2% carbon source). Cells were transformed using the lithium acetate method.[238] Media were supplemented with 2% agar for solid media. The *S. cerevisiae* W303 derived strain, *Δsua5:KanMX*(YCplac33-*SUA5*)[239], was used as the host for the complementation assay. For each pESC-TRP plasmid derivative to be tested, three independent clones were selected after transformation and grown on GLU-TRP media. Clones were then streaked onto GAL-TRP containing 0.1% 5-fluoroorotic acid (5-FOA) to counter-select the YCplac33-*SUA5* plasmid (containing *URA3*). After 2 rounds of selection, clones were checked for their acquired Ura- phenotype, their plasmid content was confirmed by sequencing after plasmid rescue before being finally evaluated for fitness by a 10 fold serial dilution spotted onto GAL-TRP minimal supplemented

media. Empty pESC-TRP, pESC-TRP-*SUA5* and pESC-TRP-*SUA5-myc* were used as negative and positive controls, respectively.

5.2.6 Quantitative real-time PCR

Total mRNA from knocked down podocytes, primary skin fibroblasts and LCLs was isolated using Qiagen Extraction RNeasy® Kit and treated with DNase I. One µg total RNA was reverse-transcribed using Superscript II, according to the manufacturer's protocol (Life Technologies). The relative expression levels of the mRNA of interest were determined by real-time PCR using Power SYBR Green ROX Mix (ThermoFisher Scientific) with specific primers listed in Supplementary Table 5.7. Samples were run in triplicate and gene of interest expression was normalized to human hypoxanthine-guanine phosphoribosyl transferase (*Hgprt*). Data were analyzed using the 2−ΔΔCt method.

5.2.7 Quantification of t6A modification

Yeast tRNAs were extracted and purified from actively growing cells (at OD_{600nm} of approximately 3.107 cells/mL) with phenol induced cell permeabilization, LiCl-selective precipitation and subsequent ion exchange-chromatography purification on an AXR-80 column (Nucleobond, Macherey-Nagel), according to the manufacturer's instructions. For human primary fibroblasts, the two-step protocol that was applied is detailed in Supplementary Methods. 10 µg of yeast tRNAs and approximately 1 µg of human fibroblast tRNAs were then enzymatically hydrolyzed into ribonucleosides with nuclease P1, phosphodiesterase and alkaline phosphatase, deproteonized by filtration, and finally dried under vacuum according to the protocol of Thuring *et al.*[240] t^6A ribonucleoside was analyzed using the quantitative LC/MS-MS protocol of Thüring *et al.*[240] Quantification of t^6A was performed by integration of the peaks of interest and expressed relative to the total area of the peaks corresponding to the four canonical unmodified ribonucleosides assessed in the same sample for normalization. tRNA extracted from three independent samples were each measured twice (two technical replicates). Detailed information are provided in Supplementary Methods.

5.2.8 Protein extraction and immunoblotting

Proteins from KD podocytes, primary fibroblasts and LCLs were extracted in lysis buffer containing 150mM NaCl, 50mM Tris-HCL pH7, 0.5% Triton-X100 with Complete™ protease inhibitors (Roche) as in Serrano-Perez *et al.*[241] Fifty micrograms of proteins were loaded onto acrylamide gels and blotted onto nitrocellulose membranes (Amersham). The membranes were blocked in 1X

Tris-buffered saline, 0.1% Tween 20 (TBST) with 5% bovine serum albumin or in Odyssey (LI-COR Bioscience) blocking buffer. Membranes were then incubated with the indicated primary antibodies, washed and then incubated with either HRP-conjugated or LI-COR IRDye secondary antibodies. Signals were detected using ECL reagents (Amersham Biosciences) and acquired in a Fusion Fx7 darkroom (Vilber Lourmat) or acquired with Odyssey CLx near-infrared fluorescent imaging system (LI-COR Bioscience). Densitometry quantification was performed either using Bio-1D software or using *Image studio lite* software (version 5.2). Uncropped and unprocessed blots are provided in the Source Data file.

5.2.9 Immunoprecipitation and cycloheximide chase experiments

For immunoprecipitation, HEK293T cells were transiently transfected with the adequate plasmids (2HA-tagged GON7, V5-tagged-LAGE3 wild-type (WT) and/or mutants) using calcium phosphate. Forty-eight hours post transfection, cells were lysed in 150mM NaCl, 25mM Tris-HCL pH8, 0.5% Triton with protease inhibitors and HA-tagged GON7 was immunoprecipitated using the µMACS™ Epitope Tag Protein Isolation Kit (Miltenyi Biotec). Briefly, fresh lysates (1-1.5 mg of protein) were incubated either with mouse anti-V5 antibodies, followed by a 30-minutes incubation with magnetic beads-coupled to protein A, or directly with magnetic beads coupled to a HA antibody. Immunoprecipitated proteins were isolated using µMACS® Separation Columns in a magnetic µMACS separator and subsequently eluted with 1X Laemmli buffer. Lysates and immunoprecipitated samples were subjected to immunoblot.[241] To assess rates of protein degradation, HEK293T cells transiently expressing either 2HA-GON7 or V5-LAGE3 alone, or co-expressing both proteins were incubated with cycloheximide at a final concentration of 100 µg/ml for the indicated time periods (0.5, 1, 2, 4 and 6 hrs). Total protein extracts and immunoblotting were performed as described above. Anti-HA and anti-V5 antibodies were used to reveal GON7 and LAGE3, respectively. Relative GON7 and LAGE3 protein amount were normalized to those of α-tubulin at each time point.

5.2.10 Cell proliferation, apoptosis and protein synthesis assays

Cell proliferation, apoptosis level and rates of protein synthesis were assessed in KD podocytes using the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (MTT) (Promega), the Caspase 3/7 Green detection Reagent (C10423, ThermoFisher Scientific) and the Click-iT HPG Alexa Fluor 488 Protein Synthesis Assays (C10428, Thermo Fisher Scientific), respectively, according to manufacturer's instructions. Detailed information are provided in Supplementary Methods.

5.2.11 Proteomic studies

Human podocyte cell lines stably expressing either 2HA-GON7 or V5-LAGE3 were used to perform proteomic studies. 2HA-GON7 and V5-LAGE3 were immunoprecipitated as described in the section above. Eluates were processed according to Braun *et al.*[232] Two groups (control IP versus IP HA or IP V5), each containing three biological replicates were used for statistical analysis. Only proteins that were identified at least three times out of six were retained. A *t*-test was performed, and the data were represented in a volcano plot (FDR <0.01, S0 = 2,250 randomizations).

5.2.12 Telomeric restriction fragment

Measurement of the length of the terminal restriction fragments was performed by Southern blotting according to Touzot *et al.*[242]

5.2.13 Expression and purification of KEOPS subunits

All structural work was done using the full length proteins of GON7, LAGE3 and OSGEP. For NMR experiments, two vectors were ordered from Genscript (Piscataway, USA) for the expression of either unlabeled his-tagged LAGE3 (vector "pET21a-LAGE3_hisTEV_op") or ^{15}N-labelled his-tagged GON7 (vector "pET24d-C14_hisTEV_op") whose sequences are shown in Supplementary Table 5.8. Expression and purification of LAGE3 and ^{15}N-GON7 and subcomplex LAGE3/15N-GON7 preparation are described in detail in Supplementary Methods.

For SAXS or Crystallogenesis experiments, preparation of unlabeled GON7 and GON7/LAGE3/OSGEP subcomplex, and co-expression and purification of the KEOPS subunits are detailed in Supplementary Methods and Supplementary Figures 12 and 13. Fractions of the heterotrimeric GON7/LAGE3/OSGEP complex eluted from size exclusion chromatography (Supplementary Table 5.9) were then re-loaded onto NiIDA and washed with lysis buffer A supplemented with increasing concentrations of NaCl (0.2 M; 0.5 M; 1 M and 2 M) in order to remove traces of contaminants. Bound proteins were eluted using 3 fractions of 2 mL of buffer A supplemented with 100, 200 and 400 mM imidazole and the 3 subunits complex was concentrated to 8.3 mg mL^{-1} for crystallization trials. A unique crystal was obtained using the sitting-drop vapor diffusion method after more than 6 months-incubation at 4°C. The successful condition was composed of 100 nL of protein solution and 100 nL of 30 % PEG 4000, 0.1 M Tris HCl pH 8.5 and 0.2 M Magnesium Chloride. The crystal was cryo-protected by quick-soaking in reservoir solution supplemented with 30% glycerol prior to flash freezing in liquid nitrogen.

5.2.14 Modeling and crystal structure determination

Modeling of YRDC: the Phyre2 and I-tasser webservers both proposed high confidence models for YRDC based on the StSua5 crystal structure despite weak sequence identity between the two species. A 3D model of YRDC was built using the MODELLER software.[243] X-ray diffraction data collection was carried out on beamline Proxima1 at the SOLEIL Synchrotron (Saint-Aubin, France) at 100K. Data were processed, integrated and scaled with the XDS program package.[244] The crystal belonged to space group $P4_3$. The OSGEP and LAGE3 subunits were positioned by molecular replacement with the programs PHASER[245] and MOLREP, implemented in the CCP4 suite[246] using the structures of MjKae1 (PDB ID: 2VWB) and ScPcc1 (PDB ID: 4WX8) as search models. Residual electron density showed clearly the presence of the GON7 subunit, which was constructed using the program BUCCANEER.[246] The initial structure was refined using the BUSTER program (Bricogne G., Blanc E., Brandl M., Flensburg C., Keller P., Paciorek W., Roversi P, Sharff A., Smart O.S., Vonrhein C., Womack T.O. (2017). BUSTER version 2.10.3. Cambridge, United Kingdom: Global Phasing Ltd.) and completed by interactive and manual model building using COOT[247]. The correctness of the assigned GON7 sequence was verified by omit mFo-DFc, 2mFo-DFc, Prime-and-switch electron density maps[248] (Supplementary Figure 5.14). One copy of the GON7/LAGE3/OSGEP heterotrimer was present in the asymmetric unit. Data collection and refinement statistics are gathered in Supplementary Table 5.5. The coordinates have been deposited at the Protein Data Bank (code 6GWJ).

5.2.15 Small-angle X-ray analysis

Small angle x-ray scattering (SAXS) experiments were carried out at the SOLEIL synchrotron SWING beamline (Saint-Aubin, France). The sample to detector (Aviex CCD) distance was set to 1500 mm, allowing reliable data collection over the momentum transfer range 0.008 $Å^{-1} < q < 0.5$ $Å^{-1}$ with $q = 4\pi\sin\theta/\lambda$, where 2θ is the scattering angle and λ is the wavelength of the X-rays ($\lambda = 1.0$ Å). To isolate the various species in solution, SAXS data were collected on samples eluting from an online size exclusion high-performance liquid chromatography (SEHPLCBio-SEC3Agilent) column and directly connected to the SAXS measuring cell. 65 µl of GON7/LAGE3/OSGEP and GON7 samples concentrated at 1.5 and 6.7 $mg.L^{-1}$ respectively were injected into the column pre-equilibrated with a buffer composed of 20 mM MES pH 6.5, 200 mM NaCl and 5 mM 2-mercaptoethanol. Flow rate was 300 µl/min, frame duration was 1.0 s and the dead time between frames was 0.5 s. The protein concentration was estimated by UV absorption measurement at 280 and 295 nm using a spectrometer located immediately upstream of the SAXS

measuring cell. A large number of frames were collected before the void volume and averaged to account for buffer scattering. SAXS data were normalized to the intensity of the incident beam and background (i.e. the elution buffer) subtracted using the program FoxTrot[249], the Swing in-house software. The scattered intensities were displayed on an absolute scale using the scattering by water. Identical frames under the main elution peak were selected and averaged for further analysis. Radii of gyration, maximum particle dimensions and molecular masses were determined using PrimusQT[250] (Supplementary Table 5.4). The BUNCH program[251] was then used to build atomic models of GON7/LAGE3/OSGEP starting from the crystal structure and by determining the optimal position of the missing regions as to fit the data. In a final step, we substituted the dummy residues of these flexible parts with all-atom descriptions using the programs PD2 and SCWRL4.[252] An ultimate adjustment was performed using the program CRYSOL.[253] The modelling was repeated 10 times and the best model was deposited in SASBDB[254] with codes SASDFK8, SASDFM8 and SASDFL8 for GON7, GON7/LAGE3 and GON7/LAGE3/OSGEP, respectively.

5.2.16 Statistical analyses

GraphPad Prism 8.0 software was used for the graphical representation and statistical analysis of cell-based data. Results are presented as mean ± s.e.m of at least *n*=3 independent experiments. For statistical analysis, data sets comparing more than 3 conditions (to a control group) were analyzed with ANOVA followed by Dunnett's multiple comparisons test or by using Kruskal-Wallis test followed by a Dunn's multiple comparisons test. Data sets with only two conditions to compare were analyzed using an unpaired t-test or a Mann-Whitney test. $P<0.05$ was considered statistically significant. A standard confidence interval of 95% was applied in all analyses. Displayed in the figures are the mean values of all technical replicates for each of the independent experiments (displayed as single data points). Black lines indicate the mean values of all independent experiments.

5.2.17 Data availability

The data generated during the current study are available from the corresponding authors upon reasonable request. The source data underlying Figs 3c-d, 4 and 6 and Supplementary Figures 1, 5b, 8b, 9 and 10 are provided as a Source Data file. Accession codes for deposited data: crystal structure of GON7/LAGE3/OSGEP (PDB ID: 6GWJ, [https://www.rcsb.org/structure/6GWJ]); SAXS model codes SASDFK8, SASDFM8 and SASDFL8 for GON7, GON7/LAGE3 and GON7/LAGE3/OSGEP, respectively.

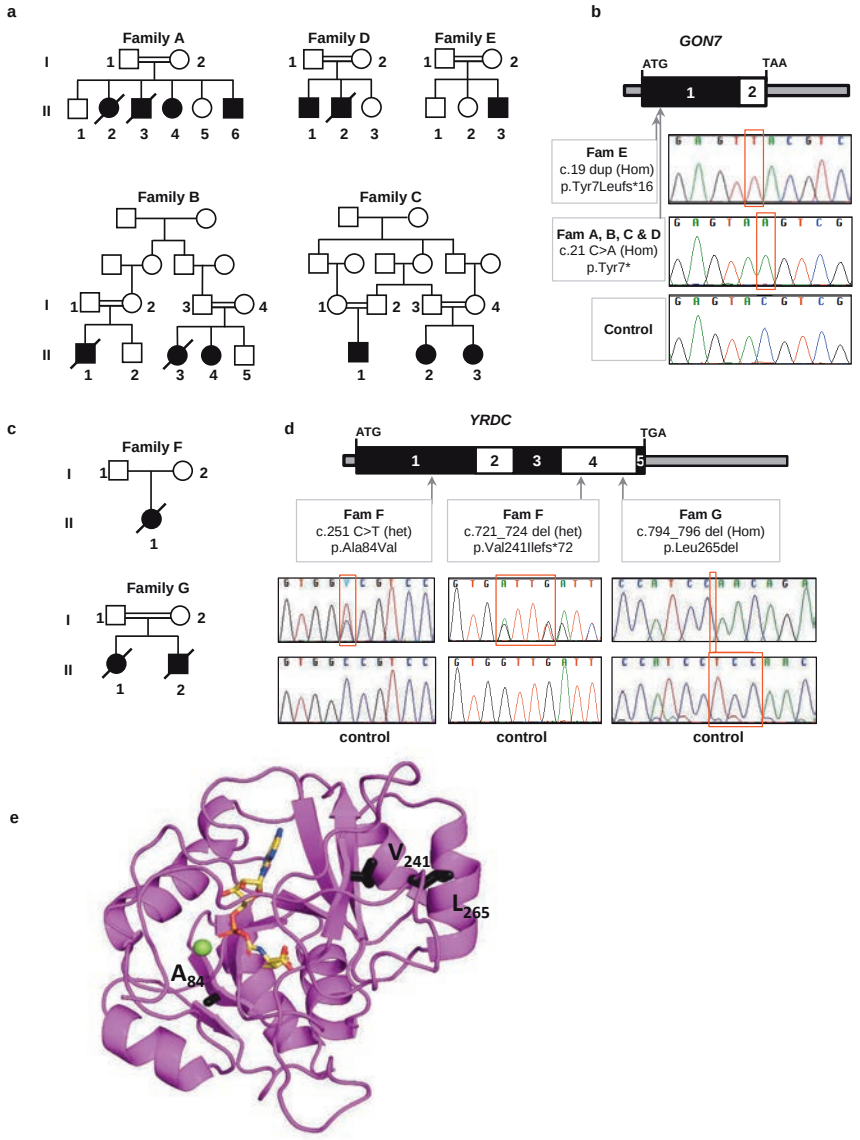


Figure 5.1 | Identification of mutations in GON7 and YRDC in patients with Galloway-Mowat syndrome. (a) and (c) Pedigrees of families with mutations in GON7 (a) and in YRDC (c). Affected individuals are in black. (b) and (d) Organization of exons of human GON7 and YRDC cDNAs. Positions of start and stop codons are indicated. Arrows indicate positions of the identified mutations. Lower panels show the sequencing traces for affected individuals with identified mutated nucleotide indicated with a red square (Hom: homozygous; het, heterozygous). (e) Representation of a 3D-model of human YRDC, bound to the reaction product threonylcarbamoyl-adenylate, in sticks. The model was constructed using the crystal structure of Sulfolobus tokodaii Sua5 (PDB code 4E1B). The side chains of the three mutated residues are in black sticks. The green sphere represents a Mg2+ ion.

5.3 Results

5.3.1 Identification of GON7 and YRDC mutations in GAMOS patients

Through whole-exome sequencing in individuals with GAMOS, we identified mutations in the *GON7* gene in eleven affected individuals from five unrelated families and in the *YRDC* gene in three affected individuals from two unrelated families (Fig.1a-d and Supplementary Table 5.1). Four of the families with *GON7* mutations (Families A to D) carried the same homozygous nonsense mutation (c.21 C>A, p.Tyr7*) which causes a stop codon at position 7 of the protein. These families, all originating from the same region of Algeria, shared a common haplotype at the *GON7* locus indicating a founder effect (Supplementary Table 5.2). The affected individual of the fifth family (Family E) carried a different mutation involving the same residue at position 7 and leading to a frameshift (c.19dup, p.Tyr7Leufs*16). Both *GON7* mutations are predicted to lead to the absence of protein expression and, as expected, no protein was detected in cells available from the affected individuals from family A, B and C (Supplementary Figure 5.1a and c). Two compound heterozygous *YRDC* mutations were identified in Family F: a missense mutation (c.251 C>T, p.Ala84Val) and a 4-base pair deletion leading to a frameshift (c.721_724del, p.Val241Ilefs*72). In Family G, we identified a homozygous in-frame deletion of Leucine 265 (c.794_796 del, p.Leu265del). For both families, western blot and qPCR analysis on cell extracts from affected children showed the presence of YRDC transcripts and proteins (Supplementary Figure 5.1b and d). To make a prediction of the effect of the *YRDC* mutations on the protein, we created a 3D structural model of human YRDC using the structure of the YRDC domain of the archaeal Sua5 (PDB 4E1B, 20 % sequence identity[255]) and mapped these mutations onto this *in silico* model (Figure 5.1e). Structures of YRDC domains are very well conserved and sequence alignment shows that the human YRDC only has minor insertions/deletions compared to Sua5 (Supplementary Figure 5.2). The replacement of Ala84, located in a hydrophobic region between a α-sheet and a connecting β-helix, by the larger amino acid valine might perturb optimal packing and destabilize the structure of the protein. The YRDC Leu265del mutation affects a highly conserved amino acid and creates a deletion in a C-terminal peptide that hangs over the active site and could have a role in enzyme activity.

Early-onset proteinuria was observed in all affected children, with first detection ranging from between birth and 5 years. All but three children reached ESRD between 1.5 months and 6 years of age. All individuals carrying *YRDC* mutations presented with congenital or infantile SRNS detected from between birth and 4 months of age and died early, whereas most of the individuals carrying *GON7*

mutations were alive at last follow up, with either a functioning graft or with normal renal function despite a mild to heavy proteinuria (Supplementary Table 5.1). Kidney biopsies, when available, typically displayed FSGS (Families A, C and E) or DMS (Families B, F and G) (Fig. 2a-d). In addition to developmental delay, primary microcephaly was present in the two affected children of one family with *YRDC* mutations, whereas the affected child in the second *YRDC* family and all *GON7* mutated individuals presented with post-natal microcephaly. Brain MRI revealed a spectrum of cerebellar and cortical hypoplasia or atrophy with thin corpus callosum and ventricular dilation, myelination delay, and in one case a simplified gyral pattern (Individual G.II-2) (Fig.2e-n, Supplementary Figure 5.3). Extra-renal features included facial dysmorphy, arachnodactyly, hiatal hernia with gastro-oesophageal reflux, congenital hypothyroidism (solely in the *YRDC* cases) and myoclonia. This clinical picture is highly reminiscent of that observed in GAMOS-affected individuals with mutations in *LAGE3*, *OSGEP*, *TP53RK* and *TPRKB*.[232] However, individuals with *GON7* mutations presented with milder neurological and renal manifestations, always with post-natal microcephaly and no gyration defects, later onset of proteinuria (median age 18 months vs. 1) and slower progression to ESRD (median age 49 months in 8/11 children vs. 5 months in 3/3 children), and a longer survival compared to the *YRDC* cases.

5.3.2 Impact of YRDC and GON7 mutations on t^6A biosynthesis

To assess the pathogenicity of *YRDC* mutations, we first used a yeast heterologous expression and complementation assay as previously performed for *OSGEP* mutations identified in GAMOS individuals.[232] Indeed, the deletion of *SUA5*, the *YRDC* orthologue in *S. cerevisiae* leads to a very severe growth defect, similarly to the deletion of each of the genes encoding the five KEOPS subunits.[223,226,256,257] We therefore heterologously expressed the human YRDC cDNAs encoding wild-type (WT) and mutant proteins in the *Δsua5* strain to evaluate their ability to rescue the slow growth phenotype. Since the catalytic activity of YRDC does not require protein partners, the WT YRDC protein efficiently complemented the *Δsua5* growth defect (Fig.3a).

Although a somewhat similar complementation was observed for the p.Ala84Val and p.Leu265del mutants, the p.Val241Ilefs*72 mutant was notably unable to improve the poor growth of the *Δsua5* strain (Fig.3a). All YRDC proteins were efficiently expressed in *Δsua5* strain, except the p.Val241Ilefs*72 mutant that was barely detecTable 5.by western blot, suggesting that it is likely being degraded by an intracellular proteolytic machinery (Fig.3b). Using mass spectrometry, we then analyzed the t^6A content of these transformed *Δsua5* yeast strains.

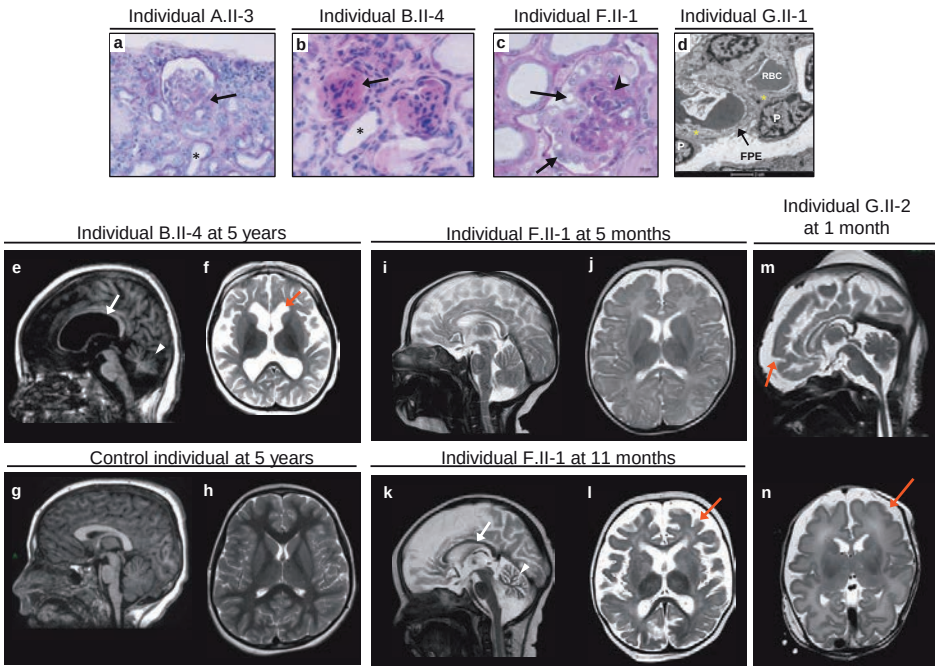


Figure 5.2 | Kidney pathology analysis and neuroimaging. Light and transmission electron microscopy (TEM) of kidney sections of patients with GON7 (a-b) or YRDC mutations (c-d). (a) Individual A.II-3 displays a retracted glomerulus with a focal segmental glomerulosclerosis lesion at the vascular pole (black arrow) and tubular dilations (black star) (PAS; 200 x magnification). (b) Individual B.II-4 displays diffuse mesangial sclerosis with tiny, retracted and sclerosed glomeruli (black arrow) with dilated tubes surrounded by flat epithelial cells (black star) and interstitial fibrosis (H&E; 400 x magnification). (c) Individual F.II-1 displays a marked glomerular tuft collapsing (arrowhead) surrounded by a layer of enlarged and vacuolized podocytes (black arrows) (PAS stain; 400 x magnification, scale bar, 10µm). (d) TEM of individual G.II-1 shows diffuse foot process effacement (FPE; black arrow), a classical hallmark of nephrotic syndrome, along a glomerular basement membrane (GBM) with abnormal folded and laminated segments (yellow stars), alternating with others with normal appearance. P, podocyte; RBC, red blood cell. Scale bar, 2µm. Brain MRI of patients with GON7 (e-f) and YRDC mutations (i-n). (e-f) Brain MRI abnormalities in individual B.II-4 at 5 years. Sagittal T1 weighted image (e) shows important cortical subtentorial atrophy as well as corpus callosal (arrow) and cerebellar atrophy (arrowhead). The axial T2 weighted image (f) shows abnormal myelination and ventricular dilatation (red arrow). (g-h) Brain MRI of a 5-year old control showing sagittal T1 (g) and axial T2 (h) weighted images. (i-l) Brain MRI abnormalities in individual F.II-1 at 5 months (i, j) and 11 months (k, l). Sagittal T2 weighted image shows normal pattern at 5 months (i) evolving to a progressive major cerebellar (arrowhead) and cortical atrophy with a very thin corpus callosum (arrowhead) at 11 months (k). The axial T2 weighted image is normal at 5 months (j) but shows a very marked abnormality of myelination and cortical atrophy (red arrow) at 11 months (l). (m-n) Brain MRI abnormalities in individual G.II-2 at 1 month. Sagittal T2 (m) and axial T2 (n) weighted images show gyral anomalies with marked frontal gyral simplification (red arrow) and myelination delay.

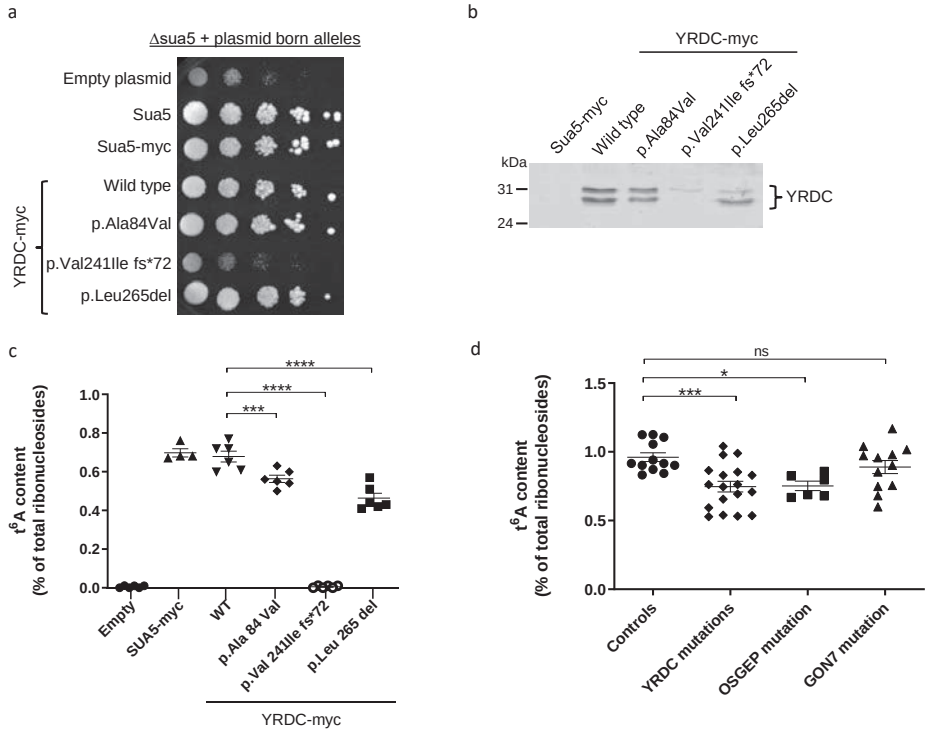


Figure 5.3 | Effects of YRDC and GON7 mutations on t6A biosynthesis. (a) Evaluation of fitness of Δsua5 yeast strains expressing human YRDC variants (spots are from 10-fold serial dilutions of cell suspensions at OD600nm=0.5, and three independent clones were evaluated) and (b) western blot analysis on total protein extracts from Δsua5 yeast cells expressing human YRDC variants using anti-hYRDC antibody. (c-d) Mass spectrometry (LC-MS/MS) quantification of t6A modification in total tRNAs extracted from Δsua5 yeast cells expressing human YRDC variants (c) (mean ± s.e.m. of 2 independent LC-MS/MS experiments (technical replicates), each measuring samples from 3 independent yeast transformants; one-way ANOVA ($F(5,28)=269.4$, $P<0.0001$), Dunnett's multiple comparisons test, ***p=0.0008, ****p <0.0001) and from cultured primary skin fibroblasts from controls (2 unaffected individuals) and affected individuals with either the p.Tyr7* GON7 mutation (2 individuals), or the YRDC mutations (3 individuals) or with the p.Arg352Gln OSGEP mutation in the homozygous state (individual « CP » described in Braun et al., 2017) (d) (mean ± s.e.m. of 2 independent LC-MS/MS experiments (technical replicates), each measuring samples from 3 independent cell culture experiments; one-way ANOVA ($F(3,44)=6.446$, $P<0.001$), Dunnett's multiple comparisons test, ns=0.4894, *p=0.0169, ***p =0.008). Source data are provided as a Source Data file.

As expected, since Sua5 is the only enzyme in yeast that generates TC-AMP, the *Δ*sua5 mutant was unable to synthetize t^6A, whereas the WT YRDC expressing strain exhibited t^6A levels comparable with those measured for WT Sua5 (Fig.3c). The p.Ala84Val and p.Leu265del mutants showed a slight, but significant decrease in t^6A levels. In contrast, like in the *Δsua5* strain, no trace of t^6A modification could

be detected in the p.Val241Ilefs*72 mutant (Fig. 3c). In line with the results of the growth complementation assay, there was a direct correlation between cell fitness and t^6A content. This allowed us to classify *YRDC* mutations into hypomorphic (encoding p.Ala84Val and p.Leu265del) and amorphic (encoding p.Val241Ilefs*72) alleles, as has been previously shown for *OSGEP* mutations.[232] A similar approach could not be applied for *GON7* mutations since GON7 failed to complement the growth defect of the *Δgon7* yeast strain (Wan *et al.*, 2017[227] and our data). We therefore measured the t^6A content in fibroblasts from two individuals with the p.Tyr7* *GON7* mutation, three individuals with *YRDC* mutations and one individual with the p.Arg325Gln *OSGEP* mutation. The t^6A levels were significantly decreased in both *YRDC*- and *OSGEP*-mutated fibroblasts, and to a lesser extent in *GON7*-mutated fibroblasts (Fig.3d) confirming the impact of these mutations on t^6A biosynthesis in affected individuals. In addition, we demonstrated that, similarly to individuals with *OSGEP* or *TP53RK* mutations[232], telomere length was not affected in individuals with YRDC and GON7 mutations (Supplementary Figure 5.4). This confirms that contrary to what has been demonstrated in yeast, human YRDC and KEOPS complex are not involved in telomere maintenance in human cells.[258–260]

5.3.3 In vitro characterization of WT and mutants YRDC

To compare the stability and structure of the WT YRDC with those of the p.Ala84Val and p.Leu265del mutants, we expressed and purified these proteins in an *E. coli* expression system (Supplementary Table 5.3). The three proteins could be purified but we noticed that both mutants were less sTable 5.and less soluble compared to the WT (Supplementary Methods). To probe the proper folding of the YRDC WT and mutants, we collect 1D ^{1}H-NMR spectra (Supplementary Figure 5.5a). All the spectra displayed well-dispersed signals for amide protons as well as several signals at chemical shifts lower than 0.8 ppm that are typical of methyl groups in the hydrophobic core of proteins, suggesting the WT and mutants were well folded. To compare their enzymatic properties, we measured their TC-AMP synthesizing activities *in vitro* by quantifying the pyrophosphate reaction product. The p.Ala84Val and p.Leu265del mutants have lost about 75 and 30% of their activities respectively compared to WT (Supplementary Figure 5.5b). The activities of these mutants are compatible with their hypomorphic nature, as suggested by the results of the yeast ΔSUA5 complementation experiments (Fig. 3a).

5.3.4 Proliferation, apoptosis and protein synthesis defects

We have previously shown that transient gene expression knockdown (KD) of human KEOPS components *OSGEP*, *TP53RK* and *TPRKB* leads to perturbations of

various cellular processes including proliferation and apoptosis.[232] Similarly here, we transiently depleted the expression of *GON7* and *YRDC*, as well as *LAGE3* and *OSGEP* as positive controls, in an immortalized human podocyte cell line.[261] We then demonstrated using a colorimetric cell proliferation assay that diminished expression of all four of these genes decreased cell proliferation, with the strongest decrease being observed in *LAGE3* KD podocytes (Fig.4a). Despite efficient *GON7* KD, cells exhibited only a slight decrease in cell proliferation compared to cells treated with the control scrambled shRNA (Fig.4a and d). The impairment of cell proliferation in *YRDC* and *OSGEP* KD cells was less marked than in *LAGE3* KD cells, which could be explained by a less efficient gene silencing (Fig.4d). By measuring Caspase-3/7 activity, we next demonstrated that apoptosis was inversely related to proliferation with *LAGE3* KD podocytes displaying the highest rate of apoptosis (Fig.4b). Since loss of t^6A modification impacts global translation in yeast[262], we also quantified the newly synthetized protein levels, which were decreased in all KD cells (Fig. 4c), even in *GON7* KD podocytes where proliferation and apoptosis rates were not drastically affected (Fig.4a-b). Altogether, these results reinforce our previous findings for the other KEOPS subunits, OSGEP, TP53RK and TPRBK, and confirm that mutations which alter t^6A biosynthesis in human cells have an impact on cell survival through decreased proliferation and protein synthesis, ultimately leading to apoptosis.

5.3.5 Structure of the human GON7/LAGE3/OSGEP subcomplex

To better understand the role of human GON7 and how its loss of function could be connected with GAMOS, we set out to determine its structure and to establish how it interacts with the other KEOPS subunits. We had either crystal structures (TPRKB) or good quality 3D-models (LAGE3, OSGEP, TP53RK) for all of the KEOPS subunits at our disposal, except for GON7.[226] Based on very weak sequence similarity, it was proposed, that GON7 is a remote homolog of yeast Gon7.[227] We first investigated the structure of GON7 in solution by collecting a 2D 1H-15N Band-Selective Optimized Flip Angle Short Transient Heteronuclear Multiple-Quantum Correlation (SOFAST-HMQC) NMR spectrum of a ^{15}N-labelled GON7 sample. The poor spectral dispersion in the ^{1}H dimension of the 2D correlation spectrum showed that GON7 lacks well defined structure, confirming the conclusions of Wan *et al.*7. Adding non-labelled LAGE3 to the sample, caused the shift and/or disappearance for many cross-peaks, suggesting GON7 interacts with LAGE3 (Supplementary Figure 5.6a). We further characterized the conformation of GON7 in solution by Small Angle X-ray Scattering (SAXS) measurements. By representing the scattering data as a dimensionless Kratky plot ($qR_g^2 \cdot I_q/I_0$ versus qR_g), one can assess qualitative information on the degree of compactness of the scattering object.[263] The plateau observed for GON7

is characteristic of a fully disordered protein, possibly with very short elements of secondary structure (Fig.5a), confirming our NMR data (Supplementary Figure 5.6a). We then purified the recombinant GON7/LAGE3/OSGEP complex and analyzed its behaviour in solution by SAXS. Our SAXS data established that the complex has a 1:1:1 stoichiometry in solution (Fig.5b, Supplementary Table 5.4). In contrast with GON7, the dimensionless Kratky plot for GON7/LAGE3/OSGEP shows a bell shaped curve with a maximum for qRg ≈ 2 (Fig.5a). This shape suggests that the complex is mainly compact, but that disordered regions are still present. In addition, the comparison of the distance distribution functions of GON7/LAGE3/OSGEP and GON7 shows that the latter alone is more extended than the complex (Supplementary Figure 5.6b). In full agreement, the ^{15}N SOFAST-HMQC NMR spectrum of the complex reveals that about 35 amino-acid residues of GON7 remains flexible and disordered in the complex whereas ~35 crosspeaks vanished upon complex formation. These latter crosspeaks likely correspond to amino-acid residues engaged in the interaction with LAGE3 and thus experiencing extensive line-broadening due to the large molecular size of the complex. We therefore deduced that GON7 is becoming partially ordered upon complex formation with LAGE3/OSGEP and set out to determine its structure by X-ray crystallography. We obtained 1.9 Å resolution diffraction data of the GON7/LAGE3/OSGEP complex (Fig.5c-d, Supplementary Table 5.5). The structure could be solved by molecular replacement using our 3D-models of OSGEP and LAGE3.[228,232,259] LAGE3 contains 60 residues at the N-terminus that are absent in the Pcc1 orthologues from yeast and archaea and that are predicted to be disordered. We did not observe any electron density for this N-terminal peptide, confirming this region indeed lacks a sTable 5.structure. We cannot exclude however that partial proteolysis removed this peptide during the long crystallization process. LAGE3 is at the centre of the complex, binding on one side to OSGEP and on the other to GON7, which does not directly interact with OSGEP (Fig.5c). The structures of the LAGE3 and OSGEP subunits are very similar to their archaeal/fungal Pcc1 and Kae1 orthologues respectively. The two helices of LAGE3 associate with the N-terminal helices of OSGEP into a helical bundle. Only 45% of the GON7 sequence adopts a well-defined structure upon binding to LAGE3, confirming our SAXS- and NMR-based conclusions (Fig.5a, Supplementary Figure 5.6a-d). The N-terminal peptide of GON7 forms a β-hairpin between Met1 and Ser20 and the region between Gly25 and Pro50 forms a helix that lies parallel against the β-hairpin (Supplementary Figure 5.7). Electron density for GON7 was absent for residues 21 to 24 and for the region beyond position 50. The C-terminal half of GON7 is highly enriched in acidic and sparse in hydrophobic amino acids and predicted to be unfolded. Despite their very

weak sequence similarity (19% identity, 34% similarity), the structures of human GON7 and yeast Gon7 are almost identical (RMSD = 1.41 Å for 45 Cα positions; yeast Gon7 PDB entry: 4WXA) (Fig.5d and Supplementary Figure 5.7). The β-hairpin of GON7 aligns with the β-sheet of LAGE3 to form a continuous 5 stranded anti-parallel sheet. The helix of GON7 packs in an anti-parallel orientation against the C-terminal helix of LAGE3. The complex is stabilized mainly by the hydrophobic packing of side chains emanating from β1 and α1 of GON7 and α2 and β1 of LAGE3. The association mode between GON7 and LAGE3 is very similar to that of the yeast Pcc1/Gon7 complex, illustrated by their superposition (RMSD= 1.4 Å; Fig.5d). Structure based sequence alignment between human GON7 and yeast Gon7 shows that only 6 out of 45 ordered residues (12%) are conserved (Supplementary Figure 5.7). Compared to human GON7, yeast Gon7 is longer by about 20 residues that were disordered in its structure. The experimental SAXS curve of the GON7/LAGE3/OSGEP complex in solution is in excellent agreement (χ^2 = 0.33) with the scattering curve calculated on the all-atom model built using the programme BUNCH from the crystal structure (see Material and Methods) (Fig. 5b). We therefore conclude that, although sharing very low sequence homology, human GON7 and yeast Gon7 are homologues that interact identically with their respective partners (LAGE3, Pcc1) in the human and yeast KEOPS complex.

5.3.6 Role of human GON7 in KEOPS complex stability in vivo

We further explored the deleterious cellular effects of the GAMOS-associated *GON7* mutations. We first confirmed by mass spectrometry analysis that in a human podocyte cell line stably overexpressing either 2HA-tagged GON7 or V5-tagged LAGE3, the four additional KEOPS subunits significantly co-purified with GON7 or LAGE3, respectively, thus confirming that a five-subunit KEOPS complex is present in these renal glomerular cells (Supplementary Figure 5.8a). We have previously demonstrated that the majority of GAMOS-associated mutations in genes encoding KEOPS complex components do not affect the intermolecular interactions between the LAGE3/OSGEP/TP53RK/TPRKB subunits.[232] In order to check whether mutations in LAGE3 affected GON7 binding, we co-expressed 2HA-tagged GON7 with WT or mutant V5-tagged LAGE3 in HEK293T cells. Our co-immunoprecipitation experiments demonstrated that the LAGE3 mutations found in GAMOS individuals do not prevent binding to GON7 (Supplementary Figure 5.8b). Intriguingly, however, we noticed that co-expression of GON7 with LAGE3 in HEK293T cells led to an increased expression level of GON7, and to a lesser extent of LAGE3, suggesting that the interaction stabilizes both proteins (Fig.6a).

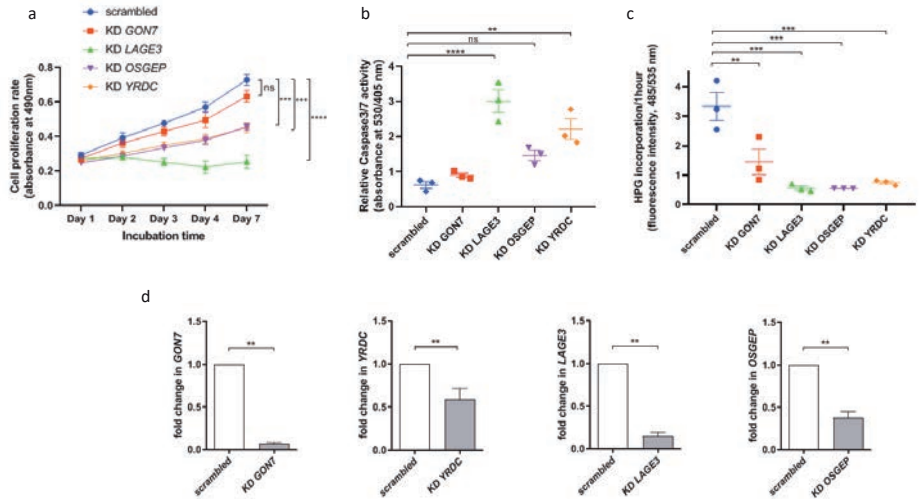


Figure 5.4 | Proliferation, apoptosis and protein synthesis defects upon GON7 and YRDC knockdown. Transient knockdown (KD) of GON7, LAGE3, YRDC and OSGEP was performed by lentiviral transduction of shRNA in immortalized human podocyte cell lines with a scrambled (non-targeting) shRNA as control. (a) Cell proliferation was assessed using a colorimetric MTT assay over 7 days, measuring absorbance at 490 nm at days 1, 2, 3, 4 and 7 (mean ± s.e.m of n=5 experiments, with each experiment performed in triplicate; two-way ANOVA (P<0.0001), Dunnett's multiple comparisons test, ns=0.2031, ***p<0.0007, ****p <0.0001). (b) Cell apoptosis was evaluated by quantification of caspase 3/7 activation on the basis of fluorescence intensity (530/405 nm). Absolute values were normalized to DAPI fluorescence intensity as an internal control and compared to non-targeting shRNA-treated control cells (scrambled) (mean ± s.e.m of n=3 experiments with each experiment performed in triplicate; one-way ANOVA (F (4,10)=21.42, P<0.0001), Dunnett's multiple comparisons test, ns=0.0556, **p=0.0012, ****p <0.0001). (c) Protein biosynthesis rates were assessed on the basis of incorporation of HPG, an alkyne-containing methionine analog. After 2 hours, alkyne-containing proteins were quantified on the basis of fluorescence intensity (485/535 nm). Absolute values were normalized to DAPI fluorescence intensity as an internal control and compared to control cells (mean ± s.e.m of n=3 experiments, with each experiment performed in triplicate, one-way ANOVA (F (4,10)=16.36, P=0.0002), Dunnett's multiple comparisons test, **p=0.0035, ****p <0.0003). (d) Relative expression of GON7, YRDC, LAGE3 and OSGEP transcripts were normalized to that of HPRT in KD podocytes compared to non-targeting shRNA control treated cells (mean ± s.e.m of n=5 experiments, with each experiment being performed in triplicate; two-tailed Mann-Whitney test, **p<0.05). Source data are provided as a Source Data file.

We therefore studied the stability of GON7 and LAGE3 in a time-course experiment using cycloheximide, an inhibitor of protein biosynthesis, in HEK293T cells transiently expressing either 2HA-GON7 or V5-LAGE3 alone or co-expressing both tagged-proteins. When expressed alone, GON7 and LAGE3 protein levels decreased, suggesting both proteins may be unsTable 5.in absence of their partner. This is particularly obvious for GON7 whose protein level decreased by half within an hour following cycloheximide addition (Fig.6b, Supplementary Figure 5.9). On the contrary, when co-expressed, we observed an increase of both GON7 and LAGE3 protein levels reflecting an increase in their stability.

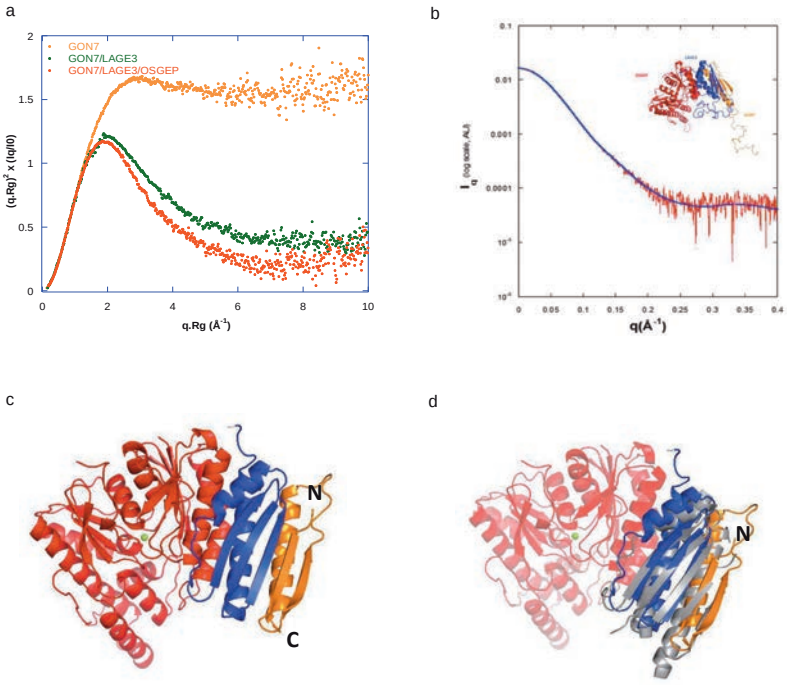


Figure 5.5 | Structure of the GON7/LAGE3/OSGEP complex. (a) Normalized Kratky plot of intensity scattering of GON7 (orange) and of the GON7/LAGE3 (green) and GON7/LAGE3/OSGEP complexes (red). q: scattering vector, R_g radius of gyration, I_q: scattering intensity, I_o: scattering intensity at zero angle. (b) Experimental x-ray scattering curve of the GON7/LAGE3/OSGEP complex (red). The blue curve represents the calculated scattering curve for the corresponding crystal structure of the complex. This yielded a good fit with the experimental data (χ^2 = 0.33). The inset shows the BUNCH model. (c) Representation of the crystal structure of the GON7/LAGE3/OSGEP complex: GON7 (gold), LAGE3 (blue), OSGEP (red). The N and C-termini of GON7 are labelled. The crystal lacked density for GON7 beyond residue 50. The active site of OSGEP is highlighted by the Mg^{2+} ion (green). (d) Superimposition of the yeast Gon7/Pcc1 complex (grey) onto GON7/LAGE3/OSGEP. GON7/LAGE3/OSGEP (same colour code as in panel c).

We wondered whether the absence of GON7 also impacts the stability of the whole KEOPS complex and indeed, we were able to demonstrate that the protein levels of the four KEOPS subunits were decreased in cells of individuals mutated for *GON7*, whereas they were not affected in cells of individuals with *OSGEP* or *WDR73* mutations, the latter being also responsible for a specific subset of GAMOS cases not linked to a t^6A biosynthesis defect (Fig.6c, Supplementary Figure 5.10a).[234] In addition, we demonstrated that this protein level decrease was not due to transcriptional regulation (Supplementary Figure 5.10b). Altogether, these results suggest that the absence of GON7 affects KEOPS stability resulting in a decreased expression level of the four other subunits, which might impact t^6A levels.

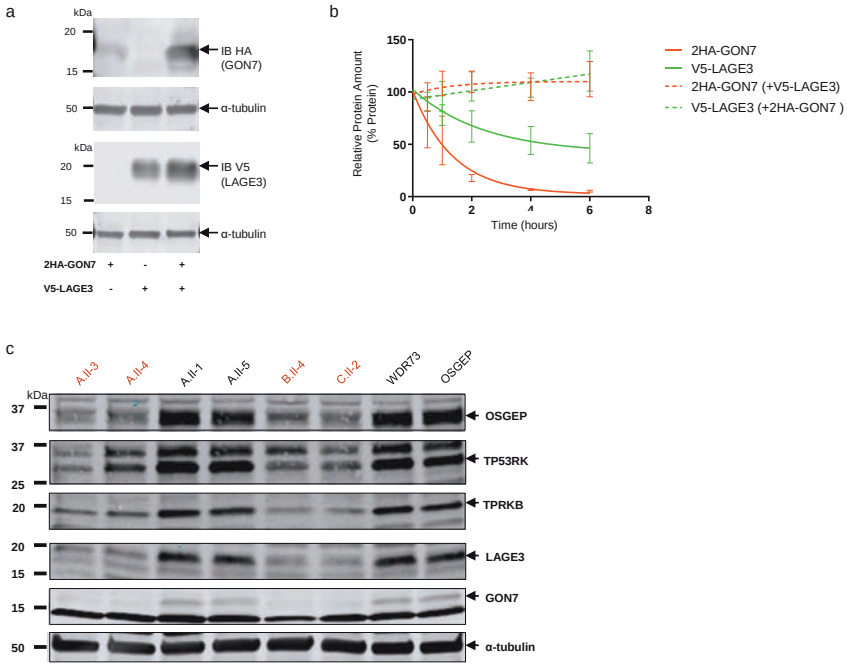


Figure 5.6 | Role of GON7 on KEOPS complex stability. (a) Immunoblot analysis of HEK293T cell lysates expressing either 2HA-tagged GON7 or V5-tagged LAGE3 alone or co-expressing both proteins. Anti-HA and anti-V5 antibodies were used to assess GON7 and LAGE3 expression, respectively; with α-tubulin used as loading control. (b) Representation of cycloheximide chase experiments by fitting a one-phase exponential decay curve to experimental data (one representative experiment is shown in Supplementary Figure 5.9) (mean ± s.e.m of n=3 experiments). HEK293T cells were transfected with either 2HA-tagged GON7 or V5-tagged LAGE3 alone or with both proteins before being subjected to treatment with 100 µg/ml cycloheximide for the indicated time points in order to assess rates of protein degradation followed by western blotting of the cell lysates for both proteins with anti-HA and anti-V5 antibodies, respectively. GON7 and LAGE3 protein levels were normalized to those of α-tubulin at each time point. (c) Western blot analysis of protein expression level of the five KEOPS subunits in lymphoblastoid cell lines from 2 unaffected relatives (A.II-1 and A.II-5), 4 individuals with the GON7 mutation p.Tyr*7, 1 individual with the OSGEP mutations p.Arg325Gln and p.Arg280His (individual « N2705 » described in Braun et al., 2017) and 1 individual with GAMOS linked to WDR73 mutations (individual A.II-4 described in Colin et al., 2014). One representative western blot is shown (three independent experiments were performed). α-tubulin was used as loading control. Source data are provided as a Source Data file.

5.4 Discussion

In this study, we identified mutations in two genes encoding proteins involved in t^6A biosynthesis in GAMOS patients: *YRDC* encoding the enzyme that synthesizes the TC-AMP intermediate used by the KEOPS complex and *GON7* encoding the fifth subunit of the KEOPS complex. Functional analysis of these specific mutations has

revealed that they directly impact t^6A modification (*YRDC*) and/or affect the stability of the KEOPS complex (*GON7*). These results complement our previous findings and establish that mutations in all the genes involved in this pathway lead to GAMOS.

All individuals bearing *GON7* or *YRDC* mutations present with the clinical features of GAMOS, similarly to the individuals previously reported to have mutations in the genes encoding the four other KEOPS subunits. In addition, we have expanded the GAMOS phenotype spectrum by describing congenital hypothyroidism to be associated with *YRDC* mutations. Although the two *GON7* mutations encode truncated non-functional proteins, we noticed that they unexpectedly result in a less severe clinical outcome compared to that of individuals affected by mutations in other KEOPS subunit genes or in *YRDC*, for which biallelic null mutations were not found. This suggests that the absence of GON7 has less severe consequences for cell life compared to the other components of the t^6A biosynthesis pathway, where four out of six are encoded by genes considered to be essential.[264] This less severe clinical outcome correlates with our data showing that GON7 loss of function and depletion in fibroblasts and podocytes, respectively, have globally a weaker effect on t^6A levels, proliferation, apoptosis and protein synthesis compared to that of other KEOPS subunits and *YRDC* mutations or depletion. However, although our data have confirmed that GON7 is a functional homolog of Gon7, the effect of their absence in human and yeast, respectively, is markedly different since in the absence of Gon7, the yeast KEOPS complex has only very low t^6A activity and cell growth is dramatically affected.[223] Altogether, our data in humans suggest that GON7 is not as essential in humans as in yeast for t^6A biosynthesis.

Our biochemical and structural data provide a molecular framework to understand the pathophysiological effects of the GAMOS-associated mutations. The structure of the GON7/LAGE3/OSGEP complex shows that GON7 is bound exclusively to the non-catalytic LAGE3 subunit, distant from the catalytic center of OSGEP. It has been shown *in vitro* that the intact human KEOPS complex has a 1:1:1:1:1 stoichiometry, in contrast with the complex lacking GON7 which has a 2:2:2:2 stoichiometry.[227] The latter stoichiometry has also been observed for the archaeal KEOPS complex, for which no fifth subunit similar to Gon7 has yet been discovered. The Pcc1 subunit constitutes the dimerization unit of archaeal KEOPS[265] and this is also very likely the case for the LAGE3 subunit of human KEOPS in absence of GON7.[227] In line with these results, our structure of the GON7/LAGE3/OSGEP complex shows that GON7 competes with LAGE3 for dimerization, explaining the different stoichiometries of the KEOPS complex observed in the absence or presence of GON7. Indeed, GON7 covers a large hydrophobic surface of LAGE3 (Supplementary Figure 5.11), which is very likely occupied by another LAGE3

subunit in the context of a homomeric dimer, as observed in the structure of Pcc1 dimer.[265] The exposure of this hydrophobic surface due to the absence of GON7 in the GAMOS patients may affect the solubility and activity of the KEOPS complex, and indeed, our data from experiments on cell lines further indicate that GON7 contributes to the stability of the KEOPS complex and/or to the maintenance of the correct (catalytically active) quaternary structure as evidenced by the decrease in KEOPS subunits protein levels observed in GON7 patient cells. Taken together, our data demonstrated that GON7 impacts the stability of the KEOPS complex therefore having an effect on its enzymatic activity. This is in line with the *in vitro* data of Sicheri's group showing that in presence of GON7, KEOPS t^6A activity is potentiated[265] and with our in vivo data showing that t^6A levels in *GON7*-mutated patient fibroblasts are slightly decreased compared to *YRDC*- and *OSGEP*-mutated fibroblasts.

Although human GON7 and yeast Gon7 have low sequence identity, their structures and interactions with LAGE3 and Pcc1, respectively are nearly identical. It is therefore surprising that GON7 could not complement the yeast *Δgon7* deletion mutant.[227] Comparison of the GON7/LAGE3 and Gon7/Pcc1 complexes shows that the hydrophobic character of the residues at the interface is very well conserved (Supplementary Figure 5.7). However quite a few amino acid substitutions between GON7 and Gon7 might create steric clashes that weaken or disrupt the interaction with Pcc1, explaining the lack of complementation. Nevertheless, the exquisite superposition of GON7 and Gon7 qualifies them as orthologues and confirms that GON7 is the functional 5th subunit of the human KEOPS complex. Such discrepancies between the protein sequence and structure conservation between distant species might be relevant in other protein complex, with their characterization helping to identify new candidate genes for human monogenic disorders.

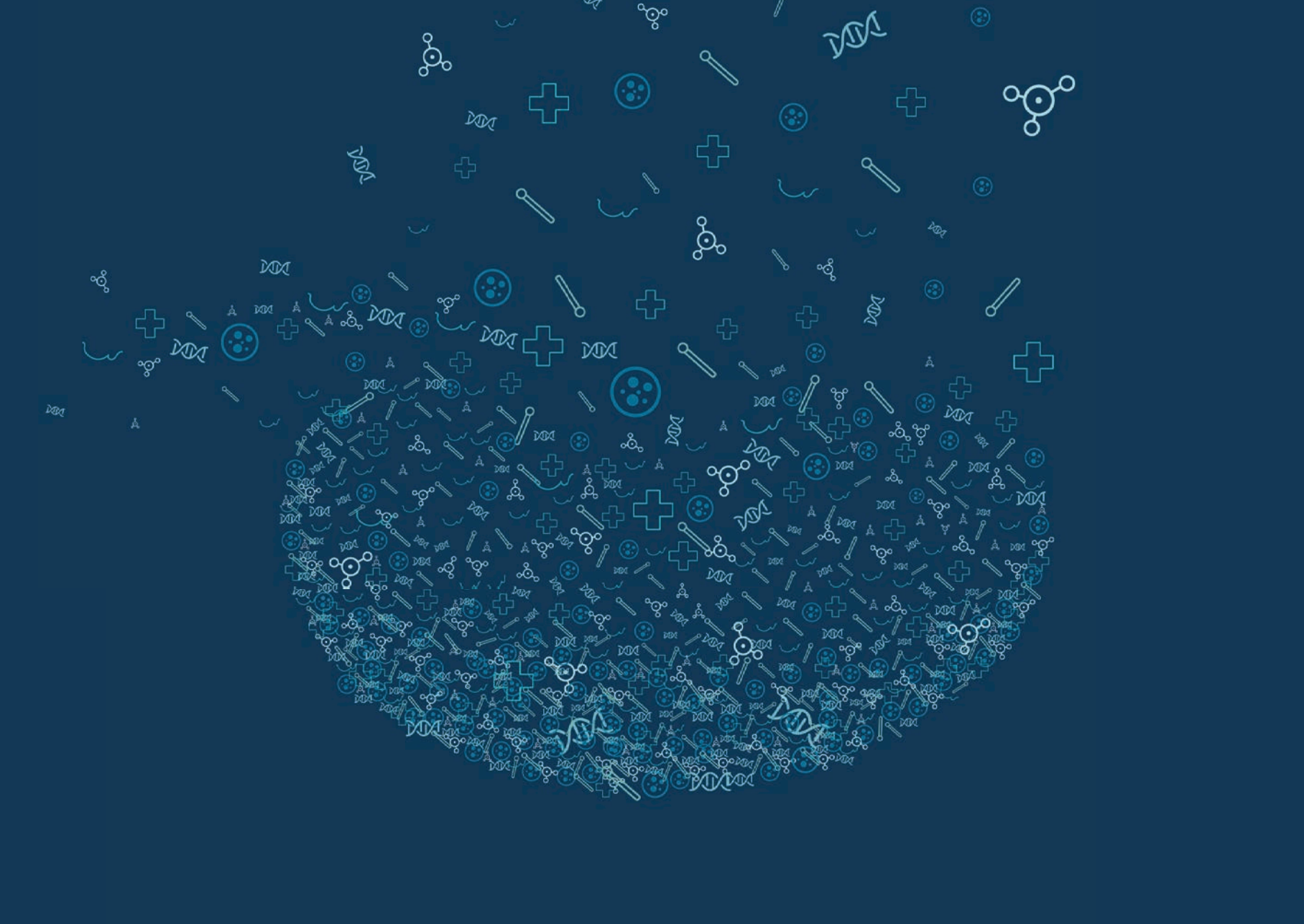
An increasing number of mutations are being identified in genes encoding tRNA-modifying enzymes that are linked to human neurodevelopmental disorders. Very recently, mutations in *WDR4*, initially described to cause a distinct form of microcephalic primordial dwarfism and brain malformations[266,267], have been identified in individuals with GAMOS[233]. WDR4 is a component of the METTL1/WDR4 holoenzyme, a N^7-methylguanosine (m^7G) methyltransferase that is responsible for the highly conserved m^7G modification on a specific subset of tRNAs.[268,269] Interestingly, it has been shown that the absence of m^7G tRNA modification leads to impaired cell proliferation, neural differentiation and a decrease in global translation with a less efficient translation of mRNAs involved in cell division and brain development, consistent with the microcephaly and brain anomalies found in individuals with *WDR4* mutations.[270] Similarly, as a consequence of the decrease in t^6A levels observed in affected individual's cells, perturbed protein translation could

impact the translation of specific mRNA involved in kidney and brain development and/or podocyte/neuron maintenance. It is likely that the requirement for t^6A-modified tRNAs levels is dependent of the cell-type and/or cell cycle as has been previously shown in *D. melanogaster* where highly proliferative cells of the wing imaginal discs are more affected by the absence of t^6A modification than fully differentiated photoreceptors.[271] Neuronal progenitors that have high mitotic activity probably have higher demands for protein translation, making them more vulnerable to any perturbation in the tight regulation of tRNA modifications. Furthermore, another potential regulatory step to spatiotemporally modulate these tRNA modifications and thus protein translation is the tissue- and developmental stage-specific expression of the tRNA-modifying enzymes.[272,273] YRDC and KEOPS subunits could be differentially expressed between specific cell-types in the brain (neural progenitors) and the kidney (podocytes) and/or during development/differentiation explaining the tissue involvement and the course of the disease as well as its clinical outcome. Further studies on neuronal/renal progenitors and neurons/podocytes differentiated from induced pluripotent stem cells obtained from individuals with mutations in YRDC and KEOPS subunits will probably provide further insights into the pathogenesis of GAMOS.

Together, our data strongly emphasize the importance and relevance of the t^6A biosynthesis pathway in the pathogenesis of GAMOS. Further investigations are needed to fully characterize all the KEOPS mutations at the biochemical, structural and enzyme activity levels to better understand their impact on KEOPS complex-dependent t^6A biosynthesis activity and how they influence the clinical phenotypes. Genes encoding components of the t^6A biosynthesis pathway have to be added to the growing list of translation-associated proteins whose loss of function are responsible for rare genetic disorders.

Supplementary material 5

For supplementary material please see the online version of this article.



NPHP1 (nephrocystin-1) gene deletions cause adult-onset ESRD

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Abstract

Nephronophthisis (NPH) is the most prevalent genetic cause for ESRD in children. However, little is known about the prevalence of NPH in adult-onset ESRD. Homozygous full gene deletions of the *NPHP1* gene, encoding nephrocystin-1, are a prominent cause of NPH. We aimed to determine the prevalence of NPH by assessing homozygous *NPHP1* full gene deletions in adult-onset ESRD.

5606 renal transplant recipients from five iGeneTRAiN consortium cohorts underwent single nucleotide polymorphism-genotyping. After quality control, autosomal copy number variants (e.g. deletions) were determined based on median log2 ratios and B-allele frequency patterns. The findings were independently validated in one cohort.

Cases are included in the analysis when they had adult-onset ESRD, defined as start of renal replacement therapy (RRT) at age 18 years or older.

We included 5606 cases with adult-onset ESRD, of which 26 (0.5%) showed homozygous *NPHP1* deletions. No donor-controls showed homozygosity for this deletion. Median age at ESRD onset was 30 (range 18-61) years for these NPH cases, with 54% of cases aged 30 years or older. Notably, only 3 (12%) cases were phenotypically classified as NPH, while most cases were defined as chronic kidney disease with unknown etiology (n=11, 42%).

Considering that other mutation-types in *NPHP1* or mutations in other NPH-causing genes were not analyzed, NPH is a relatively frequent monogenic cause of adult-onset ESRD. As 88% of cases had not been clinically diagnosed with NPH, these results warrant wider application of genetic testing in adult-onset ESRD.

6.1 Introduction

Nephronophthisis (NPH) is a Mendelian genetic disease, and a classic pediatric kidney disease. Although NPH is considered a rare disorder (incidence of 0.1 - 0.2 per 10.000 live births), NPH is the most prevalent genetic cause for end-stage renal disease (ESRD) in children, with a frequency of 15%.[17,66] The most common variant is NPH type 1 (OMIM 256100), in which patients generally present at age 13 with ESRD.[274]

The etiology of NPH lies in the primary cilium, that functions as a sensory organelle in the renal cell.[274,275] Mutations in genes coding for proteins essential to the primary cilium lead to structurally or functionally aberrant cilia, making NPH a ciliopathy.[274,275] To date, causative mutations in many genes encoding these proteins have been reported, but the largest proportion of NPH cases (20-25%) is caused by homozygous full gene deletions of the *NPHP1 gene* (OMIM 607100).[63,170] These full gene deletions are recurrent in the general population, a result of recurrent complex rearrangements at this locus due to flanking low copy repeats (elements with highly similar sequence identities).[276–278] Mutations in *NPHP1* are completely penetrant, thus the presence of the mutation always leads to a NPH phenotype.[63,170] For the other NPH genes no such recurrent full gene deletion is known.[63,170]

Clinically, NPH generally starts around age 6 with non-specific and mild symptoms due to an impaired ability to concentrate urine and retain water.[279] This leads to early symptoms such as polyuria, polydipsia and secondary enuresis.[279] If performed, renal ultrasound may show echogenicity with loss of corticomedullary differentiation.[280] The disease always progresses to ESRD, in general around age 13, with a need for renal replacement therapy (RRT, dialysis or renal transplantation).[274] In some NPH cases renal ultrasound shows corticomedullary cysts, even though the majority display small atrophic kidneys at the ESRD stage.[280] Some NPH patients (15%) also display additional extra-renal abnormalities, such as neurological anomalies (Joubert syndrome) or ophthalmological dysplasias (Senior - Løken syndrome), which can guide diagnosis.[67] However, isolated NPH may prove difficult to diagnose clinically, as the phenotype is generally non-specific, can be variable and often only becomes clinically apparent in the ESRD stage.[274] Therefore, genetic testing is the sole method to diagnose NPH with certainty.[63]

Since it almost always presents in mid-childhood, NPH is primarily considered a pediatric kidney disease. Nevertheless, anecdotal cases with adult-onset ESRD have been reported.[281–283] Although these cases suggest that the diagnosis of NPH is rare in adults, little is known about the overall prevalence of NPH in the adult-onset ESRD population.[281–286]

We set out to investigate the prevalence of NPH in adults by analyzing, as a proxy, the prevalence of homozygous *NPHP1* full gene deletions in patients with adult-onset ESRD. We purpose to do this by assessing autosomal copy number variants (CNVs, e.g. large deletions) in genomic data generated for a genome-wide association study in multiple cohorts of renal transplant recipients and the (corresponding donor-) controls from the iGeneTRAiN consortium.[287]

6.2 Methods

6.2.1 CNV analysis

We genotyped five iGeneTRAiN consortium cohorts, all consisting of renal transplant recipients and (donor-) controls.[287] The DeKAF Genomics, Gen03, TransplantLines-Genetics and Vienna cohorts were genotyped with the custom *Affymetrix Axiom Tx GWAS Array* (Affymetrix, Inc., Santa Clara, CA).[288] This array, designed for the iGeneTRAiN consortium, contains single nucleotide variants (SNV), -polymorphisms (SNP) and monomorphic markers for ~782,000 positions across the whole genome (based on human reference genome build *GRCh37*).[288] Of these, ~22,000 variants were used to cover approximately 2200 manually curated CNV regions.[288] The GoCAR cohort was genotyped with the *Infinium HumanOmniExpressExome-8 v1B* and *OmniExpressExome-8 v1.1A* (Illumina, San Diego, CA), providing whole-genome coverage with markers for ~960,000 SNPs.[287]

Quality control was performed by excluding all low-quality SNPs, leaving only high quality SNPs (call rate >0.99, Hardy-Weinberg equilibrium p>0.001, minor allele frequency <0.1), that were linkage disequilibrium (LD)-pruned to leave no pairs with r^2 >0.2. Also, we removed SNPs in regions with known long stretches of LD and non-autosomal SNPs. Only data on the 5606 adult-onset ESRD cases (start of first RRT at 18 years or older) was analyzed: n=3192 from the DeKAF Genomics and Gen03 combined cohorts, n=1230 from the TransplantLines-Genetics cohort, n=500 from the GoCAR cohort and n=684 from the Vienna cohort.

The data generated was used to determine autosomal CNVs, i.e. deletions and duplications. CNV calling was performed using the default settings of two well-published algorithms: the *BRLMM-P* algorithm designed by Affymetrix, Inc (DeKAF Genomics, Gen03, TransplantLines-Genetics) or *PennCNV* (GoCAR and Vienna).[289,290] All calls with LogR-Ratio standard deviation >0.3, B-allele frequency drift >0.01, waviness factor >0.05 or waviness standard deviation >0.15 were excluded. Loci where no definite copy number call could be made were also excluded from further analysis.

To assess *NPHP1* full gene deletions, all samples containing a CNV with an overlap of at least 1 nucleotide with the *NPHP1* region (chromosome 2: 110879888

- 110962643 based on *GRCh37*) were identified using *R Studio* (version 1.0.153 for Windows, RStudio, Inc., Boston, MA). All cases with a copy number call of zero in this entire region, i.e. homozygous full gene deletions, were selected for further analysis. We additionally assessed the number of recipients and donors with a copy number call of one, i.e. heterozygous full gene deletion carriers. The regions of the other known NPH-genes were also assessed for CNVs.[63]

All *NPHP1* deletions called by the algorithms were manually inspected to ensure only true calls were made. Furthermore, the dense SNP-arrays applied to the cohorts are well established and validated, and the *NPHP1* region is large, leading to a near zero chance of false findings.[287–290] To further validate this, the TransplantLines-Genetics samples that displayed a homozygous deletion copy number call for *NPHP1* (n=11) were independently validated with multiplex ligation-dependent probe amplification, showing 100% concordance.[170,278,283]

6.2.2. Demographic and phenotypic statistics

Demographic and phenotypic information was retrieved per cohort for all cases of adult-onset ESRD, defined as start of first RRT at 18 years or older. For adult-onset NPH cases, we studied various phenotypical characteristics, namely the age at ESRD-onset (defined as first renal replacement therapy (dialysis or transplantation, RRT), ethnicity and primary renal disease diagnosis. If patient consent (see *Ethical constraints* below) allowed for data retrieval from the patient file, data on family history, hypertension, polyuria, proteinuria and extra-renal NPH-associated anomalies was retrieved.

Ethnicity was determined by principal component analysis, using 1000 Genomes Phase 3 data (1092 samples, 14 different ancestries).[291,292] Populations were determined by visual inspection of the first two principal components (by JvS). Descriptive statistics were generated for the demographic and primary renal diagnosis variables using SPSS (version 23 for Windows, IBM Corp., Armonk, NY). Additionally, we applied a two-sided Fisher's exact test to assess these binomial variables. All samples were also analyzed on identity by state patterns to identify cases that were related up to and including the third degree (identity by state <10%).

6.2.3 Ethical constraints

The enrollment of participants for all iGeneTRAiN cohorts were approved by the institutional review board of the hospitals participants were included at. All participants signed informed consent for genomic data analysis via SNP-array. For the GoCAR cohort, TransplantLines-Genetics and Vienna cohorts the principal consent form included retrieval of relevant information from their patient file.

Participants enrolled in the DeKAF Genomics and Gen03 cohorts had to additionally consent for data retrieval from their patient file.

Furthermore, participants were not asked to consent to return of genomic study results on an individual level. Thus, we could not notify *NPHP1* homozygous or heterozygous gene deletion carriers of this finding.

6.3 Results

In total, 5606 renal transplant recipients with start of first RRT at any age equal to or older than 18 years were included. Overall descriptive characteristics of the five cohorts were reported previously by the iGeneTRAiN consortium.[287] Of these adult ESRD-onset cases, 26 cases (0.5%, Figure 6.1) displayed the same ~96,389 basepair deletion (size based on SNP-array, see example in Figure 6.2) on both alleles, including all *NPHP1* gene exons. The 26 cases were not related up to and including to the third degree.

None of the 3311 (donor-) controls displayed this homozygous deletion. None of the 5606 recipients showed a deletion of any of the other 19 known NPH-genes.[63] Markedly, we detected a higher number of heterozygous *NPHP1* deletions in transplant recipients (n=36) when compared to the transplant (donor-) controls (n=10, $p<0.001$). Even though these cases were not additionally assessed with next-generation sequencing, this finding points to the possibility of compound heterozygosity (a full gene deletion on one allele and a different pathogenic mutation on the other allele) in the recipients.

When addressing the phenotype of the cases with *NPHP1* deletions (Table 6.1 and Figure 6.1), the median age at start of RRT was 30 years (range 18-61), with 14 cases (54%) aged 30 years or older. Interestingly, the prevalence of homozygous *NPHP1* deletions was 0.9% in recipients between 18 and 50 years at start of first RRT (n=24/2794), and even higher (2.1%) in recipients aged 18 up to and until 29 years (Figure 6.1).

Regarding the clinical primary renal disease diagnosis; only three cases (12%), of whom two were younger than 30 years at start of first RRT, were classified as having NPH (Figure 6.3). The other cases (88%) were diagnosed with chronic kidney disease with unknown etiology (n=11), cystic disease (n=2), hypertensive nephrosclerosis (n=2), tubular and interstitial disease (n=2), glomerular disease (n=1), glomerulonephritis (histologically examined, n=1), sporadic primary reflux nephropathy (n=1), vascular nephropathy (n=1), urate nephropathy (n=1) and autosomal dominant polycystic kidney disease (n=1, no mutation in *PKD1* or *PKD2*).

Table 6.1 | Phenotypical information of the 26 NPHP1 homozygous gene deletion cases, including age at presentation and first renal replacement therapy, and the presence of various renal and extra-renal symptoms of nephronophthisis. Cases are sorted on age at first renal replacement therapy (youngest to eldest).

Case	Sex	Ancestry (origin)	Clinical diagnosis	Age at presentation (years)	Age at first RRT (years)	Positive family history	Hypertension (age in years)	Polyuria (age in years)	Proteinuria (age in years)	Bone disease	Eye anomalies associated with NPH	Brain anomalies associated with NPH
1	F	Caucasian (USA)	Tubular and interstitial disease	NC	18	NC	NC	NC	NC	NC	NC	NC
2	F	Caucasian (EU)	NPH	19	19	NR	-	-	+, age NR	-	-	-
3	M	Caucasian (USA)	CKDue	NC	21	NC	NC	NC	NC	NC	NC	NC
4	F	Caucasian (USA)	CKDue	NC	21	NC	NC	NC	NC	NC	NC	NC
5	M	Caucasian (EU)	Glomerular disease	22	22	-	22	NR	22	-	-	-
6	F	Caucasian (EU)	CKDue	21	23	-	21	-	-	-	-	-
7	F	Caucasian (USA)	CKDue	23	23	-	23	NR	NR	NR	-	-
8	F	Caucasian (USA)	CKDue	23	23	+	23	NR	NR	+	-	-
9	M	African-American (USA)	Hypertensive nephrosclerosis	25	25	-	25	NR	NR	NR	-	-
10	F	Caucasian (EU)	Sporadic primary reflux nephropathy	27	28	-	27	-	NR	-	-	-
11	M	Caucasian (EU)	Glomerulonephritis, histologically examined	28	28	+	-	-	NR	-	-	Severe developmental delay

Table 6.1 | Continued

Case	Sex	Ancestry (origin)	Clinical diagnosis	Age at presentation (years)	Age at first RRT (years)	Positive family history	Hypertension (age in years)	Polyuria (age in years)	Proteinuria (age in years)	Bone disease	Eye anomalies associated with NPH	Brain anomalies associated with NPH
12	F	Caucasian (EU)	NPH	29	29	-	-	-	NR	+	-	-
13	F	Caucasian (USA)	CKDue	NC	30	NC	NC	NC	NC	NC	NC	NC
14	M	Caucasian (EU)	NPH	27	30	NR	27	-	-	-	-	-
15	M	Caucasian (EU)	Vascular nephropathy	NR	30	-	+, age NR	NR	NR	NR	Congenital nystagmus	Tremor
16	M	Caucasian (USA)	Medullary cystic disease	NR	31	NR	-	NR	-	NR	NR	NR
17	M	Caucasian (EU)	CKDue	30	34	+	30	-	-	-	-	-
18	F	Caucasian (EU)	CKDue	9	40	+	9	-	-	-	-	-
19	M	Caucasian (EU)	Urate nephropathy	28	40	-	-	-	-	-	-	-
20	F	Caucasian (EU)	CKDue	39	42	-	34	-	NR	-	-	-
21	F	Caucasian (EU)	Medullary cystic disease	NR	42	NR	-	NR	-	NR	NR	NR
22	M	Caucasian (USA)	CKDue	NC	43	NC	NC	NC	NC	NC	NC	NC

Table 6.1 | Continued

Case	Sex	Ancestry (origin)	Clinical diagnosis	Age at presentation (years)	Age at first RRT (years)	Positive family history	Hypertension (age in years)	Polyuria (age in years)	Proteinuria (age in years)	Bone disease	Eye anomalies associated with NPH	Brain anomalies associated with NPH
23	M	Caucasian (USA)	CKDue	NC	44	NC	NC	NC	NC	NC	NC	NC
24	M	Caucasian (USA)	Tubular and interstitial disease	NC	46	NC	NC	NC	NC	NC	NC	NC
25	F	Caucasian (EU)	Autosomal dominant polycystic kidney disease	42	52	+	50	-	-	-	-	-
26	F	Caucasian (EU)	Hypertensive nephrosclerosis	NR	61	-	+, age NR	NR	NR	NR	Congenital blindness	-

+=symptom present, -=symptom not present, CKDue=chronic kidney disease with unknown etiology, F=female, M=male, NPH=nephronophthisis, NR=not reported in patient file, RRT=renal replacement therapy. EU=Europea, USA=United States of America. NC=For seven cases not all data was retrievable due to the patient not consenting to additional data retrieval from the patient file, see also 'Ethical constraints' in the Methods section.

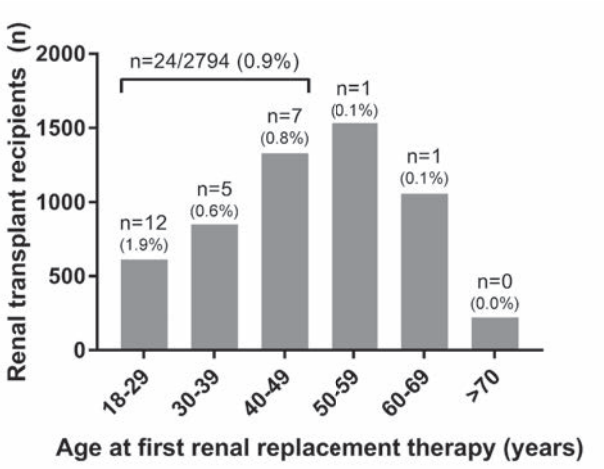


Figure 6.1 | Number of renal transplant recipients (n) in the five combined cohorts by age decade of start of renal replacement therapy. The number of homozygous NPHP1 full gene deletion carriers (n) and the percentage per specific age category (%) are displayed above each bar.

Two cases displayed extra-renal anomalies associated with NPH, namely congenital blindness (possible Senior-Loken syndrome type 1[294]) and severe neurodevelopmental delay (possibly Joubert syndrome type 4[295]). Both these cases had not been clinically diagnosed as NPH, but as hypertensive nephrosclerosis and glomerulonephritis (histologically examined) respectively (Table 6.1).

6.4 Discussion

Our data indicate that with a 0.5% prevalence of homozygous *NPHP1* full gene deletions, the frequency of NPH in adult-onset ESRD is considerably higher than previously reported.[63,284] When interpreting our results, one should note that, inherent to the method applied in our study (which only analyzes larger deletions and duplications) the overall prevalence of NPH in adult-onset ESRD we report here is very likely an underestimation (see Figure 6.4). First, with this method any combination of two smaller *NPHP1* mutations, such as (homozygous) pathogenic intragenic deletions up to ~700 basepairs and single nucleotide variants, could not be analyzed.[290] As this is outside the scope of this study, compound heterozygosity and homozygosity for other mutations were not assessed. The assumption that *NPHP1*-related disease is probably more frequent in adult-onset ESRD is supported by our recipients showing a significantly higher frequency of heterozygous deletion carriers than donors, suggesting that a subset of these deletion carriers likely carry a second mutation on the other allele.

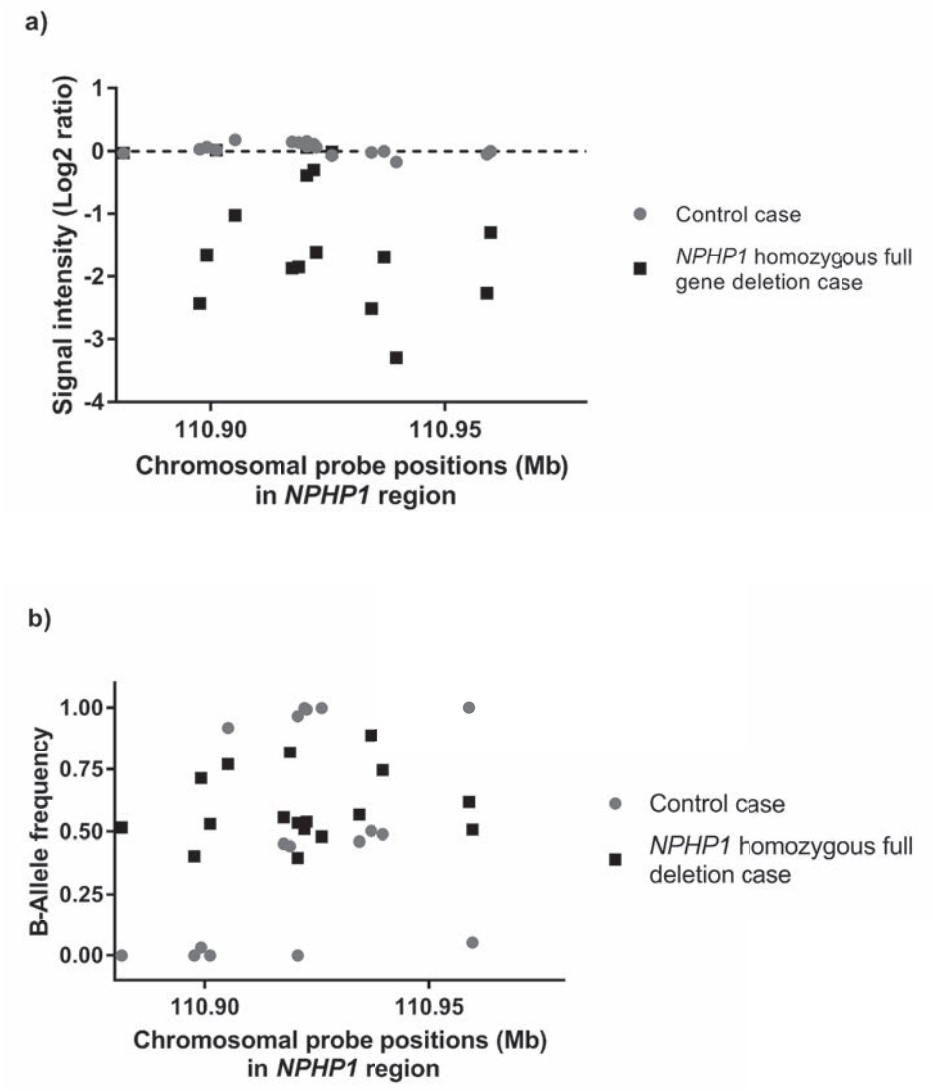


Figure 6.2 | Graphical representation of the Log2 ratio (figure 2a) and b-allele frequency (figure 2b) of all single nucleotide polymorphism markers in the NPHP1 gene region in one NPHP1 homozygous full gene deletion case in black squares (case 19, see Table 1) and one healthy control in gray circles.
The Log2 ratio (figure 2a) represents the normalized signal intensity for a specific marker, thus if there is signal for that marker the ratio is 0, while any value below 0 indicates genomic deletion. An average Log2 ratio of -2 specifically indicates a homozygous deletion. The B-allele frequency (figure 2b) displays if the particular marker is present in a homozygous state (either B-allele frequency of ~0.00 or ~1.00) or a heterozygous state (B-allele frequency of ~0.50), as is clear in the control case shown here. In cases with a homozygous deletion, the algorithm cannot accurately determine the B-allele frequency as no markers are present at all. This leads to a 'waterfall' configuration with markers being assigned B-allele frequencies anywhere between 0.00 and 1.00 at random.[293]

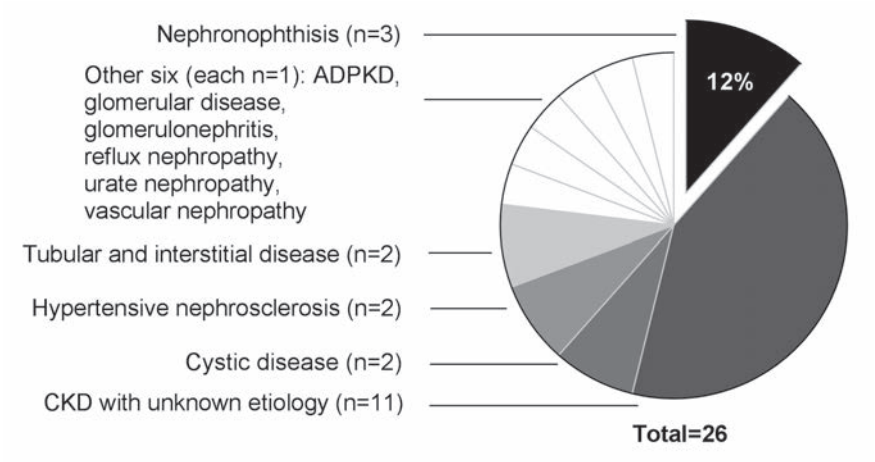


Figure 6.3 | Clinical diagnoses in the 26 cases with homozygous NPHP1 full gene deletions. Only 12% were identified as having nephronophthisis, 88% were clinically diagnosed as something other than nephronophthisis.
ADPKD=autosomal dominant polycystic kidney disease, CKD=chronic kidney disease

Second, the causative mutations in other NPH genes generally do not display recurrent full gene deletions.[63,170] This is underscored by the fact that our analysis showed no large deletions in these genes.[63] We did not yet perform any (additional) next-generation sequencing to assess the other mutations. In the light of these two considerations, our findings point to an underestimation of the number of causative NPH mutations in the overall cohort.

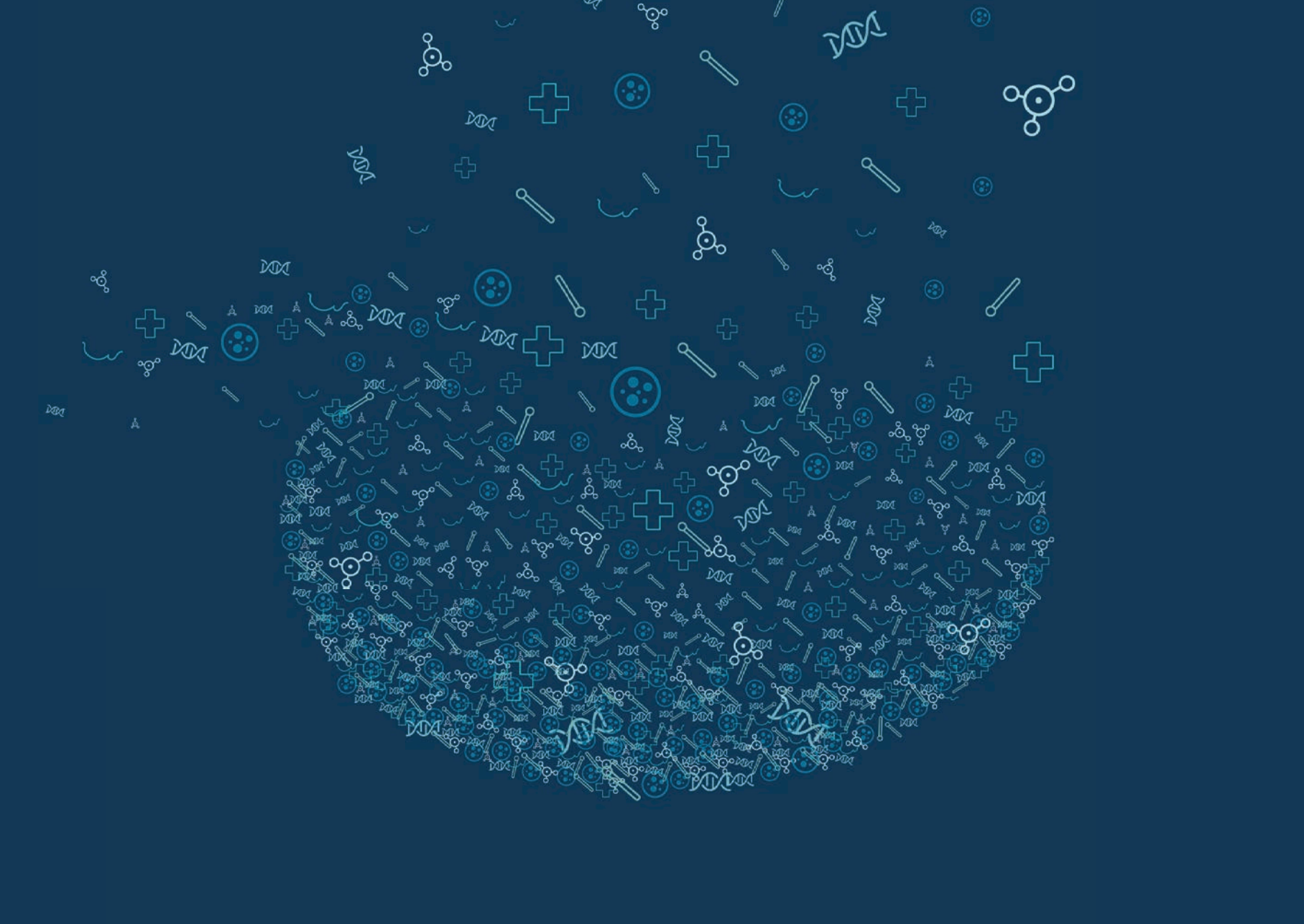
With regard to age at onset of ESRD, over half of the *NPHP1* cases in our study were 30 years or older at first onset of ESRD. Therefore, we postulate that NPH is not merely a pediatric disease entity. In the literature so far only 6 cases across 4 families have been described with *NPHP1* mutations and ESRD onset after 30 years of age, with the eldest being 56 years old.[281–283]

Our study extends this age of onset to 61 years. In the subpopulation with onset between 18 and 50 years, we observed a *NPHP1* deletion prevalence of 0.9%. The mechanism leading to phenotypic variance in age of ESRD onset remains unclear. It has been hypothesized previously that this non-pediatric onset of ESRD, especially after 30 years old, is due to influence of yet unknown modifier genes.[282,283] Due to the ancestry composition of our cohorts (the majority being Caucasian, data not shown), we could not analyze the presence of distinct ethnicity-specific modifier effects.[63,277] Nevertheless, modifier effects, whether ethnicity-specific or not, could play a role in the discrepancy in age at onset between the cases in our report and those in older literature.

Figure 6.4 | At least 1 in 200 (0.5%) of all adult-onset end-stage renal disease is due to nephronophthisis, but the overall prevalence is likely higher. The late presentation of nephronophthisis might be due to genetic modifier effects. Accurately diagnosing a monogenic disease such as nephronophthisis can have wide-ranging clinical implications.

In assessing the clinical diagnoses of the cases with *NPHP1* deletions, we show that there is likely an underdiagnosis of *NPHP1*-related disease in adult-onset ESRD in clinical practice. The underdiagnosis, which was universally present among five cohorts originating from several countries in Europe, as well as the United States, might at least in part be due to the nonspecific phenotype, as it can be especially difficult to recognize in adults presenting with advanced renal failure.[282] This is underscored by our finding that 88% of *NPHP1* cases had received a clinical diagnosis other than NPH, even in cases with NPH-associated extra-renal anomalies. Notwithstanding, it is important to accurately define the etiology of ESRD cases, not only to prevent misdiagnosis and useless treatment, but also because a monogenic disease can have a variety of clinical implications.[296] For instance, it can affect decisions related to living related kidney donation, which is contra-indicated in siblings at risk.[297] It might also influence choices regarding family planning, especially in consanguineous relationships.[28]

In summary, we are the first to show in a large cohort that NPH due to *NPHP1* homozygous full gene deletions has a prevalence of 1 in 200 cases (0.5%) in *all* adult-onset ESRD. Although the incidence was clearly higher in patients with an ESRD-onset between 18 and 50 years (prevalence of 0.9%), NPH can have an onset at up to 61 years of age. As the method we used underestimates the total number of causal mutations, we conclude that NPH is a relatively frequent monogenic cause of adult-onset ESRD that is likely underdiagnosed in current daily practice. Given the potential clinical implications of having a genetic diagnosis, our data warrant wider application of genetic testing in adult-onset ESRD.



Pregnancy in Advanced Kidney Disease: Clinical Practice Considerations on a Challenging Combination

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Abstract

Thanks to the advances in care, pregnancy is now attainable for the majority of young female CKD patients, though it is still a high-risk endeavor. Clinical decision-making in these cases is impacted by a myriad of factors, making (pre)pregnancy counselling a complex process. The complexities, further impacted by limited data and unknown risks regarding outcome, can cause discussions when deciding on the best care for a specific patient.

In this paper, we provide an overview of the considerations and dilemma's we encounter in preconception counselling and offer our perspective on how to deal with them in daily clinical practice.

The main topics we discuss in our counselling are: 1) high risk of pregnancy complications, 2) risk of permanent CKD deterioration due to pregnancy and subsequent decreased life expectancy, 3) appropriate changes in renal medication, 4) assisted reproduction, genetic testing and prenatal or preimplantation genetic diagnostics.

In our clinic we openly address moral dilemma's arising in clinical practice in pregnancy and CKD, both within the physician team as well as with the patient. We do this by ensuring an interpretive physician-patient interaction and shared decision-making, deliberating in a multidisciplinary setting and, if needed, with input from an expert committee.

7.1 Background

Pregnancy is now attainable for the majority of young female CKD patients, though it is still considered high risk.[82,298,299] We experience in our tertiary counseling and care center that clinical decision-making in advanced CKD and pregnancy is impacted by a myriad of factors.

An important aspect of (pre-)pregnancy care in advanced CKD is overlooked in literature, namely that the counseling itself can be a complex process. Limited data and unknown risks regarding outcome, as well as differences in risk perception can make providing the (pre-pregnancy) care needed to ensure a good outcome challenging.[82]

7.2 Methods

We aim to shed light on the clinical practice considerations we encounter, providing our perspective on how to deal with decision-making in daily practice. We do this by discussing the four main preconception counseling topics that we find can lead to discussion on the best care for a specific patient in our tertiary clinic: 1) the high risk of pregnancy complications, 2) the risk of permanent CKD deterioration due to pregnancy and shortened life-expectancy, 3) changes in renal medication needed in pregnancy, and 4) assisted reproduction, genetic testing and prenatal or preimplantation genetic diagnostics. Additionally, we reflect on how we deal with the clinical practice dilemmas we encounter in our clinics, to hopefully contribute to a broader discussion on the best pre-pregnancy care for CKD patients.

7.3 Results

7.3.1 - Increased prevalence of pregnancy complications in CKD

Although data is limited, and often conflicting, overall studies have shown increased risks of pre-eclampsia (odds ratio, OR, 7-14), caesarean section (OR 2-3), prematurity (OR 3-9), low birthweight (OR 2-6) and need for admittance to a neonatal intensive care unit (NICU).[300] As displayed in Figure 7.1, absolute risks of adverse outcome depends on CKD stage, and whether the patient is on dialysis or post-transplant.[84,87,89] E.g. for advanced CKD patients, pre-eclampsia rates are ~50%, caesarean section ~70%, prematurity ~90%, low birthweight 50% and need for NICU admission ~70%.[82,84,301,302] One should note that the definition of (superimposed) pre-eclampsia varies between publications and the ~50% percentage should therefore be interpreted with caution. Overall, the fetal complications could lead

to neurodevelopmental delay for the child, albeit that with the improvement in neonatal care many premature babies can lead normal lives.[303]

As the chance of complications significantly raises with advancement in CKD stage (Figure 7.1), ideally one would discuss planning a pregnancy while the patient is still in CKD stage 1-3 and risks are relatively low.[84] Risks increase once the patient is in CKD stage 4-5 or on dialysis.[84,87,89,304] Therefore, one could decide to postpone a pregnancy until after a kidney transplant. Deciding to postpone is not a clear-cut decision and depends on, among others, length of the transplantation process, risk of suboptimal kidney function post-transplant (which cannot be estimated beforehand) and the impact of a pregnancy on the graft.

Finally, though attainable with intensive dialysis schedules (e.g. nocturnal hemodialysis for 42 hours a week), pregnancy is most high risk in dialysis patients.[87,89,304] Patient and physician may face a dilemma in cases where not harming mother might harm the (future) fetus. This dilemma arises in cases where waiting until after a transplant is not preferable, due to maternal age or long waiting lists, and one has to decide whether the fetal complications of a pregnancy on intensive dialysis can be accepted.

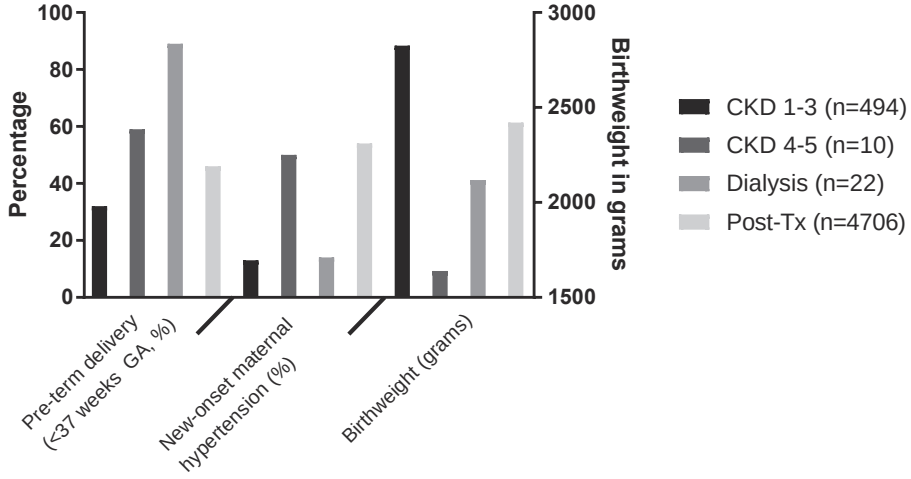

Figure 7.1 | Illustrative figure of prevalence of maternal and fetal pregnancy complications (low birthweight, preterm delivery, need for NICU and new-onset maternal hypertension) raise with advancement in CKD stage. Though data is limited, evidence shows that in advanced stages of CKD and (intensive, nocturnal) dialysis risks are high, while they decrease after a kidney transplant. Therefore, adequate timing of a pregnancy is vital. Data derived from *Piccoli et al.[84], #Hladunewich et al.[87], and ‡Deshpande et al.[89]

7.3.2 - Risk of permanent renal function deterioration due to pregnancy and limitations to life expectancy

Next to the obstetric complications, pregnancy leads to a permanent deterioration of renal function in 6-31% of women.[84,90,300] This so-called 'CKD-shift' (Figure 7.1) is complex in etiology, though likely the higher filtration rate needed in pregnancy plays a role.[84,90,300] A CKD-shift means that some stage 4-5 patients may need to start dialysis during, or in the years after pregnancy, while they otherwise might not have.

Even without a CKD-shift, life-expectancy in patients with impaired renal function is significantly shortened; women with an eGFR of 15-29 have a life-expectancy of ~13 years in comparison to healthy population.[305] Thus the maternal lifespan (or lifespan without renal replacement therapy) may not extend to her future child's adulthood. A poignant example is that of women with a renal transplant, as 12% dies within 20 years after delivery (median 6 years).[306]

The long-term consequences of a CKD-shift show that clinical practice decisions in pregnancy and CKD do not only span the pregnancy itself, but also the rest of the mother's and child's life. The poor renal outcome and limited life expectancy provides a large burden, greatly impacting quality of life for mother, partner and child.[307] As is the case for other chronic diseases, such as heart disease, decisions for child care as they pertain to shortened maternal life-expectancy ought to be addressed in a preconception stage.[308] Even though maternal survival into the child's adulthood is likely for most cases, comprehensive preparations with the patient's partner and extended social network can be useful when adverse outcomes occur.[308] Further complicating this topics is that the prognosis of CKD patients has steadily increased over the past years, a trend luckily likely to continue, making it difficult to estimate the quality of life for CKD patients 10-20 years from now.

7.3.3 - Changes in renal medication when considering pregnancy

The fetal safety of the patient's current treatment is a factor to take into account. Within the large branches of CKD treatment (antihypertensive, immunosuppressive and biological drugs) there are various safe drugs.[82] Nevertheless, many immunosuppressive drugs (including mycophenolate) and are contra-indicated in pregnancy because of teratogenicity and first trimester losses, and should therefore be discontinued timely.[82,309]

The considerations regarding maternal treatment surrender whether it is advisable to discontinue certain drugs because of teratogenicity or insufficient safety data, while discontinuation could cause adverse maternal renal disease

outcome (especially post-transplant). Additionally, the unknown fetal side-effects of certain drugs can render the decision even more complex, as one does not know if discontinuing a drug important for maternal care will even reduce fetal harm.[82]

7.3.4 - Assisted reproduction, genetic testing and preimplantation genetic diagnostics

During any preconception counseling a topic to consider is the impaired fertility that is common in women with advanced CKD.[82,300] More than healthy women, patients may require ovulation induction or assisted reproduction techniques such as in-vitro fertilization (IVF).[300]

In assisted reproduction cases, as well as spontaneous pregnancies, genetic testing should be considered. Genetic diseases are highly prevalent in the young CKD population: ~20% of all ESRD patients presenting before the age of 25 have a monogenic kidney disease.[21] A monogenic disease not only impacts the patient, for instance because different therapies might be indicated, but also her offspring, which is at risk of inheriting the kidney disease. Though not in all patients the causative genetic defect can be found, providing genetic testing opens many avenues for patients.[30]

When the causative mutation is known, invasive prenatal diagnostic (PND) testing (chorion villus biopsy or amniocentesis) can be performed, with a possibility to terminate an affected pregnancy.[310] To avoid a need for invasive diagnostics (with risk of miscarriage) and termination, preimplantation genetic diagnostic testing (PGD) has been developed for patients with a known monogenic mutation. It brings down the risk of passing on the genetic disease to the future child to 1-2%.[311] PGD entails performing genetic testing in a single cell removed from an IVF-embryo and only transferring a genetically unaffected embryo to the uterus.[311] Furthermore, the physician should realize that genetic testing in general and PGD specifically can be time-consuming (3-24 months). Thus, this is amongst the first topics to discuss with a CKD patient to ensure adequate genetic counseling and care.

Though surrounded with many large and smaller scale good clinical practice considerations, which are beyond the scope of this paper, the application of PND and PGD is widely accepted, especially in diseases that have an early onset and are severe.[81] Still, in the Netherlands a (nationwide) committee of expert physicians and bioethicists deliberate on each new gene to ensure the decision to perform PGD is morally sound for that specific gene. Whether or not PND or PGD can be considered, depends on local availability, disease severity and patient preference.[28,310]

7.4 Discussion

As stated before, the preconception counselling in CKD can be complex, causing discussions on the best practice for a specific patient. Below, we provide our perspective on how we deal with these discussions and how we ensure adequate decision-making in our clinic.

7.4.1 Physician attitude, paternalism and shared decision making

The method of preconception counseling is essential. We feel that for adequate decision-making, the patient should be fully informed about risks and potential complications of a pregnancy in CKD. Therefore, we apply a so-called "interpretive attitude" towards the physician-patient relationship, which defines the physician as being "a counsellor (...), supplying relevant information, helping to elucidate values and suggesting what medical interventions realize these values".[312] An interpretive attitude in the physician-patient relationship does not allow for 'hard paternalism' (overriding the preferences of that person), yet 'soft paternalistic' approaches or directive counseling (providing information and even advising negatively) could be applied to ensure the patient has all the input needed for decision-making.[312] The exchange of ideas between physician and patient (shared decision-making) permits the patient an independent choice, enhancing her autonomy.[313,314]

7.4.2 Reproductive autonomy and non-maleficence

Reproductive autonomy, the liberty to decide whether or not to have children, is a main principle in any discussion concerning reproduction.[315] Specifically, in every decision on pregnancy in CKD, one has to weigh the maternal reproductive autonomy against the principle to do no harm (non-maleficence) to the woman or the fetus.[316,317]

In principle, we regard the patient's autonomy as paramount in every medical decision. We apply the concept of "relational autonomy", where one includes contextual factors, such as the patient's emotional background, social and financial factors.[315] An important contextual factor is the patient's partner, who can contribute in many ways to the patient's autonomy and the decisions regarding pregnancy. This leads to an open conversation whereby the patients feel free to express themselves.

Even though CKD is a risk factor for suboptimal pregnancy outcome, in the majority of cases we feel that the desire to not harm the mother or the fetus, does not outweigh the maternal reproductive autonomy. Underscoring this is the notion that pregnancy is inherently a high risk situation, e.g. 3-5% of all pregnancies are complicated by pre-eclampsia, regardless of maternal comorbidity.[318] Therefore

the preconception counseling is aimed at the patient understanding the potential risks and working together to minimize these risks as much as possible.

Yet when there is a need for assisted reproduction we find that considerations of not harming mother and fetus are more relevant. That is to say, there is a difference between caring for a patient when she falls pregnant naturally and *assisting in* initiating a pregnancy that puts the mother and the fetus at high risks. In such cases, we argue that the physician can justifiably act more paternalistic, since he or she is actively assisting (instead of passively allowing) a situation which one highly suspects of harming a future fetus.[319,320]

7.4.1 Multidisciplinary care

One of the ways we ensure adequate decision making in our tertiary care facility is multidisciplinary care. We offer a multidisciplinary out-patient clinic where patients are counseled by a nephrologist and specialized maternal-fetal medicine specialist. A clinical geneticist specialized in hereditary kidney disease consults on genetic testing, PND and PGD if applicable. The team also confers with fertility specialists, pathologists, ethicists, and anesthesiologists to gain insight on technical care matters related to the pregnancy, as well as ethical issues that may arise. Furthermore, in cases of assisted reproduction or PGD the team is advised by local and national expert committees consisting of physicians and medical ethicists.

In conclusion, due to the advances in nephrological, fertility and obstetric care, advanced CKD patients have a myriad of choices regarding pregnancy. They should be counseled on the available factual information regarding increased pregnancy and renal complications, their long-term impact including a limited life-expectancy. Furthermore, the options regarding assisted reproductive technology, genetic testing, PND and PGD should be discussed. However, these topics and the decisions they entail can cause deliberations between physicians, and with the patient, especially since data on these issues is limited. We provide our perspective on how to deal with these situations, namely by ensuring an interpretive attitude in the physician-patient relationship and shared decision-making, additional deliberation on clinical practice dilemmas in a multidisciplinary setting and, if needed, with input from an expert committee.

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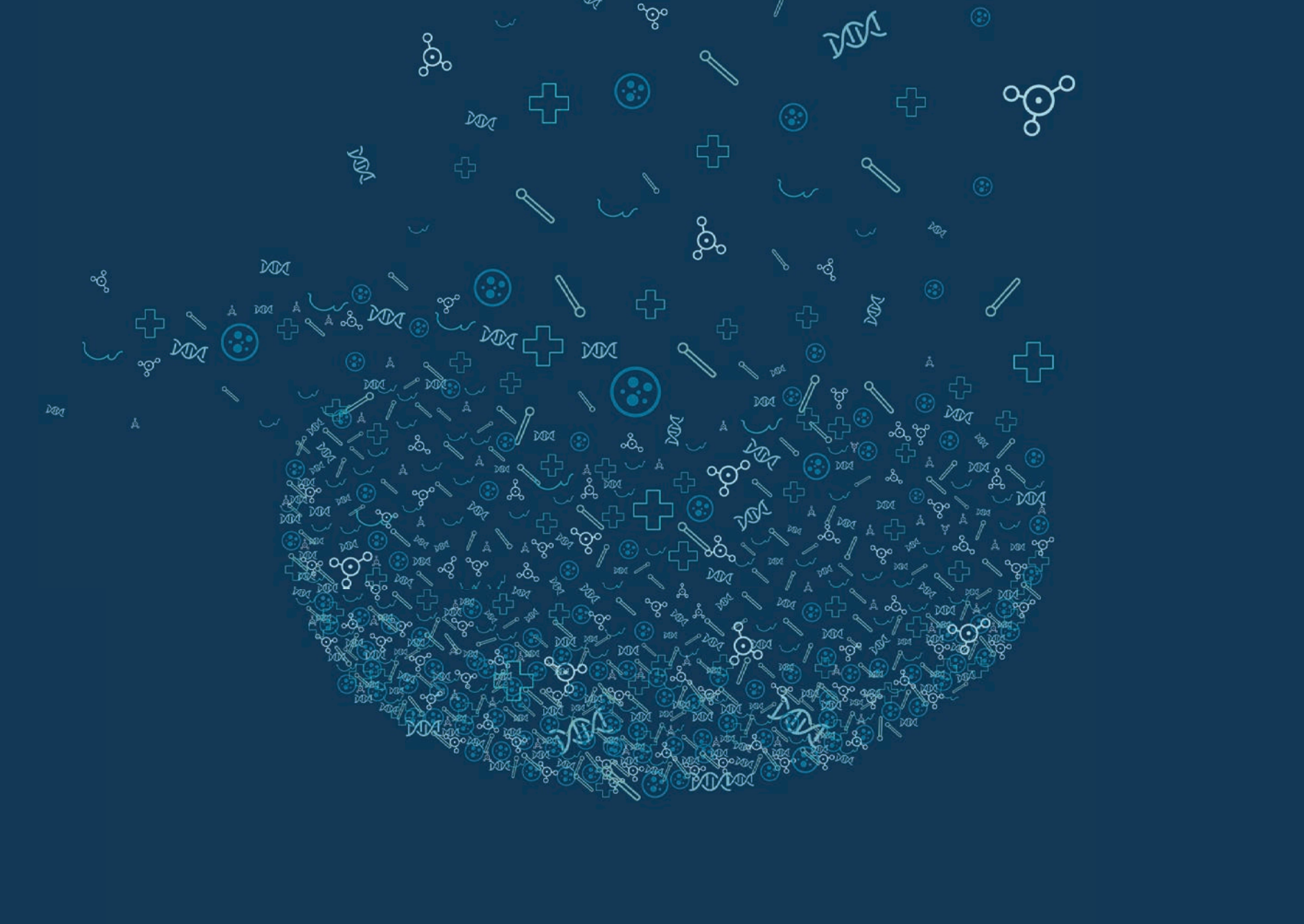

Pregnancy outcomes in women with *COL4A3-5* related disease (Alport syndrome)

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In preparation

Abstract

Chronic kidney disease (CKD) affects approximately 3% of pregnant women and increases the risk of pregnancy complications. Kidney function may deteriorate more quickly due to pregnancy, causing a (temporary) detoriation of kidney function. There is limited knowledge on pregnancy outcomes in specific renal diseases. Therefore, we studied the pregnancy and renal outcomes in 68 female patients with *COL4A3-5* related disease (Alport syndrome). Though rare, *COL4A3-5* related disease is one of the most prevalent monogenic renal diseases.

We set up a retrospective cohort study in 10 European centers, retrieving data from the medical files of women with genetically or biopsy proven *COL4A3-5* related disease. The outcomes were compared to those of 457 CKD stage 1-2 patients (a similar CKD stage as our cohort) of diverse etiology from a 2015 Italian study and 159,924 women from the general Dutch population.

In this intermediary analysis, we present data on 109 pregnancies from 68 women with *COL4A3-5* related disease. Fetal outcomes were better in *COL4A3-5* pregnancies than in pregnancies of women with CKD stage 1-2 of diverse etiology. We saw less prematurity (17% vs 36% respectively) and a higher mean birthweight of 3216 ± 663 gram compared to 2768 ± 680 in the Italian cohort. Maternal renal outcomes should be interpreted with caution (15-60% missing data): proteinuria (73%) and hypertension (30%) were more frequent in *COL4A3-5* pregnancies than in the Italian cohort. In this cohort, 10% developed severe hypertension. Median eGFR was not impacted by pregnancy and the slope declines of eGFR before and after pregnancy were not significantly different.

Fetal outcomes in pregnancies with *COL4A3-5* related disease seem to be more favorable than in a cohort with mixed cause of CKD. In this intermediary analysis, proteinuria levels and frequency of new-onset hypertension in pregnancy are higher. There is no significant eGFR loss during pregnancy or increased eGFR deterioration in the long-term. The differences between *COL4A3-5* and general CKD pregnancies underscore the importance of investigating pregnancy outcomes in specific renal disease phenotypes to ensure adequate (pre-) pregnancy counselling and care.

8.1 Introduction

Chronic kidney disease (CKD) affects approximately 3% of pregnant women, and this prevalence is expected to increase as maternal age rises.[82,321] Women with CKD have an increased risk of maternal and fetal pregnancy complications, such as pre-eclampsia, premature delivery and low birth weight.[82–84,86,87] Chance and severity of complications rise with the advancement in CKD stage, with a peak in dialysis pregnancies, while the risk lowers after receiving a transplant.[84,87,88,322] Kidney function can also deteriorate more quickly due to pregnancy, with up to 20% of patients having a so-called "CKD stage shift" to a higher CKD stage during or shortly after pregnancy.[82]

Since pregnancy in CKD was long deemed too risky, the studies that have been performed in this population have only been able to report on relatively small groups comprised of patients with heterogeneous underlying etiology.[84,87,90,323] This means that the information on pregnancy outcomes in specific diseases is limited. Thus, providing patients with tailored etiology-based pre-pregnancy counselling and care is currently not possible.[324]

One of the kidney diseases in which little is known about pregnancy outcomes is Alport syndrome (AS). With a prevalence of 1:17,000 to 1:53,000, AS is one of the most frequent genetic kidney diseases.[29,325–328] Caused by mutations in the collagen network genes *COL4A3, COL4A4* or *COL4A5*, AS is also referred to as *COL4A3-5* related disease. The aberrant collagen networks cause the main symptoms of hematuria (and proteinuria) that progresses to end-stage renal disease (ESRD), and in some cases hearing loss and ocular abnormalites.[328,329] *COL4A3-5* related disease can be diagnosed through observing a thinning or irregular thickness of the glomerular basement membrane (GBM) on renal biopsy or through genetic testing of the *COL4A3-5* genes.[138] *COL4A3-5* related disease can be inherited in an autosomal recessive, autosomal dominant, digenic or X-linked fashion.[329]

Eighty-five percent of patients have mutations in the X-linked gene *COL4A5*, making it a disease predominantly present in males.[329–331] However, due to skewed X-chromosome inactivation, female carriers of *COL4A5* mutations can also have a (highly variable) phenotype.[328,332–337] Notably, 95% of female *COL4A5* carriers develop haematuria, 75% proteinuria and 12% reach ESRD before the age of 40 years.[332]

Only 19 pregnancies in 12 *COL4A3-5* women have been reported in the literature, mainly case reports.[338–347] Of those pregnancies, 55% were complicated by preterm delivery, 26% by pre-eclampsia and the median birthweight was 2400 grams.[338–347] There was a CKD stage shift in 43%.[338–347] Due to a probable reporting and

publication bias in these case reports, it is difficult to discern the true complication rate in *COL4A3-5* pregnancies.

Here, we present the pregnancy and renal outcomes in an analysis of a part of an international cohort of 68 female *COL4A3-5* patients, where patients are currently still being enrolled in. We compare these data to pregnancies in women with CKD of any cause and to pregnancies in the general Dutch population.[84,348] The analysis of this largest cohort to date allow for more tailored (pre-) pregnancy counselling and care for patients with this prevalent monogenic renal disease.

8.2 Methods

An international retrospective cohort study, the ALPART network, was set up by the University Medical Center Utrecht (NL) in collaboration with Amsterdam University Medical Center (NL), University Medical Center Groningen (NL), Vilnius University Hospital Santaros Klinikos (LT), Fundacio Puigvert (ES), Hôpital Necker (FR), Cliniques universitaires Saint Luc (BE), Università degli Studi di Torino (IT), Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico (IT), and Azienda Ospedaliera Universitaria Senese (IT). Institutional review board approval (including informed consent to access medical records) was obtained at each center in accordance with local regulations. If not all data was available at the participating center, data was requested from the center where the patient delivered if patients provided informed consent to do so.

Data was collected retrospectively from all available female patients with a diagnosis of *COL4A3-5* related disease and at least one ongoing pregnancy (≥ 20 weeks gestational age [GA]). "*COL4A3-5* related disease" was defined as either thinning/irregular thickness of the GBM confirmed by electron microscopy of a renal biopsy or an American College of Medical Genetics (likely) pathogenic mutation in *COL4A3-5*.[39] Patients with a kidney transplant prior to the first pregnancy were excluded.

Baseline and outcome measures were defined prior to data collection. CKD stage, hypertension and (nephrotic-range) proteinuria were defined according to the KDOQI criteria.[349] Estimated glomerular filtration rate (eGFR) was calculated through the MDRD method, and if no data on proteinuria was available, CKD stage was based solely on eGFR.[350] Hypertension in pregnancy was defined as a blood pressure of >140/90 mm Hg and severe hypertension as a blood pressure of >160/100 mm Hg, HELLP-syndrome and small for gestational age (SGA) were defined according to the American College of Obstetrics and Gynaecology (ACOG) criteria.[351,352] Perinatal death includes all fetal death from a GA of 32 weeks untill 7 days postnatal.

Data on genotype, phenotype, and maternal and fetal outcomes were retrieved from the electronic patient record at the local hospital. Since data was entered at multiple centers, in some cases outcome measures had to be recalculated to fit abovementioned definitions (e.g. in case of different units of serum creatinine measurement).

COL4A3-5 patient and pregnancy data was compared to data on pregnancies in patients with CKD due to a mixed cause (n=504) and to the general Dutch pregnancy registration (n=159,924).[84,348] The CKD cohort by Piccoli et al. consists of patients with CKD stage 1-5, due to mixed causes.[84] This might therefore include *COL4A3-5* patients, though disease etiology is not mentioned in the paper.[84] The Dutch pregnancy registration includes all Dutch pregnancies, thus a small subset of the >150,000 pregnancies in this database could be CKD pregnancies.[348]

Statistical analysis was performed with SPSS (version 25, IBM, New York, NY) and GraphPad Prism (version 8.3, GraphPad Software, La Jolla, CA). All testing was performed two-sided with the probability of type I error set at 0.05. Missing variables were excluded from analysis. Groups were generally too small to allow for subgroup analysis.

8.3 Results

We included 68 women with *COL4A3-5* related disease, from 10 European centers (Supplemental Table 8.1). The baseline values on patient characteristics and disease severity are presented in Table 8.1. The diagnosis was genetically confirmed in 97%, with 2 patients being diagnosed through an abnormal GBM on renal biopsy. Most patients had a (likely) pathogenic mutation in *COL4A5*.

Prior to the first pregnancy, patients were generally in CKD stage 1-2, therefore we compared our data to the mixed cause CKD stage 1-2 cohort from Piccoli *et al.* (Table 8.1) which was weighed to match percentage of CKD stage 1-2 patients in our cohort.[84,348] One should note that for 37 patients (54%) data on CKD stage was missing, thus matching was performed based on the 31 casese where pre-pregnancy CKD stage was available for, of which 42% was in CKD stage 1 and 36% in CKD stage 2. In the *COL4A3-5* group, there is significantly more proteinuria and less hypertension at baseline than in the CKD stage 1-2 control group. Of the 68 women, 38% had more than one pregnancy and there were two twin pregnancies (Table 8.2).

Fetal pregnancy outcomes were more favorable than in the Italian CKD stage 1-2 cohort (Table 8.2 and Figure 8.1). [84,348] Prematurity (17%) and NICU admission rates (6%) were significantly lower, but higher than in the general Dutch population. Birthweight was higher than in the Italian cohort at a mean 3216 + 663 grams, which

is actually similar to birthweight in the general Dutch population.[84,348] Caesarean section (CS) was less frequent in the *COL4A3-5* group compared to the CKD stage 1-2 cohort, though more frequent than in the general Dutch population (Table 8.2).[84,348]
New-onset or doubling of proteinuria (73%) and new-onset of hypertension (30%) was more frequent in *COL4A3-5* pregnancies than in other CKD stage 1-2 pregnancies (Table 8.3 and Figure 8.1).[84] Systolic pressures ranged up to 182 mm Hg and 10% of pregnancies were complicated by severe hypertension (Table 8.3).

Proteinuria, be it pre-pregnancy or during pregnancy, was not significantly correlated with pregnancy outcomes or maternal renal outcomes (Pearson's R 0.66-0.386). Only pre-pregnancy nephrotic range proteinuria was correlated with SGA (Pearson's R 0.001). There was 59% missing data for proteinuria (striped column in Figure 8.1).

There was a non-significant loss of median eGFR during pregnancy (Table 8.3), as exemplified by the large ranges for the median eGFR values. The slope of eGFR loss was not significantly steeper post-pregnancy compared to before the pregnancy (Figure 8.2).

8.4 Discussion

We present data on the pregnancy and renal outcomes of 109 pregnancies in 68 women with *COL4A3-5* related disease from 10 centers across Europe. There seem to be more favorable fetal outcomes but also an indication for less favorable maternal renal outcomes than in other CKD stage 1-2 patients. This underscores the importance of investigating pregnancy outcomes in specific renal disease phenotypes to ensure adequate pre-pregnancy counselling and care.[324]

Our cohort mainly consisted of women with early CKD (stage 1-2) or only hematuria without proteinuria or eGFR loss, which seems to be the disease stage the majority of *COL4A3-5* women are in when they are in the reproductive age.[338–347] For 54% the data on pre-pregnancy CKD stage was missing, likely because they had subclinical or no renal symptoms. The early disease stage at the reproductive age is likely the cause for women in our cohort first being diagnosed with *COL4A3-5* related disease a median of four years after their first pregnancy. As we did not have access to specific matched controls, we chose to compare to the Piccoli *et al.* CKD stage 1-2 groups because this was the closest form of case-control matching we could attain, despite the general lack of pre-pregnancy CKD data in our cohort.[84] In a future analysis we would prefer to match patients on hypertension, renal function and proteinuria levels since these are the main determinants of fetal outcome.[82]

Table 8.1 | Baseline cohort characteristics of n=68 women with COL4A3-5 related disease, compared to the general CKD stage 1-2 population and the general Dutch population.[84,348] All values are percentages (n/n reported in medical file), thus excluding missing data, unless otherwise indicated. *=p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, without asterisk the difference is not significant.

General maternal baseline values	***COL4A3-5* related disease (n=69)**	**CKD stage 1 and 2 (n=457)**	**General Dutch population (n=159,924)**
Caucasian	94% (n=60/63)	92%	87% (n=139.133)
BMI (median [range])	21.9 (17.5-44.2)	23.3 ± 4.9	NR
Smoking	9% (n=5/56)	NR	NR
Age at first pregnancy (years, mean ± SD)	28.2 ± 5.0	31.9 ± 5.4	NR
One pregnancy Two pregnancies Three pregnancies Four pregnancies	1: 62% (n=68) 2: 29% (n=32) 3: 7% (n=8) 4: 1% (n=1)	56% first pregnancy	44% first pregnancy (n=69.794)***
Follow-up time in years after first pregnancy (median [range])	11.3 (0.2-45.8)	NR	NR
Diagnosis of COL4A3-5 related disease			
Age at diagnosis (years, mean ± SD)	34.3 ± 14.1	N/A	N/A
Genetically confirmed diagnosis	97% (n=66/68)	N/A	N/A
COL4A5 *COL4A4* (n total; n recessive inheritance) *COL4A3* (n total; n recessive inheritance) *COL4A3 and COL4A4*	70% (n=46/66) 6% (n=4/66; 3) 23% (n=15/66; 0) 2% (n=1/66)	N/A	N/A
Diagnosis made with kidney biopsy only	3% (n=2/68)	N/A	N/A
Renal function prior to first pregnancy			
No CKD CKD stage 1 CKD stage 2 CKD stage 3 CKD stage 4 Missing	19% (n= 6/31) 42% (n=13/31) 36% (n=11/31) 3: 0% (n=0/31) 3% (n=1/31) 54% (n=37/69)	*Data on the full n=504 Piccoli et al. cohort* 0% (n=0/504)*** 73% (n=370/504)*** 17% (n=87/504)* 7% (n=37/504) 2% (n=10/504, stage 4 and 5) N/A	N/A
Hematuria	100% (n=66/66)	NR	N/A
Proteinuria (gram/day, median [range]) 	0.87 (0.07-4.6) 77% (n=26/34)	0.1 (0.01-14.5)** NR	N/A
Hypertension	15% (10/68)	27% (n=138/504)**	N/A
Alport symptoms at any time			
Hearing loss	28% (n=17/61)¥	N/A	N/A
Alport specific ocular symptoms	2% (n=1/47)	N/A	N/A

BMI=body mass index, CKD=chronic kidney disease, eGFR=estimated glomerular filtration rate, ESRD=end-stage renal disease, N/A=not applicable, NR=not reported, ¥=hearing loss was observed in 3 recessive inheritance *COL4A3* cases and 14 *COL4A5* cases with X-linked inheritance

Table 8.2 | Maternal and fetal pregnancy outcomes of n=109 pregnancies in n=68 women with COL4A3-5 related disease, compared to the general CKD stage 1-2 population and the general Dutch population.[84,348] All values are percentages (n/n reported in medical file), thus excluding missing data, unless otherwise indicated. *=p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, without asterisk the difference is not significant.

	***COL4A3-5* related disease (n=109)**	**CKD stage 1 and 2 (n=457)**	**General Dutch population (n=159,924)**
Maternal outcome variables			
Singleton	98% (n=107/109)	100%**	98%
Caesarean Section	21% (n=23/109)	58%***	7.6%***
HELLP	2% (n=2/105)	NR	NR
Fetal outcome variables			
Male	49% (n=54/109)	NR	NR
Gestational age (weeks, mean ± SD)	38.6 ± 2.3	36.8	39.4
Preterm delivery (<37 weeks GA)	17% (n=18/103)	36%***	7%***
Extreme preterm delivery (<34 weeks GA)†	5% (n=5/103)	13%*	2%
Birth weight (gram, mean ±SD)	3216 ± 663	2768 ± 680***	3264
SGA (birth weight <p10)	15% (n=16/105)	15%	10%
Low Apgar (<7 after 5 minutes)	0% (n=0/79)	NR	2%
NICU admission (>48 hours)	6% (n=6/107)	18%**	3%
Perinatal death (32 weeks gestational age and 7 days post-partum)	0% (n=0/111)	NR	1%

GA=gestational age, HELLP=Hemolysis Elevated Liver enzymes and Low Platelets, NICU=neonatal intensive care unit, NR=not reported, SGA=small for gestational age.
†although extreme prematurity is defined as a gestational age (GA) <32 weeks in the Netherlands, we used the cut-off value of <34 weeks to allow for comparison with the Piccoli *et al.* cohort.

We found a lower prevalence of maternal and fetal complications such as CS, preterm delivery, SGA and NICU admission compared to CKD 1-2 patients of mixed cause.[84] Mean birth weight was significantly higher and actually similar to birthweight in non-CKD Dutch pregnancies. Though not all pregnancies were from the Netherlands and this data is thus difficult to compare to the Dutch population, the healthy birthweight is reassuring for Alport women who wish to conceive.[348] A contributor to the difference in maternal and fetal complications is that 19% of our cohort was not in CKD yet at the time of their first pregnancy. Furthermore, there are local differences in pregnancy management, e.g. CS and NICU rates are vastly different from country to country.[353] We included data from 10 European centers, limiting the impact of local pregnancy management compared with previous smaller one center studies in *COL4A3-5* patients and CKD patients with any cause CKD.[84,338–347]

Table 8.3 | eGFR and other maternal renal outcomes in n=109 pregnancies of n=68 women with COL4A3-5 related disease (excluding one post-transplant case), compared to the CKD stage 1-2 population and compared the general Dutch population.[84,348] All values are excluding missing data, unless otherwise indicated. *=p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, without asterisk the difference is not significant.

	***COL4A3-5* related disease (n=109)**	**CKD stage 1 and 2 (n=457)**	**General Dutch population (n=159,924)**
Maternal eGFR (mL/min/1.73m2, median [range])			
Prior to pregnancy	101 (24-175)	118 (6-186)	N/A
First trimester	99 (24-157)	NR	N/A
Second trimester	104 (20-181)	NR	N/A
Third trimester	99 (19-162)	NR	N/A
3 months post-pregnancy	93 (23-144)	NR	N/A
1-year post-pregnancy	94 (21-159)	NR	N/A
Maternal renal outcomes			
New onset or doubling of proteinuria	73% (n=33/45)	28%***	NR
New onset hypertension (>140/>90 mmHg)	30% (n=27/90)	12%***	5.3%***
New onset severe hypertension (>160/100 mm Hg))	10% (n=9/90)	NR	NR

CKD=chronic kidney disease, eGFR=estimated glomerular filtration rate, N/A=not applicable, NR=not reported.

Regarding maternal renal outcomes, 30% of pregnancies are complicated by new-onset hypertension. This was significant compared to other CKD stage 1-2 pregnancies, a difference that may be partly influenced by the significantly lower pre-pregnancy hypertension rates in COL4A3-5 women.[84] However, the high hypertension rates are in line with previous reports of 37% hypertension in *COL4A3-5* pregnancies.[338–347] Notably, those case reports also mention that if a patient develops hypertension during pregnancy, it is often severe or uncontrollable hypertension, which is something we saw in 10% of our patients as well.[338–347] The mechanism behind hypertension development in *COL4A3-5* pregnancies is not yet known, though it may be related to the mechanism rendering *COL4A3-5* related disease a relatively hypertensive disorder in women in general.[354]

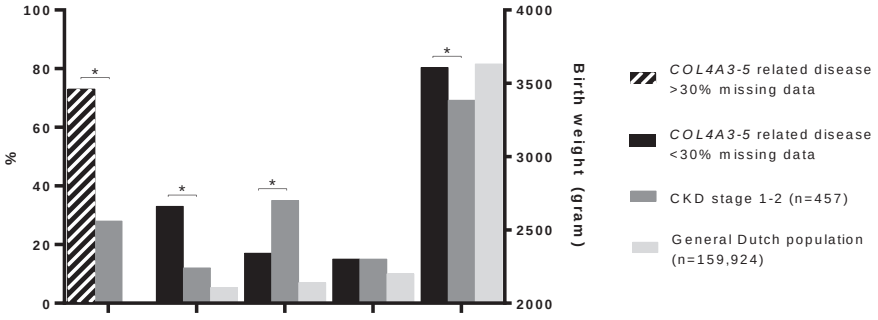
In this cohort, 73% had new-onset or doubling of proteinuria, although this is likely skewed by 59% missing data. Nevertheless, our results are in line with previous studies in *COL4A3-5* pregnancies high proteinuria rates.[338–347] In those previous reports, proteinuria at conception was proposed as a predictor for adverse pregnancy outcomes.[338–347] In our cohort we could not objectify a negative impact of pre-pregnancy and in-pregnancy proteinuria, though this may be impacted by

the number of missing data. We did find a correlation between pre-pregnancy nephrotic range proteinuria and SGA, which is a previously described relation.[355]

The development of proteinuria specifically could be related to the fragile GBM suffering relatively more from hemodynamic changes in pregnancy, making glomeruli of *COL4A3-5* patients more prone to start leaking protein.[82,134] The notion of a temporary effect of pregnancy on the GBM is underscored by our finding of a restoration of proteinuria in new-onset/doubling of proteinuria cases.

We also observed no loss of median eGFR during pregnancy and no long-term effect of pregnancy on eGFR, which is similar to previous reports.[338–347]

Due to a large number of pregnancies for which pre-pregnancy renal parameters were not reported, we could not report on CKD stage shift. We could not compare the *COL4A3-5* post-partum eGFR values to the Piccoli *et al.* cohort as this was not reported in that study.[84] It is difficult to draw conclusions on long-term eGFR decline because we were limited in these analyses by the number of reported cases with enough data points on eGFR. Since we did not have access to data of women with *COL4A3-5* related disease who had not been pregnant, we cannot gauge the effect of pregnancy on renal disease progression.



COL4A3-5 related disease cohort	New onset or doubling of proteinuria	New onset hypertension	Prematurity	SGA	Birth weight
Outcome (%) (n/n reported)	73% (33/45)	30% (27/90)	17% (18/103)	15% (16/105)	96 reported
Missing data (%) (n/total cohort)	59% (64/109)	17% (19/109)	6% (6/109)	4% (4/109)	12% (13/109)

Figure 8.1 | Renal and fetal outcomes in n=109 pregnancies of n=68 women with COL4A3-5 related disease, compared to the CKD stage 1-2 population and the general Dutch population.[84,348] The striped bars represent outcomes in the COL4A3-5 group that have a missing data percentage of >30%. The number of cases the outcome rates are based on in the COL4A3-5 group are noted in the table.
*= significant, only differences between *COL4A3-5* and general CKD stage 1-2 group are noted, for differences with general Dutch population see Table 2 and 3. CKD=chronic kidney disease. SGA=small for gestational age.

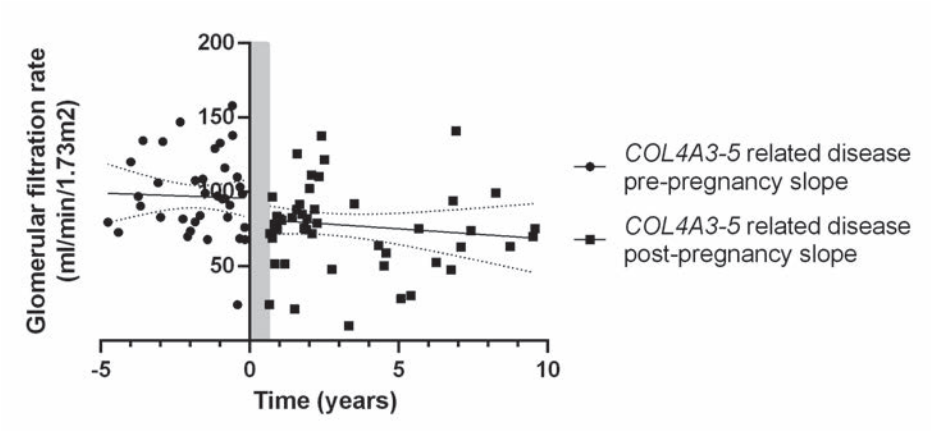


Figure 8.2 | Estimated glomerular filtration rate (eGFR) slope (ml/min/yr/1.73m2) in the 34 patients from the COL4A3-5 related disease cohort with eGFR measurements 0-5 years prior to a pregnancy (slope: -0.7030 ± 3.017), until 10 years after their pregnancy (slope: -1.366 ± 1.587). Slopes are not significantly different (p=0.85), with dotted lines indicating 95% confidence intervals. Light gray bar represents the pregnancy.

Due to the retrospective nature of data collection, there were several pregnancies with missing data on renal baseline measurements, as the patient had not yet been diagnosed with *COL4A3-5* related disease at the time of that particular pregnancy. This led to an overestimation of the prevalence of adverse renal outcomes, despite an effort to recontact patients in order to complete the dataset. Women with a more severe phenotype, such as the high percentage of our patients that developed hearing loss, are probably also more likely to be identified as *COL4A3-5* patients, causing us to possibly overestimate disease severity in this cohort in general.[328,332–337]

We hope to include more *COL4A3-5* patients in the near future, expanding the study to ~200 pregnancies. We are also establishing a detailed control group of women with CKD that is not due to *COL3A3-5* related disease, to be able to truly match cases on hypertension, renal function and proteinuria levels for an adequate case-control analysis.[82] Finally, we hope to extend this study to encompass other rare renal diseases, as we have seen that data on pregnancy outcomes in specific diseases is essential to ensure adequate patient counselling.[324]

We conclude that the fetal pregnancy outcomes in women with *COL4A3-5* related disease seem to be more favorable than in pregnancies with CKD due to any cause. We found indications for higher rates of proteinuria and (severe) hypertension, though there is no impact of pregnancy on eGFR in short-term or on eGFR decline in the long-term. With 109 pregnancies from multiple centers across Europe, this is the largest study to date in this relatively rare renal disease. The differences

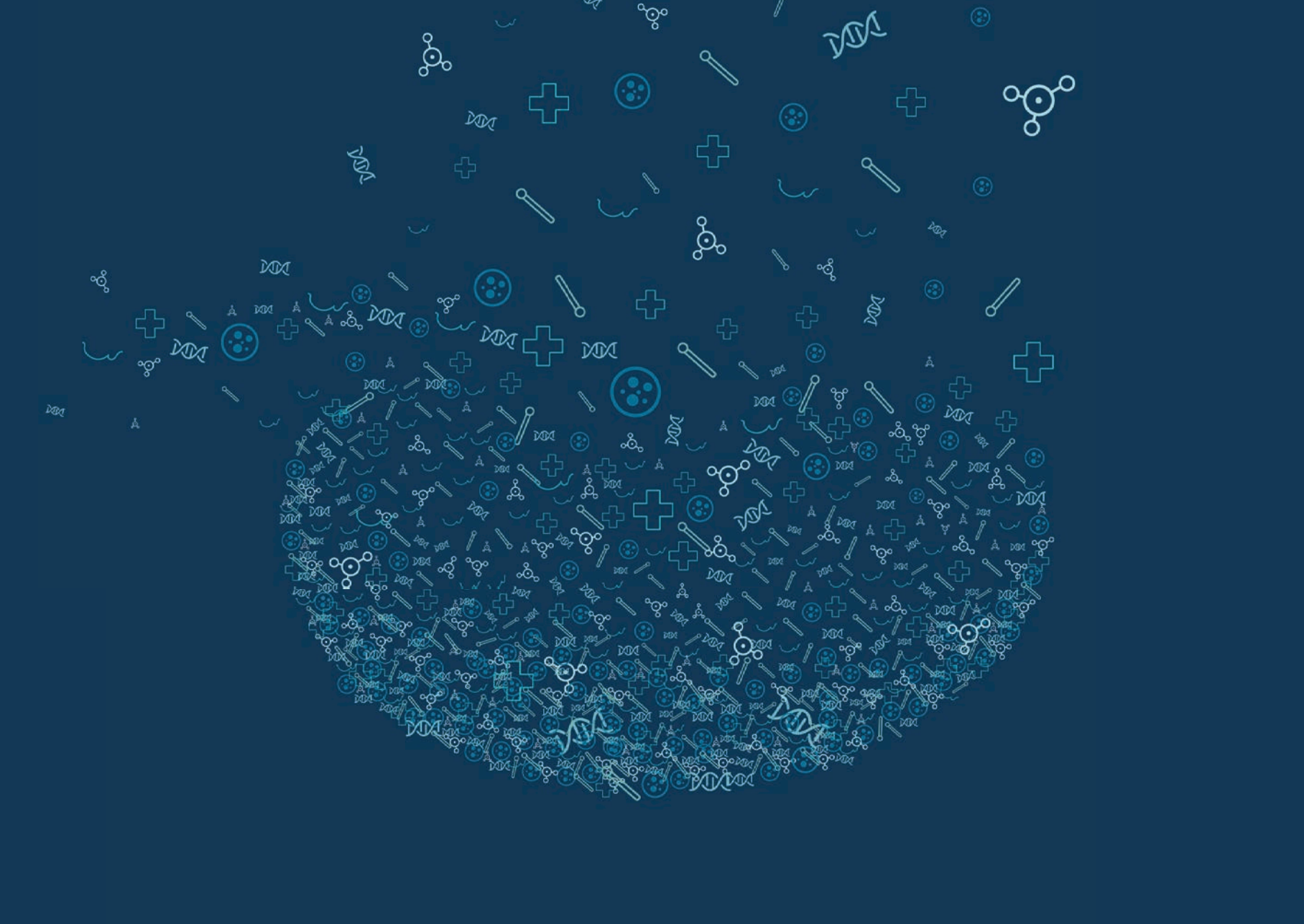
between outcomes in *COL4A3-5* related disease and general CKD pregnancies make the case for more research on pregnancy outcomes in specific renal diseases. More knowledge on the subject will help ensure adequate counselling and care for all renal disease patients with a pregnancy wish.

Supplementary material 8

Supplemental Table 8.1 | The number of pregnancies included from each participating center.

Center	Pregnancies (n, %)
University Medical Center Utrecht (NL)	30 (28%)
University Medical Center Groningen (NL)	8 (7%)
Amsterdam University Medical Center (NL)	2 (2%)
Fundacio Puigvert (ES)	6 (6%)
Hôpital Necker (FR)	12 (11%)
Cliniques universitaires Saint Luc (BE)	4 (4%)
Università degli Studi di Torino (IT)	5 (%)
Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico (IT)	4 (4%)
Vilnius University Hospital Santaros Klinikos (LT)	36 (33%)
Azienda Ospedaliera Universitaria Senese (IT)	2 (2%)

We thank the ALPART-study network collaborators who contributed data to this intermediary analysis: Agne Cerkauskaite, Antonio Mastrangelo, Aude Servais, Giorgina Piccoli, Joséphine Cornet, Liffert Vogt, Marius Miglinas, Michel van Geel, Monica Furlano, Alessandra Renieri, Rimante Cerkauskiene, Roser Torra, Rossella Attini, Valentine Gillion, Margriet de Jong. And we also thank the entire ALPART-study network for their continued collaboration on pregnancy in rare disease.



Pre-implantation genetic testing for monogenic kidney disease

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Abstract

A genetic cause can be identified for an increasing number of pediatric and adult-onset kidney diseases. Preimplantation genetic testing (formerly known as preimplantation genetic diagnostics) is a reproductive technology that helps prospective parents to prevent passing on (a) disease-causing mutation(s) to their offspring. Here we provide a clinical overview of 25-year preimplantation genetic testing for monogenic kidney disease in the Netherlands.

Retrospective cohort study of couples counselled on preimplantation genetic testing for monogenic kidney disease in the national preimplantation genetic testing expert center (Maastricht UMC+) from January 1995 until June 2019. Statistical analysis performed through χ^2 tests.

In total, 98 couples were counselled regarding preimplantation genetic testing, of whom 53% opted for preimplantation genetic testing. The most frequent indications for referral were autosomal dominant polycystic kidney disease (38%), Alport syndrome (26%) and autosomal recessive polycystic kidney disease (9%). Of couples with at least one preimplantation genetic testing cycle with oocyte retrieval, 65% experienced one or more live births of an unaffected child. Of couples counselled, 38% declined preimplantation genetic testing, for various personal and technical reasons.

Though some couples decline preimplantation genetic testing, in the couples that proceed with at least one preimplantation genetic testing cycle, the high live birth rates are hopeful for couples seeking preimplantation genetic testing. This is the first overview of the expanding indications, uptake and results of preimplantation genetic testing for monogenic kidney disease. Referrals, including for adult-onset disease, have increased steadily over the past decade.

9.1 Introduction

Chronic kidney disease (CKD) has an estimated global prevalence of 11 to 13% and is associated with high morbidity and mortality.[1–3] Recent studies have shown that a monogenic cause can be identified in 20 to 40% of patients with childhood and adult-onset CKD.[4–10]

Identifying the genetic cause in patients with monogenic kidney disease allows for counselling on prognosis and therapeutic options.[11–13] Moreover, the risk of having affected children and options regarding family planning can be discussed.[5,10] Preconception counselling encompasses everything from expected pregnancy outcomes for mother and child, to recurrence risk, invasive prenatal diagnosis and preimplantation genetic testing (formerly known as preimplantation genetic diagnostics).[14] In the Netherlands, prospective parents can be counselled on the latter two options when, due to the severity of the kidney disease in the family, they might want to prevent the birth of an affected child.

Counselling on these options enables prospective parents to make a well-informed decision regarding reproduction. When invasive prenatal diagnostic is permitted, chorionic villi sampling can be performed at 11-14 weeks, or amniocentesis at 16 weeks of gestation.[15] If genetic testing shows that the fetus is affected, the prospective parents have the option to terminate the pregnancy to prevent the birth of a child affected with monogenic kidney diseases.

Here, we focus on preimplantation genetic testing, which entails genetic testing of one or two cells derived from a six to eight cell stage day 3 embryo or of 5-10 trophectoderm cells derived from a blastocyst stage embryo at day 5-6, after in-vitro fertilization with intracytoplasmic sperm injection.[16] Only embryos without the parental mutation(s) in the biopsied cell(s) are eligible for transfer into the uterus.[16]

Preimplantation genetic testing was first performed in the Netherlands in 1995 and has been part of reimbursed medical care since 2008 (up to three cycles). The Maastricht University Medical Center+ (MUMC+) is the only center in the Netherlands licensed to perform preimplantation genetic testing. In the Dutch "*PGT the Netherlands"* consortium, the MUMC+ collaborates with multidisciplinary in-vitro fertilization teams in three tertiary center fertility clinics. In all four centers women are treated; whereas the actual preimplantation genetic testing is performed in the laboratory of the MUMC+ (so-called 'transport preimplantation genetic testing').

To date, preimplantation genetic testing has been applied for over 500 conditions worldwide.[16,17] Over the past 10 to 15 years, indications have shifted from diseases with pediatric onset and/or severe phenotype to adult-onset diseases and conditions with reduced penetrance, such as specific forms of hereditary cancer.[18] Requests for

preimplantation genetic testing in not previously requested genetic disorders are reviewed based on general disease severity by a national multidisciplinary indication committee comprised of clinical geneticists, gynecologists, medical ethicists and patient representatives. Each individual couple seeking preimplantation genetic testing will also be reviewed by a separate committee that takes into account the disease severity, genotype-phenotype correlation and the technical possibilities of not passing on the specific variant. The Dutch system of nationwide regulation and reimbursement is unique, and it has allowed the Netherlands to be one of the first and few countries where couples have the option to opt for preimplantation genetic testing for monogenic kidney disease, including adult-onset forms of kidney disease.

On preimplantation genetic testing for monogenic kidney disease specifically, there are few reports in literature. To date, 12 papers on preimplantation genetic testing for monogenic kidney disease have been published (Supplemental Item 1), mostly describing individual cases or small case series, and focusing on severe and early-onset diseases.[19,20,29,30,21–28] In recent years some reports have been published on larger series for specific diseases, such as Berckmoes *et al.*'s paper on 43 couples that underwent preimplantation genetic testing for autosomal dominant polycystic kidney disease (ADPKD).[29] However there is limited information on uptake and success rates of preimplantation genetic testing for monogenic kidney disease in general.

In this paper we show the developments in preimplantation genetic testing for monogenic kidney disease in the *PGT the Netherlands* consortium over the past 25 years, reviewing the indications, uptake, pregnancy rates and parent-related factors. We illustrate advantages and pitfalls of preimplantation genetic testing for monogenic kidney disease and provide clinical recommendations for shared decision making with regard to preimplantation genetic testing for monogenic kidney disease.

9.2 Materials and Methods

Institutional review board approval was obtained in the MUMC+ to perform a retrospective cohort study, adhering to the declaration of Helsinki. The cohort consisted of couples that were counselled in the MUMC+ on preimplantation genetic testing for monogenic kidney disease in the period of 1 January 1995 to 1 June 2019. Monogenic kidney disease was defined as a disorder with kidney disease as the main feature.

For all enrolled couples, data on baseline characteristics, genetic diagnosis, preimplantation genetic testing parameters and if applicable reasons for declining preimplantation genetic testing were retrieved from the electronic patient files. Data were retrieved from the MUMC+ patient files only. Data on preimplantation genetic testing cycles could have only been included if a treatment cycle reached

the stage of oocyte retrieval. Data was collected up to 1 June 2019. We collected data on all the pregnancies the couples had after they were first counselled on preimplantation genetic testing.

Several outcome parameters were defined: 'ongoing pregnancy' was defined as a pregnancy >12 weeks of gestation and 'live birth' was defined as the birth of a child surviving >24 hours. Whether a child was affected or unaffected with the parental monogenic kidney disease was determined based on either clinical diagnosis and/or genetic testing. If no information was available on the disease status of the child, it was noted as 'unknown'. If age at onset is <18 years, the disease was defined as having a 'paediatric-onset', if age at onset is >18 years as 'adult-onset.' Age at onset of disease was defined according to the approximate age at onset of CKD mentioned in literature.[7]

Analysis of all data was performed with SPSS for Windows (version 25, IBM, USA). The χ^2 tests were performed two-sided and the probability of a type I error was set at 0.05. All data is represented cumulatively per couple unless otherwise indicated.

9.3 Results

The cohort consists of 98 couples, of whom the baseline characteristics are presented in Table 1. The median CKD stage was stage 1, with 88% of affected prospective parents being in early CKD (stage 1-3). Nine affected prospective parents had received a kidney transplant, of whom eight were fathers. The majority of couples had autosomal dominant or X-linked disease, with the prospective mother more often being the affected parent than the father (detailed in Table 1).

Initially, preimplantation genetic testing referrals for monogenic kidney disease were incidental and indications predominantly concerned pediatric-onset kidney disease (Figure 1). The first couple with adult-onset disease (ADPKD) was referred in 2004. From 2009 onwards, the number of referrals for adult-onset disease steadily increased and became the most frequent referral indication. ADPKD was the most frequent reason for referral (38%).

Of the 98 couples who were counselled in the MUMC+, 52 (53%) chose to proceed with preimplantation genetic testing, 43 (44% of total cohort) had undergone at least one cycle that reached the oocyte retrieval stage by 1 June 2019 and 9 (9% of total cohort) were waiting for the validation of the single cell genetic test for their specific mutation (Figure 2 and Table 3). In those 43 couples, a total of 79 preimplantation genetic testing cycles reaching at least the stage of oocyte retrieval were performed, with a median two cycles (interquartile range [IQR] 1) per couple. Cumulative results per couple show a median of 10 embryos (IQR 14) available for

biopsy and a median of 3 embryos (range 1-14) genetically unaffected. Sixty-five percent (n=28) of couples who had at least one cycle had at least one live birth through preimplantation genetic testing, with four couples having had more than one unaffected live birth through preimplantation genetic testing at the time of analysis. For those who became pregnant after preimplantation genetic testing, the median duration from counselling to the first live birth was two years (IQR 1).

There were five couples who had a spontaneous pregnancy after successful (n=2) or unsuccessful preimplantation genetic testing procedures (n=3).

Thirty-seven couples (38%) chose not to proceed with preimplantation genetic testing after counselling, for various reasons, listed in Table 4. There were also 9 couples (9%) who had not yet decided whether to proceed as of 1 June 2019 (Table 3). In couples not choosing preimplantation genetic testing, the main reasons were that (prospective) parents preferred conceiving spontaneously and performing invasive prenatal diagnostics (14%) or they did not want to wait for the time-consuming preimplantation genetic testing procedure and decided to accept the risk of having an affected child (11%). Five couples (14%) had a spontaneous pregnancy between the first and second counselling session. Not proceeding with preimplantation genetic testing was not independently influenced by maternal age (p=0.13). Nor by kidney disease-related variables such as the CKD stage of the affected prospective (p=0.37) or the indication being adult-onset disease (p=0.11), nor inheritance-related factors like disease inheritance pattern (p=0.64), having an affected previous child (p=0.41

Table 9.1 | Baseline characteristics.

Characteristic	**Total couples (n=98)**
Maternal characteristics	
Maternal age at first counselling in years (median, range)	32 (22-40)
Nulliparity (n, %)	63 (64%)
Kidney disease characteristics	
Affected prospective parent CKD stage at counselling (median, range)	1 (1-5)
Affected prospective parent CKD stage >3 (n, %)	12 (12%)
Affected prospective parent post-transplantation (n, %)	9 (9%)
Genetic characteristics	
Autosomal dominant disease	53 (54%)
Autosomal recessive disease	18 (18%)
X-linked disease	27 (28%)
In case of autosomal dominant or X-linked disease	*n of total cohort*
Genetically affected parent is the father	33 (34%)
Genetically affected parent is the mother	47 (48%)

CKD=chronic kidney disease

Table 9.2 | Referral indications on preimplantation genetic testing for monogenic kidney disease.

Disease	n (%)	Gene (n)
Autosomal dominant inheritance		
ADPKD*	37 (38%)	*PKD1* (35), *PKD2* (2)
Brachio-oto-renal syndrome	5 (5%)	*EYA1* (5)
INF2-related FSGS*	3 (3%)	*INF2* (3)
Nail-patella syndrome*	3 (3%)	*LMX1B* (3)
PAX2-related disease	2 (2%)	*PAX2* (2)
aHUS*	1 (1%)	*CFH* (1)
ADTKD-*HNF1B**	1 (1%)	*HNF1B* (1)
Autosomal recessive inheritance		
ARPKD	9 (9%)	*PKHD1* (9)
Joubert syndrome	7 (7%)	*CEP290* (5), *TCTN3* (1), *C5ORF42* (1)
Bardet-Biedl syndrome	1 (1%)	*BBS7* (1)
Cystinosis	1 (1%)	*CTNS* (1)
Nephrotic syndrome	1 (1%)	*COQ2* (1)
X-linked inheritance		
Alport syndrome	25 (26%)	*COL4A5* (25)
Nephrogenic diabetes inspidus	2 (2%)	*AVPR2* (2)

ADPKD=autosomal dominant polycystic kidney disease, ADTKD=autosomal dominant tubulointerstitial kidney disease, ARPKD=autosomal recessive polycystic kidney disease, FSGS=focal segmental glomerulosclerosis, *=adult-onset disease

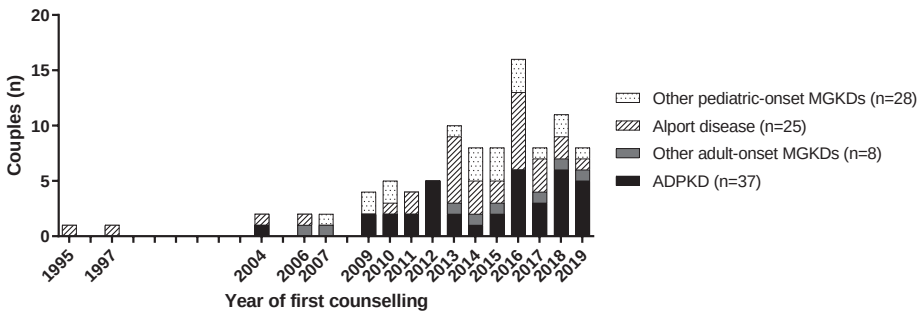


Figure 9.1 | The number of couples (n) counselled on preimplantation genetic testing for monogenic kidney disease in the Netherlands has steadily increased since the first referral in 1995, Including for adult-onset diseases.
ADPKD=autosomal dominant polycystic kidney disease, ARPKD=autosomal recessive polycystic kidney disease, PGT=preimplantation genetic testing, MGKD=monogenic kidney disease

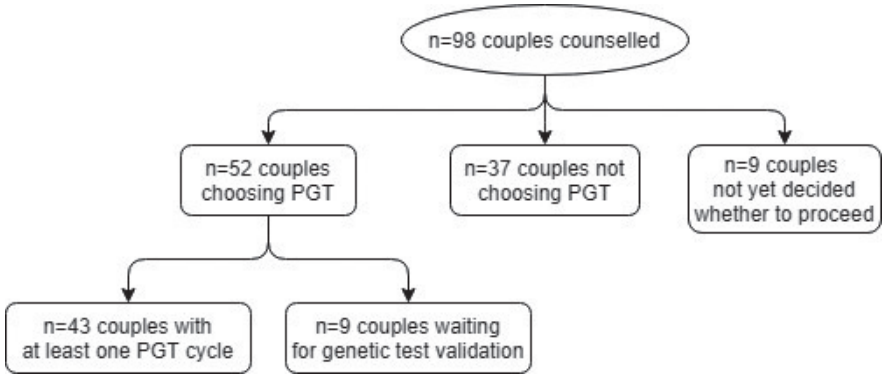


Figure 9.2 | Flowchart of the n=98 couples that were counselled on pre-implantation genetic testing for monogenic kidney disease.
PGT=pre-implantation genetic testing

9.4 Discussion

Over the past decade, preimplantation genetic testing is increasingly performed in the Netherlands for couples to prevent passing monogenic kidney disease on to their offspring. There is a strong increase in preimplantation genetic testing referrals for monogenic kidney disease since 2009. This can on the one hand be explained by increasing technical possibilities in preimplantation genetic testing, but also by an increase in referrals for adult-onset monogenic kidney disease, which reflects an increase in preimplantation genetic testing referrals for adult-onset disease in general.[31] This underscores the potential influence of disease burden, whether that be pediatric or adult-onset, on the choice for preimplantation genetic testing.[28] The importance of disease burden was recognized by the national committee of clinical geneticists, gynecologists, medical ethicists and patient representatives that decided on the allowed preimplantation genetic testing indications.[35] Through consensus discussion this committee moved to allow adult-onset disease indications in the early 2000's, incorporating them in the 3 cycle insurance reimbursement scheme, thereby offering couples with adult-onset disease the option of preimplantation genetic testing.[35] As the number of kidney diseases for which a monogenic cause can be identified continues to grow, it becomes increasingly important to counsel patients timely on their reproductive options in order to enable them to make an informed choice.[10]

In general, reasons to proceed with preimplantation genetic testing depend on the nature and the severity of the condition, the onset of symptoms and the affected

Table 9.3 | Preimplantation genetic testing and pregnancy outcomes.

	n (%)	Median per couple of cumulative cycles (range)
*Couples with at least one PGT cycle**	*43 (44%)*	
Cycles with oocyte retrieval	79	2 (1-4)
Embryos for biopsy	537	10 (2-31)
Of which embryos genetically unaffected and suitable for transfer‡	190 (35%)	3 (1-14)
Couples with one or more pregnancy going >12 weeks GA	31 (72%)	1 (0-2)
Couples with 1 or more unaffected live births	28 (65%)	N/A
Couples with 1 unaffected live birth	24 (56%)	N/A
Couples with >1 unaffected live birth	4 (9%)	N/A
Duration from referral to first live birth in years	N/A	2 (1-6)
Couples not proceeding with PGT	*37 (38%)*	
Non-PGT pregnancies going >12 weeks GA	19 (49%)	N/A
Terminations of pregnancy after diagnosis of affected fetus (either with fetal ultrasound or PND)	3 (8%)	N/A
Live births**	15 (41%)	N/A
Of which known to be affected	4 (27%)	N/A
Of which not affected	4 (27%)	
Of which affected status unknown***	7 (47%)	
Couples waiting for validation of the single cell genetic test on 1 June 2019	*9 (9%)*	**N/A**
Couples not yet decided 1 June 2019	*9 (9%)*	**N/A**

N/A=not applicable, PGT=preimplantation genetic testing, *including couples not yet finished with the maximum of 3 reimbursed cycles on 1 June 2019, **one out of 19 ongoing pregnancies resulted in a mid-trimester loss, ***not all have received genetic testing due to patient being a minor, ‡=in 2-3% embryo quality after thawing was too poor to perform the genetic test, thus were not suitable for testing and transfer

Table 9.4 | Patient reported reasons for declining preimplantation genetic testing after counselling.

	n (%)
Choice by couple	*20 (54%)*
Declined without giving a specific reason	9 (24%)
Opt for spontaneous pregnancy with PND	5 (14%)
Couple does not want to wait for PGT	4 (11%)
Couple deem parental health too poor	2 (5%)
Technical reason	*9 (24%)*
Maternal age >42 years at estimated time of start of IVF/PGT	4 (11%)
Single cell genetic test not possible	2 (5%)
Specific indication is not allowed yet	2 (5%)
Ovarian reserve does not support IVF	1 (3%)
Other	*8 (22%)*
Spontaneous pregnancy prior to first PGT cycle	5 (14%)
Couple separated	3 (8%)

IVF=in-vitro fertilization, PGT=preimplantation genetic testing, PND=invasive prenatal genetic diagnostics

status of parents and current children.[32] Other factors in choosing preimplantation genetic testing include the wish to avoid suffering for offspring and feelings of guilt related to passing on the disease to future generations.[33,34] Although reasons for choosing preimplantation genetic testing were not systematically recorded for our cohort, the relevance of the consideration to avoid the disorder in offspring is illustrated by the decision of three couples to terminate a pregnancy after prenatal testing had shown that the child was affected.

The 65% of couples that had at least one live birth through preimplantation genetic testing in our cohort is high. The *PGT the Netherlands* consortium reports that 32% of all started preimplantation genetic testing cycles resulted in a live birth (cumulative data since 1995).[35] However, a couple may start more than one cycle, so these data are difficult to compare as per couple pregnancy rates are not available for the total Dutch preimplantation genetic testing population.[31]

One should note that since we only include data from the MUMC+ pre-implantation genetic testing procedure, we cannot report on maternal factors that influence IVF success rates such as ovarian reserve, documented by the local multidisciplinary fertility teams.[36] Additionally this meant that we could only report on cycles with oocyte retrieval, meaning that there are possibly some cycles without oocyte retrieval that we could not take into account in our analysis, thus overestimating the live birth rate. Most importantly, half of the couples proceeded with preimplantation genetic testing, which could have created another sample bias. Though the 65% rate of at least one live birth per couple reported in this study is hopeful for couples seeking preimplantation genetic testing for monogenic kidney disease, this number should be used in counselling with caution because of the small sample size and possible biases.

Thirty-eight percent of referred couples did not proceed with preimplantation genetic testing, which is a higher percentage than the previously reported 17% in a Belgian cohort of 65 ADPKD cases.[29] This difference is likely due to our earlier moment of inclusion, namely at their first referral for counselling in the MUMC+, as the number of couples that opted out is similar to the nationwide *PGT the Netherlands* data.[35] We underestimate the overall decline-rate, because we could not collect information on patients that were counselled by their local geneticist or nephrologist and chose not to be referred for more extensive preimplantation genetic testing counselling in the MUMC+.

In our cohort the main patient reported reasons for declining preimplantation genetic testing was the time investment and perceived relatively low chance of a pregnancy going >12 weeks GA after preimplantation genetic testing, which is consistent with literature.[28,29,37]

Interestingly, in our cohort, time from the moment of first counselling to delivery of the first child was similar for preimplantation genetic testing and non-preimplantation genetic testing couples. The relatively healthy state of our cohort (88% in early CKD) could also have influenced decision making. The impact of index patient disease state is illustrated by a couple that did not choose preimplantation genetic testing or invasive prenatal diagnostics for their first pregnancy. Soon after having their first child, the index patient underwent a kidney transplant. This shifted their perspective on disease severity and burden in such a way that the couple subsequently decided that they did not want to pass on this disease to their further offspring and opted for preimplantation genetic testing for the next pregnancy. A case like this underscores the importance of periodic counselling on preimplantation genetic testing of patients in the reproductive age.

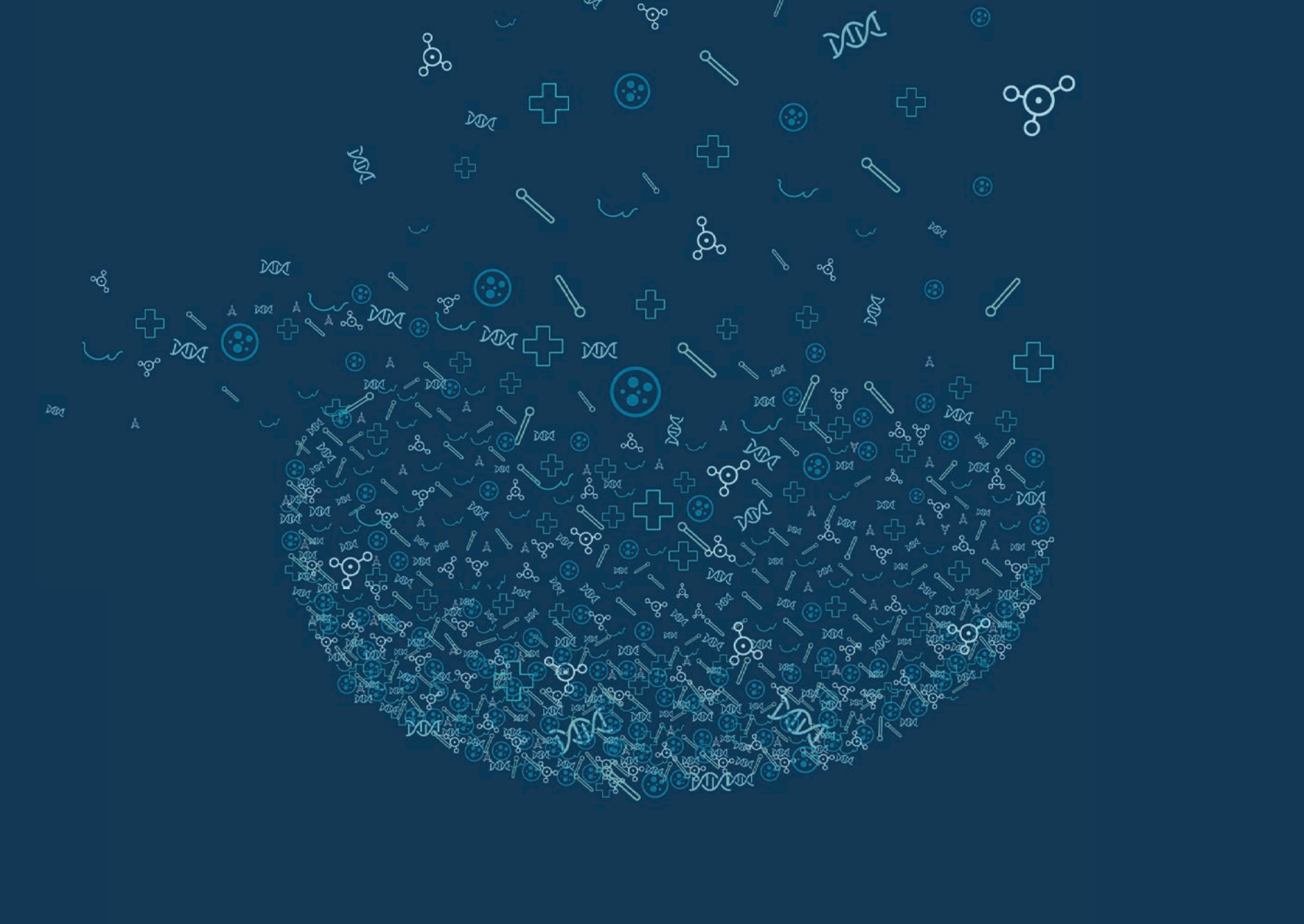
Counselling monogenic kidney disease patients on preimplantation genetic testing, its waiting time and success rates, should also include information on the technical limitations to preimplantation genetic testing. For example, preimplantation genetic testing is only an option if the disease causing mutation in the family can be identified and a single-cell genetic test can be developed.[38] Additionally, in some ADPKD families hypomorphic alleles or modifier variants complicate genetic counselling and preimplantation genetic testing because penetrance may be variable.[39–41] Finally, prospective parents are counselled on the maternal health risks related to the IVF/ICSI needed for preimplantation genetic testing, e.g. ovarian hyperstimulation syndrome and post-retrieval bleeding or infection, and risks related to pregnancy in CKD patients in general, which rise with advancement in CKD stage.[14,28,42,43]

We recommend that all prospective parents from monogenic kidney disease families are counselled on reproductive options, including preimplantation genetic testing, as a part of standard care .[44] If the couples expresses interest in preimplantation genetic testing or invasive prenatal diagnostics or asks for more in depth reproductive counselling, they should be referred to a specialised genetic counselling unit. In a study among 96 ADPKD patients in the United Kingdom, 63% of patients with kidney failure reported that they would have considered preimplantation genetic testing, and 18% would consider invasive prenatal diagnostics and termination of pregnancy.[45] In addition, 68% of patients thought preimplantation genetic testing should be offered to ADPKD patients, regardless of whether they would consider this option for themselves.[45] The fact that the majority of affected parents in our cohort had CKD stage 1, underscores the notion that patients are interested in preimplantation genetic testing regardless of their disease stage.[10]

Our recommendation is in line with the KDIGO consensus report on ADPKD stating that preimplantation genetic testing should be part of reproductive counselling of ADPKD patients as 'these decisions are for the patients and/or parents to make', although access to this technology varies across countries.[10,46] However, a study among clinicians revealed that 93% of clinical geneticists inform ADPKD patients about the option of preimplantation genetic testing, while only 41% of nephrologists and 23% of pediatric nephrologists discuss preimplantation genetic testing.[44] Increased awareness of preimplantation genetic testing for monogenic kidney disease among (pediatric) nephrologists is required, for example through checklists for patients and guidelines for doctors that include discussing genetic testing and family planning, to standardize care for monogenic kidney disease patients and families.[46]

In conclusion, we provide the first extensive overview of preimplantation genetic testing referrals for monogenic kidney disease. Our analysis includes monogenic kidney disease indications, considerations of prospective parents, and the uptake and results of the procedure. Since 2009, there has been an increase in referrals for monogenic kidney disease in particular for adult-onset conditions. The percentage of unaffected live born children resulting from preimplantation genetic testing in monogenic kidney disease is high in our cohort, likely due to sample bias. Still, the uptake of 53% could indicate that decisions regarding preimplantation genetic testing are complex for prospective parents. Our data can aid in counselling prospective parents from families with monogenic kidney disease on the option of preimplantation genetic testing. This enables couples to make informed decisions in line with their personal, cultural and moral background and beliefs.

9



10

General discussion: The case for a ‘genetics first’ approach in chronic kidney disease

This thesis presents the case for a 'genetics first' approach in chronic kidney disease (CKD), where genetic testing is integral part of the primary diagnostic toolbox.[356] The work in this book showcases the feasibility of genetic testing and the clinical impact a monogenic kidney disease (MGKD) diagnosis has on patient care. This value of an adequate MGKD diagnosis is evident in all phases of a patient's life, through the fetal stage and childhood, to adulthood and eventually conception and gestation of the next generation.

In this chapter, I discuss the results of the previous chapters, the life-long implications of an adequate diagnosis, and a vision on the transitioning field of renal genetics.

10.1 Need for identification: high prevalence, but also underdiagnosis of MGKD

Until recently, MGKDs were mainly thought to be causal in patients with very early-onset (prior to 25 years of age), unusually severe disease and familial disease.[17,21,28] Nevertheless, we and other authors have shown that MGKDs are also more prevalent in adult CKD than previously assumed.[26,27,92–95,102]

In **Chapter 6,** we showed that the classic pediatric disease nephronophthisis is a major contributor to end-stage renal disease (ESRD) at an adult age.[102] Age at onset of ESRD ranged up to 61 years, more than 30 years older than ever recorded for nephronophthisis.[102] Interestingly, almost all of the patients we identified had not been clinically diagnosed as having nephronophthisis.[102] It should be noted that ESRD itself is quite a severe phenotype, therefore these results cannot be translated one-on-one to general CKD patients of the same age.[132] Nevertheless, the fact that 1 in 200 transplant patients were clinically not recognized as having just one specific MGKD, underscores that there is severe underdiagnosis of MGKD, especially in the adult-onset renal disease population.[132]

The notion of MGKD underdiagnosis, in both children and adults, is in line with earlier studies. Depending on the extent of the genetic test(s) used and the group of patients selected for a study, diagnostic yields vary from 10-73%.[26,27,92–99] Table 10.1 displays an overview of the recent studies on diagnostic yield in the general CKD population, ciliopathy, CAKUT and SNRS patients. [26,27,92–99] Table 10.1 illustrates that in most studies investigating diagnostic MGKD yield, some sort of population selection is performed, e.g. on a specific suspected MGKD phenotype or familial disease.[26,27,92–99] Remarkably, in one in five patients the clinical diagnosis was reclassified after genetic testing.[26,27,92–99] The gain of knowledge regarding MGKD prevalence and the high reclassification percentage warrants a change of

perspective on the value of genetic testing in the renal disease population.

Currently, often only high risk patients are offered genetic testing.[26,27,92–94] We showcased the importance of genetic testing in patients with a higher likelihood of genetic disease in **Chapter 3**.[101] In correspondence with the current regular diagnostic trajectory, these focal segmental glomerulosclerosis (FSGS) patients were only referred to our tertiary expert center after most regular nephrological diagnostic tools, such as invasive renal biopsies, were exhausted.[101] We adequately diagnosed all three with a MGKD with clear benefits of the genetic diagnosis on decisions related to treatment and/or family planning.[101] Had these patients been genetically diagnosed earlier, the benefit would definitely have been even greater because they would not have received corticosteroid treatment as their physician would have known their genetic disease would be steroid resistant.[56]

10.2 Transition to a genetics first approach

The new knowledge on the high MGKD prevalence in renal disease patients and the fact that genetic testing often leads to a reclassification of the primary clinical diagnosis, indicate that genetic testing deserves a more prominent place in the diagnostic trajectory of these patients. Such a 'genetics first' approach, has been a topic of discussion for many years in the rare disease field.

To assess the diagnostic yield of a genetics first approach in ESRD patients, we performed a large WES-based multi-genepanel (379 known renal disease genes) in 113 transplant recipients (**Chapter 2**). To best reflect the ESRD patients for whom a genetics first approach would be applied for in daily practice, we only excluded patients with a clear non-genetic diagnosis (such as HIV-related CKD) or who were older than 50 years at their first renal transplant. This showed a diagnostic yield of 52%, underscoring the relevance of applying a genetics first approach in the wider CKD population.

When discussing a new diagnostic approach, diagnostic yield is not the only factor to consider. We should certainly also study the impact of a MGKD diagnosis on patient care and well-being, to unravel a potential life-long added value of an adequate MGKD diagnosis.

10.3 Life-long value of an MGKD diagnosis

Adequately diagnosing a patient with a MGKD may have a life-long value for that patient (Figure 10.1), from impacting prognostication and decisions on treatment to enabling informed choices around family planning.

Table 10.1 | Overview of recent studies investigating diagnostic yield of NGS in renal disease.

	Number of patients sequenced (n)	Familial disease (n,%)	Consanguinity (n, %)	ESRD (n, %)	Adult-onset disease (n, %)	Renal phenotype	Number of genes sequenced (n)	Diagnostic yield (n, %)
Connaughton *et al.*, 2019[26]	114 families	102 (78%)	NR	90 (79%)	117 (69%)	Familial CKD and/or extra-renal features	WES	42 (37%)
Groopman *et al.*, 2019[97]	Subcohort 1: 1128 individuals Subcohort 2: 2187 individuals	Subcohort 1: NR Subcohort 2: 619 (28% of subcohort)	NR	Subcohort 1: 1128 (100%) Subcohort 2: NR	NR for both subcohorts	Subcohort 1: ESRD at age 50-80 Subcohort 2: "genetic CKD"	625	307 (9%)
Mann *et al.*, 2019[92]	104 individuals	23 (22%)	9 (9%)	104 (100%)	0 (0%)	Pediatric transplant recipients with mostly CAKUT, SNRS and ciliopathy	396	34 (33%)
Ottlewski *et al.*, 2019[96]	50 individuals	NR	NR	50 (100%)	NR, age at first RRT mean 43 years (range 15-67 years)	Transplant waitlist patients with "Undetermined ESRD" defined as no renal histology or unspecific histology	209	6 (12%)
Bullich *et al.*, 2018[95]	305 individuals	NR	NR	NR	155 (51%)	Suspected cystic and glomerular MKD	140	222 (73%)

Table 10.1 Continued.

	Number of patients sequenced (n)	Familial disease (n,%)	Consanguinity (n, %)	ESRD (n, %)	Adult-onset disease (n, %)	Renal phenotype	Number of genes sequenced (n)	Diagnostic yield (n, %)
Lata *et al.,* 2018[94]	92 individuals	53 (58%)	NR	NR	NR, age at presentation mean 42 years (SD 17 years)	CKD of unknown cause or familial hypertension/ nephropathy	WES	22 (24%)
Van de Ven *et al.,* 2018[27]	232 families	NR	93 (40%) consanguineous	NR	0 (0%)	CAKUT, of which n=62 (27%) severe	WES	31 (14%)
Warejko *et al.,* 2018[93]	300 families	93 (31%)	146 (49%) consanguineous	NR	8 (3%)	SNRS	WES	85 (29%)
Mallett *et al.,* 2017[98]	135 families	NR	NR	NR	NR, median age at referral 19 years (range 0-71 years)	Likely genetic CKD	WES-derived phenotype based genepanels	59 (42%)
Braun *et al.,* 2016[99]	79 families	19 (24%)	60 (76%)	NR	0 (0%)	Clinical diagnosis of pediatric onset NPHP, no *NPHP1* gene deletions	WES	50 (63%)

CAKUT=congenital anomalies of the kidney and urinary tract, CKD=chronic kidney disease, ESRD=end-stage renal disease, MKD=monogenic kidney disease, NGS=next-generation sequencing, NPHP=nephronophthisis, RRT=renal replacement therapy (dialysis or renal transplantation), SD=standard deviation, SNRS=steroid resistant nephrotic syndrome, WES=whole exome sequencing

10.3.1 Non-invasive diagnostics allow for clear prognostication

Genetic testing is relatively non-invasive compared to e.g. a renal biopsy, since DNA is isolated from peripheral blood.[357] A genetics first approach would obviate the need for renal biopsies for specific patients, not exposing patients to the complications of this invasive procedure.[70] **Chapter 2** showed that the genetic test diagnosis was as good as, and in many cases more adequate, than the histological diagnosis. Especially in cases with advanced CKD, where histology is of limited value due to general tissue damage masking disease markers, genetic testing as soon as the patient presents has a clear added benefit.[14,358]

An adequate MGKD diagnosis allows for patient-specific prognostication. First, in patients in whom a likely MGKD is found through conventional diagnostics, e.g. an Alport syndrome patient diagnosed through observing a thin glomerular basement membrane (GBM) on renal biopsy.[191] For those patients, finding the specific genetic cause means a tailored prognosis, as disease progression differs depending on the gene that is mutated and the type of mutation.[138,332,359]

A genetics first approach also allows for so-called 'reverse phenotyping', where the MGKD diagnosis allows for the identification and treatment of subclinical phenotypes that one would not have been aware of without the genetic testing result.[360] An example of this is a patient with mental retardation and obesity in which a variant is found in one of the Bardet-Biedl genes, prompting the physician to look for associated renal function decline and thereby allowing for better prognostication.[361]

10.3.2 Possibility of tailored treatment

A MGKD diagnosis means that patients become eligible for certain treatments they may have not received without the diagnosis, e.g. ACE-inhibition in *COL4A3-5* related disease.[135] It can also prevent the prescription of ineffective therapies, such as steroids in genetic nephrotic syndrome and genetic FSGS.[56]

In the future, 'gene therapy' will hopefully also become an option in MGKD, making the impact of an adequate MGKD on treatment even larger. Gene therapy encompasses methods to introduce an exogeneous gene, change the endogenous DNA sequence or silence the expression of a particular endogenous gene.[362] One method to affect very specific gene editing is with the CRISPR-Cas9 method (**Chapter 1**), which offers many possibilities but has also been surrounded by ethical questions related to extensive gene editing.[363] Gene therapy for renal disease is currently focused on slowing down general disease processes such as fibrosis, e.g. by targeting mesenchymal cells with genetic constructs.[364] The main hurdles for renal gene therapy remain the variety of cell-types present in the kidney, that often are not easily transduced by regular viral vectors and the fact that most

vectors are larger than the afferent arterioles in the nephron, making it difficult to get the drug to the right cell-type.[365,366]

Therefore, it is likely the largest strides can be made in targeted gene therapies. A recent example of such targeted therapy is an exon skipping treatment (lowering the expression of *Cep290*), which was successful in a Joubert syndrome murine model.[367] Currently, there are targeted gene therapies being brought to market for a variety of genetic diseases, which probably lies ahead for MGKD as well.[368]

10.3.3 Implications for (living related) renal transplantation

When a CKD patient needs a renal transplant, it is relevant to know if there is a genetic cause for two reasons. First, because it offers information on the chance of graft failure, for example there is a ~5% chance of disease recurrence in a graft in hereditary nephrotic syndrome, contrary to non-hereditary nephrotic syndrome where the recurrence risk is ~26%. [369] This allows for better prognostication and in case of graft function deterioration.

Second, due to long transplant waiting lists, more and more patients receive a kidney from a living related donor.[370] In MGKD, family donation should be preceded by extensive screening of the possible donor, including genetic testing if applicable (e.g. in diseases with a variable age or severity of presentation like we described in **Chapter 6**).[102,139] Special attention should be payed to X-linked diseases, such as *COL4A5*-related disease, where female family members may seem not to be affected based on general laboratory testing but still can have the disease-causing mutation and may develop a phenotype later on.[96,332] Adequate screening of transplant receivers, and by extension also the donors, would improve the likelihood of a successful transplant and decrease the risk of future renal dysfunction in the donating family member.

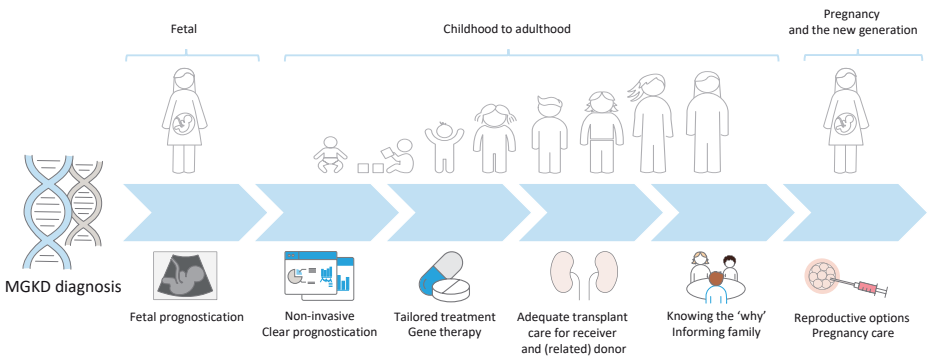


Figure 10.1 | A monogenic kidney disease (MGKD) diagnosis impacts care and counselling throughout a patient's life.
Icons used in this figure were developed by the UMC Utrecht, copyright for those images lies with the UMC Utrecht. Icons used with permission.

10.3.4 The importance of knowing the 'why'

For many patients the answer to 'why' they have developed a kidney disease is very important.[371,372] Though it can make their life more complicated in some aspects (e.g. obtaining insurance), knowing the exact etiology of their disease generally has a large positive impact on patients' lives.[371,372] . For instance, it allows patients to explain their disease to others and that it improves perceived access to appropriate health care.[371,372]

10.3.5 MGKD diagnosis allows for informing family members

Knowing that a disease is heritable, also means that family members that might be affected can be counselled on their likelihood of developing renal disease.[373] They would after all also be subject to the clinical implications of a MGKD diagnosis if they were affected. Informing family members involves allowing them the 'right to *not* know', but also offering them pre-symptomatic genetic testing if they wish.[373,374] Special care should be given to children, who in principle do not receive pre-symptomatic genetic testing for adult-onset disease until they are of legal age to make their own medical decisions, unless pediatric genetic testing allows for early treatment or preventative measures.[101]

10.3.6 MGKD diagnosis offers information useful for family planning

Having a genetic disease can complicate decisions surrounding family planning, as the disease may be passed on to the future child. In MGKD, this is further complicated by the fact that pregnancy may also bring along a high maternal and/or fetal risk in women with renal disease. In this thesis, I have addressed the three important items in family planning for MGKD patients. First, the need for appropriate counselling (**Chapter 7**), second pregnancy outcomes in a specific MGKD (Alport syndrome, **Chapter 8**) and third the option for patients with a genetic disease to have pre-implantation genetic testing performed (**Chapter 9**)

Deciding on a pursuing a pregnancy in CKD can be complex, due to the possibility of adverse maternal and fetal outcomes, deterioration of maternal kidney function, changes in renal medication and decisions around genetic testing.[82] This is especially the case in advanced CKD and dialysis when the likelihood of complications is significantly increased.[84,301,375] In **Chapter 7** we explored the clinical practice considerations that come with counselling patients on pregnancy in advanced CKD.[324] We found that counselling in a multidisciplinary setting, including a nephrologists, obstetrician and clinical geneticist, ensures that all aspects of reproduction in CKD are well discussed in the counselling session. Because patients *want* to be informed about reproductive options, even when those choices may be difficult.[210] Having CKD patients make their own decisions regarding a pregnancy in

CKD conforms with the larger transition to allowing patients with chronic diseases to choose whether they want to pursue a high risk pregnancy.[376]

The data on pregnancy outcomes is based on small cohorts consisting of CKD patients with heterogeneous underlying causes. **Chapter 8** presents the intermediary analysis of a large cohort of patients with Alport syndrome, one of the more frequent MGKDs. In the largest cohort to date, we show that pregnancy outcomes are better than in pregnancies of patients with a similar CKD stage with CKD due to any cause. This underscores the need for research in specific renal diseases, to have the data to adequately counsel patients with those diseases. We are currently expanding the dataset to include more than 200 Alport pregnancies that we can compare to detailed data of non-Alport pregnancies.

One of the reproductive options patients want to be informed on is pre-implantation genetic testing (PGT).[210] PGT involves single cell genetic testing on an in-vitro fertilized embryo, reducing the chance of passing on that genetic disease to ~1%.[377] In **Chapter 9** we present the Dutch evolution of providing PGT for MGKD. PGT was performed mainly for early-onset severe diseases, though more recently the indications have been expanding to adult-onset diseases such as ADPKD. This expansion fits within the world-wide development of extending the PGT regulations, including steps toward a government funded system (which the Netherlands has had for 25 years) and towards regulations that include PGT for adult-onset and non-syndromal conditions.[378–381] The PGT field is also on the forefront of concisely debating the ethical issues that arise with these types of reproductive techniques, shaping critical thinking on related topics such as genome editing.[378–381]

10.4 The genetic test: the opportunities and dissipating barriers

As with any diagnostic test, there are benefits and pitfalls one should consider when applying genetic tests as a first-tier diagnostic approach. Below, I will discuss how to select the appropriate genetic test, how to counsel a patient adequately and how, thanks to the advances in genetic testing, there are little financial or time-related barriers for applying a genetics first approach in CKD (Figure 10.2).

10.4.1 Opportunities of broad testing and the case for a tiered approach

With broad testing, such as WES, it is less likely that one will miss a causal variant. Another benefit is that WES-testing may yield variants in genes not yet associated with disease, expanding the knowledge on genetic heterogeneity in kidney disease.[28,29,382] This is reflected in the studies in Table 10.1, which report candidate variant yields ranging up to 27%.[27,93,94,99]

These candidate variants start out as a variant of unknown significance (VUS) or a likely pathogenic variant in a gene of unknown significance (GUS).[383] The relation of a VUS or GUS to the patient's phenotype may be investigated with *in vitro* or *in vivo* experiments.[40,48] We performed functional experiments to study causality between variants found by WES in *YRDC* and *GON7* and Galloway-Mowat syndrome in **Chapter 5**. Using *in vitro* experiments and chemistry analyses we showed that YRDC and GON7 are essential for modification of transfer RNA and that the mutations in our patients cause aberrant function of those proteins.[384] The discovery of this causal relation allowed our patients to opt for PGT when they wanted to have another child.

Dependent on the patients' phenotype, various types of functional studies can be performed.[383] As described in **Chapter 1**, this can range from measuring cell growth *in vitro* to studying genetically modified zebrafish embryos, incorporating a knockout or knock-in of the variant of interest, with use of the CRISPR-Cas9 system.[40,48] Recently, the field of organoids, *in vitro* 'mini-organs', has taken flight.[385,386] Patient-derived organoids allow for disease characterization and drug screening in a more complex and organ-resembling system than two dimensional cell cultures.[387] Organoids have even been successfully applied to test drugs for cystic fibrosis.[387] For a complex organ like the kidney, it has proven challenging to create organoids.[388] Researchers from our institute have nevertheless succeeded in culturing miniature renal tubules from patient urine, the so-called 'tubuloids', which can be used for testing possible treatments for a specific patient.[389] Ideally these techniques could be used for assessing phenotype causality of GUSses, but also VUSses in a known gene, in a routine diagnostic fashion.

Broad genetic testing may also yield findings in genes not specifically related to the phenotype the test was initially performed for. An example of such an incidental finding is the identification of a pathogenic variant in a breast cancer-related gene in a patient being tested to find a possible genetic cause for CKD.[39] As everyone is estimated to have ~50-100 genetic variants associated with human disease, when performing WES there is a ~1% chance of incidental findings.[142,143,390]

In my view a tiered WES-based diagnostic approach is most applicable in daily practice.[26,27,92–94] This is underscored by our findings in **Chapter 2**, where we showed that the diagnostic yield of using a WES-derived multi-genepanel (379 genes) in ESRD patients was high with 53%. Others have found diagnostic yields from 12% in unexplained ESRD up to 73% in specific phenotypes.[95,121,122] WES-based genepanels allow one to start with a targeted gene panel in order to limit the risk of incidental findings. When no variants are identified, it is relatively easy to 'open up' the WES backbone data and look beyond the known disease genes, maximizing the opportunity for finding the causal variant and new candidate variants.[144]

10.4.2 Financial and time-related barriers are dissipating

The technical developments have caused NGS testing to become cheaper and faster, though this naturally is influenced by locally available resources.[34] Costs for sequencing (excluding bio-informatic analysis) have significantly lowered; WES can be as cheap as 382 pounds (~400 euro) and WGS costs around 1312 pounds (~1600 euro).[391] To compare, a single WES is about 2 times less expensive than performing a single ultrasound-guided renal biopsy.[392]

Assessing cost-effectiveness of genetic testing is notoriously difficult, as estimating the costs saved by preventing other diagnostics or ineffective treatment is a challenge.[391] No research on this topic has been performed for the renal disease population specifically, albeit that studies in our center showed that WES is very cost-effective as a first-tier diagnostic test in intellectual disability patients.[393,394]

In analyzing cost-effectiveness, one has to also take time-related costs into account. In my view, the time-related financial costs may be another benefit of a tiered system. Although difficult to objectify, it likely saves costs since WES-based gene panel analysis time is shorter than analysis of the entire whole exome data. In our center, assessing a smaller WES-based genepanel takes a trained genetic labspecialist around 5 minutes, but delving deep into variants found through WES can take up to a day.[395]

Through a rapid diagnostics scheme, we were actually able to find the causal variant with WES in our Galloway-Mowat family (**Chapter 5**) within 7 days. This included sample collection, laboratory preparations, actual sequencing and bio-informatic analysis. Others have also reported quite fast turn-around times, namely 40 days from obtaining the sample to a clinical report of variants identified by WES.[396]

These data indicate that costs and turn-around time are no hurdle anymore (in high income countries at least) for the clinical application of a genetics first approach in the majority of patients.

10.4.3 The importance of appropriate pre- and post-test counselling

Appropriate counselling on the opportunities, limitations and possible results of genetic testing, is an integral part of providing the patient with adequate care. The type of professional that provides the genetic counselling depends on the setting, but should always be somebody with experience in the implications of genetic testing.

We wrote a clinical practice recommendation (**Appendix 1**, in Dutch) to provide Dutch physicians with tools to adequately counsel patients, select the right test, communicate the results and know when to consult an expert. Multidisciplinary care is central to ensure that patients receive appropriate counselling allowing them to make an informed decision on whether or not to undergo genetic testing and to understand the results of the test.

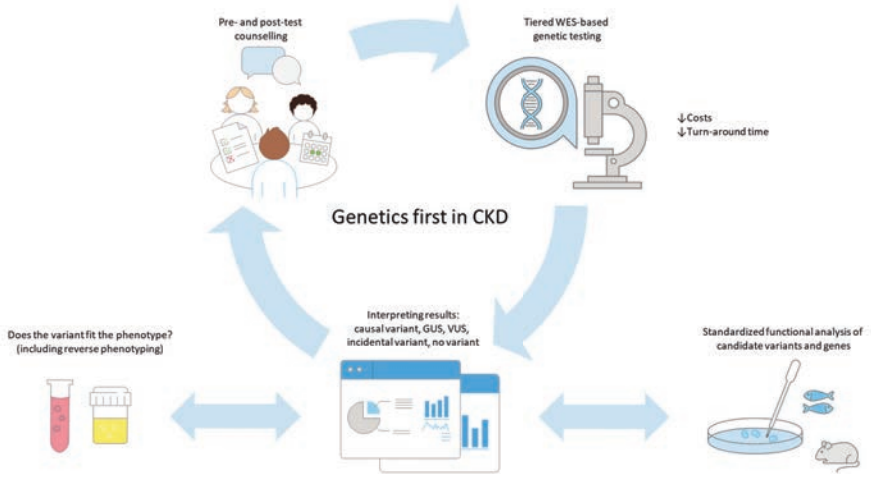


Figure 10.2 | The logistics of a genetics first approach in chronic kidney disease (CKD). Icons used in this figure were developed by the UMC Utrecht, copyright for those images lies with the UMC Utrecht. Icons used with permission.

10.5 Beyond monogenic disease

A wider application of genetics (first) in daily clinical practice will result in more knowledge on prevalence of MGKD, elucidate new genotype-phenotype relationships and find new causal genes for these disorders. Thanks to international collaborative platforms, such as GeneMatcher, an initiative to connect researchers who share an interest in the same known genes or GUSses, knowledge can be shared all over the world.[397] This platform allowed us to set up the collaboration that led to the work presented in **Chapter 5** on *GON7* and *YRDC*.

With these fast-paced advances, more and more genes are linked to human disease. The finding of new causal genes has exponentially increased since the introduction of NGS.[398] Currently, ~3200 of the ~20,000 known human genes are associated with human disease.[33,399]

It is also clear that not all heritable diseases are monogenic. Diseases can be oligogenic (caused by a mutation in more than one gene), polygenic (variants in multiple genes cause the disease), due to complex genomic rearrangements or multifactorial (variants in multiple genes along with environmental factors cause the disease) [400–402] Additionally, human disease can be caused by changes in non-protein coding regions (regulatory DNA in intronic and intergenic regions, which are substantially larger than the coding regions and are sequenced by WGS) or even outside of the DNA sequence ('epigenetic' changes).[403,404]

~5% of patients with a 'monogenic' disease in fact have mutations in more than one gene and are oligogenic diseases. [405] For renal disease specifically, oligogenic inheritance has been shown to play a role in classic monogenic ciliopathies.[406,407] The work on polygenic risk scores is still in development, but was already applied with in a genome-wide association study on the same patients we genotyped in Chapter 6.[408] Genomic rearrangements (i.e. CNVs) provide a significant part of the genomic burden in CAKUT, and even very large complex rearrangements (e.g. chromothripsis) have been associated with kidney disease.[49,409] Mutations causing dysregulation of non-coding RNA have been linked to renal cancer, diabetic nephropathy, FSGS and lupus nephritis.[410,411] Epigenetic changes, such as methylation and histone modifications, have shown to be independent factors in development of diabetic nephropathy and renal fibrosis.[412]

Though there is much work being done on non-monogenic kidney diseases, this is not yet applicable in daily practice. Developments in the monogenic field will hopefully allow to identify more patients with MGKD in an early stage.

10.6 Horizons for reproductive care in MGKD

Though the advances in genetic diagnostics are highly consequential, identifying the patient with a MGKD is only the first step in adequate care. An MGKD diagnosis has life-long value, including for fetal, reproductive and pregnancy care (Figure 10.1). Great strides have been made in the field of caring for fetuses and mothers with MGKD.

Within fetal diagnostics, there are continuous developments. In **Chapter 4** we describe how a fetal ultrasound could be a non-invasive way to assess nephron number in fetal CAKUT, in order to predict post-natal renal outcome. Fetal ultrasounds have the advantage that they are widely available and relatively cheap. They are however more suited for crude prediction, while other tools allow for more specific analysis. Fetal MRI provides more detailed assessment of the fetal kidney, showing a clear advantage over fetal ultrasound in diagnosing CAKUT severity, thereby aiding counselling.[413] Fetal genetic testing may soon also be similarly non-invasive through the sequencing of cell-free fetal DNA from maternal blood, which is currently already applied in daily practice for numerical chromosomal disorders.[414–416] To be able to predict postnatal renal outcome in CAKUT, a European consortium is setting up a study to investigate biomarkers in the amniotic fluid/fetal urine.[417] Efforts such as these will hopefully lead to new ways to diagnose fetal MGKD and offer information on the postnatal renal outcome.

Pregnancy care for MGKD patients is also fast improving. Intensive (nocturnal) peritoneal and hemodialysis schedules have resulted in relatively good outcomes

in high risk patients.[88] Nevertheless, there is still much to learn on the etiology of renal disease and the associated outcomes, to eventually better identify factors that predict outcome.[418] Validating angiogenic (placental growth factor) and antiangiogenic (soluble fms-like tyrosine kinase 1) biomarkers in CKD women can help in the prediction of adverse pregnancy outcomes.[418,419]

For early CKD-stage and post-transplant patients, the gains lie in insights on the optimal drugs to use during pregnancy.[82] However, it remains difficult to test therapeutics on pregnant women, specifically those drugs that are known to already have severe side-effects in non-pregnant subjects.[420,421] Therefore developing knowledge on this subject will likely be a challenge for years to come.

A promising new avenue involves drugs that are targeted to counteract poor outcome in CKD pregnancies. Examples are drugs that improve placental function, as poor placental function is a main factor in maternal and fetal complications of CKD pregnancies.[422] Illustrative is a recent trial that compared sildenafil to placebo in severe intra-uterine growth restriction due to placenta insufficiency. The study had to be terminated prematurely due to neonatal death in the sildenafil group.[423–425] To circumvent the fetal side-effects of maternal drugs, researchers at our institution are investigating a.o. drug-delivery systems that allow for targeted delivery of treatments to the placenta, without impacting the fetus.

Though there are still strides to be made in care for pregnant high risk patients, our and other studies have shown that with comprehensive expert care, relatively favorable pregnancy outcomes are attainable, specifically for patients in CKD stage 1-3.[82,84] Along with the promising advancements in care for advanced CKD and post-transplant patients, this is hopeful for young MGKD patients with a pregnancy wish.

10.7 Towards better science

The work in this thesis was aimed at answering clinical questions. Is a genetics first approach in CKD warranted? What is the prevalence of nephrophthisis in adults? Can we help CKD patients in making complex choices surrounding family planning and what can we tell them on the outcomes of reproductive techniques such as PGT?

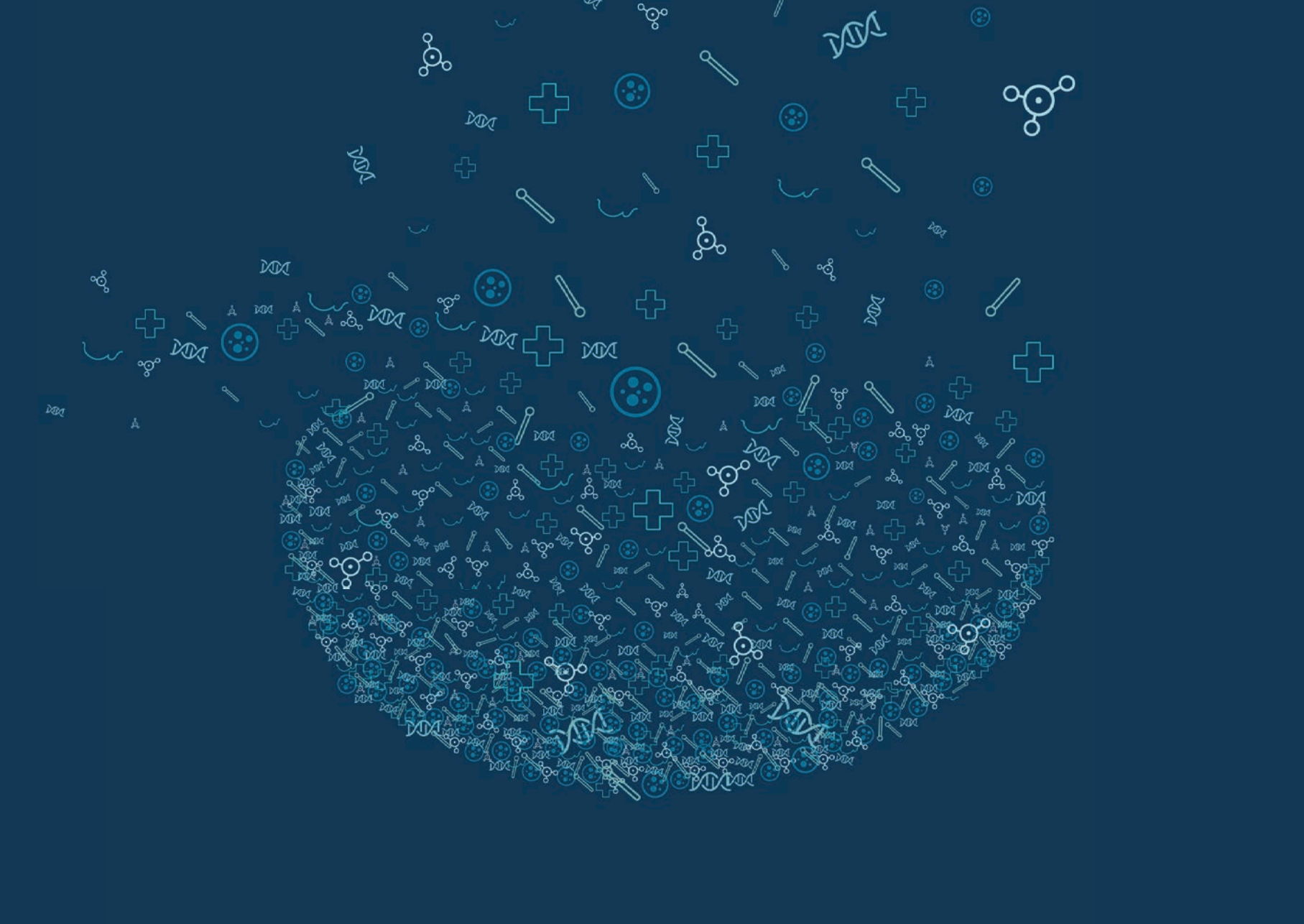
I believe that answering questions, whether clinically relevant ones or more fundamental questions like elucidating the intricate function of a specific protein complex like KEOPS, is what science should be about. Nevertheless, currently scientists are mainly evaluated on the number of publications and grants they produce. This is not necessarily wrong, at the very least this system has certainly

led to some of the largest leaps in knowledge of the modern era.[385,426,427]

However, people within the scientific system have noted that the focus on numbers does not necessarily mean that we are actually performing good science.[428,429] **Appendix 2** describes how, through a grass roots organization called 'young Science in Transition', we changed the way PhD candidates are evaluated in our institution. The evaluation form we designed is currently used by ~300 PhD candidates, and may be introduced for all Utrecht University PhD students soon. With the new form, there is less focus on bibliometrics and more room for developing research competences and other career goals. I believe this will make our PhD candidates better researchers, in turn hopefully leading to better science.

10.8 Conclusions

The work in this thesis makes the case for a genetics first approach in CKD. Though there is a high prevalence of MGKD, it is underdiagnosed in daily practice. This while an adequate MGKD diagnosis provides patients with life-long improvements in care and well-being. Next to the direct impact on patients, the outcomes of genetic testing increase knowledge on disease processes. More knowledge on MGKD will allow for the identification of more MGKD patients and the improvement of reproductive care. Now is the time to seize the opportunities presented to speed up the progress in care for our patients.



Appendix 1

Dutch Federation of Nephrology clinical practice recommendation on genetic diagnostics in renal disease (in Dutch)

Handreiking Erfelijke Nieraandoeningen en urinewegafwijkingen en Nefrologische Verwijsindicaties Klinische Genetica

Namens de NFN richtlijnencommissie verantwoordelijk:
Dr. M.B. Rookmaaker

Samenstelling werkgroep
Dr. A.M. van Eerde, klinisch geneticus, UMC Utrecht
Dr. M.B. Rookmaaker, internist-nefroloog UMC Utrecht
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Fotografie
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A.1.1 Inleiding

Genetische nieraandoeningen en aangeboren urinewegafwijkingen zijn frequente oorzaken van nierfalen in de Nederlandse populatie, met een aandeel van in totaal 26% (Tabel 1). Met name in de groep patiënten die bij aanvang van de nieraandoening jonger is dan 40 jaar, zonder etiologische of classificerende diagnose, is het aannemelijk dat erfelijke nieraandoeningen en urinewegafwijkingen nog een aanvullend percentage verklaren.

Het stellen van een genetische diagnose kan vele belangrijke implicaties hebben. Afdelingen Klinische Genetica zijn goed geoutilleerd om de consequenties voor de patiënt en familieleden te bespreken. Ten eerste kan een genetische diagnose consequenties hebben voor de individuele patiënt: bijvoorbeeld wat betreft behandelopties, inschatten van prognose, indicatie voor extrarenale follow-up, eventueel uitbreiden van transplantatiescreening voor familietransplantatie, bespreken van reproductieve keuzes bij kinderwens. Ten tweede kan een genetische diagnose implicaties hebben voor familieleden: zoals presymptomatische (DNA) diagnostiek en indien mogelijk preventieve behandeling bij bijvoorbeeld Alport syndroom. Ten slotte kunnen er implicaties zijn voor de medische wetenschap en zorg: denk aan voortgang der wetenschap, verbeterde diagnostische classificatie, etc.

Het is belangrijk om te weten dat de klinisch geneticus niet altijd DNA-diagnostiek inzet. Een patiënt kan ook verwezen worden om alleen de voor- en nadelen van genetisch onderzoek te bespreken,

In het geval van kinderwens helpt een genetische diagnose bij erfelijkheidsadvisering, wat betreft het overervingspatroon en/of het inschatten van herhalingskans. Diagnose en herhalingskans tezamen geven richting aan de noodzaak tot advies van uitgebreid echo-onderzoek in een zwangerschap en postnatale follow-up, van mogelijkheden voor prenatale diagnostiek en preïmplantatie genetische diagnostiek (embryoselectie; beide alleen mogelijk met bekende pathogene mutatie(s) in de probandus/indexpatiënt), en van de alternatieve mogelijkheden voor het inrichten van kinderwens, met name sperma-/eiceldonatie, adoptie, afzien van kinderwens.

Het belang van kennis rondom de primaire diagnose geldt niet alleen voor nieuwe patiënten, maar zeker ook voor patiënten uit nefrologische controlepopulaties (zonder duidelijke diagnose) en patiënten die reeds eindstadium nierfalen bereikt hebben.

Tabel A.1.1 | Primaire nieraandoening, diagnoses in RENINE registratie (dd. januari 2015) op basis van oude ERA-EDTA diagnosecodering, uitgezet tegen leeftijd ten tijde van eerste nierfunctievervangende therapie, bij patiënten geboren na 1950. (Zie voor de individuele aandoeningscodes die gebruikt zijn voor de indeling van de categorieën bijlage 6).

Leeftijd (in jaren) van eerste nierfunctievervangende therapie	0 t/m 9	10 t/m 19	20 t/m 29	30 t/m 39	40 t/m 49	50 t/m 59	>60	Eindtotaal
Aangeboren/erfelijk	29%	21%	10%	10%	17%	16%	11%	**15%**
Waarschijnlijk aangeboren urinewegafwijking	33%	30%	17%	10%	5%	2%	2%	**11%**
Onbekend	5%	14%	20%	17%	14%	13%	8%	**15%**
Door hypertensie (onwaarschijnlijk bij jonge patiënten)	0%	1%	5%	10%	12%	12%	11%	**9%**
Andere oorzaken	33%	34%	48%	54%	52%	57%	67%	**51%**
Eindtotaal	100% (n=452)	100% (n=977)	100% (n=2062)	100% (n=2593)	100% (n=3067)	100% (n=2312)	100% (n=242)	**100% (n=11705)**

Deze handreiking is bedoeld voor nefrologen en internisten die betrokken zijn bij de behandeling van patiënten met nieraandoeningen. Met de toenemende mogelijkheden en kennis verandert de plaats van genetisch onderzoek in de klinische praktijk snel. Hierdoor zijn er nauwelijks 'evidence based' aanbevelingen te geven ten aanzien van de opsporing van genetische nieraandoeningen. Deze handreiking poogt een toepasbaar overzicht te geven van de mogelijkheden van nefrogenetische diagnostiek en doet suggesties ten aanzien van detectie, vervolgdiagnostiek en verwijsindicaties bij patiënten met een primaire nieraandoening met mogelijk een genetische component.

A.1.2 Indelingen van genetische nieraandoeningen

In de literatuur worden indelingen aangehouden om structuur te brengen in de veelheid van nieraandoeningen met genetische etiologie. Zo kunnen ziektebeelden ingedeeld worden naar de overervingsvorm. Ook kan gekozen worden voor een patiëntgeoriënteerde benadering gebaseerd op de manier waarop de patiënt zich klinisch presenteert, of een meer pathofysiologische benadering gebaseerd op de anatomische locatie en/of histopathologische afwijkingen.

Het is van belang om onderscheid te maken tussen congenitale nier-aandoeningen en nieraandoeningen met een genetische oorzaak. Van congenitale nieraandoeningen is sprake als de functionele of morfologische afwijkingen van de nier al voor, bij of kort na de geboorte aanwezig zijn (hoewel het kan zijn dat deze zich pas op latere leeftijd openbaren). Nieraandoeningen met een genetische achtergrond kunnen bij de geboorte reeds herkenbaar zijn, maar kunnen ook op latere leeftijd gediagnosticeerd worden.

A.1.2.1 Indeling op basis van overervingspatroon

Een eerste indeling kan gemaakt worden op basis van de overerving; de aanwezigheid van een bepaald overervingspatroon kan helpen bij de identificatie van een genetische nieraandoening. Er zijn grote conceptuele verschillen tussen monogeen-genetische aandoeningen en polygeen-genetische of multifactoriële aandoeningen (zie bijlage 1).

Er hoeft niet per definitie sprake te zijn van Mendeliaanse (=monogene) overerving om wel diagnostiek naar monogene aandoeningen te verrichten. Een genetische aandoening betekent namelijk dat er vaak -maar niet altijd!- meer mensen in de familie zijn aangedaan. Het is uiteraard zinvol om een familieanamnese af te nemen (zie paragraaf A.1.4).

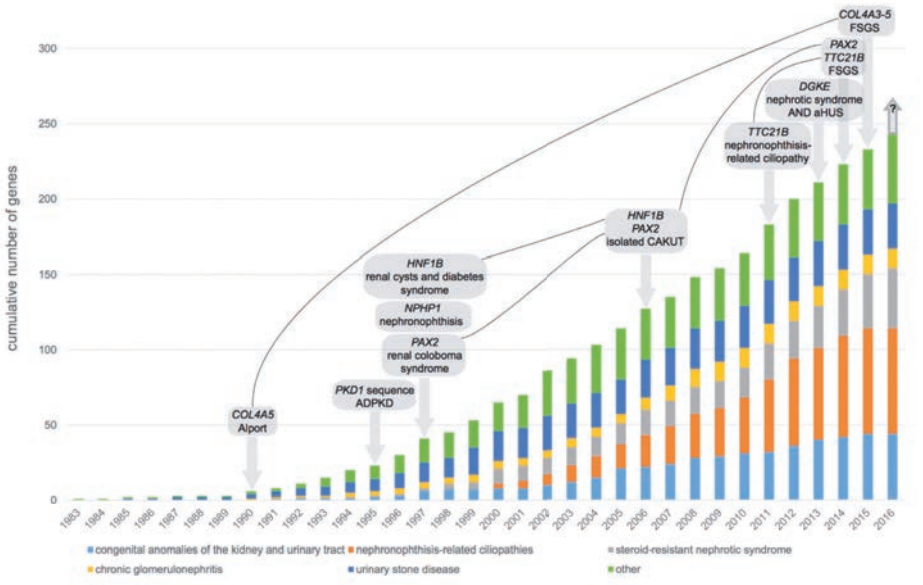
Er zijn verschillende overervingsvormen (zie *NVN Brochure Erfelijkheid* voor voorbeelden van verschillende overervingswijzen in een stamboom), namelijk:

- *Autosomaal*: dit betreft aandoeningen veroorzaakt door een mutatie in een gen dat op een niet-geslachtschromosoom ligt; zowel mannen als vrouwen kunnen aangedaan zijn.
- *Dominant:* er hoeft slechts één allel van het gen gemuteerd te zijn om de aandoening te krijgen. Nakomelingen hebben 50% kans deze mutatie te erven, hierdoor zijn vaak meerdere personen in de familie aangedaan.
- *Recessief:* beide allelen van het gen moeten gemuteerd zijn om de aandoening te krijgen. Als beide ouders drager zijn, hebben broers en zussen a priori 25% kans aangedaan te zijn. Vaak is slechts 1 generatie in de familie aangedaan.
- *X-chromosomaal*: dit betreft aandoeningen veroorzaakt door een mutatie in een gen dat op het X-chromosoom ligt. Door verschillen in X-inactivatie kunnen vrouwen wel of niet aangedaan zijn. Bijvoorbeeld bij draagsters van Alport syndroom door een *COL4A5* mutatie.[332]
- *Mitochondriële overerving*: met een mutatie in het niet-kerngecodeerd mitochondrieel DNA.1. In deze gevallen is sprake van overerving van uitsluitend moeder op kind (*Erfelijkheid: mitochondriele overerving*).
- "*De novo*": de mutatie is niet afkomstig van ouders, maar nieuw ontstaan in de indexpatiënt. Voor het nageslacht geldt dan uiteraard wel het bij dat gen passende overervingspatroon.

In bepaalde gevallen kan *incomplete penetrantie* of non-penetrantie een rol spelen. Dit betekent dat sommige patiënten die de mutatie dragen toch geen klinische verschijnselen (=fenotype) laten zien. Bij *variabele expressie* laten mutatiedragers verschillende fenotypes in aard en ernst zien. Hierdoor kan het overervingspatroon soms lastig te herkennen zijn, zoals bijvoorbeeld bij *HNF1B* mutatiedragers.

A.1.2.2 Indeling op basis van klinische presentatie

Naast de indeling naar overervingspatroon kan de (oorspronkelijke) klinische presentatie van de patiënt verder helpen bij het maken van de genetische differentiaaldiagnose. Belangrijke hoofdgroepen hierbij zijn [17]:



Figuur A.1.1 | Historisch overzicht van aantal geïdentificeerde genen dat een erfelijke nieraandoening veroorzaakt, met nefrogenetische 'mijlpalen'. Inclusief voorbeelden van uitbreiding van het fenotypisch spectrum van een aantal klassieke nieraandoeninggenen (gennaam cursief, bijbehorend aandoeningbeeld eronder). Het staafdiagram geeft het cumulatieve aantal bij nieraandoening betrokken genen over de jaren, gebaseerd op Vivante en Hildebrandt [21] en de diagnostische ervaring van de auteurs. Publicatiejaartallen zijn gebaseerd op OMIM.
Figuur overgenomen uit Van Eerde et al 2016 [28] met toestemming van Kidney International.

Proteïnurie/ hematurie

Tubulaire functiestoornis: polyurie of elektrolytstoornissen door renaal verlies, aanwezigheid van glucose, aminozuren, kleine eiwitten, of overige metabolieten in de urine. Deze kan eventueel verder worden getypeerd op basis van het aangedane nefronsegment.[20]

- Nefrolithiasis/ nefrocalcinosis
- Morfologische afwijkingen:
- Cysten
- Echodensiteit
- Tumoren
- CAKUT (congenital anomalies of the kidney and urinary tract)

Zeker bij patiënten met verder gevorderde nierinsufficiëntie kan het achterhalen van het oorspronkelijke presentatiepatroon lastig zijn. In bijlage 2 zijn tabellen te

vinden waarin genetische nieraandoeningen zijn ingedeeld in groepen met deze klinische presentaties.

A.1.2.3 Indeling op anatomisch of patho(fysio)logisch niveau

Een indeling op basis van histopathologisch niveau kan ook gebruikt worden om de differentiaaldiagnose (verder) te verkleinen.[20] Hierbij valt te denken aan verschillende erfelijke vormen van FSGS [117] of tubulo-interstitiële fibrose [430].

Het is belangrijk te weten dat met de toename van kennis duidelijk wordt dat van oudsher bekende nieraandoeninggenen meerdere nefrologische beelden kunnen veroorzaken. Voorbeelden hiervan zijn *COL4A3/4/5* (klassiek bij Alport syndroom) en *PAX2* mutaties (klassiek bij renaal coloboom syndroom) bij FSGS, en *DGKE* mutaties bij aHUS. (5,6)

Hierbij dient opgemerkt te worden dat met de snel uitbreidende kennis over de genetische bijdrage aan nieraandoeningen de overzichten met aandoeningen per definitie gedateerd zijn.[28,29] De tabellen in bijlage 2 dienen ter illustratie en zijn zeker niet volledig. De differentiaaldiagnose kan richting geven aan het genetisch onderzoek dat men inzet. Ook als het klinisch niet goed mogelijk is om een duidelijke richting aan de differentiaaldiagnose te geven, kunnen de moderne uitgebreide genetische onderzoeken, in samenspraak met een klinisch geneticus, indien gewenst uitkomst bieden.

A.1.2.4 Conclusie

In deze praktische handreiking kunnen we, mede door de snelle ontwikkelingen, geen sluitende genetische differentiaaldiagnoses geven, maar wel algemene handreikingen voor de “genetische aspecten” van de patiënt met een nieraandoening en dan met name de patiënt met, of bij wie gedacht moet worden aan, een monogene aandoening.

In het vervolg worden eerst aandachtspunten ten tijde van het consult belicht, daarna volgen verwijsindicaties en enkele bijlagen met relevante informatie. Omdat het veld zeer in beweging is en het reëel is om te verwachten dat in steeds meer patiëntengroepen DNA-onderzoek vooraan in het diagnostisch traject zal worden gestart (genotype-eerst benadering, in plaats van fenotype-eerst benadering), dient dit document regelmatig herzien te worden. In geval van twijfel, aarzel niet om te overleggen met een klinisch geneticus.

A.1.3 Aanleidingen voor (herziening) work-up primaire nieraandoening

Er zijn verschillende momenten waarop er aanleiding kan zijn voor het (opnieuw) verrichten van (familie)anamnese, lichamelijk onderzoek en (genetisch) aanvullend onderzoek.

Voorbeelden hiervan zijn:

- Bij intake of overname van een nieuwe patiënt
- Bij een patiënt zonder diagnose of twijfel over een bestaande diagnose. (Heroverweeg in dat geval regelmatig, bijvoorbeeld elke 5 jaar):
  - Bijvoorbeeld "nefrosclerose" bij jonge patiënt (cave beschrijvende diagnoses en/of atypische presentatie)
  - Klopt de diagnose met de kennis van nu?
  - Ook bij klinisch harde diagnoses is het verrichten van DNA-diagnostiek de enige manier om de uitzonderingen te vinden [431,432], en om prenatale of preïmplantatie genetische diagnostiek -indien gewenst en geïndiceerd- mogelijk te maken [28]
- Bij overweging familietransplantatie
- Bij (in de toekomst) actieve kinderwens
- Bij het beschikbaar komen van nieuwe behandelingen van genetische aandoeningen. Denk bijvoorbeeld aan Tolvaptan bij ADPKD en ACE-remming bij Alport syndroom.
- Als nieraandoeningen/ urinewegafwijkingen uit de eenvoudige familieanamnese naar voren komen. Ook in het geval van nieuw-beschikbare informatie uit de familieanamnese, bijvoorbeeld als de patiënt kinderen of kleinkinderen heeft gekregen in de afgelopen tijd.

A.1.4 (Familie)Anamnese

Om de kans op een erfelijke ziekte bij een individuele patiënt in te schatten is het afnemen van een familieanamnese door de nefroloog essentieel. Het is te adviseren dit voor elke patiënt in ieder geval éénmaal te doen en daarna regelmatig te herhalen (zie ook paragraaf A.1.3). De informatie uit de familieanamnese biedt aanknopingspunten voor een vermoeden van genetische ziekte, en is tevens ook behulpzaam bij het schrijven van een verwijzing naar een klinisch geneticus.

Een uitgebreide (familie)anamnese betekent tenminste:

- **Wie:** tot en met *tweedegraads familieleden*. Suggestie: teken een stamboom

om overzicht te krijgen van alle familieleden, stel daarna pas de 'wat'-vragen en scan complete stamboom in in een elektronisch dossier.
Dit betreft dus de volgende personen:

- o Patiënt zelf
- o Kinderen (inclusief miskramen/vroeggeboortes/ongewenste infertiliteit)
- o Kleinkinderen
- o Ouders
- o Grootouders
- o Broers/zussen
- o Kinderen van broers/zussen van patiënt
- o Ooms/Tantes
- *o 3e graads familieleden en verder: alleen op indicatie*
- o Vraag ook naar consanguïniteit: in elk geval mbt de eventuele partner van de patiënt en de ouders van de patiënt.

- **Wat:**
  - o De primaire nieraandoening
    - Leeftijd van presentatie
    - Klinische presentatie (creatinine, proteïnurie, sediment, morfologie van de nier bijvoorbeeld op echo, metabole stoornissen)
    - Beloop
  - o Hypertensie op jonge leeftijd
  - o Pyelonefritiden/ onverklaarde febriele episodes op de kinderleeftijd
  - o Aangeboren nier-/urinewegafwijkingen, ook bij patiënten bekend met niercysten
  - o Overige aangeboren afwijkingen, (zie ook enkele foto's bij 'Lichamelijk onderzoek' in paragraaf A.1.5) bijvoorbeeld:
    - Bijoortjes en/of pre-auriculaire pits (branchio-oto-renaal syndroom, Townes-Brocks syndroom)
    - Geslachtsorgaanafwijkingen (hypospadie, uterusvormafwijkingen; *HNF1B*)
    - Halsfistels (branchio-oto-renaal syndroom)
    - Hartafwijkingen (22q11 deletiesyndroom, ciliopathieën)
    - Nageldysplasie (nagel-patella syndroom)
    - Polydactylie (ciliopathieën)
    - Schisis (22q11 deletiesyndroom)
    - Encephalocele (ciliopathieën)
  - o Verstandelijke beperking
  - o Overige tracti: vraag deze uit over de indexpatiënt, mocht hij/zij hier

bijzonderheden hebben dan kan een uitgebreidere familieanamnese zinvol zijn. Denk bijvoorbeeld aan:

- Diabetes mellitus
- Gehoor
- Myopathische aandoeningen
- Neurologische aandoeningen (bijvoorbeeld hersenbloedingen, aandoening van Charcot-Marie-Tooth)
- Visus (bijvoorbeeld retinitis pigmentosa)
- Maligniteiten (inclusief leeftijd van presentatie)
- Specifiek: nierkanker (zie ook: *Richtlijn erfelijke tumoren*). Overige verschijnselen, passend bij een syndromale oorzaak van niertumor (in bijlage 3 vindt u een lijst met syndromen met niertumoren):
  - Angiomyolipomen (tubereuze sclerose complex)
  - Fibrofolliculomen, longcysten, pneumothorax (Birt-Hogg-Dubé syndroom)
  - Haemangioblastomen (CZS en retina), endolymfatic sac tumor, voornamelijk in middenoor, feochromocytomen, cysten of tumoren van pancreas of lever, cystadenomen nabij uterus of epididymis (von Hippel-Lindau (VHL) syndroom)
  - Leiomyomen van huid of uterus (hereditary leiomyomatosis and renal cell cancer (HLRCC)
  - Paragangliomen, feochromocytomen (hereditary paraganglioma and pheochromocytoma)

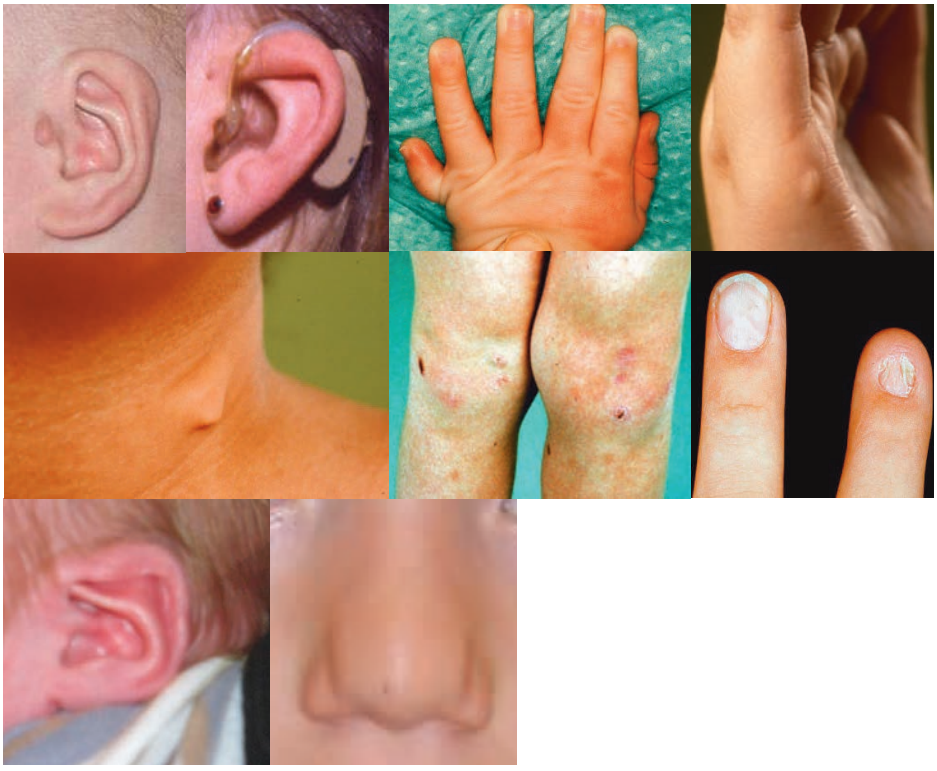
Zie ook 'Aanvullend genetisch onderzoek' (paragraaf A.1.7).

Nota Bene:

- Ook patiënten met een negatieve familieanamnese kunnen een erfelijke aandoening hebben. Bijvoorbeeld een autosomaal recessieve aandoening, of een autosomaal dominante aandoening veroorzaakt door een *de novo* mutatie of germline/kiembaan mozaïek bij één van de ouders.
- Het is aan te raden de familieanamnese op een dusdanige wijze in het elektronisch dossier te noteren dat deze eenvoudig terug te vinden en aan te vullen is. Uit privacy-overwegingen dienen er geen herleidbare gegevens (naam, geboortedatum etc.) van familieleden in het dossier van de indexpatiënt te staan, ook als de indexpatiënt zelf deze informatie heeft verstrekt.

A.1.5 Lichamelijk onderzoek

Zoals gebruikelijk dient bij elke patiënt met een nieraandoening een volledig internistisch lichamelijk onderzoek te worden verricht. Hoewel het dysmorfologisch onderzoek bij uitstek de expertise van de klinisch geneticus is, tonen wij hier een aantal dysmorfieën die kunnen voorkomen bij genetische aandoeningen die ook tot nierfunctiestoornissen kunnen leiden en makkelijk herkenbaar zijn (foto's met dank aan Prof.dr. R.C.M. Hennekam, klinisch geneticus AMC en dr. M.J. v.d. Boogaard, klinisch geneticus UMC Utrecht).



Figuur A.1.2 | Dysmorfien die kunnen voorkomen bij genetische aandoeningen die ook tot nierfunctiestoornissen kunnen leiden en makkelijk herkenbaar zijn. Foto's met dank aan Prof.dr. R.C.M. Hennekam, klinisch geneticus AMC en dr. M.J. v.d. Boogaard, klinisch geneticus UMC Utrecht.
Van links naar rechts bovenste rij: Afwijkende vorm van het oor (o.A.1. Townes-Brocks syndroom; bij patiënt op de foto ook een preauriculaire tag), preauriculaire pit (brachio-oto-renaalsyndroom (BOR), postaxiale polydactylie, inclusief postminimus of litteken na verwijdering zoals op de foto uiterst rechts (Bardet-Biedl syndroom en overige ciliopathieën).
Van links naar rechts middelste rij: Halsfistel (BOR), kleine of afwezige patellae en afwijkende nagels (beide passend bij nagel-patella syndroom).
Onderste rij: kleine, wat vierkante oren en opvallende neus met kleine alae nasi (22q11.2 deletie syndroom)

Zie voor een overzicht van gestandaardiseerde terminologie: *Elements of morphology*. Enkele niet getoonde afwijkingen zijn: colobomen (NB bij het renaal coloboomsyndroom geen iris of rentina coloboma maar juist n. opticusafwijkingen), triphalangeale duim (Townes-Brocks syndroom) en enkele huidafwijkingen zoals angiokeratomen (ziekte van Fabry) of fibrofolliculomen (Birt-Hogg-Dubé syndroom).

A.1.6 Aanvullend (niet-genetisch) onderzoek

Aanvullend onderzoek kan helpen het klinisch beeld verder in kaart te brengen. De waarde van de onderzoeken is niet alleen afhankelijk van de differentiaaldiagnose maar ook van de timing van de diagnostiek. Als men bij vergevorderd nierfalen op zoek wil naar de primaire diagnose kan het zinvol zijn om de data rondom de eerste presentatie te achterhalen. Toch kan aanvullend onderzoek ook bij gevorderde nierinsufficiëntie zinvol zijn, denk aan beeldvorming van de nieren op zoek naar aanlegstoornissen of opvallende metabole stoornissen die niet verklaard kunnen worden door gevorderde nierinsufficiëntie.

Naast een gebruikelijk lichamelijk en aanvullend onderzoek, kan breder niet-genetisch aanvullend onderzoek de differentiaaldiagnose meer richting geven. Denk bijvoorbeeld aan urinezuur en magnesium bij nierinsufficientie en cysten (mogelijk *HNF1B*-gerelateerde ziekte).

A.1.7 Aanvullend genetisch onderzoek

Bij DNA-onderzoek kan er gekozen worden uit één gerichte test voor één specifiek gen (Sanger sequencing), maar ook voor een panel genen bestaande uit meerdere genen die alle geassocieerd zijn met een groep aandoeningen met een vergelijkbaar klinisch fenotype of bijvoorbeeld voor alle genen waarvan bekend is dat zij geassocieerd zijn met nieraandoeningen. Uiteraard zijn exoom-/genoombrede ('whole exome' of 'whole genome') sequencing tests ook mogelijk. Vaak worden genpanels gefilterd van whole exome data, in dat geval is het mogelijk om in een later stadium een volgende analyse van de bestaande data te verrichten. Zie voor verdere uitleg onderstaand kader met terminologie.[30]

Terminologie

Onderzoeksmethoden

- Single Nucleotide Polymorphism (SNP) array en -in het verleden veel gebruikt- Array Comparative Genomic Hybridization (Array CGH): methodes om genoombreed te screenen op aanwezigheid van kopienummervarianten vanaf ongeveer 150 Kb groot. SNP array kan daarnaast informatie opleveren met betrekking tot consanguïniteit bij de ouders van patiënt of het bestaan van uniparentale disomie.
- Sanger sequencing: gericht DNA-onderzoek per individueel gen.
- Next Generation Sequencing (NGS): DNA-onderzoek naar meerdere/veel/alle genen ("exoom") of het hele genoom tegelijk. Het gaat hierbij om een andere techniek waardoor de mutatiedetectie van een specifiek gen niet altijd even hoog is als met Sanger sequencing van datzelfde gen. Bij genpaneldiagnostiek worden vaak op de achtergrond meerdere panels of zelfs het hele exoom gesequenced, maar wordt voor de uitslag alleen naar het specifiek opgegeven panel gekeken.
- Multiplex ligation-dependent probe amplification (MLPA): methode om voor een individueel gen te onderzoeken of er sprake is van een deletie of duplicatie. Voor dat specifieke gen is de methode gevoeliger dan SNP array, maar niet altijd beter; bij MLPA van *HNF1b* gen zal een genomvattende deletie kunnen worden opgespoord maar om vervolgens de exacte grootte van de deletie vast te stellen verdient SNP array de voorkeur.

Typen uitslag

- Kopienummervarianten (*copy number variants*; CNVs): submicroscopische deleties of duplicaties van een groter stuk DNA.1.
- Dragerschap: als een patiënt heterozygoot is voor een mutatie die in homozygote/, compound heterozygote of hemizygote vorm een autosomaal recessieve of een geslachtsgebonden aandoening veroorzaakt.
- Verklarende pathogene mutatie, ook wel klasse 4 of 5 varianten genoemd (waarschijnlijkheid van ziektecausaliteit respectievelijk 95%-99% en >99%).
- Variant of unknown significance (VUS) of klasse 3 variant: Een variant in het DNA, waarvan de relatie met een aandoening niet (genoeg) is bewezen om als pathogeen geclassificeerd te worden (waarschijnlijkheid van ziektecausaliteit 5%-95%). De detectie van een VUS betekent dat de diagnose niet bevestigd is, soms nog wel bevestigd kan worden. Dit is dus niet gelijk aan een "toevalsbevinding".

Overig

- Presymptomatische diagnostiek: diagnostiek bij (ogenschijnlijk) gezonde familieleden/ familieleden zonder evidente verschijnselen, naar een in de familie bekende aandoening (kan zowel genetische als niet-genetische diagnostiek betreffen)
- Toevalsbevindingen: een pathogene mutatie in een gen, dat niet verantwoordelijk is voor een aandoening die de reden was voor het onderzoek, maar voor een andere aandoening

A.1.7.1 Overwegingen bij zelf aanvragen van genetische diagnostiek door nefroloog

Genetische diagnostiek van enkele genen of kleinere fenotypegerichte genpanels kan, maar hoeft niet, door een goedgeïnformeerde nefroloog zelf aangevraagd worden. Iedere (kinder)nefroloog is bevoegd om DNA-onderzoek aan te vragen bij patiënten **met symptomen** van een erfelijke ziekte, waarbij bekwaamheid met betrekking tot pre- en posttest counseling en vertrouwdheid met de interpretatie van de uitslagen doorslaggevend zijn. Voorafgaand aan het inzetten van genetisch onderzoek is het essentieel de implicaties die genetische diagnostiek kan hebben in overweging te nemen (zie ook paragraaf A.1.7.3 en A.1.7.6) en mee te nemen in de beslissing wel of geen genetisch onderzoek in te zetten. Goedgeïnformeerd houdt in dat **de nefroloog al het onderstaande beheerst**:

- Genen in het betreffende pakket kennen, zowel qua fenotype als overervingswijze. In de praktijk zullen dit over het algemeen kleinere genpanels betreffen (tot ~75 genen).
- Comfortabel zijn bij pretest counseling; men dient te bespreken:
    - De mogelijkheid van VUS (zie box).
    - De mogelijkheid van nevenbevindingen (zie box). Bijvoorbeeld enkele oncogenen (*RET*, *WT1*) hebben ook niergerelateerde functies, en kunnen daarom onderdeel zijn van genpanels die niet-oncologiegeoriënteerd zijn.
    - Dragerschap van een (bijvoorbeeld autosomaalrecessief)erfelijke aandoening, kan in het geval van kinderwens leiden tot onderzoek bij de partner (afhankelijk van de dragerschapsfrequentie) of andere familieleden, terwijl het nierfenotype niet verklaard wordt.
    - De (psycho)sociale implicaties van genetisch onderzoek. Een goedgeïnformeerde nefroloog is in staat de patiënt hier adequaat in te begeleiden tijdens de pretest counseling. Bijvoorbeeld: voor een patiënt met eindstadium nierfalen zal de verzekerbaarheid niet veranderen met een diagnose Alport syndroom, voor een patiënt met een spoortje hematurie mogelijk wel. (Zie ook: *Verzekeringen*).
    - Momenteel is het beleid ten aanzien van het al dan niet nodig zijn van getekende informed consent voor genpanelonderzoek per centrum verschillend (met name bij kleinere genpanels).
- Inzicht hebben in efficiënte en rendabele inzet van genetisch onderzoek. Het is bijvoorbeeld niet zinvol om bij broers/zussen tegelijk genpanelonderzoek in te zetten. Het is ook niet zinvol en met name niet kosteneffectief om allerlei panels achter elkaar aan te vragen. Andersom is een groot panel aanvragen bij een specifieke verdenking van een mutatie in een bepaald gen niet doelmatig

en kan het leiden tot onnodige nevenbevindingen. Bovendien, bij een heel specifieke verdenking is de 'single gene' test vaak nog net iets preciezer, omdat er dan nog strengere eisen zijn aan sensitiviteit en er geregeld ook een deletietest wordt verricht (MLPA). Een voorbeeld waarin een single gene test nog de voorkeur verdient is bij cystinose. Het kan wel kosteneffectief zijn om na te gaan of er in de test die ten grondslag ligt aan een eerdere genpaneluitslag meer genen of het hele exoom zijn gesequenced, zodat wellicht een nieuwe vraag vanuit de al beschikbare data beantwoord kan worden.

- Weten dat degene die het onderzoek inzet ook de eerste is die de uitslag met patiënt bespreekt, en daartoe bekwaam moet zijn. Bij twijfel: overleg voor het uitslaggesprek met een klinisch geneticus.
- Weten wat de impact van een mogelijke genetische diagnose op familieleden kan zijn, en de indexpatient en familie daarin begeleiden.
- Weten wat de doorlooptijden van de verschillende testen zijn.
- Voor al het bovenstaande verdient het aanbeveling aansluiting te vinden bij een multidisciplinair overleg waar, al dan niet op ad hoc basis, casuïstiek besproken wordt.

A.1.7.2 Overwegingen om te verwijzen naar een klinisch geneticus voor/ vóór het inzetten van aanvullende diagnostiek

(zie ook paragraaf A.1.8: Verwijsindicaties)

Een patiënt kan uiteraard altijd verwezen worden vanwege een verdenking op een genetische aandoening, bijvoorbeeld om genetisch aanvullend onderzoek in het kader van genetische counseling plaats te laten vinden (zie ook paragraaf A.1.8.2: Verwijs-/overlegredenen: nog niet gestelde genetische diagnose). Tevens kan laagdrempelig overlegd worden met een multidisciplinair expertisecentrum, waar ingeschat kan worden of een verwijzing geïndiceerd is en zo ja of deze het beste naar een gespecialiseerde nefroloog of direct naar een klinisch geneticus of naar een combinatiespreekuur kan zijn. Een verwijzing naar een klinisch geneticus verdient de voorkeur in de volgende gevallen:

- Vóór het verrichten van grote genpanels, waar de kans op nevenbevindingen groter is. Overigens is de toekomstvisie van de VKGN dat goed onderwezen orgaanspecialisten deze grotere genpanels ook vaker zelf aan zullen vragen.
- Vóór het verrichten van exoombreed of genoombreed onderzoek, waarvoor per definitie academische counseling aangewezen is.
- Presymptomatische diagnostiek.
    - Niet-genetisch presymptomatisch onderzoek: Het valt te overwegen voor al het presymptomatisch onderzoek, inclusief bijvoorbeeld beeldvorming

in het kader van ADPKD of urineonderzoek bij Alport syndroom, patiënten te verwijzen naar een klinisch geneticus voor counseling van medische, psychologische en maatschappelijke implicaties van deze diagnostiek (zie ook paragraaf A.1.7.6).
    - Genetisch presymptomatisch onderzoek: het aanvragen van deze DNA-diagnostiek is voorbehouden aan een klinisch geneticus en wordt slechts verricht na adequate genetische counseling.
    - Een gezond familielid/familielid zonder verschijnselen kan zich op het gesprek met de klinisch geneticus voorbereiden middels een keuzehulp te bereiken via: *Keuzehulp presymptomatische diagnostiek*
- Bij complexe stambomen, bijvoorbeeld:
    - Grote families. De geneticus kan bijvoorbeeld gemakkelijk in het familiedossier andere leden opsporen en is getraind in het omgaan met familiedynamiek.
    - Gevallen waarin mogelijk de genetische afwijking ergens in de familie al bekend is en het niet altijd nuttig en kosteneffectief is die mutatie in elke patiënt apart aan te tonen.
    - In families waarin er consequenties van familie-onderzoek zijn voor mensen die het niet willen weten. Een voorbeeld van dat laatste: grootmoeder is aangedaan, nog levende moeder is niet getest, dochter komt voor presymptomatisch onderzoek. Met een positieve uitslag is de tussenliggende moeder obligaat drager.
- Alle bovenstaande situaties vergen genetische counseling. Op die manier komt de kennis van voorkomen van een familiaire mutatie en de gevolgen daarvan terecht bij familieleden voor wie deze (medisch) relevant is.

A.1.7.3 Timing van genetische diagnostiek

De timing van genetische diagnostiek kent twee aspecten: aan de ene kant de plaats van genetische diagnostiek in het uitwerken van de differentiaaldiagnose, en aan de andere kant de patiëntfactoren die aanleiding kunnen geven tot het verrichten van genetisch onderzoek.

Waar genetische diagnostiek voorheen met name werd verricht nadat de klinische diagnose praktisch al was gesteld, zijn er steeds meer redenen om de genetische DNA-diagnostiek naar voren te halen in het diagnostische traject:

- Ten eerste kan een genetische diagnose invasieve diagnostiek met name het nierbiopt overbodig maken. Dit geldt zeker in populaties met een vooraf hoge trefkans op een genetische nieraandoening. Denk hierbij o.A.1. aan een positieve familieanamnese voor genetische nieraandoeningen of nierfalen op

jonge leeftijd.[28] (Suggestie: stadium 4/5 CKD vóór de leeftijd van 40 jaar zonder evidente diagnose.)

- Ten tweede zijn de kosten van genetische diagnostiek aan het dalen en daarmee wordt het steeds meer concurrerend met andere klinische diagnostiek.
- Ten derde kan in sommige gevallen een genetische oorzaak van een op het oog niet-primair genetische nierziekte niet worden uitgesloten op basis van alleen klinisch onderzoek.

De verschillende beweegredenen om DNA-diagnostiek te verrichten zijn ook nauw verbonden met de timing van deze diagnostiek in het leven van patiënten:

- Ten eerste kan DNA-diagnostiek natuurlijk helpen bij het stellen van een diagnose. Vaak is verdenking op een diagnose voldoende en verandert DNA-diagnostiek weinig aan de behandeling. Desondanks is in de afgelopen jaren steeds meer wetenschappelijk bewijs gevonden voor de toegevoegde waarde van een bewezen genetische diagnose in het sturen van therapeutische beslissingen. Een genetische diagnose kan voorkomen dat zinloze medicatie wordt gestart:
  - Bijvoorbeeld bij patiënten met een FSGS-beeld in het nierbiopt en mutaties in podocytgenen waarbij behandeling met steroïden geen toegevoegde waarde heeft.[136]
  - Ook is er een rol voor genetische diagnostiek bij de specifieke behandeling van patiënten met ADPKD met tolvaptan en mogelijk gaat deze rol toenemen in de komende jaren.[432–434].
  - Andere voorbeelden van genetische diagnoses die behandelconsequenties zouden kunnen hebben zijn Alport syndroom (*COL4A3-5*-gerelateerde aandoening, overwegen starten ACE-inhibitie) en sommige gevallen van aHUS.
  - Genetische diagnoses kunnen ook inzicht geven in de kans op recidief-aandoening in het transplantaat of oorspronkelijkeaandoening-gerelateerde transplantatie-problematiek, bijvoorbeeld anti-GBM bij Alport syndroom.
- Ten tweede kan DNA-diagnostiek relevante informatie geven bij patiënten die invulling willen geven aan een kinderwens. Ook bij aandoeningen die (nog) geen duidelijke behandeling kennen kan een genetische diagnose relevant zijn voor patiënten die gebruik zouden willen maken van prenatale diagnostiek of preïmplantatie genetische diagnostiek. Een genetische diagnose zou ook kunnen leiden tot afzien van kinderwens of het overgaan tot adoptie. In het verlengde hiervan kunnen ook broers of zussen van patiënten met een erfelijke nieraandoening duidelijkheid krijgen of ze de aandoening kunnen krijgen of doorgeven aan (toekomstige) kinderen. Een diagnose opent de deur naar adequate en heldere genetische familiecounseling.

- Ten derde kan genetische diagnostiek relevant zijn bij nierdonatie door een familielid. De aanwezigheid van een erfelijke aanleg die ook bij een potentiële donor uit de familie alsnog tot expressie kan komen kan een argument zijn bij het maken van de keuze om een familielid wel of niet te laten doneren.
- Ten slotte dient vermeld te worden dat los van alle medisch-inhoudelijke overwegingen, het voor sommige patiënten heel belangrijk kan zijn om inzicht te hebben in de oorzaak van een vaak zeer ingrijpende aandoening.

A.1.7.4 Logistiek

DNA-diagnostiek waarvan de uitslagen gebruikt moeten kunnen worden als basis voor prenatale diagnostiek, preïmplantatie genetische diagnostiek of presymptomatische diagnostiek kan enkel worden verricht in gecertificeerde DNA-laboratoria; de meeste zijn verbonden aan de academische centrA.1.

Voor gerichte gendiagnostiek kan nagekeken worden op *www.DNAdiagnostiek.nl* welke centra een specifieke test verrichten. Via deze site zijn ook de aanvraagformulieren te downloaden. Door de snelle ontwikkelingen lopen de aanvraagformulieren op de website nog wel eens iets achter op het actuele aanbod. Bij twijfel kunt u overleggen. U kunt overleggen met de laboratoriumspecialist klinische genetica verantwoordelijk voor de specifieke test (telefoonnummer via *www.DNAdiagnostiek.nl*), bij meer klinische vragen kunt u overleggen met een klinisch geneticus.

Zodra de uitslag bekend is wordt deze alleen naar de aanvrager (en eventuele kopiehouders) verstuurd. Andere behandelaren kunnen deze uitslag alleen met expliciete toestemming van patiënt opvragen (telefoonnummers via *www.DNAdiagnostiek.nl*).

Na het onderzoek blijft het overgebleven DNA tenminste 30 jaar- hoewel in de praktijk de levensduur van 3 generaties (~100 jaar) wordt aangehouden- bewaard in het betreffende laboratorium en kan gebruikt worden voor vervolgdiagnostiek en/of wetenschappelijk onderzoek indien de patiënt daar schriftelijke toestemming voor geeft. Er is een procedure om het materiaal te vernietigen indien gewenst. De aanvraag en de uitslagbrief dienen 115 jaar bewaard te worden.

A.1.7.5 Kosten

Counseling door een klinisch geneticus en DNA-onderzoek zijn voor de verzekeraar aparte verrichtingen. DNA-onderzoek valt buiten de DOT-structuur. Beide behoren tot het basispakket, maar gaan wel ten koste van het eigenrisicobedrag (https://www.umcutrecht.nl/nl/Ziekenhuis/Afdelingen/Genetica).

Voor het onderzoeken van één gen wordt een standaardtarief van ongeveer

€800,- in rekening gebracht. Voor het verrichten van NGS-diagnostiek wordt het dubbele standaardtarief gerekend. Gericht DNA-onderzoek naar een al in de familie bekende DNA-variant is goedkoper dan een enkel standaardtarief (prijspeil 2017).

A.1.7.6 Maatschappelijke aspecten

Een (presymptomatische) diagnose kan consequenties hebben voor het afsluiten van verschillende verzekeringen. Er wordt in Nederland onderscheid gemaakt tussen te verzekeren bedragen onder en boven de zogenoemde 'vragengrens'. De vragengrens ligt voor levensverzekeringen op 268.125 euro, voor het eerste jaar arbeidsongeschiktheid op 38.877 euro (d.d. 2017).

Boven en onder de vragengrens moet iemand altijd klachten, verschijnselen en aandoeningen melden (zie voor presymptomatische DNA-diagnoses én een levensverzekering onder de vragengrens, hieronder).

Als iemand gezondheidsproblemen heeft van een erfelijke aandoening is die persoon wettelijk verplicht dit aan de verzekeraar te melden. Als de specialist de symptomen ziet als duidelijke kenmerken van de erfelijke aandoening moet iemand deze symptomen melden aan de verzekeraar. Ook als hij of zij verder nog geen klachten heeft. Of iets wel of niet een kenmerk is van een (erfelijke) aandoening is aan de expertise van een medicus. Zie ook: *Melden aan verzekeraar*

Bij bedragen onder de vragengrens:

- Hoeven gezonde mensen die een (presymptomatische) DNA-diagnose hebben dat niet te melden (er mag ook niet naar gevraagd worden).
- Mag er niet gevraagd worden naar aandoeningen in de familie.
- Hoeft een informatief gesprek bij een klinisch geneticus of andere medisch specialist niet gemeld te worden.

Bij bedragen boven de vragengrens:

Boven de vragengrens mag dit wel worden gevraagd, maar verzekeraars gaan daar verschillend mee om. Het is belangrijk voor patiënten om zich goed te laten informeren maar ook voor dokters die hierin adviseren om op de hoogte te zijn. Patiënten kunnen hierover voorgelicht worden bij een klinisch genetisch centrum. Meer informatie: *http://erfelijkheid.nl/special/verzekeren, vanatotzekerheid.nl.*

Bij autosomaal dominante aandoeningen die leiden tot eindstadium nierfalen is het - in het kader van gevolgen voor eigen verzekerbaarheid en consequenties voor kinderen - te overwegen om DNA-onderzoek te doen in de oudst beschikbare generatie in de familie, omdat daar vaak minder/geen nieuwe verzekeringen

aangegaan hoeven te worden en het eigen risicoprofiel, als er eenmaal gevorderd nierfalen is, niet erg meer zal veranderen met een genetische diagnose. De gestelde genetische diagnose helpt bij het adequaat informeren van familieleden en gerichte diagnostiek kan dan zo nodig worden ingezet.

A.1.8. Verwijsindicaties

A.1.8.1 Waarheen verwijzen?

Alle afdelingen klinische genetica (zijn academisch, maar via buitenpoliklinieken worden ook veel perifere ziekenhuizen bediend) leveren algemene nefrogenetische zorg (zie ook *Poliklinieken klinische genetica*).

Sinds 2015 zijn door de NFU expertisecentra voor zeldzame aandoeningen erkend, welke beschikbaar zijn voor overleg en verwijzingen. Zowel het UMC Utrecht (*Expert Centre Hereditary and congenital nephrologic and urologic disorders*), als het Radboudumc (*Radboud Center Renal Disorders*) heeft multidisciplinaire poliklinieken voor kinderen en volwassenen met complexe nierbeelden en/ of een verdenking op een erfelijke nieraandoening. Bovendien zijn aan deze expertisecentra een multidisciplinair overleg (*Radboud Center Renal Disorders)* en een multidisciplinaire polikliniek zwangerschap en nieraandoeningen verbonden: voor pre-conceptieadvies, maar ook zwangerschapsbegeleiding (*Polikliniek zwangerschap en nieraandoeningen UMC Utrecht*). Het ErasmusMC heeft een multidisciplinair nefrogeneticaoverleg. Er zijn meer landelijk erkende expertisecentra op het gebied van specifieke zeldzame nieraandoeningen, de meest recente lijst staat *op de website van Orphanet*.

Begin 2017 is ERKNET, het Europese referentienetwerk voor zeldzame nieraandoeningen erkend door de Europese Unie (*https://erknet.org*). Binnen dit referentienetwerk kan informatie worden ingewonnen, bijvoorbeeld over individuele casus, van experts in die specifieke aandoening.

A.1.8.2 Verwijs-/overlegredenen: nog niet gestelde genetische diagnose bij verdenking genetische nieraandoening

Overleg met of verwijzing naar een klinisch geneticus is altijd mogelijk. Het is belangrijk om de eventuele klinische implicaties mee te nemen in de afweging om wel of niet genetisch onderzoek te verrichten. Redenen voor contact kunnen zijn de verdenking op een erfelijke nieraandoening of urinewegafwijking, bijvoorbeeld op basis van:

- Specifiek passend (biochemisch) fenotype
- Twijfel aan een klinische diagnose, bijvoorbeeld: “ADPKD”, maar kleine nieren
- Opvallend jonge/ opvallend ernstige casus

  - Suggestie: stadium 4/5 CKD vóór de leeftijd van 40 jaar zonder evidente diagnose
- Een nieraandoening in combinatie met:
  - Verstandelijke beperking
  - Dysmorfieën
  - Aangeboren afwijkingen
  - Aandoeningen van andere tracti (zie paragraaf A.1.4, tenzij duidelijk verklaarbaar of prevalent)
  - Meerdere miskramen bij patiënt, partner, of moeder; kan een aanwijzing zijn voor een chromosoomafwijking; voornamelijk relevant in het kader van kinderwens
- >1 patiënt in de familie
  - Liefst binnen tweedegraads verwanten (zie overzicht bij familieanamnese)
  - Afhankelijk van grootte van stamboom en prevalentie van aandoening:
    - "Onverwacht hoge frequentie". Bijvoorbeeld: Bij twee keer IgA nefropathie in grote familie: vooralsnog geen reden voor verwijzen, maar bij bijvoorbeeld 3 of meer aangedane eerstegraads familieleden, zou u dit wel kunnen overwegen. Dan moet overwogen worden, of er toch een (zeldzame) erfelijke variant van IgA nefropathie bestaat, of dat wellicht de diagnose in de familie bijgesteld kan worden.
    - Bij een duidelijk monogene aandoening, zoals in het geval van een Mendeliaanse of mitochondriële stamboom, is de kans groter een genetische diagnose te kunnen stellen.
    - Ook wanneer er geen duidelijke Mendeliaans of mitochondriëel overervingspatroon is, maar wel een herhaling van een zeldzaam beeld: denk dan aan een erfelijke oorzaak.
- Nierkanker i.c.m (zie ook: *Richtlijn erfelijke tumoren*)
  - Leeftijd:
    - 1 persoon (1e-graads) met nierkanker <45 jaar
    - 2 personen (1e en 2e-graads) met nierkanker <70 jaar
    - 3 personen (1e en 2e-graads) met nierkanker (*https://www.erfelijkekanker.nl/zakkaartjes*)
  - Bilaterale of multifocale maligniteit
  - Andere maligniteiten bij patiënt of familie (zie paragraaf A.1.4)
  - Bijkomende verschijnselen bij patiënt of familie (zie paragraaf A.1.4)
- Andere vormen van kanker (praktische zakkaartjes voor verwijscriteria bij vormen van kanker: *https://www.erfelijkekanker.nl/zakkaartjes*)
- Vragen met betrekking tot familieleden/kinderwens: extra argument

- Voor counseling ten behoeve van presymptomatische screening van gezonde familieleden, ook bij een verdenking op –maar nog niet bewezen- erfelijke aandoening

A.1.8.3 Verwijsredenen: een (door een nefroloog) reeds gestelde (genetische) diagnose

In principe bij elke afwijkende bevinding, dus een VUS/klasse-3-variant of diagnose/ klasse-4- of 5-variant*

- Bij een diagnose: voor het informeren van de patiënt met betrekking tot de genetische aspecten van de aandoening, maar ook voor het in gang zetten van het informeren van familieleden en het bespreken van consequenties bij een eventuele kinderwens. De klinisch geneticus beschikt over goede infrastructuur hiervoor en heeft ervaring hiermee.
- Bij een VUS: de klinisch geneticus zal nagaan of er aanvullende onderzoeksmogelijkheden (o.A.1. segregatie-analyse) zijn waarna de VUS definitief als pathogeen beschouwd mag worden. Ook als dat niet lukt en er dus onzekerheid betreffende pathogeniciteit blijft, zal geadviseerd worden m.b.t. zowel genetische als nefrologische follow-up voor patiënt, maar zo nodig ook voor familieleden.

In het algemeen is het aan te raden om counseling door een klinisch geneticus aan te bieden bij een (nieuw vastgestelde) erfelijke aandoening. Uiteraard kunt u ook patiënten verwijzen die opnieuw vragen hebben, bijvoorbeeld in het kader van kinderwens, of die de erfelijkheidsaspecten van hun diagnose niet volledig begrijpen.

Verder kunt u verwijzen bij:

- Vragen over presymptomatische (DNA-)diagnostiek bij familieleden
  - Zeker bij minderjarigen
  - Let extra op aandoeningen waarvoor presymptomatische behandeling mogelijk geïndiceerd is, zoals Alport syndroom en ADPKD
- Kinderwens: informatie rondom de herhalingskans en de mogelijkheden voor het invullen van de kinderwens. De opstellers van deze handreiking zijn van mening dat, ook bij autosomaal dominante aandoeningen met een onset op volwassen leeftijd, patiënten en familieleden met 50% kans in de vruchtbare leeftijd in elk geval geïnformeerd dienen te worden omtrent mogelijkheden voor het invullen van een kinderwens en daartoe zeer laagdrempelig verwezen dienen te worden.
  - Vragen over herhalingskans
  - Vragen over prenatale diagnostiek
  - Vragen over preïmplantatie genetische diagnostiek (PGD; zie voor korte toelichting bijlage 4 en *www.PGDNederland.nl)*

    - Dragerschapsonderzoek: voorlichting over testen van partner op dragerschap van een mutatie in het gen dat verantwoordelijk is voor de autosomaal recessieve aandoening van de patiënt. Denk er hierbij aan om te vragen naar consanguïniteit.
- NB als een aanstaande ouder – dus ook een aanstaande vader – (mogelijk) een aangeboren afwijking van de urinewegen heeft (gehad) is dat een indicatie voor een GUO (Geavanceerd Ultrageluid Onderzoek) gedurende de zwangerschap (rond 18-20 weken, met herhaling rond 32 weken). Dit is een uitgebreid echoscopisch onderzoeken. Dit is niet hetzelfde als het Structureel Echoscopisch Onderzoek (SEO - de reguliere 20 weken echo). Dit onderzoek kan door een verloskundige in gang worden gezet, maar patiënt moet hier wel op geattendeerd worden.
- Familiedonatie bij onduidelijke/mogelijk genetische nieraandoening van ontvanger
- Bij opvallend grote fenotypische variabiliteit binnen een familie met één Mendeliaanse diagnose: speelt er nog een extra erfelijke aanleg doorheen? Zie bijvoorbeeld Bergmann *et al.,* 2011.[435]

A.1.8.4 Verwijsredenen: Uitslagen van genetische diagnostiek die buiten de expertise van de aanvrager vallen

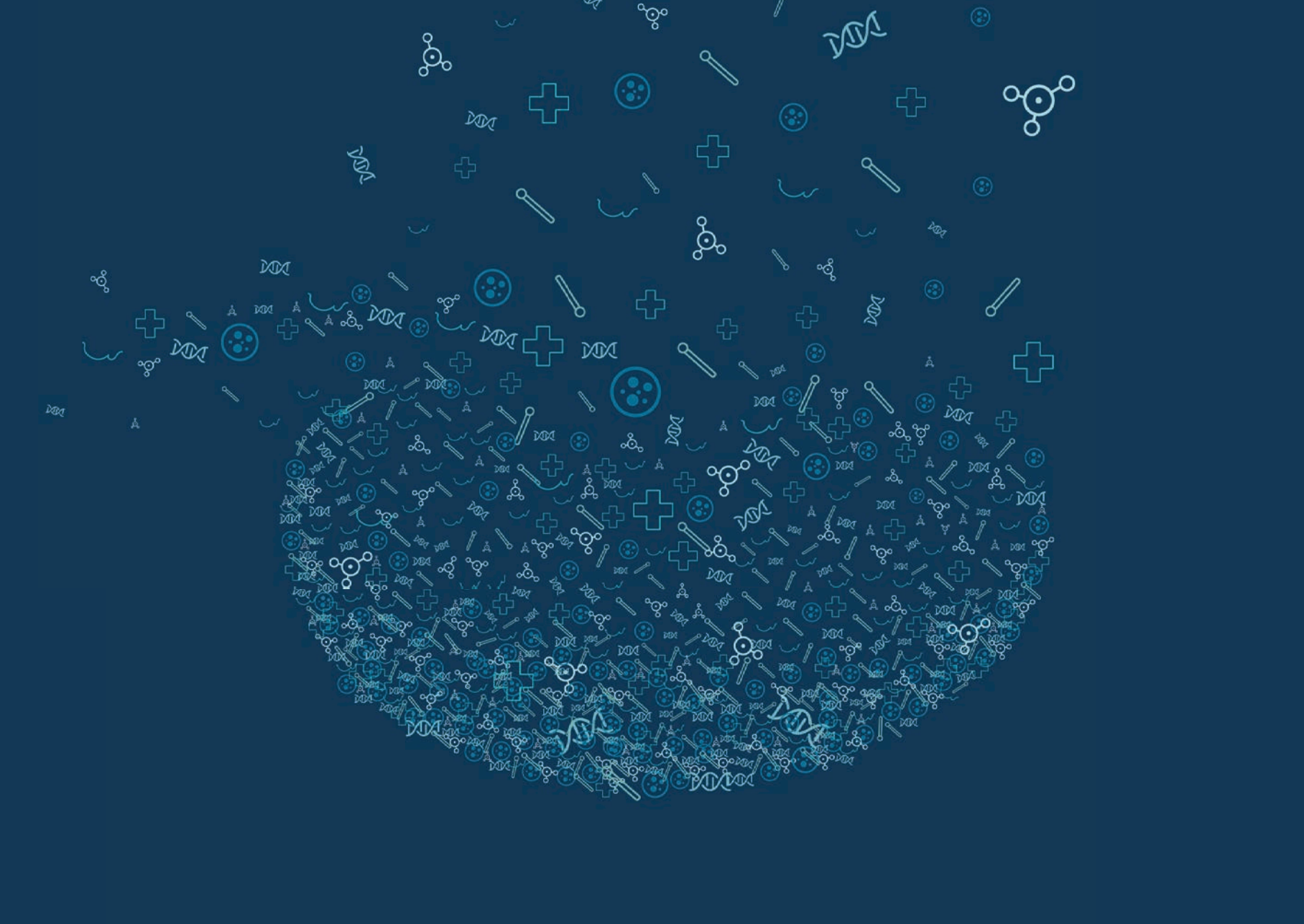
Wanneer een nefroloog genetische diagnostiek heeft aangevraagd, is het in onderstaande gevallen in elk geval aan te raden naar een klinisch geneticus te verwijzen:

- Een klinische diagnose van een autosomaal recessieve aandoening, echter er wordt maar op één allel een mutatie gedetecteerd*. Dan is verwijzing raadzaam omdat het soms toch mogelijk is om een variant op het andere allel aan te tonen en omdat ook alleen dragerschap genetische counseling nodig kan maken in het kader van bijvoorbeeld kinderwens en consanguïniteit.
- Een klinisch sterke verdenking op een erfelijke nieraandoening of urinewegafwijking, maar waar bij de gekozen genetische diagnostiek geen afwijkingen worden gevonden. In dat geval is het misschien mogelijk dat met inzetten van bredere/andere diagnostiek wel een genetische diagnose wordt gevonden.
- Als er geen pathogene mutatie, maar een VUS (zie box) wordt gevonden*, hetzelfde geldt voor CNVs met onbekend effect. NB een VUS mag niet uitgelegd worden als een diagnose.
- Als er een nevenbevinding wordt gedaan* (zie box).
- Als er aanwijzingen zijn voor een grote(re) CNV (zie box), die meer bevat dan alleen het specifieke gen van interesse* (zie ook 'Aanvullend onderzoek').

*Uitgaand van het adagium dat elke afwijkende uitslag een verwijsindicatie vormt, is deze opsplitsing enigszins arbitrair.

Bijlagen Appendix 1

Voor bijlage zie de online versie van deze handreiking.



Appendix 2

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How young researchers can and should be involved in re-shaping research evaluation

Annemijn M. Algra*, Inez Koopman*, Rozemarijn Snoek*, on behalf of Young SiT

* These authors contributed equally to this work.

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PhD students are experiencing a major evaluation gap: they are being assessed on criteria that do not match their own research goals, activities, or the role they wish to play in society.

To promote high-quality research, we need to change the incentives and rewards for career advancement in science.

It's not easy to shift deeply ingrained practices, but finding an alternative to publication numbers as a measure of scientific quality has finally become a priority for research policy.

In 2018, our thinktank, Young SiT, started a grassroots movement at our institution, the University Medical Center Utrecht (UMC Utrecht) in the Netherlands, to reduce our focus on output metrics and promote better science.

As a result, PhD candidates are no longer being evaluated primarily on bibliometrics, but also on their research competencies and professional development.

A.2.1 Involve young scientists

Young SiT takes its name from the Science in Transition (SiT) movement launched at UMC Utrecht in 2013. Its motto is "Fewer numbers, better science."

SiT has spurred a broadening of assessment criteria at our institution, for instance, by also weighing the societal impact of a scientist's work. Dutch universities as a whole have recently embraced a similar change,but they mainly impact senior scientists, allowing little involvement from young researchers.

As young scientists, we want to be involved in shaping the landscape that determines our day-to-day work, since we are the ones who will benefit most (namely, for the rest of our careers).

Young SiT's goal is to improve and speed up a transition towards more open and responsible research evaluation. Our thinktank consists of 15 young scientists, ranging from PhD candidates to associate professors, all from different disciplines.

Through consensus discussion, we choose themes that we deem both relevant and feasible to tackle, such as responsible research evaluation, public engagement, and open science.

For each theme, we organize a mini-symposium for thinktank members and invited speakers from inside and outside science, such as people working in (medical) companies, to learn from best practices and to brainstorm on how changes can be implemented in young scientists' daily research practices.

During a mini-symposium on the theme, 'Career reward systems', we concluded that young scientists would benefit from an evaluation method that promotes professional diversity and growth, instead of mainly output metrics.

A.2.2 Change the evaluation form

Dutch universities often expect PhD dissertations in medical research to consist of at least four publications. At our institution, the PhD candidate evaluation form, which lists publications, abstracts, and prizes, was the main focus of the annual assessment of PhD candidates by their supervisors.

We devised a new evaluation form, based partly on an existing PhD competence model developed by Dutch university medical centers.

Our new form asks the PhD candidate to describe their two best accomplishments, motivated by personal or societal impact. Accomplishments can range from dealing with setbacks to speaking at a conference or a patient organization event.

The new evaluation form also asks PhD candidates to self-evaluate their professional growth in research-related competencies, using the online Dutch PhD Competence Model tool.This tool focusses on competencies such as responsible conduct of science, communication, and leadership.

PhD candidates can create goals based on these competencies, which can serve as a conversation starter and a framework for their annual evaluation.

A.2.3 From idea to implementation

For the implementation of our new evaluation form, we chose the graduate program, Clinical and Experimental Neuroscience, where two of us are currently enrolled, as a starting point.

In the spring of 2019 we pitched our idea to the director, coordinator, and a representative body, and used their feedback to fine-tune and implement our evaluation form.

As of January 2020, around 200 PhD candidates in the graduate program now use this new evaluation form. This has motivated other graduate programs in the university to implement it also.

The Board of Studies of Utrecht University and PhD Council of the Utrecht Graduate School of Life Sciences are both showing interest in implementing our new PhD candidate evaluation form for all 1,800 of their PhD candidates.

Although a new evaluation form might seem like a small change, it empowers PhD candidates to discuss their professional development as part of their assessment. We believe this shows how science can benefit from grassroots movements like our Young SiT initiative.

There are many more themes and ideas that can be tackled like this, as was recently shown by the journal club initiative, ReproducibiliTea, which has set up journal clubs for researchers around the world to promote local open science communities and facilitate discussions about reproducibility of scientific work.

These initiatives show that we do not have to wait for institutions, funders, or journals to involve us. We, as young researchers, can start transforming science now.

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List of Abbreviations

±	Standard deviation
1D	One dimensional
^{1}H-NMR	Proton nuclear magnetic resonance
2D	Conventional / two dimensional
2HA	Two human influenza hemagglutinin tags
3D	Three dimensional
95%CI	95% Confidence interval
ACE	Angiotensin-converting enzyme
ACMG	American College of Medical Genetics
ADPKD	Autosomal dominant polycystic kidney disease
aHUS	Atypical Hemolytic-uremic syndrome
APTT	Activated partial thromboplastin time
AS	Alport syndrome
BMI	Body mass index
BOR	Brachio-oto-renal syndrome
CAKUT	Congenital anomalies of the kidney and urinary tract
CKD	Chronic kidney disease
CKDue	Chronic kidney disease with unknown etiology
CNV	Copy number variant
CSFK	Congenital solitary functioning kidney
Del	Deletion
DMS	Diffuse mesangial sclerosis
DNA	Deoxyribonucleic acid
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
eGFR	Estimated glomerular filtration rate
EM	Electron microscopy
ESRD	End-stage renal disease
EU	Europe
F	Female
FSGS	Focal segmental glomerulosclerosis
fUS	Fetal ultrasound
GA	Gestational age
GAMOS	Galloway-Mowat syndrome
GBM	Glomerular basement membrane
GFR	Glomerular filtration rate

HEK293T	Human embryonic kidney cells that express SV40 large T antigen
HGNC	HUGO gene nomenclature committee
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HRP	Horseradish peroxidase
IDP	Intrinsically disordered protein
IF	Immunofluorescence
Ig	Immunoglobulin
IP	Immunoprecipitation
IQR	Interquartile range
KD	Knockdown
LC/MS-MS	Liquid chromatography–mass spectrometry with two mass spectrometers
LCL	Lymphoblastic cell line
LM	Light microscopy
M	Male
m^7G	N7-methylguanosine
MCD	Minimal change disease
MGKD	Monogenic kidney disease
MLPA	Multiplex ligation-dependent probe amplification
MRD	Monogenic renal disease
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
N/A	Not applicable
NC	No consent for data retrieval
NGS	Next-generation sequencing
NICU	Neonatal intensive care unit
NOS	Not otherwise specified
NPH	Nephronophthisis
NR	Not reported
OD	Optical density
OMIM	Online Mendelian inheritance in man
OR	Odds ratio
PGD	Pre-implantation genetic diagnostics
PGT	Pre-implantation genetic testing (previously preimplantation genetic diagnostics [PGD])
PND	Invasive prenatal diagnostics

PT	Prothrombin time
RNA	Ribonucleic acid
RPN	Renal papilla number
RRT	Renal replacement therapy
SAXS	Small angle X-ray scattering
SD	Standard deviation
shRNA	Short hairpin ribonucleic acid
SiT	Science in Transition
SNP	Single nucleotide polymorphism
SNRS	Steroid-resistant nephrotic syndrome
SNV	Single nucleotide variant
SOFAST-HMQC	^{1}H-^{15}N band-selective optimized flip angle short transient heteronuclear multiple-quantum correlation
t^6A	Threonylcarbamoylation of the N6 nitrogen of the adenosine at position 37 of most ANN-accepting transfer RNAs
TC-AMP	Threonylcarbamoyl-AMP intermediate
TGP	Targeted genepanel
tRNA	Transfer ribonucleic acid
USA	United States of America
V5	Simian virus five tag
VUS	Variant of unknown significance
WES	Whole exome sequencing
WT	Wild-type
YNB	Yeast nitrogen base

A4

Nederlandse samenvatting

Ook voor niet ingewijden

De nieren zijn twee boonvormige organen die afvalstoffen uit het bloed filteren. Deze afvalstoffen vormen samen met uitgescheiden water de urine. Wanneer de nieren langzaam hun filtratievermogen verliezen, spreekt men van een chronische nierziekte. Chronische nierziekten zijn vaak progressief, dat betekent dat de nierfunctie langzaam steeds verder achteruit gaat. Uiteindelijk leidt dat bij een deel van de patiënten tot eindstadium nierfalen, dan is de nierfunctie dusdanig slecht dat een machine het bloed moet filteren (dialyse) en de patiënt een nieuwe nier nodig heeft (niertransplantatie).

Een chronische nierziekte kan veel verschillende oorzaken hebben. Het diagnosticeren van de onderliggende oorzaak van een nierziekte kan ingewikkeld zijn, omdat de verschillende oorzaken allemaal op elkaar kunnen lijken als je onderzoek doet in het bloed of een stukje nierweefsel (nierbiopt) onder de microscoop bekijkt. Het is belangrijk om de onderliggende oorzaak te diagnosticeren, omdat deze vaak bepaalt wat het verloop (prognose) van de ziekte zal zijn en welke medicatie kan helpen om het ziekteproces af te remmen.

De oorzaken van chronische nierziekte zijn zowel veel voorkomende oorzaken, bijvoorbeeld schade aan de kleine vaten in de nier bij suikerziekte, als meer zeldzame oorzaken. Een deel van de zeldzame oorzaken zijn erfelijke ziekten. Deze ziekten ontstaan door een fout in het erfelijk materiaal (genen), waardoor een eiwit (bouwsteen van de nier) niet goed of helemaal niet gemaakt kan worden. Deze fout kan doorgegeven worden aan kinderen, met als gevolg dat er soms meerdere mensen in de familie ziek zijn. Hoewel deze erfelijke ziekten op zichzelf zeldzaam zijn, vormen ze samen een belangrijke oorzaak van chronische nierziekte.

Het is belangrijk om patiënten met een erfelijke nierziekte te herkennen, zodat zij en hun familieleden de juiste zorg kunnen krijgen. Momenteel worden alleen patiënten die een verhoogde kans hebben op een erfelijke ziekte, bijvoorbeeld omdat er meer mensen in de familie ziek zijn, getest op erfelijke nierziekten (genetische test).

De toegevoegde waarde van genetisch testen bij mensen met een chronische nierziekte in de dagelijkse praktijk

Deel één van dit boek richt zich op de toegevoegde waarde van erfelijkheidsonderzoek bij mensen met een chronische nierziekte in de dagelijkse praktijk. In **Hoofdstuk 2** onderzochten we hoe vaak erfelijke nierziekten voorkomen. We toonden aan dat

ze veel vaker voorkomen dan gedacht, en dat minimaal één op de vijf mensen die voor de leeftijd van 50 jaar eindstadium nierfalen ontwikkelt een erfelijke nierziekte heeft. Één op de 15 patiënten in onze studie was door hun dokter gediagnosticeerd met een andere ziekte dan de genetische test aantoonde. Om die reden stellen we in **Hoofdstuk 2** dat het belangrijk is om bij alle patiënten waarvan verwacht wordt dat ze eindstadium nierfalen bereiken voor de leeftijd van 50 jaar op tijd een genetische test te overwegen.

Om de nierdokter in de dagelijkse praktijk handvatten te bieden voor het herkennen van patiënten met een erfelijke nierziekte en het aanvragen van de juiste genetische test, schreven we een handreiking voor gebruik in de spreekkamer (**Bijlage 1**).

Hoofdstuk 3 is een overzicht van enkele volwassen patiënten met focaal segmentale glomerulosclerose. Genetische testen bij deze patiënten lieten zien dat hun ziekte erfelijk was. We onderzochten wat de impact van de genetische diagnose was op het leven van deze patiënten. Het bleek dat er bijvoorbeeld een patiënt was die andere medicatie kon krijgen nu duidelijk was wat echt de oorzaak was van de nierziekte. Ook konden we de patiënten vertellen dat ze langer met hun transplantatienier konden doen. Normaal gesproken kan focale segmentale glomerulosclerose opnieuw actief worden na een niertransplantatie, maar bij de erfelijke vorm gebeurt dit niet.

De diagnose en prognose bij mensen met een erfelijke nierziekte

In deel twee van dit proefschrift ligt de focus bij het diagnosticeren en voorspellen van de prognose van mensen met een erfelijke nierziekte. **Hoofdstuk 4** beschrijft een nieuwe methode om met een gewone echo tijdens de zwangerschap te voorspellen wat de kans is op nierfunctieverlies na de geboorte bij baby's die in aanleg maar één werkende nier hebben. We lieten zien dat bij deze baby's er een toename is van het aantal nefronen (de kleinste functionele eenheid van de nier), en dat de nefronen niet, zoals eerder gedacht, groter worden. Het aantal nefronen heeft invloed op de nierfunctie. Door met echo een indicatie te kunnen geven van het aantal nefronen, kunnen we mogelijk in de toekomst al terwijl de baby nog in de buik van de moeder zit voorspellen hoe goed de nierfunctie zal zijn van het kind.

Voor een specifieke erfelijke nierziekte, Galloway-Mowat syndroom, zochten we in **Hoofdstuk 5** uit waar de fout zit in het erfelijk materiaal dat deze ernstige ziekte veroorzaakt. Bij meerdere patiënten vonden we fouten in *GON7* en *YRDC*. Deze genen coderen voor bouwstenen (tRNA) die belangrijk zijn voor het functioneel maken van weer andere eiwitten. Met experimenten in het laboratorium toonden we aan dat de fouten die onze patiënten hadden, ervoor zorgen dat het proces van

tRNA functioneel maken niet meer goed gaat en uiteindelijk daarmee tot Galloway-Mowat syndroom leiden.

Door het onderzoek in **Hoofdstuk 6** weten we nu dat de erfelijke nierziekte nefronoftise niet, zoals altijd gedacht, alleen maar voorkomt bij kinderen maar juist ook een belangrijke oorzaak is van nierfalen op de volwassen leeftijd. We kwamen hier achter door een genetische test te doen bij meer dan 5500 niertransplantatiepatiënten. Daarbij bleek bij één op de 200 patiënten een belangrijk nefronoftise-gen helemaal verdwenen te zijn uit het erfelijk materiaal. Dat deze 'kindernierziekte' ook bij oudere volwassenen voorkomt, is een belangrijk bewijs dat erfelijke nierziekten op alle leeftijden kunnen voorkomen.

Impact van een erfelijke nierziekte op het invullen van een kinderwens

Als je dokter weet dat je een erfelijke nierziekte hebt, kan hij de prognose soms beter voorspellen, andere medicatiekeuzes maken en er bijvoorbeeld voor zorgen dat je alleen een transplantatienier krijgt van een familielid dat niet zelf ook ziek is. Het hebben van een erfelijke nierziekte betekent geregeld ook dat je de ziekte door kan geven aan je kinderen. Om die reden heeft een erfelijke nierziekte een impact op hoe nierpatiënten kiezen hun kinderwens in te vullen.

Een chronische nierziekte verhoogt de kans op complicaties tijdens de zwangerschap. Zo heeft een vrouw met een nierziekte een grotere kans op zwangerschapsvergiftiging, een te klein kind bij de geboorte, maar ook kan de nierfunctie achteruitgaan voor een zwangerschap. Nierpatiënten met een kinderwens kunnen daardoor voor ingewikkelde keuzes komen te staan. Bijvoorbeeld: accepteer ik de kans op achteruitgang van mijn nierfunctie, waardoor ik misschien wel moet dialyseren, zodat ik zwanger kan zijn? Het is de taak van artsen patiënten goed te informeren over de risico's, en in **Hoofdstuk 7** wordt beschreven welke thema's daarbij aan bod dienen te komen en voor welke dilemma's ook de dokter kan komen te staan. We beschrijven welke dingen de arts kan doen, bijvoorbeeld wat betreft gesprekshouding en dat overleg met verschillende andere specialisten kan helpen om de patiënte een goed geïnformeerde keuze te laten maken.

Het mooiste is wanneer een patiënte op haar situatie toegespitste informatie kan krijgen over haar kans op zwangerschapscomplicaties. Er is echter nog te weinig onderzoek gedaan naar de zwangerschapsuitkomsten bij specifieke erfelijke nierziekten om daar voor die patiënten wat over te kunnen zeggen. **Hoofdstuk 8** is het resultaat van een onderzoek dat we deden naar de zwangerschapsuitkomsten bij vrouwen met Alport syndroom. We ontdekten dat voor vrouwelijke Alport patiënten de kans op zwangerschapscomplicaties lager is dan bij vrouwen met

andere chronische nierziekten, waardoor we in de toekomst deze groep patiënten beter kunnen informeren voorafgaand aan een zwangerschap en hen tijdens een zwangerschap beter kunnen begeleiden.

Patiënten met een erfelijke nierziekte en een kinderwens hebben de mogelijkheid om te voorkomen dat ze deze nierziekte doorgeven aan hun toekomstig kind. Dit kan op verschillende manieren, in **Hoofdstuk 9** geven we een overzicht van de pre-implantatie genetische test (PGT) voor erfelijke nierziekten in Nederland. Met PGT wordt een reageerbuisembryo getest op de aanwezigheid van de erfelijke ziekte, en alleen embryo's die de erfelijke ziekte niet hebben worden in de baarmoeder geplaatst. Hierdoor is de kans dat de baby de erfelijke ziekte draagt bijna nul. Voor dit hoofdstuk onderzochten we 98 paren die voorgelicht waren over PGT, waarvan ongeveer de helft uiteindelijk ook koos om PGT te doen. Een groot deel daarvan was succesvol en resulteerde in een kind zonder de erfelijke ziekte. Ongeveer de andere helft van de voorgelichte patiënten besloot om niet voor PGT te kiezen, met als belangrijkste reden dat ze niet wilden wachten op de lange PGT procedure, die gemiddeld zo'n 2 jaar duurt. Ook zagen we een toename in PGT voor nierziekten die pas op volwassen leeftijd de eerste verschijnselen geven. We concludeerden dat het belangrijk is dat patiënten goed geïnformeerd worden over de mogelijkheid van PGT, zodat ze de keuze kunnen maken die het beste bij hen past.

Conclusies van dit proefschrift

Het werk in dit proefschrift laat zien dat er een toegevoegde waarde is van (op tijd) genetisch testen bij patiënten met een chronische nierziekte, wat ik uitgebreid bediscussieer in **Hoofdstuk 10**. Ondanks het feit dat erfelijke nierziekten vaak voorkomen, worden ze vaak niet goed of niet tijdig gediagnosticeerd. Dit terwijl de diagnose van een erfelijke ziekte een levenslange impact heeft op het welzijn van en de zorg voor de nierpatiënt. Meer kennis over erfelijke nierziekten zal er hopelijk toe leiden dat meer patiënten herkend worden, waardoor zij betere zorg kunnen krijgen, ook rondom het invullen van een kinderwens.

Tot slot: ik heb veel geleerd van het uitvoeren van de onderzoeken beschreven in dit boek. Hoewel dit boek bestaat uit de artikelen die voortvloeiden uit mijn werk, ben ik ook als persoon gegroeid. Helaas worden onderzoekers momenteel vaak alleen beoordeeld op het aantal artikelen, en staat de persoonlijke ontwikkeling minder centraal. Om dat te veranderen, heeft een groep jonge onderzoekers, waar ik deel van uitmaakte, een nieuw beoordelingsformulier ontwikkeld voor promovendi aan onze universiteit (Universiteit Utrecht). Dit formulier biedt de ruimte aan het ontwikkelen van bredere competenties. We hopen dat dit gaat bijdragen aan de persoonlijke ontplooiing van promovendi (**Bijlage 2**).

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*These authors contributed equally to this work.

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Curriculum vitae

Rozemarijn Snoek was born on December 22nd 1992 in Alkmaar. At three weeks old she moved with her parents to Managua, Nicaragua for the purposes of her parents' work. She enjoyed Kindergarten at the German primary school Colegio Alemán Nicaragüense.

After moving back to the Netherlands with her parents and her sister, they settled in Utrecht. Rozemarijn graduated *cum laude* from grammar school at the Christelijk Gymnasium Utrecht, where she was student body president for three years.

In 2010 Rozemarijn started her study of Medicine at Utrecht University. She was student body president and obtained her Bachelor's degree in 2013. During her Masters, she did several clinical internships at the department of Obstetrics in the Wilhelmina Children's Hospital and was an extracurricular research assistant at that department for two years, under the supervision of dr. Roel de Heus. Rozemarijn obtained a *cum laude* Master's degree in Medicine in September 2016.

In October 2016 Rozemarijn started her doctoral research, which resulted in this thesis, at the department of Clinical Genetics of the University Medical Center Utrecht. The aim of these studies was to elucidate genetic causes for renal disease, in order to improve their diagnostic trajectory and provide reproductive options when patients want to start a family. During this time, she was supervised by prof. dr. Nine Knoers, dr. Albertien van Eerde and dr. Titia Lely. Rozemarijn took part in a two-year PhD curriculum (*TULIPS*), focused on developing personal leadership in science. She was also a member of the *Young Science in Transition* thinktank, which aims to provide young scientists with the tools to make changes to the scientific landscape.

After the completion of this thesis, Rozemarijn continues her clinical training as a resident (ANIOS) in Gynaecology and Obstetrics at the Elizabeth Tweesteden Hospital in Tilburg. Rozemarijn currently lives in Utrecht with her husband Maarten.