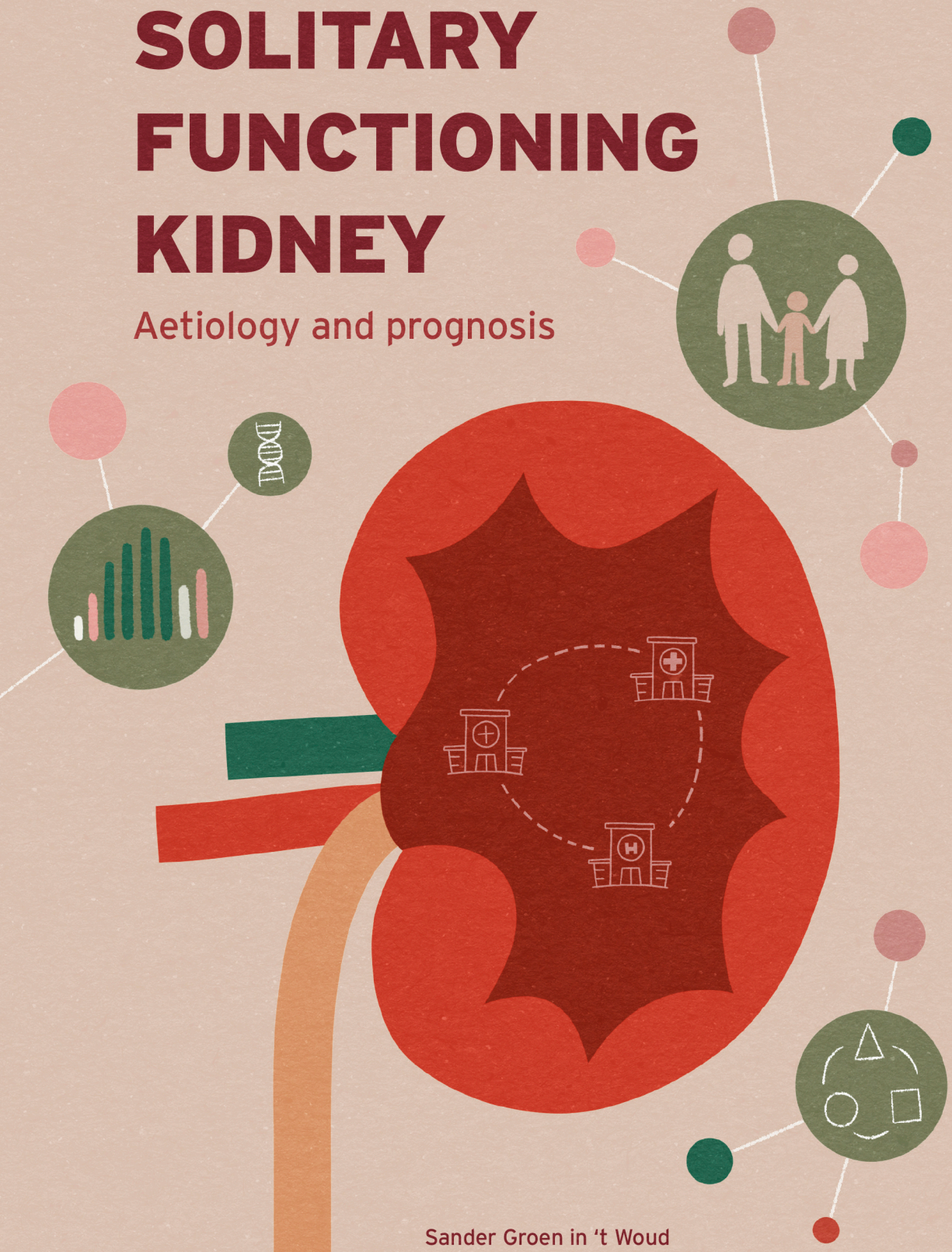
SOLITARY FUNCTIONING KIDNEY

Aetiology and prognosis



Sander Groen in ‘t Woud

Solitary functioning kidney: Aetiology and prognosis

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The work in this thesis was carried out within the Radboudumc Graduate School, at the department for Health Evidence and the department of Paediatric Nephrology of the Radboud university medical center in Nijmegen, the Netherlands

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CHAPTER 1

General introduction and outline of this thesis

BACKGROUND

Normally, children are born with two functioning kidneys, which are important for fluid homeostasis, electrolyte levels, and filtration and excretion of waste products. These functions are mainly carried out by nephrons, functional units of which each kidney contains between 200,000 and 2,500,000.[1] Nephrons consist of a glomerulus, from which blood is filtered, and a tubule, in which the filtrate is processed into urine using excretion and resorption.[2] An important measure of kidney function is the glomerular filtration rate (GFR), the amount of blood plasma that is cleared of a substance (often creatinine) per time unit. To maintain a normal GFR, crosstalk between the tubuli and glomeruli (*i.e.* tubuloglomerular feedback) is vital.[3] The kidneys are also important hormone secreting organs, a function that is largely independent of the number of nephrons. Malfunctioning of the kidneys will lead to chronic kidney disease (CKD) over time, and may also result in cardiovascular disease and other complications.[4,5]

Embryology

Development of the kidneys and urinary tract stretches from the third to the 36th week after conception.[6-9] First, the pronephros is formed, which regresses and is replaced by the mesonephros. Development of the final kidney or metanephros starts in week five after conception with the appearance of the ureteric bud on day 28, followed by the invasion of the metanephric mesenchyme on day 32.[7] Both processes are crucial and disruption may lead to agenesis of the kidney.[8] After invasion of the metanephric mesenchyme, branching morphogenesis (*i.e.* repetitive bifurcation) of the ureteric bud results in formation of the collecting system and ureter.[7,10] Cells from the metanephric mesenchyme develop into nephrons and fuse with the developing collecting system into functional nephric unit.[7] Disturbances in branching morphogenesis or its interaction with the metanephric mesenchyme may result in multicystic dysplastic kidney (MCDK) or kidney hypo/dysplasia (KHD).[11,12]

Unilateral kidney agenesis (UKA), MCDK, and KHD may all result in a congenital solitary functioning kidney (CSFK). CSFK is part of a spectrum of congenital anomalies referred to as congenital anomalies of the kidney and urinary tract (CAKUT). Children may also acquire a solitary functioning kidney (SFK) after a nephrectomy, which can be required because of benign (*e.g.* malfunctioning kidney leading to hypertension) or malign (*e.g.* Wilms tumour) conditions.

Prevalence

The prevalence rates of the most common causes of CSFK, which are UKA and MCDK, are 1:2,200 live-births[13-18] and 1:4,200 live-births,[19-30] respectively, while the prevalence of CSFK regardless of the cause is estimated to be 1:1,500 live-births.[31] The prevalence

of an acquired solitary functioning kidney (ASFK) in children is less well known. Each year, more than 2,000 nephrectomies in children are performed in the US.[32] With approximately 3.6 million childbirths annually, this comes down to a prevalence of 1:1,800 children. If we extrapolate these numbers to The Netherlands, approximately 100 children are born with CSFK annually and approximately 100 children will undergo a nephrectomy resulting in ASFK.

Aetiology of congenital solitary functioning kidney

The aetiology of CSFK has not been studied thoroughly so far, as most studies include multiple phenotypes from the CAKUT spectrum simultaneously. This is driven by the observation that identical genetic mutations can lead to different subtypes,[33] which indicates a shared aetiology with variable expressivity, by practical considerations, such as higher statistical power to detect associations with larger cohort sizes, and by insufficient clinical information to differentiate among subtypes. All anomalies of the CAKUT spectrum are hypothesized to have a multifactorial aetiology, because both genetic and environmental causes have been identified.[34] Although the heritability of CSFK is unclear, studies on children with vesicoureteral reflux (VUR), which is also part of the CAKUT spectrum, showed higher concordance rates among monozygotic twins (80%) compared to dizygotic twins (35%), supporting a strong genetic component.[35]

The lack of studies focussing on CSFK only could be misleading, since our group and others have shown that both environmental and genetic risk factors may differ among CAKUT subtypes, which necessitates stratified analyses.[36-38] If different mechanisms play a role, studies combining subtypes of CAKUT may have lower statistical power to detect specific associations and/or may detect associations that do not pertain to all subtypes. Therefore, all aetiological studies presented in this thesis were focussed on patients with CSFK only. To align with other studies, we included patients with UKA, MCDK, and KHD in our genetic studies, whereas we focussed on patients with UKA or MCDK in our study on environmental risk factors.

Genetic aetiology

Studies into the genetic aetiology of CSFK have led to the awareness that multiple inheritance pathways and aetiological mechanisms may play a role.[33,34,39-41] As stated before, CSFK is often studied in the context of CAKUT, and several observations that have been important for understanding CAKUT aetiology can also be applied to CSFK. Firstly, CSFK occurs relatively frequently, suggesting that the developmental process of the kidney is either prone to disturbance by factors that are relatively common, or sensitive to many different disturbances that may or may not be rare. Secondly, most CSFK cases are sporadic, but familial occurrence is not uncommon. Whereas Belk *et al.* found that none of 94 first-degree relatives of 29 patients with MCDK had congenital kidney abnormalities,[42] Wu *et al.* reported a family history of CSFK in 9/86 patients

with UKA,[43] and McPherson found that 20% of patients with UKA or KHD had at least one affected family member, with the highest risk (12%) for offspring of patients.[44] In a Turkish cohort with a high rate of consanguinity, Bulum *et al.* identified that 11/19 (58%) patients with KHD had an affected first degree relative, compared to 1/10 patients with UKA (10%) and 1/11 (9%) patients with MCDK.[45] This suggests that monogenic causes play a role in at least part of the CSFK cases. Another important observation is that CSFK mostly occurs in isolated form, but extrarenal anomalies may be found in up to one third of patients[17] and CAKUT anomalies can be part of over 200 syndromes.[41] This broadens the list of candidate genes for CSFK beyond those directly involved in kidney and urinary tract development. Lastly, CAKUT penetrance is incomplete and several subtypes can be seen within families, which suggests that genetic modifiers and interactions with environmental factors may be relevant for the aetiology of all CAKUT subtypes.[33,34,40]

A few studies have attempted to identify rare monogenic causes in CSFK-specific cohorts or reported their results stratified per CAKUT subtype. Wu *et al.* identified a pathogenic variant in 9/86 (11%) patients with UKA using targeted exome sequencing of 25 genes.[43] Using whole-exome sequencing in 163 CAKUT trios, Lei *et al.* found *de novo* pathogenic or likely pathogenic variants in 4/36 (11%) foetuses with MCDK and 1/16 (6%) foetuses with UKA.[46] Lastly, Liu *et al.* solved 1/21 (5%) of foetuses with isolated MCDK by performing whole-exome sequencing after chromosomal microarray analysis had returned negative results.[47] The number of solved cases in these CSFK-specific cohorts is in line with results from cohorts including a broader range of CAKUT subtypes (6-20%).[48] Copy-number variations (CNVs) also play an important role in the aetiology of CSFK. This was first identified in a cohort of 192 patients with KHD and later confirmed in a different cohort of CSFK patients, with known copy-number disorders identified in 11% and 8% of patients, respectively.[49,50] Although future studies will undoubtedly identify additional rare monogenic causes of CSFK, the low number of patients for whom a monogenic cause can be found indicates that other genetic mechanisms are also likely to be involved.

Indeed, the group of Sanna-Cherchi showed that common variants may also play a role in the aetiology of CAKUT, when they performed a genome-wide association study (GWAS) in almost 1,400 patients with VUR and discovered three genome-wide significant loci.[51] This group also published preliminary results of a GWAS on almost 3,000 cases with several CAKUT subtypes.[52] One statistically significant association within the *CHODL* gene was reported, as well as several loci reaching thresholds for suggestive statistical significance. Interestingly, the effect sizes of the variants in the CAKUT-wide study varied between 1.2 and 1.4, whereas the VUR-specific study found effect sizes in the range of 1.4-6.9.[51,52] This could indicate that larger effect sizes can be found when homogeneous study populations are used. Nonetheless, a GWAS on CSFK-specific populations has not yet been published.

All of the studies described above focussed on germline genetic variants (*i.e.* variants that were present at the conception and found in all cells), whereas postzygotic genetic factors (*i.e.* variants that occurred after conception and are present in only part of the cells) could be involved as well. In a case-report on a 30-year old monozygotic twin pair discordant for UKA, Jin *et al.* found differential methylation for 10 pathways and 25 genes, which prompted the authors to suggests that environmental factors may have led to the defect in the affected twin.[53] The authors also searched for differential single-nucleotide polymorphisms, postzygotic mutations, and CNVs, but did not discover any. Since discordant monozygotic twins are a promising but rare opportunity to study aetiological mechanisms, finding and studying such twin pairs could further elucidate CSFK aetiology.

Environmental risk factors

Several previous investigations focussed on the role of environmental risk factors in the aetiology of CSFK and identified maternal diabetes,[54-61] obesity,[56] alcohol use,[55,62] and age[55] as potential risk factors. For maternal diabetes and obesity, the causal pathways presumably include a high glucose level during embryonic kidney development.[56,63] As such, diabetes has to be present in early pregnancy, which means that pre-existing diabetes is the strongest suspect. When considering the effect of maternal alcohol use, the dose seems to be an important factor. Both Moore *et al.* and Martinez-Frias *et al.* found a dose-response relationship between the amount of alcohol consumed and the occurrence of kidney defects, with no effect or even a slightly decreased risk for low doses and an increased risk for higher doses of alcohol or binge drinking.[62,64] For maternal age, results in the literature are conflicting, with one study identifying an increased risk for mothers <18 years of age,[55] whereas others found the lowest risk for mothers younger than 20 years.[65]

Researchers investigating environmental risk factors for congenital anomalies face several major challenges, which include identifying a sufficiently large number of cases with a similar diagnosis, classifying exposures in the aetiologically relevant time window adequately, identifying and adjusting for the relevant confounders, and handling missing data.[66] Differences in methods to deal with these challenges are likely responsible for the heterogeneity in the environmental risk factors reported to be associated with CSFK so far. Therefore, more research in larger populations with detailed data collection is needed to clarify the exact role of environmental risk factors in the aetiology of CSFK.

Gene-environment interactions

Lastly, interactions between genetic and environmental factors are expected to explain some of the complex aetiology of congenital anomalies,[67] although such interactions have not been studied in the aetiology of CSFK or other CAKUT phenotypes. Successful examples in the field of congenital anomalies include interactions between genetic variants and multivitamin use in the aetiology of cleft lip/palate[68] and interactions

between genetic variants and maternal smoking in the aetiology of congenital heart defects.[69] To study the role of gene-environment interactions in CSFK aetiology as well, large cohorts of children with CSFK are needed with access to DNA and data on exposure to environmental factors.

Long-term outcomes of CSFK

In healthy adults, the absolute risk of living with an SFK remains low enough to allow for donation of a kidney.[70,71] For a long time, this was extrapolated to children and living with an SFK from childhood was thought not to lead to substantial long-term consequences.[72,73] This paradigm changed with reports of hypertension and microalbuminuria in children with CSFK[74] and was strengthened by a study which showed that 30% of children with CSFK had developed kidney failure by the age of 30.[75] Subsequent studies confirmed the higher prevalence of hypertension, (micro)albuminuria or proteinuria, and a decreased GFR (summarized under the term kidney injury) in children with CSFK, although large differences remained in the estimated risks.[76-83]

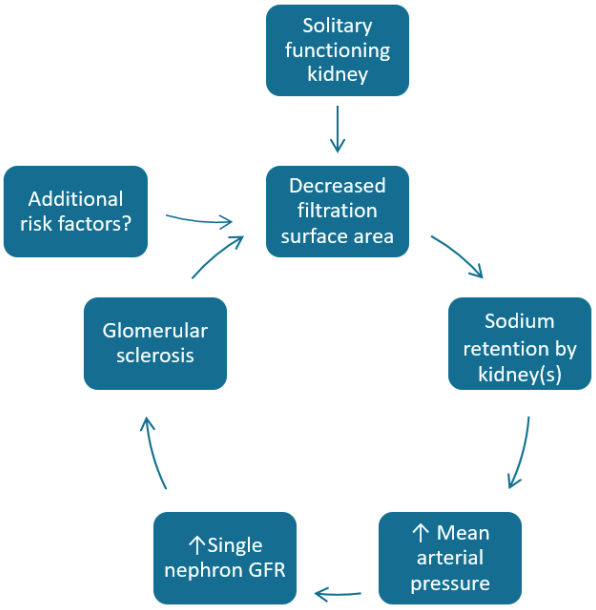


Figure 1 Mechanisms leading to hyperfiltration injury according to the Brenner hypothesis[84]

Kidney injury in children with SFK or other causes of a low nephron number is thought to be driven by glomerular hyperfiltration. This hypothesis was introduced by Brenner *et al.* in 1988 based on several lines of evidence.[84] A crucial observation for the Brenner hypothesis was that nephrogenesis is completed at birth and no additional nephrons can be formed afterwards.[85] Furthermore, they incorporated findings by Hayman *et*

al., who noted that all their patients with a normal blood pressure had at least 700,000 glomeruli per kidney.[86] Brenner *et al*. postulated that in individuals with a low nephron number, the filtration surface area is decreased, leading to sodium retention and a subsequent increase in single nephron GFR (Figure 1). Although this is beneficial for maintaining the overall GFR in the short-term, it also leads to glomerular sclerosis and a further reduction in filtration surface area. This vicious cycle could ultimately lead to kidney failure, although the speed of progression is dependent on several determinants. The Brenner hypothesis is still applied to explain the high prevalence of kidney injury in SFK patients. It remains unknown, however, which factors influence the risk of kidney injury in children with SFK and how these risk factors can be used to tailor clinical management. As the amount of literature on long-term outcomes of SFK is very limited, new studies focussing on risk factors and clinical management are highly warranted.

AIMS, DATA SOURCES, AND OUTLINE OF THIS THESIS

Aims

Parents of children with SFK often face a lot of uncertainty. In case of CSFK, the underlying aetiology is unknown in 80-90% of patients,[39] which may leave parents with questions regarding the risk for future offspring and whether they can influence this risk, *e.g*. with their health behaviour or lifestyle. Therefore, the first aim of this thesis was to get more insight into the genetic and environmental causes of CSFK.

Both CSFK and ASFK can lead to long-term complications, with higher rates of hypertension, proteinuria, and a reduced estimated GFR (eGFR) reported.[76-83] The proportion and characteristics of patients that will develop these complications are unclear, which may add to the uncertainty of parents and patients. Additionally, both under- and overtreatment may occur, leading to avoidable health damage and/or unnecessary costs. Therefore, the second aim of this thesis was to obtain more knowledge on the long-term consequences of living with SFK from childhood, with the ultimate goal to improve the clinical management of these children.

Research questions

To achieve the aims of this thesis, the following research questions were formulated:

Research question	Chapter
Part I: Aetiology of congenital solitary functioning kidney	
Are common single nucleotide variants involved in the aetiology of congenital solitary functioning kidney?	2
Can postzygotic mutations be identified in monozygotic twins discordant for congenital solitary functioning kidney?	3
Which environmental risk factors are involved in the aetiology of congenital solitary functioning kidney?	4
Can gene-environment interactions explain part of the unknown aetiology of congenital solitary functioning kidney?	5
Part II: Outcomes and management of children with solitary functioning kidney	
What is the optimal management of children with a congenital solitary functioning kidney based on the current literature?	6
Is the rate of kidney injury different between children undergoing a nephrectomy for a benign or a malign indication?	7
What is needed for the development of risk-based care for children with a solitary functioning kidney?	8
Which are the risks of and risk factors for kidney injury in children with a solitary functioning kidney?	9

Data sources

To answer these research questions, a large cohort of children with SFK was needed, for which the AGORA (Aetiologic research into Genetic and Occupational/environmental Risk factors for Anomalies in children) data- and biobank was an excellent starting point.[87] The AGORA data- and biobank was initiated in 2004 by researchers and clinicians from the Amalia children's hospital, the Department for Health Evidence, and the Department of Human Genetics at the Radboud university medical center to facilitate research into the causes and consequences of congenital anomalies. It currently contains clinical information, DNA samples, and questionnaire data for more than 5,000 patients with a congenital malformation and their parents, while data collection is still ongoing. Recruitment of patients takes place at the Radboud university medical center (from 2004 onwards) and the University Medical Center Utrecht (from 2013 onwards). Furthermore, healthy controls were recruited in 2011 and 2021 by asking multiple Dutch municipalities to draw a random sample of children in the age range of the cases. Parents of these children were asked to fill out the AGORA questionnaire and DNA samples were collected from a subset of 750 healthy children and their mothers.

Since the number of patients with SFK in the AGORA data- and biobank was insufficient for the intended studies in this thesis, the number of participating patients was increased by recruiting patients with SFK from the medical registries of the Radboud university medical center and 35 other academic and non-academic hospitals throughout the Netherlands (Figure 2). This led to the SOFIA (SOlitary Functioning kIdney: Aetiology and prognosis) study, which included almost 1,000 children with SFK used for the studies described in this thesis.

Figure 2 Hospitals participating in patient recruitment for the SOFIA study

Outline

Part I: Aetiology of congenital solitary functioning kidney

In the first part of this thesis, the aetiology of CSFK is subject of investigation. A strong genetic component is expected in the aetiology of CSFK and many studies have searched for rare variants or CNVs, but none investigated the role of common variants. Therefore, we decided to use our study population for a genome-wide association study, described in **Chapter 2**. For this study, we selected a homogenous subgroup of CSFK patients to search for single nucleotide polymorphisms associated with the development of CSFK. In addition, the inclusion of two monozygotic twins discordant for CSFK in the SOFIA study facilitated a study on postzygotic mutations. Using deep exome sequencing, we searched for variants present in the affected child but not in

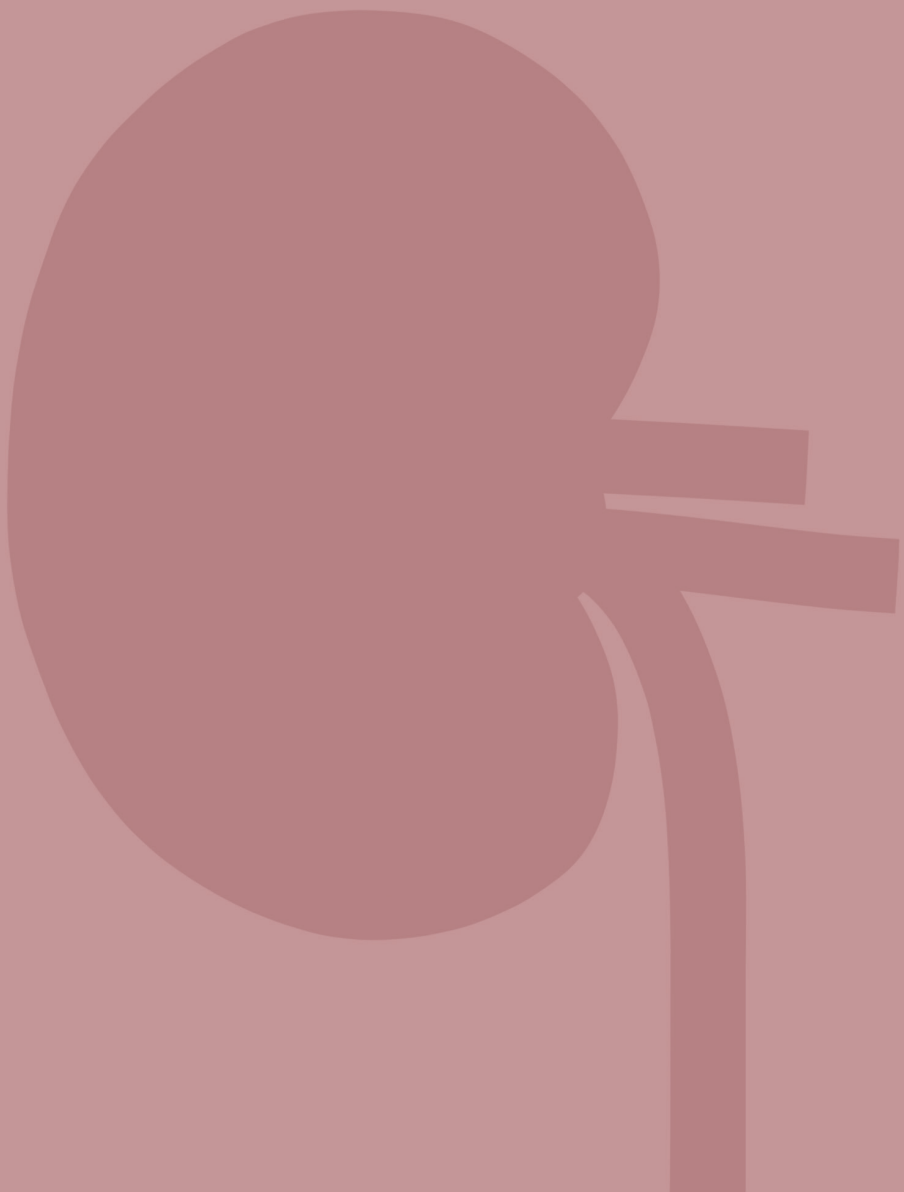
the unaffected sibling. The results and possible implications of this study are described in **Chapter 3**.

As described previously in this introduction, the exact role of environmental risk factors in the aetiology of CSFK is still unknown. In **Chapter 4**, the goal was to identify environmental and other relevant risk factors for CSFK. Replication of some of the previously reported associations was attempted, while several risk factors not studied in relation to CSFK before were investigated using a case-control study with maternal questionnaire data. After separately investigating the role of genetic and environmental risk factors for CSFK in chapters 2 and 4, respectively, these data were used as input for the gene-environment interaction analyses described in **Chapter 5**. For this study, a number of relevant environmental factors was selected and combined with the available genetic data.

Part II: Outcomes and management of children with solitary functioning kidney
Part two of this thesis focusses on the consequences of living with SFK from childhood and optimizing the management strategy. Since the prevalence of kidney injury differs greatly between cohorts of SFK patients, various guidelines with different strategies for long-term management have appeared.[88-90] In **Chapter 6**, the current literature on outcomes of children with CSFK is summarized to provide recommendations for initial clinical management and long-term follow-up of these children. At the same time, limited evidence was available for the long-term management of children with ASFK. In the systematic review and meta-analysis described in **Chapter 7**, the aim was to find out whether kidney injury rates differed based on the indication for nephrectomy, and to learn whether this could be a factor for stratification of follow-up care.

Appropriate care for children with SFK can only be delivered with continuous efforts to identify factors that influence the risk of kidney injury. Ideally, this care is tailored to an individual patient's risk profile, instead of the one-size-fits-all approach that is still common. **Chapter 8** highlights our vision on the care for children with SFK in the future, and describes potential risk factors that could be involved in the risk of kidney injury in these children. One of the important steps towards more personalized care for patients with SFK can be made by investigating risk factors in large cohorts with a long duration of follow-up. Such a cohort was created in the SOFIA study, which includes the largest cohort of children with SFK gathered worldwide. In **Chapter 9**, the risk of and risk factors for kidney injury are investigated using this population.

In the final chapter of this thesis, **Chapter 10**, some key methodological aspects of the studies in this thesis are discussed. Furthermore, the findings are put into the context of previous research, including a discussion on their clinical implications. Lastly, directions for both clinical care and future research are provided.



PART I

Aetiology of congenital solitary functioning kidney

CHAPTER 2

A genome-wide association study into the aetiology of congenital solitary functioning kidney

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ABSTRACT

Congenital solitary functioning kidney (CSFK) is a birth defect which occurs in 1:1,500 children and predisposes to kidney injury. Its aetiology is likely multifactorial. In addition to known monogenic causes and environmental risk factors, common genetic variation may contribute to susceptibility for CSFK. We performed a genome-wide association study among 452 patients with CSFK and two control groups of 669 healthy children and 5,363 unaffected adults. Variants in two loci reached the genome-wide significance threshold of $5x\ 10^{-8}$ and variants in 30 loci reached the suggestive significance threshold of $1x\ 10^{-5}$. Of these, an identified locus with lead single nucleotide variant (SNV) rs140804918 (odds ratio 3.1 , p-value = $1.4x10^{-8}$) on chromosome 7 was most promising due to its close proximity to *HGF*, a gene known to be involved in kidney development. Based on their known molecular functions, both *KCTD20* and *STK38* could explain the suggestive significant association with lead SNV rs148413365 on chromosome 6. Our findings need replication in an independent cohort of CSFK patients before they can be established definitively. Our analysis suggests, however, that common variants play a role in CSFK aetiology. Future research could enhance our understanding of the molecular mechanisms involved.

INTRODUCTION

Living with a solitary functioning kidney from childhood predisposes to kidney injury later in life, with up to 80% of children with a solitary functioning kidney showing signs of kidney injury at 18 years of age.[91] Moreover, 20-40% of these children may develop end-stage kidney disease by the age of 30.[75] Even in less severely affected individuals, signs of kidney injury that develop during childhood can result in a higher risk of cardiovascular disease later in life.[5]

A congenital solitary functioning kidney (CSFK) is most often the result of developmental kidney anomalies, such as unilateral kidney agenesis (UKA), multicystic kidney dysplasia (MCDK), or kidney hypo/dysplasia (KHD). These anomalies fall within a spectrum of anomalies referred to as congenital anomalies of the kidney and urinary tract (CAKUT), which are thought to partly have a shared aetiology.[92] Several causative mechanisms may play a role in the aetiology of CAKUT: environmental risk factors, monogenic causes, pathogenic copy number variants (CNVs), and common genetic variants.[34]

Environmental risk factors, such as maternal diabetes, overweight, and use of artificial reproductive technologies have been shown to play a role in CAKUT aetiology.[36,93,94] For CSFK specifically, our group identified important roles for maternal stress and infections during pregnancy, as well as a protective effect of folic acid supplementation, in addition to previously identified factors such as conception using in vitro fertilization/ intracytoplasmic sperm injection, maternal smoking during pregnancy, and older maternal age.[95] Monogenic causes for CAKUT have been discovered in over 150 different genes and this number keeps rising due to increased availability and decreased costs of exome sequencing.[48,96] Approximately one third of these genes have been associated with isolated forms of CAKUT, whereas the other genes with defects in multiple organ systems.[48] Studies performing whole exome sequencing (WES) in patients with sporadic and isolated CSFK are rare, and the yield is relatively low compared to non-isolated or familial CSFK.[47] Wu *et al.* and Lei *et al.* identified causative variants in 11% of mostly sporadic and isolated patients with UKA and MCDK, respectively, and Liu *et al.* solved 7% of patients with isolated UKA or MCDK using a combination of chromosomal microarray analysis and WES.[43,46,47] CNVs also play an important role in the aetiology of CAKUT. This was first identified in a cohort of 192 patients with KHD and later extended to other CAKUT phenotypes, including CSFK.[38,49,50] As CAKUT is in between common and rare disorders, low-frequency variants with intermediate effects may explain an important fraction of its genetic aetiology.[97] Nonetheless, genome-wide association studies (GWAS) have rarely been performed for CAKUT because of the large cohorts needed. A GWAS including almost 1,400 patients with vesicoureteral reflux (VUR) identified three loci that reached genome-wide significance, with odds ratios ranging from 1.4 to 3.7,[51] whereas a previous GWAS on 500 VUR patients and a GWAS including 756 patients with posterior urethral valves (PUV) failed to identify

genome-wide significant loci.[98,99] In contrast, two genome-wide significant variants were identified in a GWAS on whole genome sequencing data from 132 PUV patients: a common variant with a minor allele frequency (MAF) of 0.2 and odds ratio (OR) of 0.4 near *TBX5*, and a rare variant with a MAF of 0.05 and OR of 7.2 near *PTK7*.[100] Up to now, no GWAS has been published for CSFK patients. To fill this knowledge gap and investigate the role of common genetic variants in the aetiology of CSFK, we performed a genome-wide association study in a Dutch cohort of patients with CSFK.

PATIENTS AND METHODS

Patients

Patients with CSFK, defined as a solitary functioning kidney resulting from UKA, MCDK or KHD, were derived from the AGORA (Aetiologic research into Genetic and Occupational/environmental Risk factors for Anomalies in children) data- and biobank.[87] This data- and biobank is coordinated from the Radboud university medical center Amalia children's hospital and contains clinical information, DNA samples, and questionnaire data from patients with congenital malformations and their parents. Patients were recruited prospectively during a visit to the Department of Paediatric Nephrology or Division of Pediatric Urology of the Amalia children's hospital or University Medical Center Utrecht, or retrospectively in one of 36 Dutch hospitals participating in the SOFIA (Solitary functioning kidney: aetiology and prognosis) study.[91] For the current GWAS, we selected patients born from January 1st 1993 through December 31st 2020 from whom DNA was available. Patients with either a known genetic cause or a syndrome (with or without molecular diagnosis) were excluded from the analyses.

Two healthy control populations were used. In 2011, 39 Dutch municipalities selected random samples of 150 or 300 inhabitants born in 1990-2010 for the AGORA data- and biobank. These children and their parents were invited to participate in AGORA via regular mail. Over 2,000 families filled out a questionnaire, while mothers and children from a subsample of 748 families donated a saliva sample for DNA isolation as well. Because of this relatively small control population available in the AGORA data- and biobank, we included a second control population of 6,468 participants who donated blood samples for the Nijmegen Biomedical Study (NBS).[101] NBS is a population-based study carried out by the Departments for Health Evidence, Laboratory Medicine, and Human Genetics of the Radboud university medical center together with the community health service and municipality of Nijmegen. Participants were recruited via random sampling of inhabitants of Nijmegen who were 18 years or older. Participants filled out one or more questionnaires and donated blood samples. All study protocols were

approved by the Regional Committee on Research Involving Human Subjects and informed consent was obtained from all participants and/or legal representatives.

Genotyping

Genotyping of all patients' DNA samples was performed by deCODE genetics (Reykjavik, Iceland) using Infinium Global Screening Array deCodeGenetics_V3 (Illumina, San Diego, CA, USA), which contains variants present on the Infinium Global Screening Array (GSA) v3.0 BeadChip as well as 50,000 high-quality markers from the OmniExpress bead chips in order to make data derived from these two chips more compatible. The AGORA controls were genotyped using Infinium Global Screening Arrays, while the NBS controls were genotyped using OmniExpress bead chips (Illumina, San Diego, CA, USA).

Quality control

Quality control (QC) was performed separately for all three cohorts using the PLINK toolset (https://www.cog-genomics.org/plink/2.0/).[102] First, a sex check was performed to identify sex discrepancies. Next, variant QC was carried out, removing variants with a call rate <98%, a deviation from Hardy-Weinberg equilibrium with a p-value $<1x10^{-10}$ (for patients) or $<1x10^{-6}$ (for controls), or a MAF <0.001. Samples were removed if the call rate was <98%. We used the Kinship-based INference for Gwas (KING) toolset to check family relationship within the cohort and to remove individuals related up to the third degree.[103]

Imputation

Before imputation, the datasets containing patients and controls were merged. *A priori*, we decided to use a two-stage approach for the analyses. Since the patients and AGORA controls were genotyped using similar chips, we started our analyses with the patients and AGORA controls. Due to a limited number of controls, however, power to detect an association in this dataset was limited. We had 80% power to detect variants with an allelic OR of 3.2 for a MAF of 0.05, an OR of 2.5 for MAF 0.10, and an OR of 2.1 for MAF 0.25 with genome-wide significance. In contrast, the NBS cohort had more power given the higher sample size (80% power to detect variants with an OR of 2.9 for MAF of 0.05, OR of 2.3 for MAF 0.10, and OR of 1.9 for MAF 0.25), but could be more prone to array-specific batch effects.

To enable this approach, we created two datasets: the patients were merged with the controls from the AGORA data- and biobank to form the AGORA dataset, and the patients were merged with the controls from the NBS to form the NBS dataset. Imputation was based on the overlap in variants genotyped in patients and controls (n= 524,412 in the AGORA cohort and n= 207,692 in the NBS cohort). Genotypes of

the samples were phased using Eagle (v2.4.1) and imputed using Minimac (v4) with 1,000 Genomes Project Phase 3 data as reference panel.[104-106] After imputation, ancestry estimation was performed using data from 1,000 Genomes Project Phase 3, by assigning individuals to closest reference super- and subpopulations according to the geometric distance in the 10 principal component space.[107] Further analyses were performed using individuals with predicted GBR or CEU ancestry only to create a homogeneous study population.[105]

Statistical analyses

Using PLINK, ORs were calculated for each SNV using logistic regression models. Analyses were limited to variants with a Minimac imputation quality score (r2) of 0.6 and minor allele count of 20 (for patients and controls combined). The first four principal components and sex were included as confounders. Inflation was visually inspected using quantile-quantile (QQ) plots and results were depicted in Manhattan plots, which were created in RStudio using the 'qqman' package. Variants with a p-value $<5x10^{-8}$ were considered statistically significant on a genome-wide level, while variants with a p-value $<1x10^{-5}$ were considered suggestively significant. FUMA (https://fuma.ctglab.nl/) was used to infer independent genomic risk loci and we selected loci with at least 5 SNVs showing suggestive significance.[108] To further filter our results and select only the most promising loci, we checked the MAF of the lead SNP in the AGORA and NBS controls and compared these with the MAF in openly available reference sets, such as the Dutch GoNL population[109] or, if GoNL data was not available, data for individuals with a European ancestry from the 1,000 Genomes Project.[105] We restricted to loci with a lead SNV that: 1) reached genome-wide statistical significance, or 2) had a MAF >0.05 in our cases and this MAF in our cases was closer to the MAF in the controls of our discovery cohort than to the MAF in the reference database (*i.e.* using the MAF of the reference database as control would result in a stronger effect of the SNV). For the selected loci, we used LocusZoom to visualise results,[110] checked the location (intergenic, intronic, or exonic), and used the Open Target Genetics platform (https://genetics.opentargets.org/) to estimate relatedness with nearby genes. Furthermore, we obtained information on topologically associated domains from TADKB (http://dna.cs.miami.edu/TADKB/)[111], while the RegulomeDB (https://regulomedb.org/)[112] was used to assess functional importance of loci. Lastly, spatiotemporal and species-specific gene expression was visualized using the web-based application developed by the Kaessmann group (https://apps.kaessmannlab.org/evodevoapp/).[113]

RESULTS

Participants

The AGORA data- and biobank contained DNA from 184 prospectively collected patients with a solitary functioning kidney and saliva samples from 660 patients that were collected for the SOFIA study (Figure 1). For 10 participants, both a DNA sample and a saliva sample was retrieved, meaning that 844 samples from 834 unique patients were available for genotyping. Of these 834 patients, 32 were excluded due to failed genotyping, while 35 patients were excluded because of a known genetic cause or syndrome and 315 patients presented with a phenotype other than UKA, MCDK, or KHD. This resulted in 452 successfully genotyped CSFK patients. For 669 AGORA controls and 5,363 NBS controls, genotyping was performed successfully at an earlier stage.

AGORA dataset results

After quality control and imputation, the AGORA GWAS dataset consisted of 9,956,431 SNVs in 403 patients and 622 controls. The lambda values (1.03) and visual inspection of the QQplot (Figure 2) showed no signs of inflation. None of the SNVs reached genome-wide significance, but several peaks reached suggestive significance (Figure 3). In total, 43 SNVs had a p-value below $1x10^{-5}$ and 11 genomic risk loci were identified. After restricting to loci with at least five SNVs, six were visualized using LocusZoom and listed in Table 1.

NBS dataset results

In the NBS dataset, 403 patients and 4,366 controls were available for analysis after QC and 9,313,318 SNVs were present after imputation. Again, no signs of inflation were found based on the lambda value (1.04) and QQplot (Figure 4). Sixteen SNVs from two independent loci reached genome-wide significance (Figure 5). Additionally, 335 SNVs from 42 loci had a p-value below $1x10^{-5}$. Twenty-four of these loci contained at least five SNVs and were further investigated (Table 1).

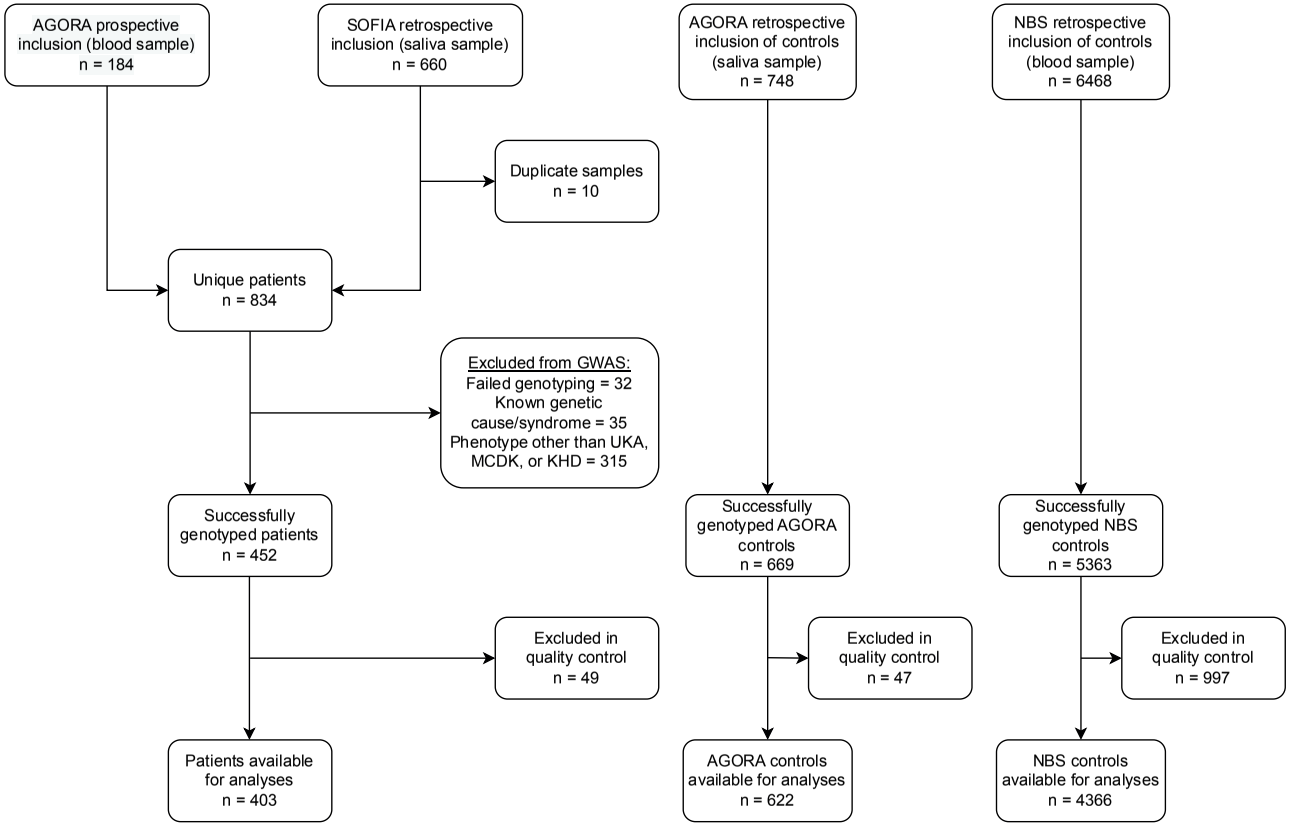


Figure 1 Flowchart of the number of included patients and controls after recruitment, genotyping and quality control. GWAS genome-wide association study, UKA unilateral kidney agenesis, MCDK multicystic dysplastic kidney, KHD kidney hypo/dysplasia

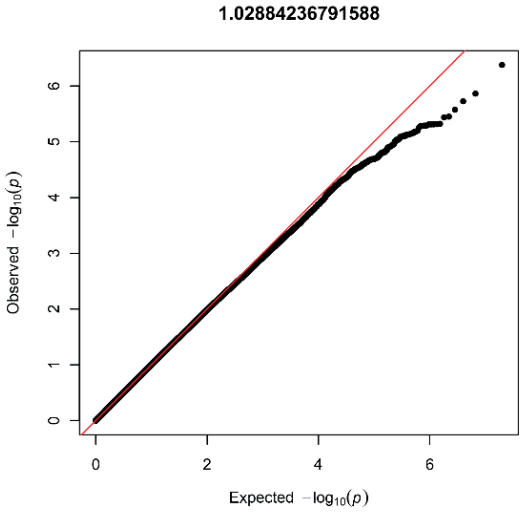


Figure 2 Quantile-quantile plot with lambda value for the AGORA dataset (403 patients and 622 controls).

Loci with a genome-wide statistically significant p-value

Two loci, located on chromosome 7 and 18, reached genome-wide significant p-values (Table 1). The locus on chromosome 7, with lead SNV rs140804918, was found to have a MAF of 0.05 in the patients, 0.02 in the NBS controls, and 0.03 in the AGORA controls and the GoNL database, and a corresponding p-value of 1.4×10^{-8} in the NBS dataset. The resulting ORs in the NBS and AGORA datasets were 3.1 and 1.9, respectively, which are usual effect sizes for a SNV associated with a congenital malformation.[97,114] The location of this SNV is promising, given its proximity to the Hepatocyte Growth Factor (*HGF)* gene (Figure 6A). Moreover, this genomic risk locus is in the same topologically associated domain as the *HGF* gene, and several of the variants in this locus are located in the regulatory region of *HGF*, with RegulomeDB scores of 0.75-1.0 indicating that they are likely to be a regulatory variants.[112] *HGF* gene expression in human kidney tissue was found to be highest in weeks five and six post conception,[113] which aligns with two important events in embryonic kidney development: the appearance of the ureteric buds on day 28 and invasion of the newly developed metanephric mesenchyme on day 32, followed by the start of branching morphogenesis.[7]

The lead variant on chromosome 18 was rs184382636, which had allelic odds ratios of 5.9 and 12.9 in AGORA and NBS controls, respectively, and a p-value of 1.2×10^{-10} in the NBS dataset. The MAF in cases was 0.017, but only 0.002 in the NBS controls and the GoNL database and 0.003 in the AGORA controls, which increases the likelihood of a false positive association. This variant is located in an intergenic region approximately

500 kbp from the *SMIM21* gene and has a RegulomeDB score of 0.07 (Figure 6B). *SMIM21* mRNA tissue expression is highest in the testis, with no expression in developing or mature kidney tissue. The SMIM21 protein is thought to be part of the cell membrane. [113] Five other genes are located in the same topologically associated domain, but none could be linked to kidney development.

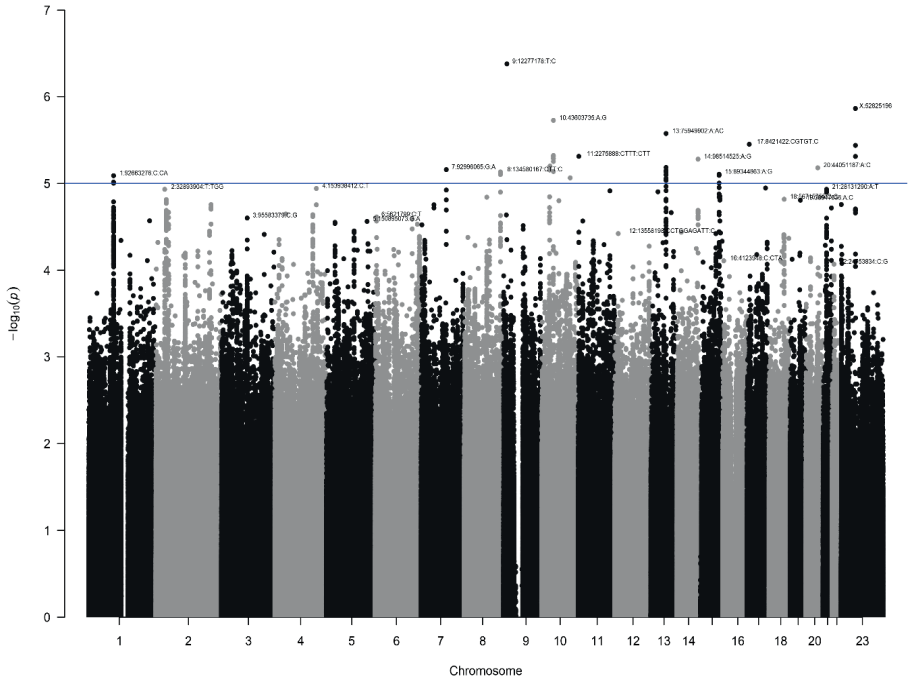


Figure 3 Manhattan plot for the AGORA dataset (403 patients and 622 controls). The horizontal line indicates the threshold for suggestive statistical significance ($p<1x10^{-5}$)

Table 1 Genomic risk loci with at least five SNVs with a p-value below $1x10^{-5}$ identified in a genome-wide association study of congenital solitary functioning kidney, with the lead SNV for each locus being shown

Chr	Position	rsID	Discovery dataset	MAF patients	MAF reference	AGORA dataset			NBS dataset			n SNVs
						MAF controls	OR	p-value	MAF controls	OR	p-value	
Genome-wide significant SNVs												
7	81228890	rs140804918	NBS	0.048	0.031	0.029	1.91	8.4E-03	0.020	3.10	1.4E-08	51
18	73636290	rs184382636	NBS	0.017	0.002	0.003	5.93	2.4E-03	0.002	12.9	1.2E-10	42
SNVs selected based on MAF in discovery and reference populations												
3	189912222	rs10433490	NBS	0.092	0.169	0.153	0.59	4.0E-04	0.150	0.53	4.3E-06	6
6	36451716	rs148413365	NBS	0.106	0.188*	0.156	0.94	7.0E-01	0.166	0.51	1.9E-06	84
13	38000591	rs9547854	NBS	0.087	0.047	0.052	1.83	1.6E-03	0.051	1.97	2.1E-06	66
14	98514525	rs148251525	AGORA	0.127	0.048	0.075	2.08	5.2E-06	0.106	1.22	8.2E-02	5
15	89344863	rs111283115	AGORA	0.333	0.449	0.499	0.65	7.8E-06	0.387	0.71	3.5E-04	24
21	28122146	rs2830456	NBS	0.281	0.370	0.410	0.64	2.5E-05	0.350	0.63	4.5E-06	46
Other suggestively significant SNVs												
1	79317005	rs140198115	NBS	0.025	0.009	0.011	2.37	1.9E-02	0.010	3.79	7.8E-06	17
1	92663276	rs11406716	AGORA	0.319	0.283*	0.246	1.64	8.1E-06	0.277	1.27	8.4E-03	76
3	22488640	rs182382581	NBS	0.025	0.012	0.009	4.10	1.5E-03	0.008	5.04	8.6E-08	7
3	6328767	rs7629003	NBS	0.009	0.003	0.003	4.68	4.9E-02	0.002	14.48	7.6E-06	20
4	106051876	rs190397903	NBS	0.020	0.007	0.013	1.97	8.5E-02	0.007	5.23	2.0E-06	9
4	70836149	rs146356251	NBS	0.018	0.008	0.008	2.79	2.1E-02	0.006	4.84	7.4E-06	7
5	78674386	rs138487999	NBS	0.034	0.019	0.016	2.33	5.5E-03	0.014	2.84	7.4E-06	125
5	139646076	rs145922969	NBS	0.030	0.025	0.013	3.15	1.6E-03	0.015	3.20	2.5E-06	8

Table 1 Genomic risk loci with at least five SNVs with a p-value below $1x10^{-5}$ identified in a genome-wide association study of congenital solitary functioning kidney, with the lead SNV for each locus being shown (continued)

Chr	Position	rsID	Discovery dataset	MAF patients	MAF reference	AGORA dataset			NBS dataset			n SNVs
						MAF controls	OR	p-value	MAF controls	OR	p-value	
5	96015902	rs181945740	NBS	0.018	0.001	0.008	4.20	7.0E-03	0.005	5.90	1.4E-06	7
5	134199058	rs73282857	NBS	0.019	0.006	0.005	4.68	9.4E-03	0.006	7.00	5.6E-08	150
7	10014656	rs190937828	NBS	0.090	0.063	0.066	1.76	1.3E-03	0.054	2.30	1.7E-06	14
8	134595232	rs10112722	AGORA	0.437	0.366	0.347	1.55	7.5E-06	0.370	1.34	2.3E-04	13
10	44117049	rs60001082	NBS	0.085	0.069*	0.065	1.46	4.3E-02	0.054	2.00	7.3E-06	17
11	2275888	rs1325019515	AGORA	0.380	n/a	0.305	1.61	4.9E-06	0.312	1.40	1.1E-04	19
11	58767505	rs11229723	NBS	0.022	0.006	0.011	2.23	3.4E-02	0.007	4.41	1.8E-06	64
11	95161244	rs192152837	NBS	0.018	0.004	0.004	10.2	2.4E-04	0.005	6.35	3.6E-07	8
12	43256387	rs181072352	NBS	0.012	0.005	0.008	1.44	4.8E-01	0.002	10.40	1.2E-06	5
12	385846	rs554290784	NBS	0.015	0.005	0.004	4.23	1.1E-02	0.003	5.59	6.8E-06	5
13	75949902	rs144419778	AGORA	0.267	0.212*	0.19	1.71	2.7E-06	0.205	1.47	1.9E-05	24
14	60728107	rs146557102	NBS	0.041	n/a	0.018	2.75	4.8E-04	0.017	2.96	5.5E-07	8
18	50196707	rs77493404	NBS	0.022	0.009	0.014	2.25	1.2E-02	0.008	4.23	7.5E-06	22
20	44453790	rs201841698	NBS	0.033	0.029	0.020	2.42	6.1E-03	0.013	3.40	2.1E-06	7
20	51885312	rs6126804	NBS	0.030	0.008	0.016	2.58	7.3E-03	0.013	3.30	6.0E-06	8
22	32945612	rs73172143	NBS	0.095	0.078	0.068	1.47	2.7E-02	0.059	1.88	7.0E-06	6

*MAF from European reference population due to absence in the GoNL database. Chr chromosome, rsID Reference SNV cluster ID, MAF minor allele frequency, OR odds ratio, n SNVs, amount of single nucleotide variants in associated genomic risk locus, n/a not available.

Other selected loci

Of the thirty other loci (6 from the AGORA dataset and 24 from the NBS dataset, Table 1), we selected the six loci with a lead SNV with a MAF >0.05 in the cases and in which the effect estimate using the reference population MAF would be stronger than the effect estimate identified in the discovery dataset. The MAFs varied between 0.05 and 0.50 and odds ratios ranged between 0.5 and 2.1 (Table 1). Four of the six loci were located in an intergenic region, whereas one was intronic and one was located just upstream of a gene (Table 2, Figure 6C-D, Supplementary Figure 1A-D).

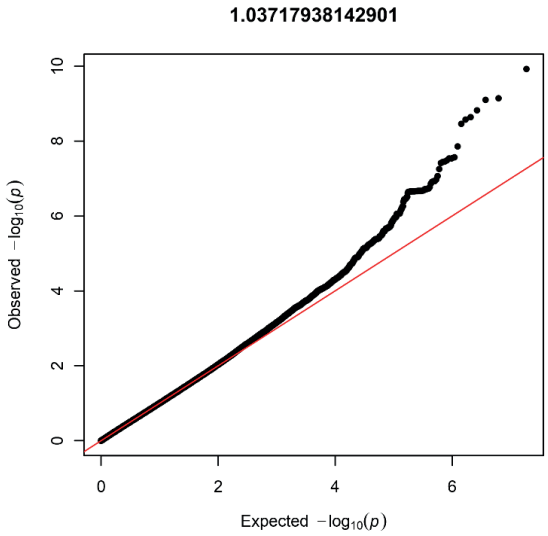


Figure 4 Quantile-quantile plot with lambda value for the NBS dataset (403 patients and 4,366 controls).

Although two of the intergenic SNVs shared a topologically associated domain with genes involved in kidney development (rs9547854 with *FREM2* and rs148251525 with *VRK1*), these SNVs had low RegulomeDB scores (<0.20, Table 2), suggesting no regulatory effect on the nearby genes. The other two intergenic SNVs could not be linked to genes involved in kidney development. Only two variants were located in or close to protein-coding genes: rs148413365 on chromosome 6 is an intronic variant of *KCTD20*, but also in closely proximity of *STK38* (also known as *NDR1*) (Table 2, Figure 6C). Both of these genes show high expression in embryonic kidney tissue and are involved in pathways linked to kidney development. Lastly, rs111283115 on chromosome 15 is a 2kbp upstream variant of *ACAN* (Table 2, Figures 6D). The encoded protein is an important part of the extracellular matrix in cartilage and causal *ACAN* gene variants have been found in patients with short stature.[115] It has relatively low expression in kidney tissue compared to other genes and could not be linked to kidney development, making it unlikely that variants in this gene are involved in the aetiology of CSFK.

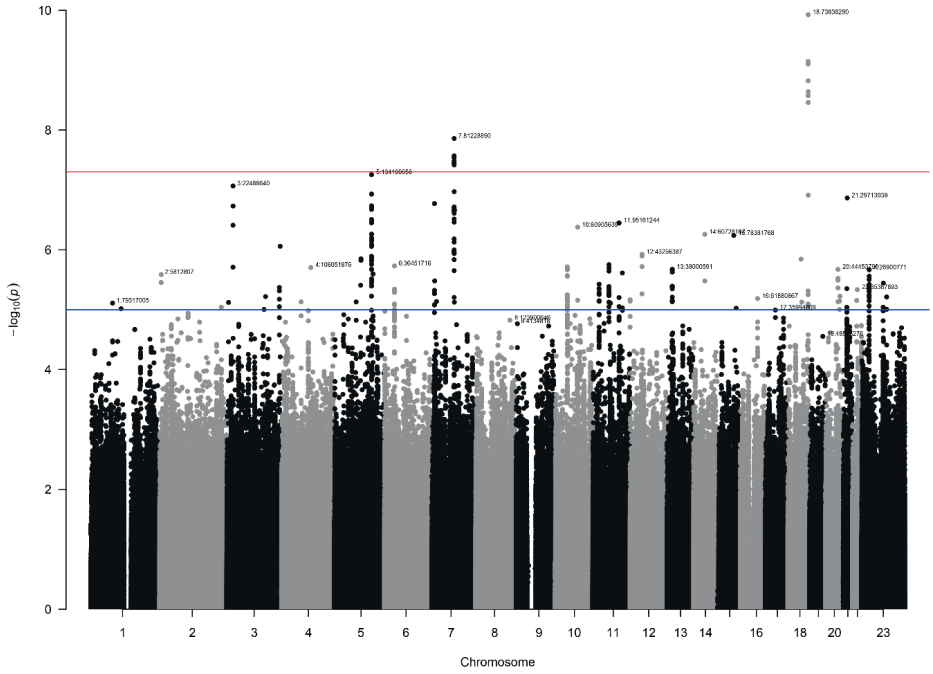


Figure 5 Manhattan plot for the NBS dataset (403 patients and 4,366 controls). The lower horizontal line indicates the threshold for suggestive statistical significance (p-value <1x10^{-5}) and the upper horizontal line indicates the threshold for genome-wide statistical significance (p-value <5x10^{-8}).

DISCUSSION

We performed the first GWAS in a phenotypically homogeneous population of patients with CSFK and identified several loci that may be linked to kidney development. Despite the relatively small number of patients included in the GWAS, variants in two loci reached genome-wide statistical significance. In addition, variants in thirty loci reached suggestive significance, of which six were selected as most promising based on the minor allele frequencies seen in our study population and acquired from reference databases. Although we lacked an independent replication cohort, we were able to identify two genomic loci which are most likely to be involved in kidney development based on the result of our GWAS and *in silico* evaluation.

Of the two loci with genome-wide significance, the locus on chromosome 7 with lead SNV rs140804918 is most interesting because of its MAF, OR, and location close to the *HGF* gene. Involvement of HGF and its receptor Met in kidney development was already suggested by Santos *et al.* in 1994.[116] Both Hgf and Met proteins were identified

in mouse kidneys from embryonic day 11.5 onwards and branching morphogenesis in cultured kidney cells could be decreased using anti-HGF serum. Although complete Hgf or Met knockout is lethal, conditional knockout models showed decreased ureteric bud branching and nephron numbers.[117] Furthermore, Met protein was shown to be involved in branching morphogenesis together with the epidermal growth factor receptor (EGFR),[117] while activation of Met through glial cell line-derived neurotrophic factor (GDNF) was hypothesized to create a positive feedback loop further stimulating branching morphogenesis.[109] Variants affecting HGF protein levels could thus influence branching morphogenesis through activation of Met.

Only one of the loci with suggestive significance could be linked to kidney development based on *in silico* analyses. The lead SNV rs148413365 of this locus on chromosome 6 is an intronic variant of the *KCTD20* gene. The molecular function of the encoded KCTD20 protein is not well-characterized, but is thought to be within the AKT-mTOR-p70 S6k signalling cascade because of protein-protein-interactions with MAP3K8, PPP1CA, and MARK4.[118] This signalling cascade is crucial for compensatory kidney hypertrophy after a reduction in nephron number,[119] and as such, it may also be involved in an adequate response to maldevelopment of one kidney by the contralateral kidney. In normal human embryonic kidney tissue, activated AKT, mTOR, and P70 S6 kinase are constantly expressed in the ureteric bud.[120] Embryonic tissue from MCDK patients showed different expression patterns in a recent study, indicating that inappropriate mTOR pathway activation may be involved in cyst formation in MCDK.[120] Whether this could be a primary cause of MCDK or a response secondary to *e.g.* ureteral blockage is still unclear. The other gene that could possibly explain an association between rs148413365 and CSFK is *STK38*. The STK38 protein was recently identified as part of the Hippo pathway and is able to inhibit YAP/TAZ signalling.[121] Deletion of LATS1/2, which has a similar effect on YAP/TAZ expression as STK38, leads to upregulated YAP/TAZ signalling and kidney agenesis, which could be rescued by reducing YAP/TAZ levels.[122] This suggests an important role for YAP/TAZ signalling in kidney development, which could be affected by *STK38* gene variants. Both *KCTD20* and *STK38* are expressed throughout embryonic kidney development and are in the 10% of genes with the strongest expression in foetal kidney tissue.[123] Combined with the evidence of functional relevance, both may be involved in the aetiology of CSFK and should be topic of further investigation.

An important feature of a GWAS is the unbiased testing of many common variants across the genome. This approach has the advantage that it enables identification of genes or pathways not previously linked to a certain trait, but it has the inherent risk of false positive results as well. To reduce this risk, a strict threshold for genome-wide significance ($5x10^{-8}$) is generally applied, and replication in an independent cohort is required before an association is established. In our study, two variants reached the genome-wide significance threshold.

Table 2 A selection of genomic risk loci with candidate affected protein-coding genes

Chr	rsID	Dataset	Candidate affected gene	Distance from SNV to TSS	Regulome score[112]	Variant effect predictor[124]	Gene function*
Genome-wide significant SNVs							
7	rs140804918	NBS	*HGF*	171 kbp	0.247	Intergenic	Regulates cell growth, motility and morphogenesis in various cell types and tissues
18	rs184382636	NBS	*SMIM21*	497 kbp	0.071	Intergenic	Integral membrane component
SNVs selected based on MAF in discovery and reference populations							
3	rs10433490	NBS	*P3H2*	72 kbp	0.154	Intergenic	Posttranslational modifier of collagen IV
6	rs148413365	NBS	*KCTD20*	41 kbp	0.163	Intronic	Member of AKT-mTOR-p70 S6k signalling cascade
			STK38	64 kbp			YAP/TAZ inhibitor in hippo pathway
13	rs9547854	NBS	*POSTN*	172 kbp	0.112	Intergenic	Induces cell attachment and spreading and plays a role in cell adhesion
14	rs148251525	AGORA	*BCL11B*	1224 kbp	0.000	Intergenic	T cell transcription factor
15	rs111283115	AGORA	*ACAN*	1.8 kbp	0.145	Upstream gene variant	Major component of extracellular matrix of cartilaginous tissues
21	rs2830456	NBS	*ADAMTS1*	96 kbp	0.184	Intergenic	Has antiangiogenic activity, involved in inflammation and cancer cachexia

*Based on https://www.ncbi.nlm.nih.gov/gene/. Chr chromosome, rsID Reference SNV cluster ID, SNV single nucleotide variant, TSS transcription start site, kbp kilo base pair, MAF minor allele frequency.

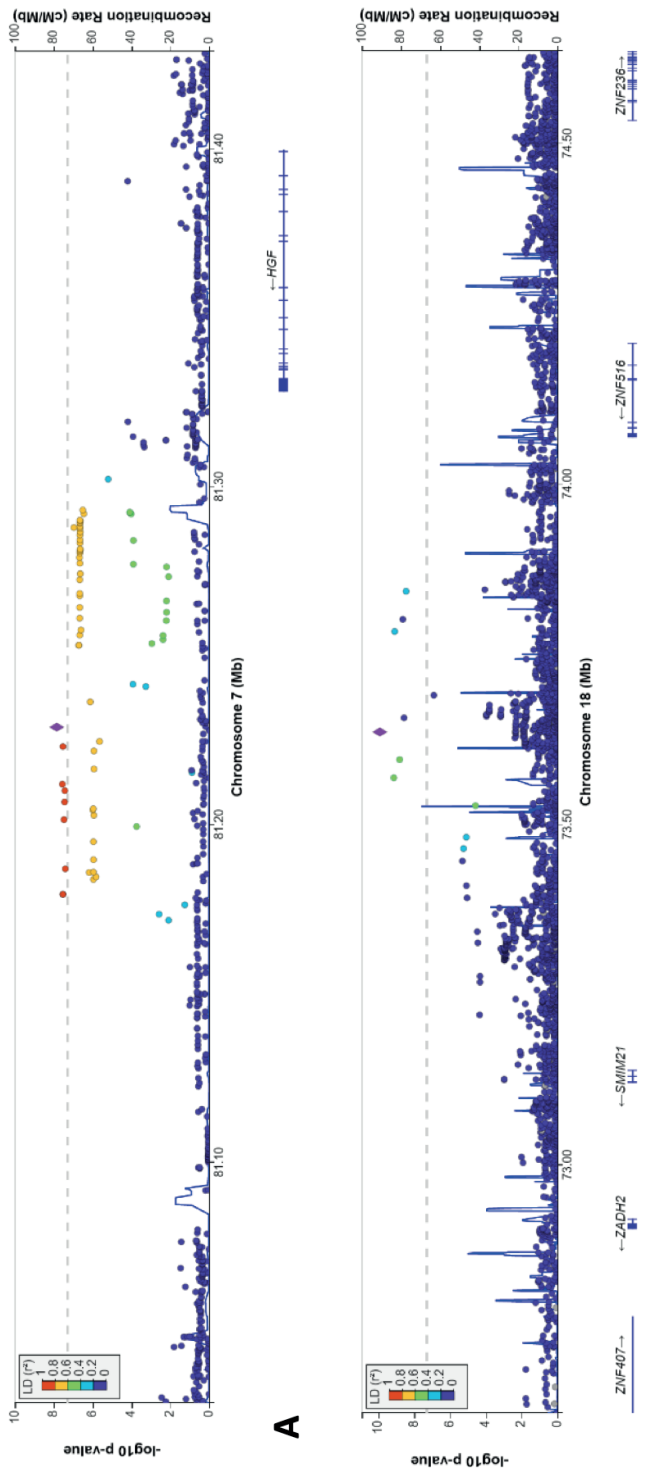


Figure 6 LocusZoom plots of: (**A**) SNV rs140804918 on chromosome 7 (**B**) SNV rs184382636 on chromosome 6 (D) SNV rs111283115 on chromosome 15.

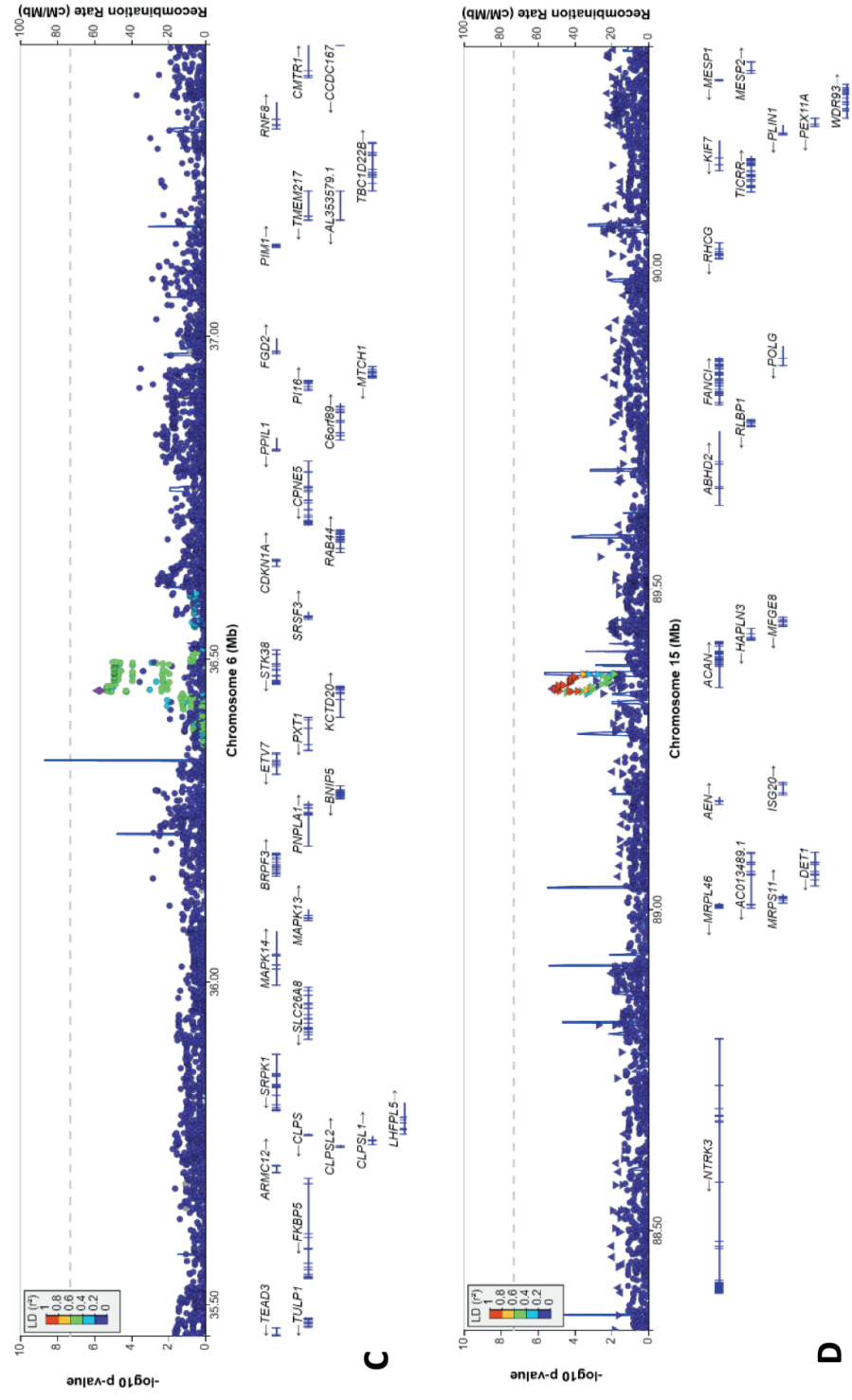


Figure 6 LocusZoom plots of: (A) SNV rs140804918 on chromosome 7 (B) SNV rs184382636 on chromosome 18 (**C**) SNV rs148413365 on chromosome 6 (**D**) SNV rs111283115 on chromosome 15. (continued)

Unfortunately, we were unable to find an independent cohort with a large enough number of CSFK patients to replicate our findings, which is an important limitation of our study. Although replication in other populations remains needed, the fact that the MAFs of the identified SNVs were consistent across different control populations reduces the chance of type one errors.

Ideally, our analyses would have been further stratified based on subtypes (*i.e.* UKA, MCDK, and KHD analysed separately), but this would have led to insufficient power to identify relevant associations. Although patients with known disease-causing mutations or syndromes were excluded, we were unable to investigate the presence of monogenic causes or pathogenic CNVs in most patients (~75%), which would have increased our ability to identify SNVs involved in CSFK aetiology. A strength of our study was the large and homogeneous study population including only patients with CSFK due to UKA, MCDK or KHD, which are phenotypes likely sharing a common aetiology. Furthermore, we restricted our analyses to participants with the two most common ancestral backgrounds and were able to use two large geographically matched control populations.

Our study provides important knowledge about the role of common variants in the aetiology of CSFK. The limitations of our study illustrate, however, that establishing large, well-characterized patient cohorts remains important to replicate GWAS findings and identify additional variants. As an example, at least 3,000 genotyped patients would be needed for 80% power to identify a variant with an OR of 1.5 and a MAF of 0.05 in case of a 1:5 case-control ratio. As such, unravelling the aetiology of relatively rare congenital anomalies, such as CSFK, will only be possible if large international collaborations are created. The establishment of the European Reference Networks (*e.g.* ERKNet and eUROGEN) and the intended foundation of a European Health Data Space will hopefully facilitate such initiatives.

In conclusion, we identified several variants that reached or approached genome-wide significance in our GWAS of CSFK. The role of *HGF* in kidney development could be larger than expected so far and further research into this gene is needed. Furthermore, both *KCTD20* and *STK38* could explain a suggestively significant association with rs148413365 based on their molecular functions. Because of the lack of an independent replication cohort, these findings need replication before they can be established, and future research is warranted to enhance our understanding of the molecular mechanisms involved in the origin of CSFK.

SUPPLEMENTARY MATERIAL

Quality control

AGORA cases

Of 834 unique patients, genotyping was successful in 802. After excluding cases with a known genetic cause or syndrome (n = 35) and phenotypes other than unilateral kidney agenesis, multicystic dysplastic kidney or kidney hypo/dysplasia (n = 315), 452 cases remained. In QC, two cases were removed because of sex discrepancies. In the 450 remaining cases, 686,487 variants were genotyped. During marker quality control (QC), 25,642 variants were removed due to a call rate <98%, 250 due to a failed Hardy-Weinberg test and 98,755 because of a minor allele frequency (MAF) <0.001. Two samples were excluded due to a call rate <98%, resulting in a dataset of 448 samples and 561,840 variants.

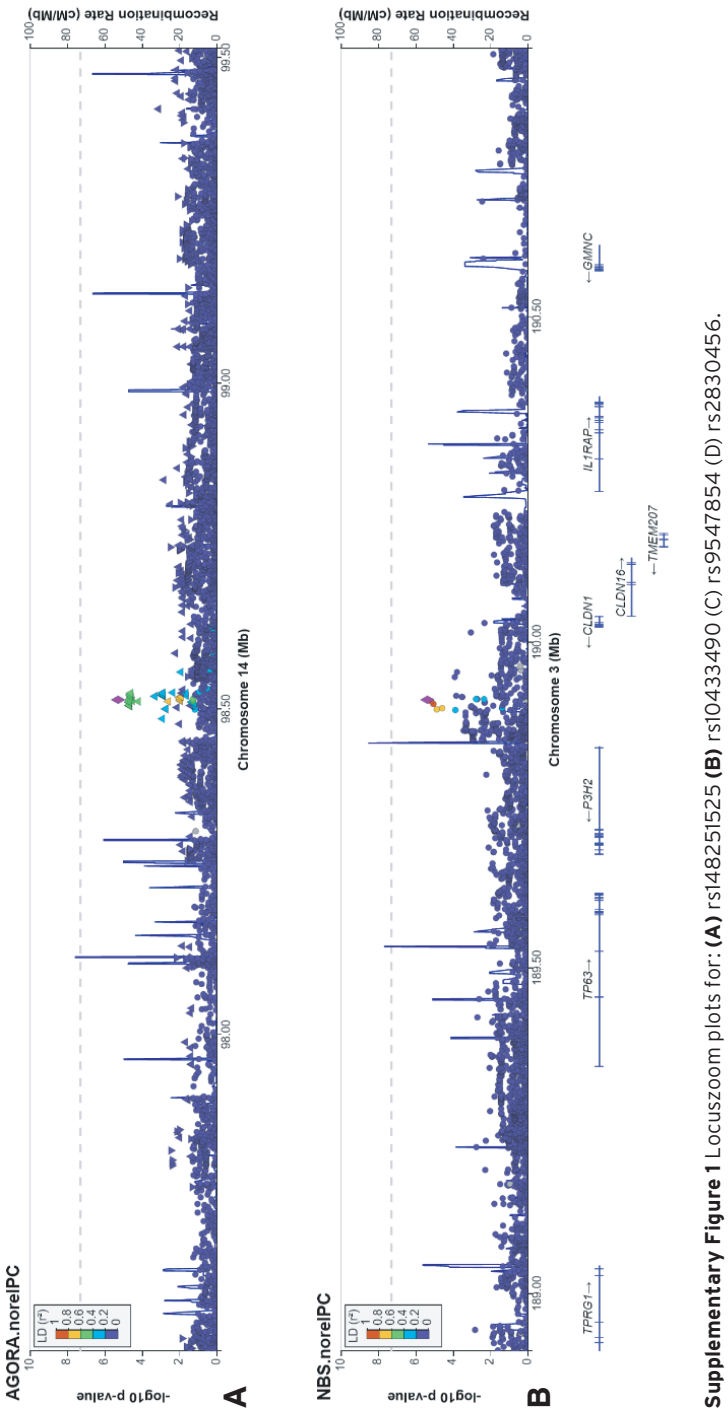
AGORA controls and AGORA dataset

Four AGORA controls were removed because of sex discrepancies. In the resulting 665 AGORA controls, 692,998 variants were genotyped. Variants were removed due to a genotype call rate <98% (n=39,376), a failed Hardy-Weinberg test (n=1,082), or a MAF <0.001 (n=97,753), resulting in 554,787 variants passing QC. Five samples had a call rate <98% and were removed. Merging the cases with the AGORA controls resulted in a dataset with 524,412 shared variants in 1,108 individuals. Imputation yielded a database with 9,956,431 with a Minimac imputation quality score (r2) of ≥0.6 and minor allele count (MAC) of 20. After imputation, six cases and 12 controls were excluded due to relatedness and 39 cases and 26 controls were excluded because of non-European ancestry, leaving 403 cases and 622 controls for analysis in the AGORA dataset.

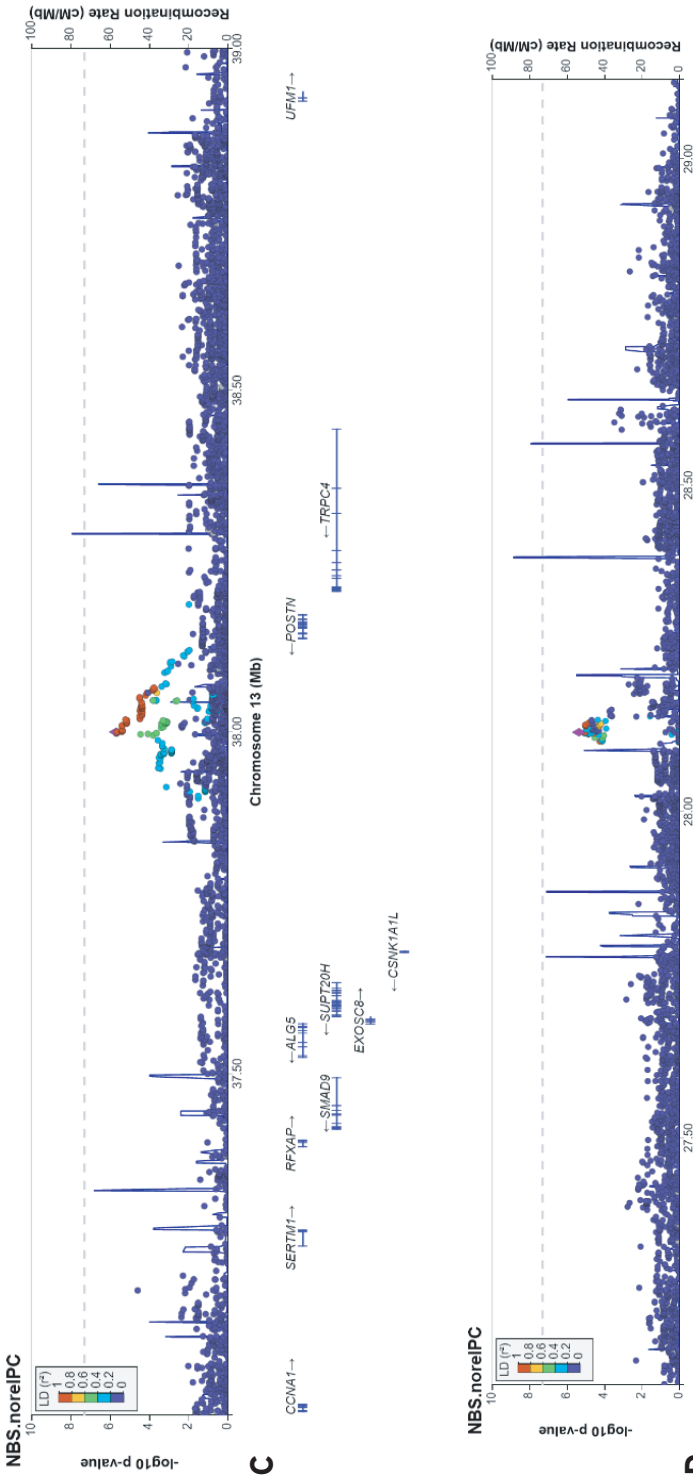
NBS controls and NBS dataset

In the NBS control group, samples with sex discrepancies and variants with a call rate <95% had been excluded previously. Of 670,542 remaining variants, 13,646 were removed due to a call rate <98%, 8,678 due to a failed Hardy-Weinberg test and 15,364 because of a MAF <0.001, leaving 632,854 variants. Eighty-five samples were removed because of a sample call rate <98%. Only 207,692 variants were shared with the case dataset and used for imputation, which resulted in 9,313,318 SNPs with an r2 of ≥0.6 and MAC of 20. After imputation, six cases and 884 controls were excluded due to relatedness and 39 cases and 28 controls because of non-European ancestral backgrounds, resulting in 403 cases and 4,366 controls available for analyses in the NBS dataset.

Supplementary figures



Supplementary Figure 1 Locuszoom plots for: **(A)** rs148251525 **(B)** rs10433490 (C) rs9547854 (D) rs2830456.



Supplementary Figure 1 Locuszoom plots for: (A) rs148251525 (B) rs10433490 **(C)** rs9547854 **(D)** rs2830456. (continued)

CHAPTER 3

A genetic investigation of monozygotic twins discordant for congenital solitary functioning kidney

Sander Groen in 't Woud, Alexander Hoischen, Richarda M. de Voer, Marcel Nelen, Wout F.J. Feitz, Nel Roeleveld, Loes F.M. van der Zanden, and Michiel F. Schreuder

Congenital anomalies of the kidney and urinary tract (CAKUT) are among the most frequent congenital anomalies and represent the leading cause of end-stage kidney disease in children. [125] This spectrum of phenotypes can range from relatively mild conditions such as a duplicate ureter to conditions with several long-term sequela, such as congenital solitary functioning kidney (CSFK). [34] The majority of CSFK cases occurs sporadically, which implicates a role for *de novo* genetic or non-genetic causes. [40] Monogenic causes, however, can be identified in only 10-15% of patients with CSFK, and copy-number variations explain an additional 5-15%.[39] The fact that some monozygotic (MZ) twins are discordant for CSFK implicates that mechanisms other than germline genetic variation, such as postzygotic mutations or epigenetic differences, may play a role in the aetiology. [53] Postzygotic mutations may occur at any time from early embryonic stages through adult life, and are being implicated in several diseases. [126] We investigated the presence of postzygotic mutations in two MZ twin pairs discordant for CSFK to determine whether postzygotic mutations could play a role in the aetiology of CSFK.

Two monozygotic twin pairs, each consisting of one child with congenital SFK and a healthy sibling, were identified in the SOFIA (SOlitary Functioning kIdney: Aetiology and prognosis) study. [91] From all four children, DNA was isolated from saliva samples and exomes were captured using Twist Biosciences enrichment kits. Next, whole exome sequencing (WES) was performed with a targeted sequencing depth of 300x. For both twin pairs, variants present in at least two of the reads of the affected but not the unaffected child, as identified with GATK's MuTect2 tool, were classified as postzygotic variants. Coding postzygotic variants were ranked for genetic and biological plausibility based on sequencing results and literature, respectively. Variants were classified as biologically plausible when located in a gene reported as CAKUT candidate gene, and as genetically plausible when exonic, non-synonymous, and present in more than four reads. A selection of variants was visualized using the Integrative Genomics Viewer (IGV) and validated using long-read sequencing (LRS) on the PacBio Sequel sequencer. [127]

In twin pair I, consisting of two 15 year old boys, the affected child was incidentally diagnosed with an SFK on an abdominal ultrasound for an unrelated indication. Although antenatal ultrasounds were reported to be normal, no acquired cause of the SFK could be identified, leading to a suspected diagnosis of CSFK. In twin pair II, consisting of two eight year old girls, the affected child was diagnosed with CSFK antenatally. Ultrasound evaluation of the kidneys and urinary tract was unremarkable in both healthy siblings and the family history was negative for CAKUT in both twin pairs. Exome sequencing was successful in all four samples, with mean sequencing depths of 120-281x. In twin pair I, 17 postzygotic variants were called, of which two were classified as biologically plausible cause of CSFK and none were genetically plausible (Figure 1). In twin pair II, 108 postzygotic variants were called, with five fulfilling the

criteria for biological plausibility and eight for genetic plausibility. Sixteen of the most promising variants from both twins, based on the number of reads with the variant, were selected for visualization in the IGV. Only one of these variants was considered to be a true positive result. This variant constitutes a synonymous variant in *VGLL4*, but was not considered to be a biological or genetically plausible variant. Validation using LRS was performed for six variants, selected on a combination of the visualization in the IGV, biological plausibility, genetic plausibility, and expert opinion of a laboratory specialist in genetics (AH). Primers could be successfully created for four of the six variants. For three of these variants, including the synonymous variant in *VGLL4*, LRS showed 100% reference alleles with a sequencing depth between 2,724x and 38,219x, indicating that all were technical artifacts in the initial sequencing step. Reads for the fourth variant could not be mapped to the reference genome, so no conclusion could be drawn for this variant. However, considering that the only variant that was likely to be a true positive result based on the initial visualization in the IGV was in fact a technical artefact, we conclude that all called postzygotic mutations identified were technical artefacts.

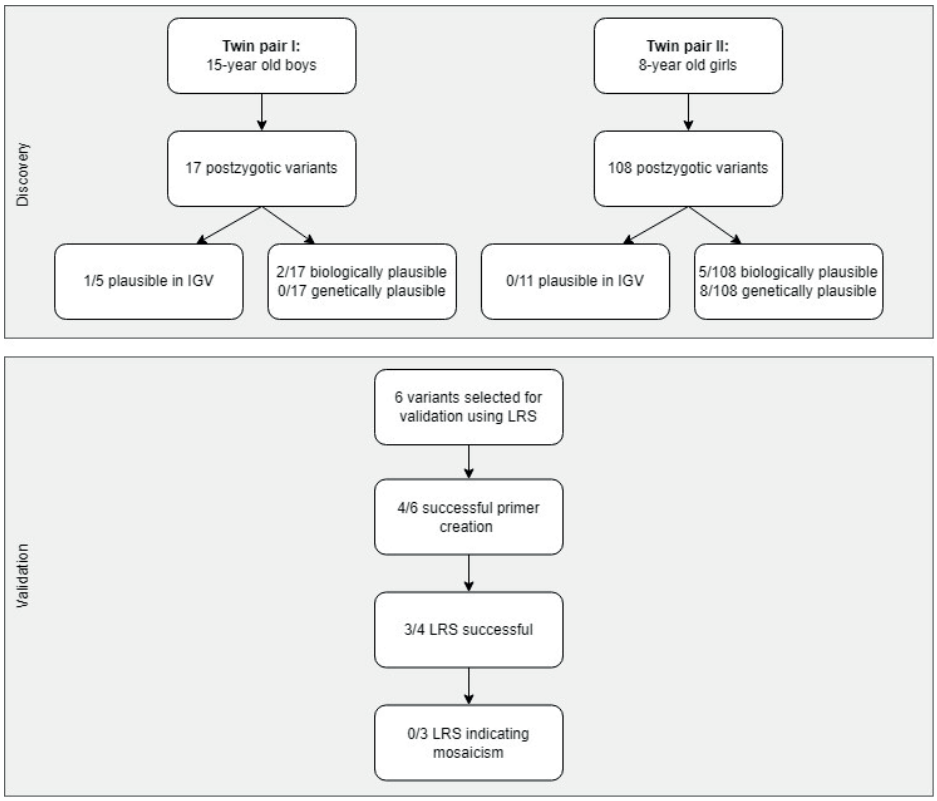


Figure 1 Flowchart of analysis. IGV Integrative Genomics Viewer, LRS long-read sequencing.

Of all *de novo* mutations, 5-10% is estimated to occur post-zygotically.[126] Therefore, it is not unlikely that in previous CAKUT studies, some variants considered to be germline disease-causing mutations were in fact postzygotic mutations with a high frequency mosaicism, whereas some individuals in whom no disease-causing variant was identified may carry a postzygotic disease-causing variant in a low level mosaicism. Deep exome sequencing of patients may identify such low level mosaicisms and could explain the discordance in MZ twins with different disease phenotypes. Recently, MZ twins were shown to differ on average by 14 postzygotic mutations using whole genome sequencing, of which five are likely to have occurred in early development.[128] As the exome only constitutes ~1% of the genome, only few exonic postzygotic mutations are to be expected when sequencing a small series of MZ twins. Although a relatively high number of suspected postzygotic mutations was identified in our two twin pairs, none of the six selected mutations could be validated using LRS. A previous genetic and epigenetic investigation of an adult MZ twin pair discordant for CSFK also failed to identify a cause.[53] Since a substantial number of CSFK patients harbours large CNVs[38] and CNVs can also occur post-zygotically,[129] postzygotic CNVs may explain the difference between the affected and unaffected siblings. Alternatively, the postzygotic mutation could be present in the affected tissue, but not in the investigated tissue (saliva), or other mechanisms such as epigenetic differences could play a role.

In conclusion, we presented two MZ twin pairs discordant for CSFK, in which we attempted to identify disease-causing postzygotic mutations. Although several suspected postzygotic mutations were identified, none could be validated with an alternative sequencing technique. Differences in the (epi)genome that were not investigated with the current techniques may explain the discordance in these twin pairs and, more broadly, some of the unexplained CSFK cases.

CHAPTER 4

Environmental and parental risk factors for congenital solitary functioning kidney – a case-control study

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ABSTRACT

Background

The aetiology of congenital solitary functioning kidney (CSFK) is largely unknown, but likely includes various risk factors. We performed a case-control study to compare exposure to environmental and parental risk factors during embryonic kidney development between children with CSFK and healthy controls.

Methods

We included 434 children with CSFK and 1,302 healthy controls from the AGORA data- and biobank matched on year of birth. Exposure to potential risk factors was investigated using parental questionnaire data. Crude and adjusted odds ratios (aORs) with 95% confidence intervals (CIs) were estimated for each potential risk factor. Multiple imputation was used to deal with missing values. Confounders for each potential risk factor were selected using directed acyclic graphs.

Results

Maternal stress was newly identified as risk factor for CSFK (aOR 2.1, 95% CI 1.2-3.5). Known associations with conception using in vitro fertilization/intracytoplasmic sperm injection (aOR 1.8, 95% CI 1.0-3.2), maternal infections during pregnancy (aOR 2.5, 95% CI 1.4-4.7), smoking during pregnancy (aOR 1.4, 95% CI 1.0-2.0), and parental CAKUT (aOR 6.6, 95% CI 2.9-15.1) were confirmed, but previous associations with diabetes and obesity could not be replicated. Folic acid supplement use and younger maternal age seemed to reduce the risk of CSFK (aORs 0.7, 95% CI 0.5-1.0, and 0.8, 95% CI 0.6-1.0, respectively).

Conclusions

Environmental and parental risk factors are likely to be involved in the development of CSFK and future studies should combine genetic, environmental and gene-environment interaction analyses. Women wanting to become pregnant should consider optimizing their health and lifestyle.

INTRODUCTION

Human kidney development is a process that can be divided into three stages: in the third week after conception, the pronephros is formed, which regresses and is replaced by the mesonephros from week four. Development of the final kidney (metanephros) starts in week five and formation of the kidney is complete around gestational week 36.[7] A series of crucial events take place in week five, starting with the appearance of the ureteric bud on day 28 followed by invasion of the metanephric mesenchyme on day 32.[7] When either of these processes is disrupted, agenesis of the kidney will occur.[8] After invasion of the metanephric mesenchyme, the ureteric bud starts to bifurcate, with each branch subsequently bifurcating in a repetitive process known as branching morphogenesis, which forms the collecting system.[7] Disturbances in branching morphogenesis may result in multicystic kidney dysplasia (MCDK).[11]

Unilateral kidney agenesis (UKA) and MCDK are the most common causes of a congenital solitary functioning kidney (CSFK), which is a birth defect with an estimated prevalence of 1 in 1,500 live births.[31] Based on the results of an animal model, children with CSFK only have an estimated 70% of the number of nephrons of individuals with two kidneys,[130] which results in an increased risk of kidney injury and necessitates long term follow-up.[91] Despite the increased use of genetic screening, a monogenic cause for the CSFK can be found in only 10-20% of patients.[33] Therefore, the aetiology is thought to be multifactorial, with both genetic and environmental factors involved.[33]

Several studies investigated environmental risk factors for CSFK, often as part of cohorts of patients with other congenital anomalies of the kidney and urinary tract (CAKUT) and sometimes stratified by specific anomalies. Previously reported risk factors for CSFK specifically include maternal diabetes,[54-56] obesity,[56] alcohol use,[55,62] and both younger and older age.[55] Other factors, such as maternal smoking,[131] infections during pregnancy,[132,133] and use of assisted reproductive technologies,[36,134] increase the risk of CAKUT, but were not studied for CSFK separately. Results for folic acid supplementation vary with some studies showing a protective effect on CAKUT,[135,136] while others found no effect,[137] and our group reported an increased risk in a previous study.[36]

Studies investigating environmental causes of birth defects are important, since knowledge about these causes may lead to improved preventive efforts and can help answer the causality questions that parents of children with birth defects often have. Many challenges are present when conducting such studies, however, including the classification of birth defects, exposure assessment, correction for confounders, and dealing with small numbers and missing values. To fill the paucity of knowledge in the aetiology of CSFK and overcome some of these challenges, we created a large database with detailed information on both the clinical phenotype and exposures to

potential environmental and parental risk factors for CSFK cases and healthy controls. We performed a case-control study to get more insight into the role of these risk factors in the aetiology of CSFK.

METHODS

Study participants

The AGORA (Aetiologic research into Genetic and Occupational/environmental Risk factors for Anomalies in children) data- and biobank contains clinical information, DNA samples, and questionnaire data for patients with birth defects.[87] Parents of patients with a birth defect visiting the Radboud university medical center (from 2004) or University Medical Center (UMC) Utrecht (from 2013) were asked to participate in AGORA and received a paper questionnaire regarding environmental, parental, and other relevant exposures before and during the pregnancy of their child. To recruit healthy controls for the AGORA data- and biobank, 39 Dutch municipalities provided a random sample of children born between 1990 and 2010 in 2011. Parents of the selected children were invited to participate in the AGORA data- and biobank by filling out the same questionnaire as the parents of patients. In 2021, a similar approach was used to obtain data for children born from 2011 through 2021. All patients with CSFK, defined as a solitary functioning kidney resulting from UKA or MCDK, and part of the controls were included in the current study (Figure 1).

As the number of patients with CSFK in the AGORA data- and biobank was limited, the SOFIA (Solitary Functioning kIdney: Aetiology and prognosis) study was initiated in 2018, with the aim to study the aetiology and prognosis of patients with SFK. For this study, which was embedded in AGORA, new CSFK patients were recruited in 36 hospitals in The Netherlands, including the Radboud university medical center and UMC Utrecht.[91] Parents of participating patients previously treated in these hospitals received a shortened version of the original AGORA questionnaire and could choose between online or paper completion. They were also asked to invite unrelated parents of healthy children to fill out this shortened questionnaire online.

For the current analyses, all patients with CSFK born between January 1st 1993 and December 31st 2020, diagnosed with UKA or MCDK before their 18th birthday, and with a completed original or shortened AGORA questionnaire were included. Children without major birth defects (defined using EUROCAT guidelines[138]) recruited via municipalities in 2011 or 2021 or via parents of children included in the SOFIA study were eligible to serve as healthy controls. The AGORA data- and biobank and the SOFIA study were approved by the Regional Committee on Research Involving Human Subjects.

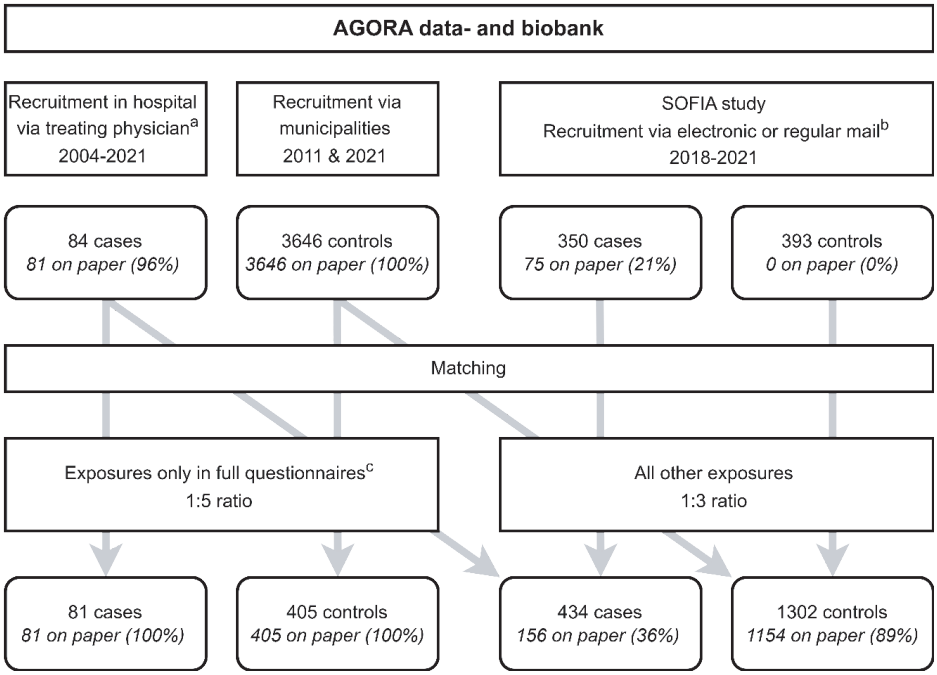


Figure 1 Flowchart of recruitment and selection of cases and controls. [a]Recruitment via treating physician was performed in the Radboud university medical center (2004-2021) and University Medical Center Utrecht (2013-2021). [b]Recruitment for the SOFIA study was performed via 36 Dutch hospitals, including the Radboud university medical center and University Medical Center Utrecht. Only patients not yet included in the AGORA data- and biobank were invited to participate. [c]Exposures only in full questionnaires were infection, diet, and stress.

Risk factors

A selection of relevant questions from the AGORA questionnaire (available in Supplementary Material) was used to assess exposure to the risk factors under study. We considered the following potential risk factors for CSFK: family history of CAKUT (defined as maternal and/or paternal CAKUT), season of conception, parental age at conception, and parental subfertility and/or conception using artificial reproductive technologies (ART). In addition, the following maternal risk factors were studied: gravidity, body mass index (BMI) at conception, pre-existing or gestational diabetes mellitus (DM), pre-existing hypertension, infections during pregnancy, medication use during pregnancy, smoking, alcohol consumption, use of folic acid or folic-acid containing multivitamins, diet, and self-reported stress during pregnancy.

Maternal and paternal age were studied as both continuous and categorized (<25, 25-29, 30-34, 35-39, ≥ 40 years) variables. Couples were considered subfertile

if one of the parents was ever diagnosed with subfertility by a doctor or if time to conception was >12 months.[139] We grouped ARTs hierarchically into conception using intra-uterine insemination only (IUI), hormonal treatment, or in-vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI). Gravidity was categorized into first or subsequent pregnancy. Body mass index was classified as underweight (<18.5 kg/m^2), normal (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), or obese (>= 30 kg/m^2). Diabetes was considered pre-existing when reported from the start of pregnancy and gestational when first diagnosed during the index pregnancy. Pre-existing hypertension was any hypertension discovered before the 20th week of gestation, in accordance with guidelines from the American College of Obstetricians and Gynaecologists.[140] Maternal infections during pregnancy were divided into cystitis and other episodes of fever or infection. Medications of interest included diabetes medications (including insulin), antihypertensive medications, inhalation corticosteroids, and anti-epileptic drugs. Maternal smoking and alcohol use were divided into three exposure groups: exposed during the aetiologically relevant period (defined as exposure from the 6th week of pregnancy onwards), exposed but not in the aetiologically relevant period (defined as exposure in the three months before and/or during the first five weeks of pregnancy), and not exposed at all in the three months before or during pregnancy. Similarly, use of folic acid supplements or folic acid-containing multivitamins was classified in a three-level variable: "use as recommended" for women who used folic acid supplementation during the entire advised period (initiation before pregnancy and continued through at least the eighth week of pregnancy), "suboptimal use" (only part of the advised period), or no use during the advised period. Sensitivity analyses were performed in which only prenatal multivitamins were taken into account. Lastly, maternal diets (vegetarian or low-salt) and perceived psychological stress were investigated as potential risk factors. All exposure data were self-reported.

Detailed questions regarding infection, diet, and stress were only asked in the original questionnaire, but not in the shortened version. Therefore, analysis of these factors was limited to subjects filling out the original questionnaire. Since mothers carrying a child with CSFK might indicate higher levels of stress in late pregnancy, resulting in reversed causation, we also performed analyses while only including women who reported stress in the first trimester. Year of childbirth, maternal ethnicity (European or other), and maternal education level (low, medium, or high) were considered as potential confounders but not as potential risk factors.

Statistical analyses

Each case was matched to three controls born in the same year to account for differences in year of childbirth between cases and controls. For the analyses limited to cases and controls that filled out the original questionnaires, we used a 5:1 ratio for additional power. Crude odds ratios (ORs) with 95% confidence intervals (CIs) were

estimated for all risk factors mentioned above using conditional logistic regression. To facilitate analyses of all participants, even in case of missing data, multiple imputation was used. Ten imputed datasets were created in which missing values were imputed using information of all variables listed as potential risk factors, as well as the potential confounders and the time between birth and filling out the questionnaire. Multiple imputation was performed using the Markov Chain Monte Carlo method with predictive mean matching, under the assumption that data was missing at random. Adjusted ORs were estimated using conditional logistic regression corrected for a minimally sufficient sets of confounders for each potential risk factor separately, derived from directed acyclic graphs (DAGs, available in supplementary material) created in DAGitty.[141] We used DAGs, because this method requires making assumptions about causal associations between variables explicit.[142] Moreover, it allows for selection of minimally needed sets of confounders, which increases statistical efficiency and avoids overcorrection introducing bias.[142] Adjusted ORs were not estimated when less than five cases were exposed. Matching, data imputation, and statistical analysis were performed using IBM SPSS statistics version 25.0.

RESULTS

The total study population consisted of 434 CSFK patients and 4,039 healthy controls for whom completed questionnaires were available. The cause of the CSFK was UKA in 151 patients and MCDK in 283 patients (detailed clinical description provided in Supplementary Table 1 and [91]). Of the 4,039 controls, 1,302 were matched to cases for the main analyses and 405 controls were matched for the analyses limited to the original questionnaire (Figure 1). Part of the study population (40 patients and 677 controls) was included in a previous study by our group.[36] Table 1 shows that matching eliminated any differences in year of childbirth, with less than 10% of the study population born before 2000. Patients and controls were also comparable with respect to maternal self-reported ethnical background, but mothers of controls were slightly higher educated and more likely to have completed the questionnaire on paper. The median time between childbirth and completion of the questionnaire was slightly shorter in controls. Only 3% of the questionnaires were filled out by the father.

Table 1 Characteristics of patients with congenital solitary functioning kidneys as well as healthy population-based controls matched on year of childbirth

	Controls (n=1,302)	Cases (n=434)
Year of childbirth		
<2000	111 (9%)	37 (9%)
2000-2004	168 (13%)	56 (13%)
2005-2009	318 (24%)	106 (24%)
2010-2014	390 (30%)	130 (30%)
≥2015	315 (24%)	105 (24%)
Maternal ethnicity		
European	1,216 (93%)	406 (94%)
Other	71 (6%)	26 (6%)
Missing	15 (1%)	2 (1%)
Maternal education level		
Low	100 (8%)	53 (12%)
Intermediate	472 (36%)	161 (37%)
High	712 (55%)	218 (50%)
Missing	18 (1%)	2 (1%)
Type of questionnaire		
Paper	1,154 (89%)	156 (36%)
Online	148 (11%)	278 (64%)
Time to completion[a]		
Years	6.2 (3.7-8.8)	6.9 (2.8-11.8)

[a]Time between date of childbirth and date of filling out the questionnaire (median, interquartile range)

Several factors were associated with CSFK development in both univariable and multivariable analyses (Table 2). The strongest risk factor was a family history of CAKUT (aOR 6.6, 95% CI 2.9-15.1), while other risk factors included maternal infections during pregnancy (aOR 2.5, 95% CI 1.4-4.7), maternal stress (aOR 2.1, 95% CI 1.2-3.5), and conception after IVF/ICSI (aOR 1.8, 95% CI 1.0-3.2). Smoking during the aetiological period seemed to be associated with an increased risk of CSFK as well (aOR 1.4, 95% CI 1.0-2.0), whereas a decreased OR was observed for alcohol use outside the aetiological period (aOR 0.7, 95% CI 0.6-0.9). Other factors presumably associated with a decreased risk of CSFK included correct use of folic acid supplementation (aOR 0.7, 95% CI 0.5-1.0) and a young maternal age (25-30 years, compared to 30-35 years; aOR 0.8, 95% CI 0.6-1.0). High maternal BMI was not associated with CSFK development, but a low maternal BMI pointed in the direction of an increased risk (aOR 1.6, 95% CI 0.9-2.9).

Our study showed no evidence for an effect of gravidity, season of conception, pre-existing or gestational diabetes, pre-existing hypertension, and paternal age. The low numbers of exposed participants prevented us from drawing conclusions for medication use and maternal diet.

Detailed analyses into folic acid supplementation showed that the type of supplement (*i.e.* folic acid only or folic acid containing multivitamins) did not influence the effect size substantially (Table 3), in contrast to earlier studies by our group.[36] Limiting our analyses to prenatal supplements only did not change these results (Supplementary Table 2). Lastly, sensitivity analyses showed that the results of multivariable analyses were generally robust even in case of missingness not at random (Supplementary Table 3).

Table 2 Crude and adjusted odds ratios for the associations between potential risk factors and congenital solitary functioning kidney

	Controls (n = 1,302)	Cases (n = 434)	cOR	aOR[a]	95% CI low	95% CI high
Gravidity						
First pregnancy	550 (42%)	179 (41%)	1.0	1.0	ref	ref
Subsequent pregnancy	716 (55%)	236 (54%)	1.0	1.0	0.8	1.2
Missing	36 (3%)	19 (4%)	-	-	-	-
Season of conception						
Spring	321 (25%)	98 (23%)	1.0	1.0	ref	ref
Summer	305 (23%)	112 (26%)	1.2	1.2	0.9	1.6
Fall	335 (26%)	115 (27%)	1.1	1.1	0.8	1.5
Winter	327 (25%)	100 (23%)	1.0	1.0	0.7	1.4
Missing	14 (1%)	9 (2%)	-	-	-	-
Maternal age						
=< 24 year	57 (4%)	25 (6%)	1.2	1.2	0.6	2.2
25-29 year	388 (30%)	108 (25%)	**0.8**	**0.8**	**0.6**	**1.0**
30-34 year	558 (43%)	195 (45%)	1.0	1.0	ref	ref
35-39 year	243 (19%)	89 (21%)	1.0	1.1	0.8	1.5
>= 40 year	36 (3%)	6 (1%)	0.5	0.5	0.2	1.3
Missing	20 (2%)	11 (3%)	-	-	-	-

Table 2 Crude and adjusted odds ratios for the associations between potential risk factors and congenital solitary functioning kidney (continued)

	Controls (n = 1,302)	Cases (n = 434)	cOR	aOR[a]	95% CI low	95% CI high
Maternal BMI						
Underweight (<18.5 kg/m^2)	37 (3%)	21 (5%)	1.8	1.6	0.9	2.9
Normal (18.5-24.9 kg/m^2)	857 (66%)	270 (62%)	1.0	1.0	ref	ref
Overweight (25-29.9 kg/m^2)	258 (20%)	93 (21%)	1.2	1.1	0.9	1.5
Obese (>=30 kg/m^2)	90 (7%)	35 (8%)	1.2	1.1	0.7	1.8
Missing	60 (5%)	15 (4%)	-	-	-	-
Subfertility						
Fertile	947 (80%)	337 (80%)	1.0	1.0	ref	ref
Subfertile without ART	149 (13%)	53 (13%)	0.9	1.0	0.7	1.4
IUI without hormones	16 (1%)	5 (1%)	0.9	0.8	0.3	2.4
Hormonal without IVF/ICSI	36 (3%)	9 (2%)	0.7	0.7	0.3	1.5
IVF/ICSI	33 (3%)	20 (5%)	**1.7**	**1.8**	**1.0**	**3.2**
Maternal diabetes						
No diabetes	1,248 (96%)	416 (96%)	1.0	1.0	ref	ref
Pre-existing diabetes	4 (0%)	1 (0%)	0.8	^	^	^
Gestational diabetes	32 (3%)	12 (3%)	1.1	1.1	0.5	2.2
Missing	18 (1%)	5 (1%)	-	-	-	-
Pre-existing hypertension						
No	1,259 (97%)	423 (98%)	1.0	1.0	ref	ref
Yes	22 (2%)	6 (1%)	0.8	0.7	0.3	1.8
Missing	21 (2%)	5 (1%)	-	-	-	-
Maternal infections*						
No infection	314 (78%)	52 (64%)	1.0	1.0	ref	ref
Cystitis	37 (9%)	9 (11%)	1.4	1.5	0.6	3.6
Other infection/fever	52 (13%)	23 (27%)	**2.6**	**2.5**	**1.4**	**4.7**
Missing	8 (2%)	0 (0%)	-	-	-	-
Anti-diabetic medication						
No	1,265 (97%)	426 (98%)	1.0	1.0	ref	ref
Yes	10 (1%)	5 (1%)	1.5	1.7	0.4	7.6
Missing	27 (2%)	3 (1%)	-	-	-	-

Table 2 Crude and adjusted odds ratios for the associations between potential risk factors and congenital solitary functioning kidney (continued)

	Controls (n = 1,302)	Cases (n = 434)	cOR	aOR[a]	95% CI low	95% CI high
Anti-hypertensive medication						
No	1,209 (93%)	419 (97%)	1.0	1.0	ref	ref
Yes	50 (4%)	12 (3%)	0.7	0.7	0.4	1.3
Missing	43 (3%)	3 (1%)	-	-	-	-
Inhalation corticosteroids						
No	1,243 (96%)	418 (96%)	1.0	1.0	ref	ref
Yes	14 (1%)	8 (2%)	1.5	1.6	0.7	4.0
Missing	45 (4%)	8 (2%)	-	-	-	-
Anti-epileptic medication						
No	1,255 (96%)	430 (99%)	1.0	1.0	ref	ref
Yes	7 (1%)	1 (0%)	0.4	^	^	^
Missing	40 (3%)	3 (1%)	-	-	-	-
Smoking						
No smoking	1,080 (83%)	348 (80%)	1.0	1.0	ref	ref
Smoking aetiological period[b]	116 (9%)	50 (12%)	**1.4**	**1.4**	**1.0**	**2.0**
Smoking other period	93 (7%)	31 (7%)	1.0	1.1	0.7	1.7
Missing	13 (1%)	5 (1%)	-	-	-	-
Alcohol						
No alcohol	646 (50%)	249 (57%)	1.0	1.0	ref	ref
Alcohol aetiological period[b]	102 (8%)	29 (7%)	0.7	0.7	0.5	1.2
Alcohol other period	533 (41%)	151 (35%)	**0.7**	**0.7**	**0.6**	**0.9**
Missing	21 (2%)	5 (1%)	-	-	-	-
Folic acid supplementation						
No supplementation	153 (12%)	59 (15%)	1.0	1.0	ref	ref
Use as recommended[c]	708 (57%)	192 (47%)	**0.7**	**0.7**	**0.5**	**1.0**
Suboptimal use[d]	384 (31%)	155 (38%)	1.1	1.0	0.7	1.5
Missing			-	-	-	-

Table 2 Crude and adjusted odds ratios for the associations between potential risk factors and congenital solitary functioning kidney (continued)

	Controls (n = 1,302)	Cases (n = 434)	cOR	aOR[a]	95% CI low	95% CI high
Maternal diet*						
No diet	384 (95%)	75 (93%)	1.0	1.0	ref	ref
Vegetarian	8 (2%)	2 (2%)	1.4	^	^	^
Low salt	9 (2%)	2 (2%)	1.4	^	^	^
Missing	4 (1%)	2 (2%)	-	-	-	-
Maternal stress*						
No	310 (77%)	51 (63%)	1.0	1.0	ref	ref
Yes	90 (22%)	29 (36%)	**1.9**	**2.1**	**1.2**	**3.5**
Missing	5 (1%)	1 (1%)	-	-	-	-
Family history of CAKUT						
No	1,070 (82%)	330 (76%)	1.0	1.0	ref	ref
Yes	13 (1%)	32 (7%)	**12.1**	**6.6**	**2.9**	**15.1**
Missing	219 (17%)	72 (17%)	-	-	-	-
Paternal age						
=< 24 year	24 (2%)	9 (2%)	1.2	0.7	0.3	1.8
25-29 year	185 (14%)	58 (13%)	1.0	1.0	0.7	1.4
30-34 year	471 (36%)	163 (38%)	1.0	1.0	ref	ref
35-39 year	340 (26%)	117 (27%)	1.0	0.9	0.7	1.2
>= 40 year	152 (12%)	42 (10%)	0.8	0.8	0.5	1.2
Missing	130 (10%)	45 (10%)	-	-	-	-

*Assessed in paper questionnaires only (n = 486). ^Not calculated since less than 5 cases were exposed. [a]Adjusted for minimal set of confounders determined using directed acyclic graphs (DAGs; available in supplementary materials). [b]Use during the etiological period was defined as exposure from the 6th week of pregnancy onwards. [c]Use as recommended is initiation before pregnancy and continued use through at least the 8th week of pregnancy. [d]Suboptimal use was defined as usage during only part of the recommended period. cOR crude odds ratio, aOR adjusted odds ratio, CI confidence interval, ref reference, BMI body mass index, ART artificial reproductive technique, IUI intrauterine insemination, IVF in vitro fertilization, ICSI intracytoplasmic sperm injection, CAKUT congenital anomalies of the kidney and urinary tract.

DISCUSSION

In this study of 434 CSFK patients and 1,302 matched population-based controls, we identified several environmental and parental risk factors for CSFK development. We newly identified maternal stress as risk factor for CSFK, and confirmed previously reported associations with conception using IVF/ICSI, maternal infections during

pregnancy, smoking during pregnancy, and a positive family history of CAKUT. Use of folic acid supplements and a younger maternal age seemed to reduce the risk of CSFK in our study, whereas previously reported associations with diabetes and obesity could not be confirmed.

Maternal stress during pregnancy is reported in the literature by up to 75% of women and has been associated with a range of adverse child-related outcomes, such as preterm birth, congenital heart defects, and even reduced reproductive function in the offspring later in life.[143-145] Although an association between maternal stress during pregnancy and CAKUT seems biologically plausible, we were unable to identify studies investigating this relationship. One study included urinary tract anomalies as outcome after depression without medication use during pregnancy and found no increased prevalence of these anomalies.[146] There may be differences, however, between acute stress and chronic stress or depression. Stress is mainly thought to exhibit its negative effects via an increased production of corticosteroids, which are known to interfere with kidney development[147] and result in lower nephron endowment.[148] Although natural glucocorticosteroids are more efficiently broken down by the 11β-hydroxysteroid dehydrogenase type 2 enzyme in the placenta then synthetic variants,[147] chronic stress has been shown to reduce the activity of this enzyme, thereby exposing the foetus to higher levels of cortisol.[149] As such, acute stress on top of chronic stress may be especially damaging for the foetus. Many difficulties exist when studying adverse outcomes related to stress in pregnancy: stress caused by different sources may influence outcomes differently,[145] differences in maternal resilience and social support may interact with the likelihood of certain outcomes,[143] and the amount of stress may be difficult to quantify using objective measurements. In our study, we defined stress based on self-reported data. This has the advantage of taking the perception of stress into account, rather than relying on the assumption that certain life events cause stress, but carries a risk of recall bias. Although we investigated stress in the first two months of pregnancy separately to avoid reverse causation, recall bias cannot be ruled out since questionnaires were filled out after birth.

In this study, we found that conception using IVF/ICSI was likely to be associated with an increased risk of CSFK. In a previous study by our group, with an overlap of 40 patients and 677 controls, we also found a possible association between IVF or ICSI and CAKUT in 562 CAKUT patients and 2139 controls.[36] Additionally, conception using ART resulted in a slightly elevated risk of urogenital abnormalities (OR 1.3, 95% CI 1.0-1.6) in an Australian registry study, but no subdivision into specific techniques was reported.[134] In two other large studies, an increased prevalence of birth defects was observed in children born after ART, but they did not investigate CAKUT specifically.[150,151] The effect size found in our study, however, was comparable to the effect sizes in these studies, supporting our findings.

Associations between maternal febrile illnesses and congenital kidney malformations and bilateral kidney agenesis have been reported by two previous studies.[132,133] In our study, we found an association with fever or other illnesses, but not with urinary tract infections. This may be explained by the amount of inflammation, which could interfere with kidney development and is usually low in lower urinary tract infections.[152] Our results also suggest that continued smoking during the aetiological period may be a risk factor for CSFK, which is in line with a recent meta-analysis.[131] The fact that a family history of CAKUT was the strongest risk factor found in our study supports the hypothesis of a multifactorial aetiology in which genetic factors are also involved.

Table 3 Comparison of the effect of folic-acid supplements and folic-acid containing multivitamins in prevention of congenital solitary functioning kidney

	Controls (n = 1,302)	CSFK cases (n = 434)	cOR	aOR[a]	95% CI low	95% CI high
No vitamins at all	**153 (12%)**	**59 (15%)**	**1.0**	**1.0**	**ref**	**ref**
Any use[b]						
Folic acid only	599 (48%)	191 (47%)	0.8	0.8	0.6	1.2
Multivitamins only	68 (6%)	21 (5%)	0.8	0.8	0.4	1.6
Both	425 (34%)	135 (33%)	0.8	0.8	0.5	1.2
Use as recommended[c]						
Folic acid only	462 (37%)	133 (33%)	0.7	0.7	0.5	1.1
Multivitamins only	60 (5%)	13 (3%)	0.5	0.6	0.3	1.2
Both	186 (15%)	46 (11%)	**0.6**	**0.6**	**0.4**	**1.0**
Suboptimal use[d]						
Folic acid only	251 (20%)	88 (22%)	0.9	0.9	0.6	1.3
Multivitamins only	31 (3%)	14 (3%)	1.2	1.2	0.6	2.8
Both	102 (8%)	53 (13%)	1.4	1.3	0.8	2.2

[a]Adjusted for minimal set of confounders determined using directed acyclic graphs (DAGs; available in supplementary materials). [b]Defined as use as recommended or suboptimal use. [c]Defined as initiation before pregnancy and continued use through at least the 8th week of pregnancy. [d]Usage during only part of the recommended period. cOR crude odds ratio, aOR adjusted odds ratio, CI confidence interval, ref reference.

Surprisingly, we did not find an association between maternal overweight or obesity and risk of CSFK. Several other studies, including a large meta-analysis, reported a mild to moderately elevated risk of CAKUT in mothers with a high BMI.[56,61,153,154] The effect estimate for obesity that we observed, however, is similar to that the very small risk identified in the meta-analysis, but our confidence interval was much larger because of fewer participants in our study. It is hypothesized that the effect of maternal weight is mainly a consequence of higher glucose levels transmitted to the developing foetus.[56] Because undiagnosed hyperglycaemia is probably less likely in The Netherlands

compared to countries with less accessible healthcare systems, others may also have found larger effect sizes. Similarly, we did not observe associations for pre-existing and gestational diabetes, which is partly due to small numbers but may also reflect stricter control of blood glucose in The Netherlands or our inability to select only mothers with gestational diabetes present during the aetiologically relevant time period. Interestingly, the analyses for women with underweight points in the direction of an increased risk of CSFK, which is as yet unexplained. Lastly, we found a protective effect of alcohol consumption, even when studying continued drinking in pregnancy. It seems unlikely that alcohol reduces the risk of CSFK, however, especially since several others found no effect or increased ORs. [36,55,62,154]

One of the most successful preventive measures against congenital malformations so far is the use of folic acid supplements. Although primarily advised for prevention of neural tube defects, lower numbers of CAKUT in children of mothers who used folic acid supplements have also been found. [135,136] In a previous study by our group, we observed a difference in effect on CAKUT between folic acid supplements and folic acid containing multivitamins. [36] In the current study, which was larger and focused specifically on CSFK, the effect was similar for both types of supplements (aORs 0.7 and 0.6 for folic acid supplements and folic acid containing multivitamins, respectively). This reaffirms the recommendation given to all Dutch women who want to become pregnant to use folic acid and vitamin D supplements consistently, and to use other vitamins or supplements only on indication. [155]

Maternal age was weakly associated with the risk of CSFK in our study, with an aOR of 0.8 (95% CI 0.6-1.0) for mothers between 25-30 years old versus mothers of 30-35 years old. Results in the literature were inconsistent: whereas Parikh *et al.* found higher risks of UKA for mothers <18 years of age, [55] Tain *et al.* reported a higher risk of CAKUT in mothers aged 20-29, 30-39, or >40 years, compared to <20 years. [65] In two other studies, no statistically significant results were found. [156,157] Therefore, if an effect of maternal age exists, it is likely to be small.

Similar to many studies investigating environmental and parental causes of congenital malformations, our study was designed as a case-control study, with the inherent potential limitation of selection bias and recall bias due to its retrospective nature and the long time between childbirth and completion of the questionnaire. To reduce the possibility of selection bias, we randomly selected the majority of controls from the geographical areas were the cases came from in the same age range. In addition, we matched cases and controls conditional on year of birth to rule out selection bias or confounding by year of childbirth. We believe that the effect of recall bias on our exposures is likely to be limited as well, since most factors studied represent important lifestyle habits, chronic conditions, or events that are not easily forgotten, especially not in the well-remembered period of pregnancy. If recall bias did occur, it was most

likely nondifferential leading to slight underestimation of the effect estimates (*i.e.* bias towards no effect), since time to completion of the questionnaire was comparable among mothers of cases and controls. If parents of cases would have underreported exposure due to, for instance, feelings of guilt, differential bias would have occurred, also resulting in underestimation of effect estimates. Our analyses were also hampered by missing values, although this was limited to ≤10% for all variables except family history. To facilitate analyses of all participants, even in case of missing data, multiple imputation was used under the assumption of missingness at random. For BMI, this assumption was supported by a comparison with population data. Although we cannot exclude with absolute certainty that some information may have been missing not at random, most results proved stable when simulating such patterns of missingness. Our study benefitted from the large number of patients with a well-defined phenotype, in contrast to others who often studied the entire CAKUT spectrum. Still, aetiological differences between UKA and MCDK cannot be ruled out, and the degree of association may vary between these diagnoses. In addition, information on many potential risk factors and confounders was available, and DAGs were created for each prespecified risk factor in order to correct for confounding in the most optimal fashion. No relevant unmeasured confounders were identified by the DAGs. Nonetheless, several lines of evidence are needed before a causal relationship can be established using observational data, so our results should be supported by others before they can be firmly established.

Our results reaffirm the positive effects of folic acid supplementation in pregnancy and suggest that the form in which folic acid is taken does not influence effectiveness. Furthermore, we again highlighted the potential harmful effects of smoking and identified stress as risk factor for CSFK. The identification of stress as novel risk factor warrants re-examination by others, but given the well-established range of negative effects of stress during pregnancy, stress reduction strategies could already be incorporated into public health advices. Several non-pharmacological measures that improve coping and reduce potential negative effects of stress during pregnancy are available.

As illustrated by the current study, the aetiology of CSFK is likely to consist of a combination of genetic and environmental factors. Although both factors may cause CSFK independently, interactions are likely to play a role, given the low number of genetically solved cases and the relatively modest effect sizes of most environmental factors. Nevertheless, only few successful gene-environment interaction studies are available. For cleft lip/palate, one of the most common birth defects, investigators found evidence for an interaction between genetic variants and both multivitamin supplements and exposure to environmental tobacco smoke.[68] We are not aware of any studies that have investigated whether similar interactions play a role in kidney development, however, but large cohorts integrating genetic and environmental data are needed to do so in the future.

In conclusion, we show that several environmental and parental risk factors are likely to play a role in the aetiology of CSFK. Government agencies and healthcare workers should focus on optimizing lifestyle and other targetable factors in women wanting to become pregnant, while further studies should incorporate environmental factors when studying the aetiology of CSFK.

SUPPLEMENTARY MATERIAL

Supplementary Tables

Supplementary Table 1 Comparison of the effect of folic-acid supplements and folic-acid containing multivitamins in prevention of congenital solitary functioning kidney, limited to multivitamins specifically for women trying to get pregnant or being pregnant.

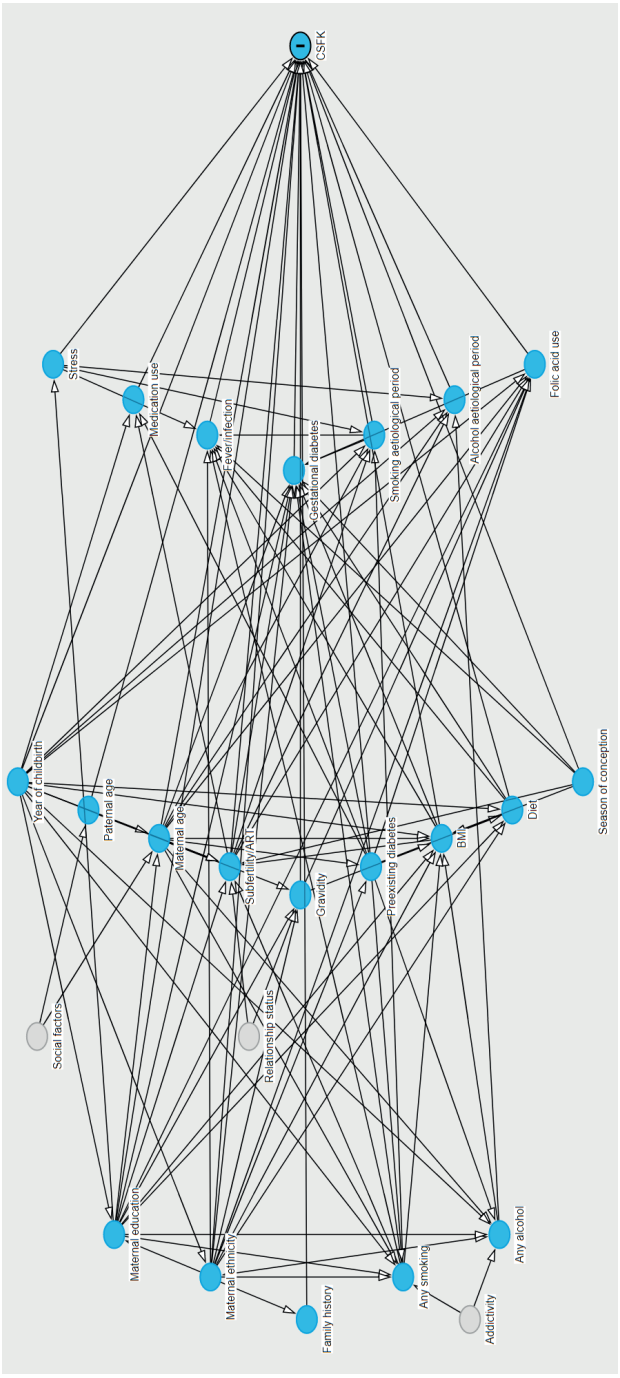
	Controls (n = 1,302)	CSFK cases (n = 434)	cOR	aOR[a]	95% CI low	95% CI high
No vitamins at all	**153 (12%)**	**59 (15%)**	**1.0**	**1.0**	**ref**	**ref**
Any use[b]						
Folic acid only	620 (50%)	202 (50%)	0.8	0.8	0.6	1.2
Multivitamins only	58 (5%)	20 (5%)	0.8	0.9	0.5	1.8
Both	404 (33%)	124 (31%)	0.8	0.8	0.5	1.2
Use as recommended[c]						
Folic acid only	471 (38%)	136 (34%)	0.7	0.7	0.5	1.1
Multivitamins only	48 (4%)	8 (2%)	0.4	**0.4**	**0.2**	**1.0**
Both	177 (14%)	43 (11%)	**0.6**	**0.6**	**0.4**	**1.0**
Suboptimal use[d]						
Folic acid only	255 (21%)	91 (23%)	0.9	0.9	0.6	1.4
Multivitamins only	27 (2%)	14 (4%)	1.3	1.4	0.6	3.2
Both	98 (8%)	50 (13%)	1.4	1.3	0.8	2.2

[a]Adjusted for minimal set of confounders determined using directed acyclic graphs (DAGs; available as supplementary table 2 and supplementary figure 1). [b]Defined as use as recommended or suboptimal use. [c]Defined as initiation before pregnancy and continued use through at least the 8th week of pregnancy. [d]Usage during only part of the recommended period. cOR crude odds ratio, aOR adjusted odds ratio, CI confidence interval, ref reference.

Supplementary Table 2 Minimal set of confounders obtained from directed acyclic graphs.

Exposure	Minimal set of confounders
Gravidity	Maternal ethnicity, education level, and age
Season of conception	No adjustment needed
Maternal age	Year of childbirth, maternal education level, and paternal age
Maternal BMI	Year of childbirth, gravidity, season of conception, maternal ethnicity, education level, age, alcohol use, smoking, and diet,
Subfertility	Year of childbirth, gravidity, season of conception, maternal ethnicity, education level, age, BMI, and smoking, and paternal age
Pre-existing diabetes	Maternal ethnicity and age
Gestational diabetes	Season of conception, maternal ethnicity, education level, age, subfertility, BMI, pre-existing diabetes, smoking, and folic acid supplementation, and subfertility
Pre-existing hypertension	Year of childbirth, gravidity, season of conception, maternal ethnicity, education level, age, BMI, subfertility, pre-existing diabetes, gestational diabetes, alcohol use, smoking, and folic acid supplementation
Maternal infections	Season of conception, maternal ethnicity, BMI, pre-existing diabetes, smoking, diet, and stress
Medication use during pregnancy	Year of childbirth, maternal pre-existing diabetes, subfertility, and infections
Smoking	Year of childbirth, maternal age, subfertility, smoking, and stress
Alcohol	Year of childbirth, maternal age, subfertility, alcohol use, and stress
Folic acid supplementation	Year of childbirth, Maternal ethnicity, education level, gravidity, age, subfertility, and pre-existing diabetes
Maternal diet	Year of childbirth, maternal ethnicity, education level, and pre-existing diabetes
Maternal stress	Maternal education level
Family history	Maternal ethnicity
Paternal age	Year of childbirth, maternal education level, and age

Supplementary Figures



Supplementary Figure 1 Directed Acyclic Graph containing all potential risk factors and confounders with their mutual relations. Factors with grey circles represent unobserved variables. BMI body mass index, CSFK congenital solitary functioning kidney.

	Maternal education	Maternal ethnicity	Family history	Any smoking	Any alcohol	Year of childbirth	Paternal age	Maternal age	Subfertility/ART	Gravidity	Preexisting diabetes	BMI	Diet	Season conception	Stress	Medication	Fever/infection	Gestational diabetes	Smoking aetiological period	Alcohol aetiological period	Folic acid use
Maternal education	X	->	-	^	^	^	-	^	^	^	-	^	^	-	^	-	-	^	-	-	^
Maternal ethnicity	->	X	^	^	^	^	-	^	-	^	^	^	^	-	-	-	^	^	-	-	^
Family history	-	^	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Any smoking	^	^	-	X	-	-	-	-	-	-	^	^	^	-	-	-	^	^	-	-	-
Any alcohol	^	^	-	<- ->	X	-	-	-	-	-	^	^	^	-	-	-	^	^	-	-	-
Year of childbirth	->	->	-	->	->	X	-	^	^	^	^	^	^	-	-	-	^	^	^	^	^
Paternal age	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Maternal age	^	-	-	->	->	^	<-->	X	-	-	-	-	-	-	-	-	-	-	-	-	-
Subfertility/ART	^	-	-	^	-	^	^	^	X	-	-	-	-	-	-	-	-	-	-	-	-
Gravidity	^	^	-	-	-	-	-	^	<- ->	X	-	-	-	-	-	-	-	-	-	-	-
Preexisting diabetes	-	^	-	->	->	-	-	^	-	-	X	-	-	-	-	-	^	^	-	-	^
BMI	^	^	-	^	^	^	-	^	->	^	-	X	-	-	-	-	^	^	-	-	^
Diet	^	^	-	-	-	^	-	-	-	-	^	->	X	-	-	-	^	^	-	-	^
Season conception	-	-	-	-	-	-	-	-	->	-	-	->	-	X	-	-	^	^	-	-	^
Stress	^	-	-	-	-	-	-	-	^	-	-	-	-	-	X	-	^	^	^	^	^
Medication	-	-	-	-	-	^	-	-	^	-	^	-	-	-	-	X	->	-	-	-	^
Fever/infection	-	^	-	^	-	-	-	^	-	-	^	^	^	^	^	->	X	-	->	-	^
Gestational diabetes	^	^	-	^	-	-	-	^	^	-	^	^	^	^	-	-	-	X	->	-	^
Smoking aetiological period	-	-	-	^	-	^	-	^	^	-	-	-	-	-	^	-	->	->	X	-	-
Alcohol aetiological period	-	-	-	-	^	^	-	^	^	-	-	-	-	-	^	-	-	-	-	X	-
Folic acid use	^	^	-	-	-	^	-	^	^	^	^	-	-	-	-	-	-	->	-	-	X

Supplementary Figure 2 Matrix containing all associations between potential risk factors and confounders. Double arrows (<- ->) indicate a shared unobserved parent variable.

CHAPTER 5

The role of gene-environment interactions in the aetiology of congenital solitary functioning kidney

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ABSTRACT

Background

Congenital solitary functioning kidney (CSFK) is an anomaly predisposing to hypertension, albuminuria and chronic kidney disease. Its aetiology is complex and includes genetic and environmental factors. The role of gene-environment interactions (GxE), although relevant for other congenital anomalies, has not yet been investigated. Therefore, we performed a genome-wide GxE analysis with six preselected environmental factors to explore the role of these interactions in the aetiology of CSFK.

Methods

In the AGORA data- and biobank, genome-wide single-nucleotide variant (SNV) data and questionnaire data on prenatal exposure to environmental risk factors were available for 381 CSFK patients and 598 healthy controls. Using a two-step strategy, we first selected independent significant single-nucleotide variants associated with one of the six environmental risk factors. These SNVs were subsequently tested in GxE analyses using logistic regression models, with Bonferroni-corrected p-value thresholds based on the number of SNVs selected in step one.

Results

In step one, 7 to 40 SNVs were selected per environmental factor, of which only rs3098698 reached statistical significance ($p=0.0016$, Bonferroni-corrected threshold 0.0045) for interaction in step two. The interaction between maternal overweight and this SNV, which results in lower expression of the ARSB gene, could be explained by lower insulin receptor activity in children heterozygous for rs3098698. Eight other GxE interactions had a p-value <0.05, of which two were biologically plausible and warrant further study.

Conclusions

Interactions between genetic and environmental factors may contribute to the aetiology of CSFK. To better determine their role, large studies combining data on genetic and environmental risk factors are warranted.

INTRODUCTION

A congenital solitary functioning kidney (CSFK) is a congenital anomaly which occurs in approximately 1:1500 children and results in hypertension, albuminuria and chronic kidney disease in up to 80% of patients at 18 years of age.[31,91] Moreover, early signs of kidney injury, such as high blood pressure, can increase the risk of cardiovascular disease later in life.[5] The most important causes of CSFK are unilateral kidney agenesis, multicystic kidney dysplasia (MCDK), and kidney hypo-/dysplasia, which all fall within the spectrum of congenital anomalies of the kidney and urinary tract (CAKUT). Anomalies within the CAKUT spectrum are thought to share part of their aetiology because of the occurrence of different CAKUT phenotypes in family members with the same mutation.[33] Genetic, environmental, and epigenetic factors may all be involved in CAKUT.[34]

More than 150 monogenic causes for CAKUT have been reported.[158] The majority is associated with syndromic CAKUT, whereas only 23 genes are known to cause isolated CAKUT.[48] For CSFK, the diagnostic yield in studies aimed at identifying monogenic causes is usually low (7-11%), suggesting that CSFK may have multiple causes.[43,46,47] Copy number variants (CNVs) contribute to the aetiology of CSFK, with 14-17% of patients harbouring a rare CNV.[49,50] In addition, common variants are likely to play a role, and we recently identified two candidate loci in a genome-wide association study (GWAS).[159]

Environmental risk factors for CAKUT have also been studied for many years, with an increasing focus on differences between CAKUT phenotypes.[36,37] Among the risk factors implicated in the aetiology of CSFK are conception using *in vitro* fertilization (IVF)/intracytoplasmic sperm injection (ICSI) and maternal diabetes, obesity, smoking, infections, and stress.[37,54-56,95] Genetic and environmental factors explain the aetiology of many congenital anomalies, including CSFK, insufficiently, however. Part of this missing insight into the aetiology may be explained by gene-environment (GxE) interactions.[67] Although evidence for interactions between genetic variants and environmental factors is available for other congenital anomalies,[68,69,160] no studies on GxE interactions have been performed for CSFK yet. Therefore, we combined data on common genetic variants and exposure to environmental factors to explore the role of GxE interactions in the aetiology of CSFK.

MATERIALS AND METHODS

Study participants

Study participants were derived from the AGORA (Aetiologic research into Genetic and Occupational/environmental Risk factors for Anomalies in children) data- and biobank[87] and were included in a previous GWAS[159] and a study on environmental risk factors. [95] Briefly, parents of patients with CSFK, defined as a solitary functioning kidney resulting from unilateral kidney agenesis, MCDK, or hypo/dysplasia, were asked to participate in AGORA when visiting the Radboud university medical center (from 2004 onwards) or University Medical Center Utrecht (from 2013 onwards). Additional recruitment to increase the number of CSFK patients in AGORA was initiated in 2018, when CSFK patients from 36 hospitals throughout The Netherlands were enrolled in the SOFIA study. [91] Biological parents were asked to fill out a questionnaire on environmental exposures shortly before and during the pregnancy of their child. DNA of the patients was extracted from blood (when available) or saliva. Patients with a known or suspected genetic or syndromic aetiology were excluded from the current study. Healthy controls were recruited through a random sample of children living in 39 collaborating Dutch municipalities. All parents of these children were also asked to fill out the questionnaire and part of them to provide a saliva sample from their child. Only children without major birth defects (defined using EUROCAT guidelines[138]) were eligible as controls. The AGORA data- and biobank was approved by the Regional Committee on Research Involving Human Subjects.

Environmental risk factors

A full description of the assessment of environmental exposures was published previously. [95] From this larger set of potential risk factors, six were selected as most likely to interact with genetic variants by a panel of experts in reproductive and genetic epidemiology: parental subfertility, use of assisted reproductive techniques (ART), maternal overweight/obesity, maternal folic acid supplementation, maternal smoking, and maternal alcohol consumption during the etiologically relevant time period. To study parental subfertility, couples reporting a diagnosis of subfertility, defined as a time to pregnancy of more than 12 months, or conception using ART, were compared to parents who conceived naturally within 12 months. To study ART specifically, we compared the subgroup of patients who conceived using ART to the same control group, excluding parents reporting subfertility but no use of ART from the analysis. Maternal overweight/obesity was defined as a body mass index (BMI) >25 kg/m^2 at the time of conception. Folic acid supplementation was considered as use of folic acid supplements or folic acid-containing multivitamins during the entire advised period (initiation before pregnancy and continued through at least the eighth week of pregnancy). Maternal

smoking and alcohol use were both defined as exposure in the three months before and/or during pregnancy.

Genotyping, quality control, and imputation

The genetic data and procedures on quality control and imputation were derived from a previous GWAS into the aetiology of CSFK.[159] Genotyping was performed by deCODE genetics (Reykjavik, Iceland) using Infinium Global Screening Arrays (Illumina, San Diego, CA, USA) in separate batches for patients and controls. During quality control, variants with a call rate <98%, a deviation from Hardy-Weinberg equilibrium with a p-value $<1x10^{-10}$ (for patients) or $<1x10^{-6}$ (for controls), or a minor allele frequency <0.01 were removed. In addition, samples were excluded if they had a call rate <98% or if individuals were related up to the third degree or were of non-European ancestry (ancestries other than 1,000 genomes population codes GBR or CEU).[105] Imputation was performed for patients and controls together based on the overlap in variants genotyped (n = 524,412) using Minimac (v4), with 1,000 Genomes Project Phase 3 data as reference panel.

Statistical analyses

We applied the two-step analytical strategy proposed by Murcray *et al.*,[161] in which the p-value threshold in the main GxE interaction analysis (step two) is based on a weighting step (step one). This method was shown to be more powerful compared to a one-step strategy when the exposure or the disease allele are rare.[161] In step one, we estimated the associations between all single-nucleotide variants (SNVs) and the selected environmental factors among the combined sample of patients and controls. We used FUMA to select independent ($r^2 \leq 0.6$) SNVs with a p-value $\leq 1x10^{-5}$ for the association with exposure status.[108] For the each of the selected SNVs, we fitted logistic regression models for case-control status in step two, with the following terms: exposure main effect, SNV main effect, and the exposure x SNV interaction term. We selected the first four principle components, maternal level of education, and sex of the child as potential confounders. To properly adjust for confounding,[162] covariate main effects, covariate x environment interaction terms, and covariate x gene interaction terms were added to the models. For the analyses in step two, the p-value threshold for statistical significance was determined as 0.05 divided by the number of SNVs selected in step one for the exposure of interest, but SNVs with a p-value <0.05 were also selected as suggestive loci for further inspection. The selected loci were visualized using LocusZoom[110] and assessed for functional relevance using the RegulomeDB.[112] Lastly, expression quantitative trait loci (eQTLs) were determined using the GTEx portal (https://gtexportal.org/). Genetic quality control and the analyses in step one were performed with PLINK 2.0,[102] while the analyses in step two were performed in R version 4.1.0.[163]

RESULTS

In total, 560 CSFK patients and 4,104 controls were available in the AGORA data- and biobank. Questionnaires were available for 501 patients and 4,039 controls, whereas DNA was collected from 503 patients and 725 controls. From 467 patients and 721 controls, both questionnaires and DNA were present. Genotyping was successful in 446 of these patients and 667 controls, but 21 patients were excluded because of a known or suspected genetic or syndromic aetiology and 22 controls because of a major birth defect. After genomic quality control and imputation, information on 9,956,431 SNVs from 381 patients and 598 controls was available for use in the GxE interaction analyses. A flowchart of the inclusion is presented in Figure 1. Patients were more often male and their mothers were more often highly educated compared to controls (Table 1). Patients were also more likely to be conceived using ART and to have a mother who was overweight or obese, whereas maternal age and parity as well as the frequency of the other exposures were comparable between patients and controls.

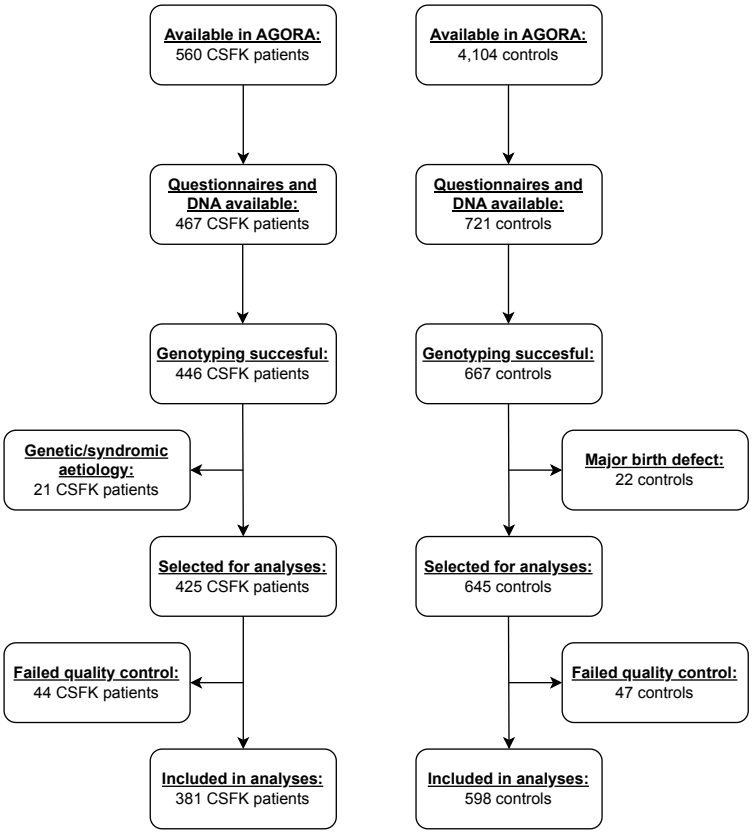


Figure 1 Flowchart of inclusion of patients and controls

Table 1 Overview of maternal and pregnancy characteristics among patients with congenital solitary functioning kidneys as well as population-based control subjects

	Patients n=381	Controls n=598
Maternal age group		
≤25 years	20 (5%)	22 (4%)
25-34.9 years	275 (72%)	468 (78%)
≥35 years	77 (20%)	108 (19%)
Missing	9 (2%)	0 (0%)
Parity		
Nulliparous	156 (41%)	232 (39%)
Multiparous	210 (55%)	364 (61%)
Missing	15 (4%)	2 (0%)
Child sex		
Male	245 (64%)	291 (49%)
Female	136 (36%)	307 (51%)
Maternal education level		
Low	45 (12%)	94 (16%)
Intermediate	149 (39%)	289 (48%)
High	187 (49%)	214 (36%)
Missing	0 (0%)	1 (0%)
Parental subfertility		
No subfertility	293 (77%)	458 (77%)
Any subfertility	84 (22%)	88 (15%)
Missing	4 (1%)	52 (9%)
Conception using ART		
No subfertility	293 (77%)	458 (77%)
Conception using any ART	34 (9%)	17 (3%)
Missing	54 (14%)	123 (21%)
Maternal BMI		
≤25 kg/m^2	252 (66%)	431 (72%)
>25 kg/m^2	119 (31%)	139 (23%)
Missing	10 (3%)	28 (5%)
Maternal folic acid supplementation		
As recommended	173 (45%)	245 (41%)
Other	187 (49%)	317 (53%)
Missing	21 (6%)	36 (6%)

Table 1 Overview of maternal and pregnancy characteristics among patients with congenital solitary functioning kidneys as well as population-based control subjects (continued)

	Patients n=381	Controls n=598
Maternal smoking		
No	308 (81%)	470 (79%)
Yes	72 (19%)	128 (21%)
Missing	1 (0%)	0 (0%)
Maternal alcohol use		
No	218 (57%)	319 (53%)
Yes	160 (42%)	279 (47%)
Missing	3 (1%)	0 (0%)

ART artificial reproductive technology; BMI body mass index

In step one, the number of independent SNVs with a p-value $\leq 1x10^{-5}$ varied per environmental factor between seven (maternal folic acid supplementation) and 40 (conception through ART) (Table 2). The lowest p-values for associations with exposure to the environmental factors ranged between $1.4x10^{-6}$ (maternal alcohol use) and $1.0x10^{-8}$ (maternal smoking). All SNVs selected in step one were carried forward to the GxE interaction analysis in step two. Of these SNVs, one reached the Bonferroni-corrected threshold for statistical significance (rs3098698, p-value 0.0016, threshold 0.0045). This variant is an intronic variant of the Arylsulfatase B (*ARSB*) gene, with a RegulomeDB score of 0.13 and an odds ratio (OR) for the corresponding GxE interaction of 3.2 (95% confidence interval (CI) 1.5-7.2) (Table 3). Odds ratios for CSFK comparing mothers with overweight/obesity to normal-weighted mothers stratified per genotype of the child were 1.9 (95% CI 1.3-2.6) in children homozygous for the reference allele and 0.6 (95% CI 0.3-1.3) for heterozygous children (Table 4). Too few children (2 patients and 5 controls) carried two alternative alleles to calculate the OR for this group. Eight other SNVs reached a p-value <0.05 (Table 3), of which two are located in regulatory regions of CTCF (CCCTC-Binding Factor) binding sites, five are located in an intron, and one is located between genes. RegulomeDB scores varied between 0.01 and 0.80, reflecting a very low and high probability of being a regulatory variant, respectively.[164] The point estimates for the OR of GxE interaction varied between 0.1 and 51.4. For only two variants (rs13409540 and rs5796570), ORs for the corresponding environmental factor (maternal overweight/obesity and folic acid supplementation, respectively) could be assessed in all three genotypes, with a dose-response visible for rs5796570 but not for rs13409540 (Table 4).

Table 2 Number of independent single nucleotide variants associated with each environmental risk factor in complete case analyses

	Number of independent significant SNVs[1]	Lowest p-value in step one	Bonferroni-corrected p-value threshold in step two
Any subfertility	12	$9.1x10^{-8}$	0.0042
Conception using ART	40	$1.3x10^{-7}$	0.0013
Maternal BMI	11	$7.3x10^{-7}$	0.0045
Maternal folic acid supplementation	7	$1.4x10^{-7}$	0.0071
Maternal smoking	20	$1.0x10^{-8}$	0.0025
Maternal alcohol use	10	$1.4x10^{-6}$	0.0050

[1]Independent significant SNVs were those with an $r^2 \leq 0.6$ and a p-value $\leq 1x10^{-5}$ for the association with the environmental risk factor of interest. SNV single nucleotide variant, ART artificial reproductive technology, BMI body mass index.

DISCUSSION

Our study is the first genome-wide GxE interaction study in patients with CSFK and identified one statistically significant interaction between the genetic variant rs3098698 and the environmental factor maternal overweight/obesity. Eight other interactions reached a p-value <0.05, but were above the Bonferroni corrected p-value threshold. Despite the small sample size, this study illustrates the potential value of gene-environment interaction studies in the field of CAKUT.

To explain the statistically significant interaction between variant rs3098698 on chromosome 5 and maternal overweight/obesity, functional consequences of this SNV were studied. The variant is located in an intron of the *ARSB* gene (Supplemental Figure 1), a gene that codes for a sulfatase (Arylsulfatase B). Arylsulfatase B controls degradation of chondroiting4-sulfate and dermatan sulphate, which may in turn influence intracellular signalling, cell-cell communication, and transcription.[165] When expression of the *ARSB* gene is reduced, chondroiting4-sulfate is more abundant, which leads to increased cellular and nuclear galectin-3 levels.[166] Galectin-3 binds to the insulin receptor and causes insulin resistance.[167] The GTEx Portal indicates that the rs3098698 variant is associated with lower expression of *ARSB*, with the most profound effect observed in kidney cortex cells (Figure 2). Therefore, children heterozygous for the rs3098698 variant are expected to be more resistant to insulin. Maternal overweight/obesity did not increase the risk of CSFK in these children, possibly because the increased maternal glucose levels do not result in increased cellular uptake of glucose in children with the SNV because of their lower insulin receptor activity. Experimental work should further investigate this and other potentially relevant mechanisms involved and could provide insight into whether the rs3098698 variant is the causal SNV or acts as proxy for other variants that were not directly tested.

Table 3 Location and predicted effect of selected single nucleotides with a gene-environment interaction p-value <0.05.

Environmental factor	Chr	Position[1]	Ref	Alt	rs number	OR GxE (95% CI)	p-value GxE	Variant effect predictor	Gene	RegulomeDB score[2]
ART	Chr 4	99731659	C	A	rs75166568	0.1 (0.0-0.4)	0.0055	Intergenic	-	0.22
ART	Chr 4	99749971	A	G	rs35047415	0.1 (0.0-0.6)	0.0087	Regulatory region variant	*CTCF* binding site	0.57
ART	Chr 13	26755829	T	C	rs77612507	51.4 (1.4-501)	0.0298	Intronic variant	*RNF6*	0.32
BMI	Chr 2	67196296	A	G	rs13409540	0.5 (0.3-0.8)	0.0057	Intronic variant	*ETAA1*	0.61
BMI	**Chr 5**	**78174969**	**G**	**A**	**rs3098698**	3.2 (1.5-7.2)	**0.0016**	**Intronic variant**	***ARSB***	**0.13**
BMI	Chr 15	52266922	T	C	rs367873445	2.6 (1.2-5.8)	0.0177	Intronic variant	*MAPK6*	0.01
Smoking	Chr 1	37900415	C	T	rs11264047	0.4 (0.2-0.9)	0.0208	Regulatory region variant	*CTCF* binding site	0.80
Smoking	Chr 7	8559913	T	A	rs75063030	5.2 (1.4-22.3)	0.0125	Intronic variant	*NXPH1*	0.50
Folic acid	Chr 12	14123620	A	AAC	rs5796570	0.6 (0.4-0.9)	0.0251	Intronic variant	*GRIN2B*	0.13

Bold values indicate associations with a p-value below the Bonferroni-corrected threshold for statistical significance. [1]Genomic locations are based on dbSNP and represented in genome build GRCh37/Hg19. [2]The RegulomeDB score ranges from 0 to 1 and represents the likelihood of being a regulatory variant [164]

Chr chromosome, Ref reference allele; Alt alternative allele, OR odds ratio, GxE gene-environment interaction, ART artificial reproductive technology, BMI body mass index.

Table 4 Odds ratios with 95% confidence intervals for the association between environmental factors and congenital solitary functioning kidneys stratified per single nucleotide variant genotype

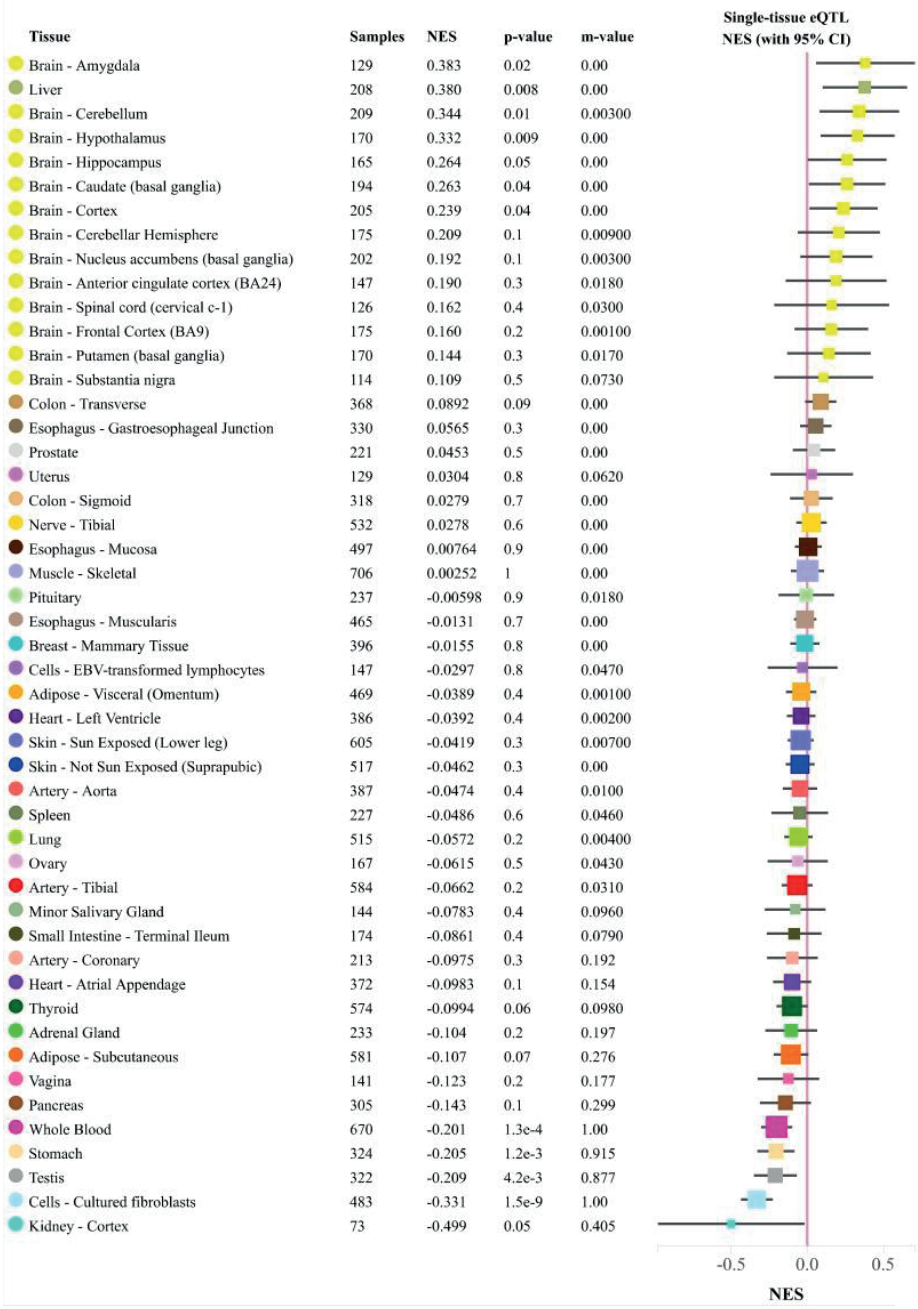
Environmental factor	rs number	Reference genotype			Heterozygous genotype			Variant genotype		
		N (%) patients	N (%) controls	OR (95% CI)	N (%) patients	N (%) controls	OR (95% CI)	N (%) patients	N (%) controls	OR (95% CI)
ART	rs75166568	295 (77%)	457 (76%)	1.9 (0.8-4.5)	81 (21%)	133 (22%)	6.2 (2.3-17)	2 (1%)	7 (1%)	n/a
ART	rs35047415	314 (82%)	473 (79%)	2.3 (1.1-5.3)	64 (17%)	117 (20%)	6.7 (2.3-19)	2 (1%)	6 (1%)	n/a
ART	rs77612507	367 (96%)	584 (98%)	4.2 (2.1-8.4)	13 (3%)	14 (2%)	0.8 (0.1-4.8)	0	0	n/a
BMI	rs13409540	221 (58%)	333 (56%)	1.1 (0.7-1.6)	137 (36%)	221 (37%)	2.5 (1.6-4.1)	23 (6%)	44 (7%)	2.0 (0.6-6.5)
BMI	rs3098698	312 (82%)	509 (85%)	1.9 (1.3-2.6)	67 (18%)	84 (14%)	0.6 (0.3-1.3)	2 (1%)	5 (1%)	n/a
BMI	rs367873445	317 (83%)	527 (88%)	1.7 (1.3-2.4)	62 (16%)	70 (12%)	0.7 (0.4-1.5)	2 (1%)	1 (0%)	n/a
Smoking	rs11264047	322 (85%)	497 (83%)	0.8 (0.5-1.1)	57 (15%)	94 (16%)	2.8 (1.3-5.9)	2 (1%)	7 (1%)	n/a
Smoking	rs75063030	351 (92%)	561 (94%)	1.1 (0.8-1.6)	28 (7%)	35 (6%)	0.3 (0.1-0.9)	2 (1%)	2 (0%)	n/a
Folic acid	rs5796570	161 (42%)	270 (45%)	0.6 (0.4-0.9)	177 (47%)	275 (46%)	1.0 (0.7-1.6)	43 (11%)	50 (8%)	1.4 (0.6-3.5)

OR odds ratio, CI confidence interval, ART artificial reproductive technology, BMI body mass index.

Eight other GxE interactions reached a p-value <0.05. Three of the variants involved (rs35047415 on chromosome 4 interacting with ART, rs13409540 on chromosome 2 interacting with maternal BMI, and rs11264047 on chromosome 1 interacting with maternal smoking) showed RegulomeDB scores above 0.5. One other variant (rs367873445 on chromosome 15, which showed an interaction with BMI) was a statistically significant eQTL. Therefore, these four variants are most likely to have functional consequences as well. The highest RegulomeDB score was observed for rs11264047, a variant that showed an interaction with maternal smoking and results in higher expression of LINC01137. Similar to smoking,[168] LINC01137 inhibits formation of miR125-b,[169,170] thereby possibly influencing the balance between transforming growth factor beta and Wnt signalling, which are important signalling pathways during kidney development.[171,172]

Variant rs35047415 on chromosome 4 was predicted as regulatory variant with an eQTL indicating slightly increased expression of *ADH5* in several tissues but not in kidney tissue. The encoded ADH5 protein is involved in aldehyde clearance, which can be linked to congenital anomalies,[173] but not to use of ART. The third variant with a high RegulomeDB score was rs13409540 on chromosome 2, which showed an interaction with maternal BMI. This variant is located in an intron of *ETAA1*, a gene involved in maintaining genomic stability.[174] No effect on gene expression was found based on eQTL data, however, making functional consequences less likely. Lastly, rs367873445 on chromosome 15 showed an interaction with maternal BMI as well. The GTEx Portal indicates that this variant may lead to higher expression of *SCG3* and *MYO5C* in kidney tissue, which have been linked to insulin secretion and overweight, respectively.[175,176] The interaction between rs367873445 on chromosome 15 and BMI, as well as the interaction between rs11264047 on chromosome 1 and maternal smoking, are promising to investigate in future studies.

Our study used data from a previous GWAS and a previous study on environmental risk factors for CSFK.[95,159] As such, the limitations of these studies are also relevant for the current GxE interaction study. Most importantly, the number of patients, although large for a study into a specific type of congenital anomaly, limits the power to detect associations. Power calculations using Quanto[177] indicated a power of approximately 80% to detect a GxE interaction, assuming a disease prevalence of 1:1,500, an SNV with a log-additive mode of inheritance, a minor allele frequency of 0.05 with an OR of 3.0, and an exposure with a prevalence of 20% and an OR of 1.5. Power to detect an interaction with an OR of 2.0 was estimated to be only 40%, although the two-step approach likely increased our power substantially.[161] Nonetheless, the limited number of subjects prevented us from further stratification into specific causes of CSFK (*i.e.* agenesis, MCDK, and hypo-/dysplasia), which may be relevant because of potential differences in aetiology.



Tissue	Samples	NES	p-value	m-value
Brain - Amygdala	129	0.383	0.02	0.00
Liver	208	0.380	0.008	0.00
Brain - Cerebellum	209	0.344	0.01	0.00300
Brain - Hypothalamus	170	0.332	0.009	0.00
Brain - Hippocampus	165	0.264	0.05	0.00
Brain - Caudate (basal ganglia)	194	0.263	0.04	0.00
Brain - Cortex	205	0.239	0.04	0.00
Brain - Cerebellar Hemisphere	175	0.209	0.1	0.00900
Brain - Nucleus accumbens (basal ganglia)	202	0.192	0.1	0.00300
Brain - Anterior cingulate cortex (BA24)	147	0.190	0.3	0.0180
Brain - Spinal cord (cervical c-1)	126	0.162	0.4	0.0300
Brain - Frontal Cortex (BA9)	175	0.160	0.2	0.00100
Brain - Putamen (basal ganglia)	170	0.144	0.3	0.0170
Brain - Substantia nigra	114	0.109	0.5	0.0730
Colon - Transverse	368	0.0892	0.09	0.00
Esophagus - Gastroesophageal Junction	330	0.0565	0.3	0.00
Prostate	221	0.0453	0.5	0.00
Uterus	129	0.0304	0.8	0.0620
Colon - Sigmoid	318	0.0279	0.7	0.00
Nerve - Tibial	532	0.0278	0.6	0.00
Esophagus - Mucosa	497	0.00764	0.9	0.00
Muscle - Skeletal	706	0.00252	1	0.00
Pituitary	237	-0.00598	0.9	0.0180
Esophagus - Muscularis	465	-0.0131	0.7	0.00
Breast - Mammary Tissue	396	-0.0155	0.8	0.00
Cells - EBV-transformed lymphocytes	147	-0.0297	0.8	0.0470
Adipose - Visceral (Omentum)	469	-0.0389	0.4	0.00100
Heart - Left Ventricle	386	-0.0392	0.4	0.00200
Skin - Sun Exposed (Lower leg)	605	-0.0419	0.3	0.00700
Skin - Not Sun Exposed (Suprapubic)	517	-0.0462	0.3	0.00
Artery - Aorta	387	-0.0474	0.4	0.0100
Spleen	227	-0.0486	0.6	0.0460
Lung	515	-0.0572	0.2	0.00400
Ovary	167	-0.0615	0.5	0.0430
Artery - Tibial	584	-0.0662	0.2	0.0310
Minor Salivary Gland	144	-0.0783	0.4	0.0960
Small Intestine - Terminal Ileum	174	-0.0861	0.4	0.0790
Artery - Coronary	213	-0.0975	0.3	0.192
Heart - Atrial Appendage	372	-0.0983	0.1	0.154
Thyroid	574	-0.0994	0.06	0.0980
Adrenal Gland	233	-0.104	0.2	0.197
Adipose - Subcutaneous	581	-0.107	0.07	0.276
Vagina	141	-0.123	0.2	0.177
Pancreas	305	-0.143	0.1	0.299
Whole Blood	670	-0.201	1.3e-4	1.00
Stomach	324	-0.205	1.2e-3	0.915
Testis	322	-0.209	4.2e-3	0.877
Cells - Cultured fibroblasts	483	-0.331	1.5e-9	1.00
Kidney - Cortex	73	-0.499	0.05	0.405

Figure 2 Single-tissue eQTL normalized expression scores of ARSB for rs3098698 in different cell types. NES normalized expression score. Figure created using GTEx portal (https://gtexportal.org/).

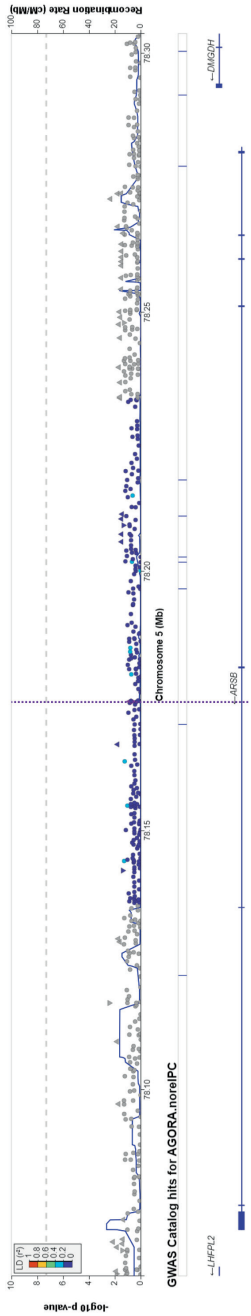
Furthermore, participants were not systematically screened for monogenic causes of CSFK or pathogenic CNVs, although patients with known or suspected genetic or syndromal causes were excluded. All environmental factors were assessed using self-reported questionnaires completed after delivery. This could have introduced the risk of recall bias for some lifestyle factors, although the period around pregnancy may be expected to be well-remembered by mothers. Similar to SNVs detected in a regular GWAS, the SNVs identified in our study may act as proxies for other causal but untested genetic variants. Functional models could provide more insight into this. The main strength of this study consists of the well-defined population in which a combination of data on genetic and environmental risk factors was available. In addition, a genome-wide search was possible due to the efficient design of the study, maximizing power in the available population.

The current study explored the possibility of GxE interactions in the aetiology of CSFK and found indications for several of such interactions. Limited statistical power and unavailability of replication cohorts mitigate the current implications of our results, which warrant confirmation before firm conclusions can be drawn. The study illustrates, however, that the aetiology of CSFK is complex. Integration of clinical data, genetic data, and information on environmental risk factors is needed for an integral assessment of relevant aetiological mechanisms, while large numbers of patients are needed to facilitate adequately powered studies. Future studies should aim for collection of all of these types of data to avoid research confined to only one aspect of the aetiology of CSFK.

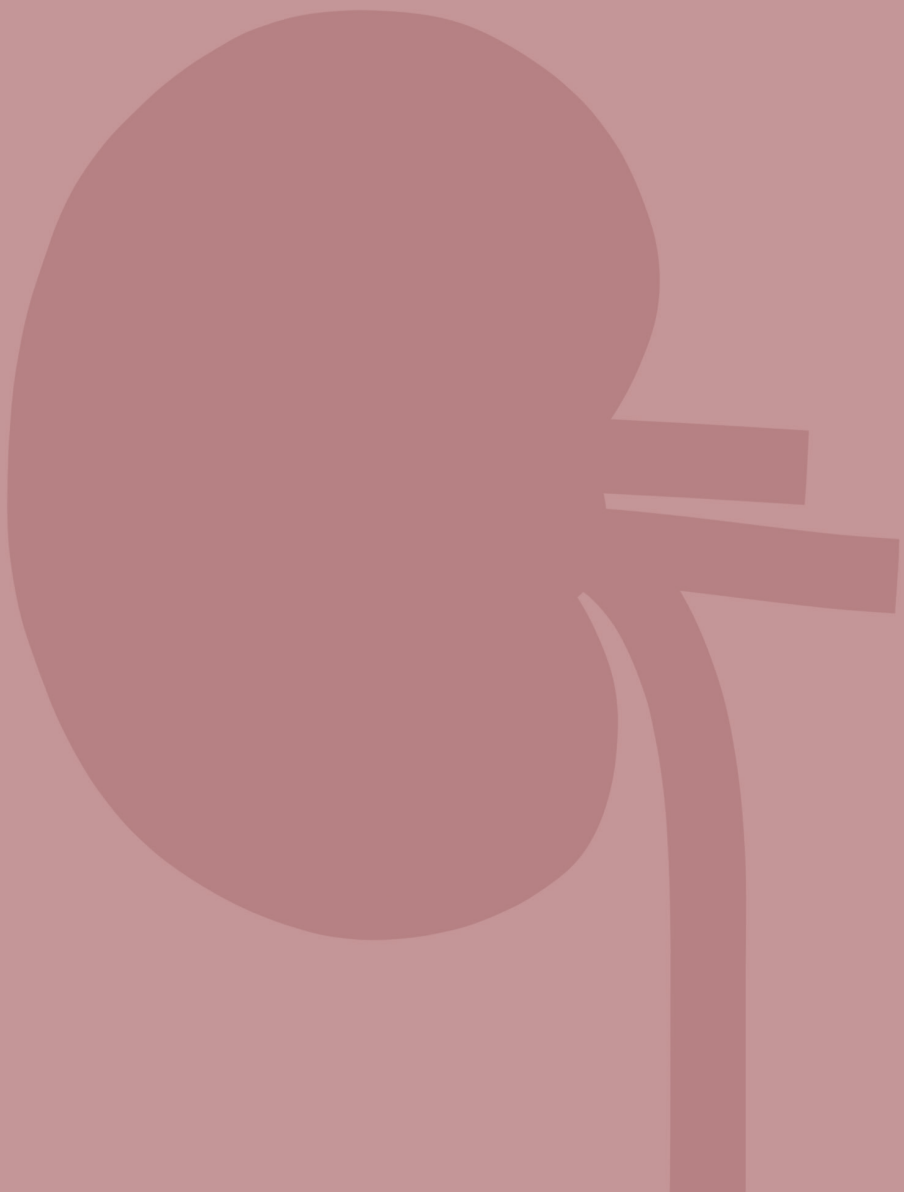
In conclusion, we identified GxE interactions as a relevant aetiological mechanism for CSFK, warranting further studies on this topic. We found an important interaction between the rs3098698 variant and the effect of maternal overweight/obesity, which provides novel leads for the pathophysiology of CSFK. This and several other potentially relevant combinations of genetic variants and environmental factors may contribute to CSFK development, although replication of our findings is highly warranted. Future studies should focus on genetic and environmental factors, as well as their interaction, to allow for a comprehensive view on the aetiology of CSFK.

SUPPLEMENTARY MATERIAL

Supplementary Figure



Supplemental Figure 1 Plot of genomic location variant rs3098698 on chromosome 5. The purple dotted line indicates the lead SNV in the GxE analysis (rs3098698). Figure created using Locuszoom (https://my.locuszoom.org/).



PART II

Outcomes and management of children with solitary functioning kidney

CHAPTER 6

Clinical management of children with a congenital solitary functioning kidney: Overview and recommendations

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ABSTRACT

Context

A Congenital Solitary Functioning Kidney (CSFK) is a common developmental defect which predisposes to hypertension and chronic kidney disease (CKD) as a consequence of hyperfiltration. Every urologist takes care of patients with a CSFK, since some will need lifelong urological care or will come with clinical problems or questions to an adult urologist later in life.

Objective

We aim to provide clear recommendations for the initial clinical management and follow-up of children with a CSFK.

Evidence acquisition

PubMed and EMBASE were searched to identify relevant publications, which were combined with guidelines on related topics and expert opinion.

Evidence synthesis

Initially, CSFK diagnosis should be confirmed and risk factors for kidney injury should be identified using ultrasound. Although more research into early predictors of kidney injury is needed, additional congenital anomalies of the kidney or urinary tract and absence of compensatory kidney hypertrophy have repeatedly been associated with a worse prognosis. The role of voiding cystourethrography and antibiotic prophylaxis remains controversial and is complicated by the exclusion of children with CSFK from studies.

A yearly follow-up for signs of kidney injury is recommended for children with CSFK. Because masked hypertension is prevalent, annual ambulatory blood pressure measurement should be considered. During puberty, an increasing incidence of kidney injury is seen, indicating that long-term follow-up is necessary. If signs of kidney injury are present, angiotensin converting enzyme inhibitors are the first line drugs of choice.

Conclusions

This overview points to the urological and medical clinical aspects and long-term care guidance for children with a CSFK, who are at risk for hypertension and CKD. Monitoring for signs of kidney injury is therefore recommended throughout life. Large, prospective studies with long-term follow-up of clearly defined cohorts are still needed to facilitate a more risk-based and individualized clinical management.

INTRODUCTION

Every urologist takes care of patients with a solitary functioning kidney (SFK) and within the field of paediatric urology, transition of care and adult urologists taking over the care of patient with a congenital anomaly, clear clinical management tools are needed. These guidelines points to the urological and medical clinical aspects and provides long term care guidance for children with a congenital solitary functioning kidney (CSFK), which is a developmental defect with an estimated prevalence of 1 in 1,500 new-borns.[17,29,31] Annually, >5,000 children are born with a CSFK in the USA and EU alone and in most cases, a CSFK is the consequence of unilateral renal agenesis (URA) or multicystic dysplastic kidney (MCDK). Two systematic reviews estimated the prevalence of URA and MCDK to be approximately 1 in 2,000 and 1 in 4,300 new-borns, respectively, and this appears to be stable in more recent cohorts (supplementary Table 1).[17,29] More males than females are affected, and a left-sided CSFK seems slightly more prevalent (supplementary Table 2).[17,29]

Living with a CSFK predisposes to hypertension, proteinuria and kidney function loss.[75,82,178] However, long term follow-up was considered unnecessary for a long time and currently large differences exist in the management of this condition. Therefore, we aim to provide practical clinical recommendations for the initial investigations, as well as indications for further diagnostics, treatment initiation, and long term follow up by a urologist, general practitioner or medical specialist in children with a CSFK, based on the currently available evidence.

EVIDENCE ACQUISITION

We searched PubMed and EMBASE using the search strategies of previously reported systematic reviews on URA and MCDK to identify publications on cohorts of patients with CSFK (supplementary methods).[17,29] Furthermore, we searched for systematic reviews (with or without meta-analysis), randomized-clinical trials, and observational studies on the different topics addressed in these guidelines, with a preference for systematic reviews. Existing guidelines on related topics were used when appropriate. When insufficient evidence was available, recommendations were formulated in consensus meetings among the authors.

EVIDENCE SYNTHESIS

Pathophysiology

Disturbances in several pathways involved in kidney development can lead to the congenital absence or reduced function of a kidney.[12] The most common causes of CSFK are renal aplasia, URA and unilateral MCDK, but other congenital anomalies of the kidney and urinary tract (CAKUT) may also lead to unilateral loss of kidney function.[82] Because kidney development continues until the 36th week of pregnancy, a CSFK can increase in size due to both hyperplasia (i.e. an increase in nephron number) and hypertrophy (i.e. an increase in nephron size).[179,180] Hyperplasia could lead to a nephron number that is greater than 50% of a person with two kidney and as such could reduce the risk of glomerular hyperfiltration and kidney injury.[180,181] Animals models show a ~50% increase in nephron numbers in the CSFK, leading to a total nephron number that equals ~70% of the total number of nephrons in an individual with two kidneys.[182]

In response to a lower number of nephrons, compensatory mechanisms in the remaining nephrons result in an increase in glomerular perfusion, leading to glomerular hyperfiltration and maintenance of a stable glomerular filtration rate (GFR).[183-185] Although beneficial in the short term, an increase in glomerular perfusion (in particular glomerular hypertension) can lead to detrimental structural changes in kidney morphology in the long term.[183-185] Following a vicious circle, glomerular hypertension leads to glomerulosclerosis with further loss of functional nephrons, which in turn increases single nephron glomerular filtration and worsens glomerular hypertension in the remaining nephrons. Glomerular hyperfiltration has been implicated as common disease pathway shared by diabetic nephropathy, focal segmental glomerulosclerosis, SFK, and other causes of low nephron numbers, such as premature birth and low birth weight.[184,186]

Based on the hyperfiltration theory, glomerular hypertension is the intermediate between a low nephron endowment and progressive kidney damage. As a consequence, signs of glomerular hyperfiltration such as albuminuria/proteinuria or systemic hypertension are expected to precede a decline in kidney function. Moreover, preventing glomerular hypertension would also prevent ongoing kidney injury,[184] creating an opportunity for treatment when diagnosed early.

Clinical presentation

Since the introduction of structured ultrasound screening during pregnancy, an increasing number of CSFKs are detected prenatally. Antenatal diagnosis of MCDK is usually possible at the 20-week routine ultrasound, since presentation with multiple cysts at 20 weeks is rare in other diagnoses.[187] In later stages of pregnancy, an MCDK

may have regressed and can be difficult to distinguish from URA.[31] Other conditions in the differential diagnosis of MCDK include severe hydronephrosis and other abdominal masses. An ectopic kidney may wrongly be diagnosed as URA on foetal ultrasound, whereas an enlarged adrenal may impose as kidney and therefore result in missing the diagnosis of URA. Repeated antenatal ultrasound can help confirm the diagnosis and can be used to monitor development of the unaffected kidney. In all cases, postnatal evaluation remains necessary to confirm an antenatally suspected diagnosis.

Assessment and diagnosis

All children with an antenatally suspected CSFK should be referred to a paediatric urologist, paediatrician, paediatric nephrologist, or urologist depending on the local and national referral patterns for postnatal evaluation of the kidneys and urinary tract. The timing of evaluation depends on the prenatal findings; in case of suspected anomalies in the remaining kidney, early postnatal evaluation is indicated (Figure 1).

Ultrasound

Ultrasound screening of the kidneys and urinary tract is the main diagnostic tool for evaluation of a patient with CSFK, given its non-invasive nature and high accuracy for diagnosing CSFK (Table 1).[188-190] At the first postnatal ultrasound, an attempt should be made to confirm prenatal findings and establish a definitive diagnosis. In addition, the presence of early compensatory hypertrophy with a kidney length of >2 standard deviations (SD) above the reference value for age could identify patients with a more favourable prognosis, although follow-up studies are needed to determine the clinical significance of this finding.[181] The status of the remaining kidney and urinary tract is also highly important for the prognosis. Approximately one in three children with an CSFK have additional urogenital anomalies, including vesicoureteral reflux (VUR) in ~20% and ureteropelvic junction obstruction (UPJO) in ~5% of patients (supplementary Table 3).[17,29] When such additional anomalies are found, urological advice should be sought to discuss diagnostic and treatment options.

Voiding cystourethrogram (VCUG)

A VCUG is the most sensitive way to detect VUR, and has frequently been used in CSFK patients given the high rate of VUR. However, as indicated in the latest guidelines for children with urinary tract infection (UTI), a high risk of VUR alone is not a proper indication for an invasive procedure such as VCUG.[191,192] CSFK patients could be considered as having an extra indication for a VCUG as high grade VUR appears to be a risk factor for kidney scarring[193-195] and kidney scarring can be considered to pose an additional risk in patients with an already reduced kidney mass. However, abnormalities on ultrasound are a major predictor for kidney scarring,[195] and the sensitivity of kidney ultrasound to detect high grade VUR is relatively high (60-100%). Furthermore, the number of patients with CSFK that need to undergo VCUG to diagnose one patient with

dilating VUR is 14 and increases to 43 considering only patients that underwent ureteral re-implantation.[196] Since ultrasound is also a cheaper and less invasive approach than VCUG, we recommend performing an ultrasound as first screening method in CSFK patients. When high grade VUR is suspected on ultrasound or UTIs occur, we suggest to use VCUG as second line investigation to help decide whether continuous antibiotic prophylaxis or surgical correction is indicated.

Scintigraphy

Kidney scintigraphy using radioactively labelled dimercaptosuccinic acid (DMSA) or mercapto acetyl tri glycine (MAG-3) can be used to visualize functioning kidney tissue. These studies are not needed routinely when URA or MCDK is suspected, since these diagnoses can be made accurately using sonographic studies of the kidney in >95% of cases.[188-190] Although a DMSA scan is more time consuming for the patients and parents involved, it may be indicated to visualize kidney scarring after a pyelonephritis. When ectopic kidney tissue is suspected, a DMSA scan is also indicated and preferred over a MAG-3 scan, since early bladder filling in combination with reduced/slow uptake of an ectopic kidney may result in missed ectopic kidney tissue in the bladder region using MAG-3 scintigraphy.[197] A MAG-3 scan is advised in case of significant urinary tract dilatation to exclude obstructions such as UPJO.

Magnetic resonance imaging

Magnetic resonance imaging of the kidney and urinary tract (MRU) is a technique that can provide detailed anatomical information. When used with gadolinium as contrast agent, functional information such as differential kidney function can be obtained simultaneously. Disadvantages of MRU include the need to lie still for a considerable amount of time and its considerable costs.[198] Furthermore, use of a bladder catheter and intravenous administration of contrast agents may be needed, and questions about gadolinium retention in the body have not yet been answered.[198] Current use of MRU is mostly limited to patients with unexplained symptoms after extensive imaging or when detailed anatomical information is needed, for instance for surgical planning, and in all instances the potential harm and benefit should be weighted. Use of gadolinium-based contrast agents is mainly guided by the kidney function and there is no apparent reason to withhold this from CSFK patients for other reasons.[199] The indications for MRU may be expanded in the future, however, especially if its potential to assess inflammation and fibrosis or count nephron number is confirmed.[198,200,201]

Laboratory measurements

To confirm adequate function of the CSFK, initial screening of GFR, blood pressure, and albuminuria/proteinuria is recommended. In patients with anomalies of the CSFK on the prenatal or first postnatal ultrasound, we recommend a first serum creatinine measurement within 1-2 weeks. The exact timing is a balance between the estimated reduction of kidney function, which may necessitate early evaluation, and the postnatal

functional development of the kidneys, for which measurement may be postponed to the 2nd week of life. In patients without anomalies of the CSFK, creatinine measurement can take place after 2-3 months.

Figure 1 Flowchart of urological or medical management of children with a congenital solitary functioning kidney (CSFK) for whom no evidence of structural kidney anomalies is seen in the CSFK on antenatal ultrasound. US kidney ultrasound, SD standard deviation, eGFR estimated glomerular filtration rate, RAAS renin angiotensin aldosterone system, ABPM ambulatory blood pressure monitoring. [a] Clinical problems or risk factors were defined as urinary tract infection, preterm birth <36 weeks, dysmaturity <p10, or low birthweight (<2,500g). [b] A first screening can take place after approximately 3 months with yearly follow-up afterwards.

Preliminary analyses of >100 CSFK patients from our own cohort show that none of the 46 children with a kidney size >2SD above the mean for age (for an individual with 2 kidneys) have a reduced kidney function within the first year of life (unpublished data). Therefore, in absence of additional indications (clinical problems, signs of obstructive uropathy, urinary tract infections, preterm birth (<36 weeks), or low birthweight (<2,500g or <p10 for gestational age)), it seems reasonable to refrain from an initial creatinine measurement in CSFK patients with compensatory hypertrophy.

Genetic screening

With advancing knowledge on the genetic aetiology of CSFK and decreasing costs for next generation sequencing, these techniques became available for more widespread diagnostic use. Currently, targeted sequencing studies for CAKUT seems the best option to balance the possible advantage of obtaining a specific diagnosis, such as HNF1β related nephropathy, with the small risk of incidental findings.[33] Since screening children with a sporadic CSFK has a success rate of 10-20%,[202] we currently suggest limiting genetic screening to children with additional anomalies or a positive family history.

Screening for Mullerian anomalies

Due to the embryological relatedness of the paramesonephric (Müllerian) and mesonephric (Wolffian) ducts, children with CSFK often show associated anomalies of the reproductive organs.[17,203] Since Müllerian duct anomalies can have severe and preventable complications, such as endometriosis, ultrasound screening of the internal genital organs is indicated in girls with CSFK.[204,205] This is possible within the first months of life due to stimulation by maternal oestrogen. When CSFK is detected in a girl, parents should be informed about the possibility of co-occurring Müllerian duct anomalies, particularly obstructed hemivagina and ipsilateral renal anomaly (OHVIRA) syndrome. After the onset of breast development, physicians should ask about menarche and cyclic abdominal pain during follow-up visits. In case of severe abdominal pain after menarche or when menarche is expected, OHVIRA and Mayer-Rokitansky-Küster-Hauser syndromes should be excluded by ultrasound.

Table 1 Diagnostic tools for patients with a congenital solitary functioning kidney

Modality	Advantages	Disadvantages	When indicated	When to consider
Kidney and bladder ultrasound	Non-invasive, cheap, widely available, high sensitivity and specificity for CSFK diagnosis	(Low grade) VUR or UPJO may be missed. Sensitivity lower in early postnatal period and other periods of dehydration	Within 1-2 weeks after birth. At 1 year follow-up. In case of UTI.	At 5, 10 and 15 year follow-up (especially when compensatory hypertrophy has not been shown)
Voiding cystourethrogram	Gold standard for VUR	Need for catheterization, risk of UTI, exposure to radiation	Dilated ureter on ultrasound, UTI	
MAG-3 renography	Simultaneous visualization of split kidney function and excretion	Requires intravenous injection, ectopic kidney tissue behind bladder may be missed	Suspected UPJO (high grade hydronephrosis without VUR)	
DMSA scintigraphy	Detection of focal parenchymal abnormalities (kidney scars), split kidney function, and ectopic kidney tissue	Requires intravenous injection, time consuming	Suspected ectopic kidney	Suspected kidney scarring after pyelonephritis
Magnetic resonance urography	Detailed anatomic information, functional information can be obtained using gadolinium contrast	May require intravenous injection, catheterization, and sedation. Time consuming and expensive	Unexplained symptoms after combinations of ultrasound, VCUG and renography (e.g. suspected ectopic ureteral implantation)	For surgical planning
Creatinine measurement to estimate GFR	Widely available, cheap	Invasive, influenced by maternal creatinine in postnatal period, late marker of kidney injury	After 1-2 weeks or 3 months (depending on ultrasound findings). Every 5 years afterwards.	When hypertension or proteinuria is found. Anomalies of SFK on imaging.
Urine albumin creatinine ratio measurement	Early marker of hyperfiltration, non-invasive, cheap, widely available	Risk of contamination, may be difficult to obtain in young children	Yearly follow-up visit	
Genetic screening (whole exome sequencing with kidney gene panel)	More specific diagnosis, risk of recurrence in next pregnancy of parents	Risk of incidental findings, low yield, not always available	Multiple associated anomalies	Strong positive family history, parental wish for pregnancy counselling in future
Office blood pressure measurement	Screening for hypertension, readily available	May be difficult in young children, risk of masked or white coat hypertension	Yearly in all children with CSFK	
Ambulatory blood pressure measurement	Identification of masked and white coat hypertension	Burdensome, no reference values for children <120cm, not always available	Yearly in CSFK patients with a history of or current hypertension or CKD	All other CSFK patients

CSFK congenital solitary functioning kidney, VUR vesicoureteral reflux, UPJO ureteropelvic junction obstruction, UTI urinary tract infection, MAG-3 mercapto acetyl tri glycine, DMSA dimercaptosuccinic acid; GFR glomerular filtration rate, CKD chronic kidney disease.

Treatment and Prognosis

Antibiotic prophylaxis

In some centres, antibiotic prophylaxis was administered to CSFK patients based on the assumption that it would reduce the number of UTIs and thereby kidney scarring, especially in children with VUR or a dilated urinary tract on imaging. However, there is no evidence that administering antibiotic prophylaxis to children with a CSFK without VUR or UTIs has clinical benefits.[206] Therefore, there seems to be little ground to prescribe antibiotic prophylaxis to all children with a CSFK.

Although antibiotic prophylaxis reduces the risk of UTIs in children with VUR, a statistically significant reduction in the number of kidney scars on DMSA has not been shown.[207-209] Based on these observations, the current AAP and NICE guidelines do not recommend routinely administering antibiotic prophylaxis following a first UTI.[191,192] However, children with CSFK were often excluded from studies into the effects of antibiotic prophylaxis and the long-term effects of UTI.[209] We recommend a precautious approach for CSFK patients, in order to prevent scarring as an additional loss of nephrons and/or to limit the additional risk of hypertension. Therefore, we suggest to administer antibiotic prophylaxis and perform a VCUG in a CSFK patient with a dilated ureter on ultrasound or after a first UTI. In addition, constipation and dysfunctional voiding should be addressed promptly, fluid intake should be encouraged, and clean toilets should be made available.[191,192] In case of VUR and recurrent UTIs under antibiotic prophylaxis, surgical interventions can be considered.

Follow-up ultrasounds

During follow-up, ultrasounds of the CSFK can identify compensatory hypertrophy, which has been identified as a favourable prognostic marker.[82,181,210,211] The value of repeated ultrasound is unclear, however, especially after compensatory hypertrophy has been observed. The risk of malignancy in MCDK does not seem to be elevated and is not a valid reason for ultrasound screening.[212] A reasonable approach is to perform a second kidney ultrasound at the age of one year, and once every five years afterwards. Especially after compensatory kidney hypertrophy >2 SD for age has occurred, cessation of ultrasound screening can be considered.

Screening for hyperfiltration

AAP guidelines recommend a blood pressure measurement at every medical encounter in children with CKD, including children with structural kidney anomalies such as CSFK.[213] Since blood pressure measurement in neonates is often imprecise, a first measurement could be performed after 3 months, and should be repeated at least yearly afterwards.

Ambulatory blood pressure monitoring (ABPM) is recommended in children with CKD due to the risk of masked hypertension.[213] Evidence for masked hypertension has also been shown in SFK specific studies (Table 2). Lack of reference values in children <120cm and technical difficulties limit its use in children younger than 5 years.[213] Therefore, ABPM should be considered in all children ≥5 years of age with CSFK. In CSFK patients ≥5 years of age with a history of or current hypertension or CKD ABPM should be performed yearly.

Based on the ESCAPE trial, the target blood pressure for children with CKD is a 24-h mean arterial pressure (MAP) <50th percentile, which was associated with a lower risk of kidney function decline.[213,214] In children with hypertension without CKD, the treatment goal is prevention of end organ damage, for which the target blood pressure is <90th percentile.[215,216] Since the risk of kidney function decline is most relevant for children with CSFK, we recommend a target blood pressure between the 50-75th percentile and if tolerated <50th percentile on 24-h MAP (Table 3). Reference values for blood pressure in children are provided in the latest AAP guidelines on hypertension.[213]

Besides hypertension, albuminuria is another early marker of glomerular hyperfiltration.[183] Thus, urinalysis should also be performed during yearly screening visits. In healthy children, measurement of urinary albumin-to-creatinine (UAC) ratio from a first morning sample was more reliable than from a random sample and better reflected results from 24-h urine samples.[217, 218] Since 24-h urine collection is cumbersome, especially in young children, a first morning void UAC seems the best screening tool. In line with guidelines for patients with diabetes and patients with CKD, treatment should be considered in case of modestly elevated UAC ratios (30–299 mg/g) and is strongly advised in case of a UAC ratio ≥300 mg/g.

Since a decrease in kidney function is expected later in the course of hyperfiltration injury, serum creatinine measurement can be performed less frequently than screening for hypertension and albuminuria. Indeed, CSFK cohorts published to date showed that an isolated decrease in eGFR occurred in only 0.3-8% of their population, and may especially occur during puberty.[78,80,82,219,220] Therefore, it seems reasonable to monitor eGFR once every 5 years until the onset of puberty and every 2 years afterwards. When the eGFR decreases, a more precise estimate combining creatinine and cystatin C can be obtained, and referral to a paediatric nephrologist is indicated.[221]

Table 2 Results of ambulatory blood pressure monitoring and office blood pressure readings in published cohorts of children with a (congenital) solitary functioning kidney

Author	Year	Number of patients	Normal OBP and ABPM	Masked hypertension	White coat hypertension	ABPM confirmed hypertension
Mei- Zahav[239]	2001	18 URA	18[a] (100%)	0	0	0
Seeman[240]	2006	15 URA	10 (67%)	0	4 (27%)	1 (7%)
Dursun[241]	2007	22 URA	17[a] (77%)	0	0	5 (23%)
Westland[242]	2014	28 cSFK	21 (75%)	5 (18%)	0	2 (7%
Tabel[243]	2015	49 SFK[b]	28 (57%)	15 (31%)	0	6 (12%)
Lubrano[244]	2017	38 cSFK	27 (73%)	0	0	11 (30%)
Zambaiti[245]	2018	50 cSFK	27 (54%)	13 (26%)	0	10 (20%)
La Scola[246]	2020	81 cSFK	47 (58%)	21 (25%)	7 (9%)	6 (7%)
Total		**301**	**195 (65%)**	**54 (18%)**	**11 (4%)**	**41 (14%)**

OBP office blood pressure, ABPM ambulatory blood pressure monitoring, URA unilateral renal agenesis, CSFK congenital solitary functioning kidney, NR not reported. [a] Results of OBP were not reported separately [b] 5 children with acquired SFK included.

Medication use

Hypertension and proteinuria are treatable risk factors for kidney function decline in children.[214,222,223] Although calcium-channel blockers and RAAS inhibitors show similar reduction in blood pressure, the combined antihypertensive and antiproteinuric properties of RAAS inhibitors make them the recommended first line treatment in children with CSFK and hypertension or albuminuria.[213,224] A decreased eGFR should not be a reason to withhold or discontinue RAAS inhibitors prescribed for hypertension and/or albuminuria. Recent data in children showed an accelerated decline of eGFR after discontinuation of RAAS inhibitors as well as an increase in albuminuria and blood pressure, suggesting that stopping RAAS inhibition might accelerate progression to kidney failure.[225]

Combined use of RAAS inhibitors and non-steroidal anti-inflammatory drugs (NSAIDs) should be avoided. In general, we recommend to use alternatives such as acetaminophen instead of NSAIDs and to refrain from NSAIDs in patients with an eGFR <60ml/min/1.73m^2 (prolonged use) or <30ml/min/1.73m^2 (any use).[226] Gentamicin use should be weighted carefully and serum levels should be monitored.[226] If possible, use of other potentially nephrotoxic drugs should also be minimized and careful monitoring of kidney function and/or drug levels may be needed.

Table 3 Indications for treatment with renin-angiotensin aldosterone system inhibitors

	When indicated	When to consider	Target	Comment
Blood pressure (office or ABPM)	Repeated blood pressure >90th percentile for height and gender		<50th percentile for height and gender (if tolerated); <75th percentile otherwise	Perform ABPM when office blood pressure is elevated to rule out white coat hypertension
Urine albumin creatinine ratio	>300 mg/g in first morning or 24h urine sample	30–299 mg/g in first morning or 24h urine sample	<30mg/g in first morning or 24h urine sample	
Estimated glomerular filtration rate	No data available	No data available	>90 ml/min/1.73m^2	Consult paediatric nephrologist when eGFR decreases more than 5 ml/min/ 1.73m^2 over 2 years, or to <90 ml/min/1.73m^2

ABPM ambulatory blood pressure monitoring, eGFR estimated glomerular filtration rate.

Duration of follow-up

In analogy to other hyperfiltration-related kidney problems, such as diabetic nephropathy, long-term follow-up is crucial and no endpoint for follow-up can be given based on scientific evidence. Moreover, epidemiological studies have shown that the higher risk of kidney injury in patients with SFK persists in adulthood.[178] There are even periods later in life in which stricter follow-up is needed than in childhood. Data from the ItalKid project indicates that puberty is a period with a higher risk of onset or progression of kidney injury.[220] After puberty, transition to adult care is important and efforts should be made to ensure follow-up is continued. Since the risk of gestational hypertension and preeclampsia was 2.5-fold higher in women living with a SFK due to URA or after donor nephrectomy, pregnancy is another time when vigilance is needed.[227,228]

Lifestyle

Because children with a CSFK are at increased risk of hypertension and kidney injury, maintaining a healthy lifestyle is of great importance. Key aspect are the avoidance of excess salt intake and obesity.[229-231] Protein restriction slowed kidney function deterioration in animal models of SFK and adults with CKD.[232,233] However, a meta-analysis of randomized control trials testing a low protein diet in children with CKD failed to show benefits over a normal diet.[234] Children may need high protein intake to meet demands for development and growth, and more research into optimal protein intake in children with CSFK and/or CKD is needed before recommendations can be given.

Physical activity is an important part of a healthy lifestyle for both healthy children and children with chronic conditions. Participation in contact sports by children with CSFK was long discouraged in fear of trauma to the remaining kidney. However, kidney injury during sports participation is extremely rare, with only 9 cases per million athlete activities for American Football and even less in other sports. [235] Furthermore, none of these injuries resulted in kidney loss. [235,236] Based on these data, it seems clear that the benefits of physical activity outweigh the low risk of severe kidney injury and participation by children with a CSFK should be encouraged.

Future perspectives

Urologists are increasingly asked to weigh the costs of their actions (for both the individual patient and society) against the potential benefits. Since studies have shown large variation in kidney injury rates in children with CSFKs, it is likely that subgroups of higher and lower risk children exist. Identification of these subgroups would allow for more tailored strategies to be used, and thus for a better cost-to-benefit ratio. [210] In addition, it would create an opportunity to select high risk patients for future trials of new therapies. Performing trials in these children would be more efficient, more ethical, and more cost-effective. Potential strategies that have been explored to identify the high and low risk subgroups are by using biomarkers, by counting nephrons in-vivo using MRI, and by combining already available clinical information in a prediction model. [210,237,238] However, all of these methods need further research to be useful in clinical practice.

CONCLUSIONS

These guidelines points to the urological and medical clinical aspects and long term care guidance for children with CSFK, who are at risk of kidney injury based on glomerular hyperfiltration. After initial confirmation of the diagnosis, mainly using ultrasound, caregivers should focus on early identification of kidney injury. A yearly follow-up with checks on blood pressure and albuminuria is important, and ABPM can be a useful tool to detect masked hypertension. Estimation of the GFR should take place once every 5 years until puberty, and every two years afterwards. When detected, kidney injury should be treated with RAAS inhibition and strict blood pressure control should be targeted. Since the risk of kidney injury seems increased during puberty and in pregnancy, extra checks are needed in these time periods. Adequate transition to adult care should result in continued screening in adulthood.

SUPPLEMENTARY MATERIAL

Supplementary Methods

Search strategy for studies with cohorts of patients with unilateral renal agenesis adopted from Westland *et al.*[17]

PubMed search

Search	Query
#5	#4 Filters: Publication date from 2012/01/01
#4	#3 NOT (animals [mh] NOT humans [mh])
#3	#1 NOT #2
#2	"Case Reports" [Publication Type]
#1	"Hereditary renal agenesis" [Supplementary Concept] OR single kidney*[tiab] OR absent kidney*[tiab] OR solitary functioning kidney*[tiab] OR solitary kidney*[tiab] OR unilateral renal agenesis[tiab] OR hereditary renal agenesis[tiab] OR unilateral renal aplasia[tiab] OR hereditary renal aplasia[tiab] OR aplastic kidney*[tiab]

OVID search

Search	Query
#6	5 Filters: Publication date from 2012/01/01
#5	#4 NOT #3
#4	#1 NOT #2
#3	'animal'/exp OR 'animal experiment'/exp NOT 'human'/exp
#2	'case report'/exp
#1	exp 'solitary kidney'/ or (single adj3 kidney*).ab,ti. or (absent adj3 kidney*).ab,ti. or ('solitary functioning' adj2 kidney*).ab,ti. or ('unilateral renal' adj2 agenesis).ab,ti. or ('hereditary renal' adj2 agenesis).ab,ti. or ('unilateral renal' adj2 aplasia).ab,ti. or ('hereditary renal' adj2 aplasia).ab,ti. or (aplastic adj3 kidney*).ab,ti.

Search strategy for studies with cohorts of patients with multicystic dysplastic kidney adopted from Schreuder *et al.* [29]

Pubmed search

Search	Query
#2	Filters: Publication date from 2008/01/01
#1	((("multicystic dysplastic kidney") OR ("multicystic kidney dysplasia")) OR "Multicystic Dysplastic Kidney"[Mesh])

EMBASE search

Search	Query
#2	Filters: Publication date from 2008/01/01
#1	'multicystic' AND 'dysplastic' or 'dysplasia' AND 'kidney'

Supplementary Tables

Supplementary Table 1a Prevalence of unilateral renal agenesis in studies published after the previous meta-analysis was performed

Author	Year	Country	Age at diagnosis	Patients with URA	Size screened population	Prevalence
Laurichesse Delmas[14]	2017	France	Prenatal with postnatal confirmation	177	447,885	1 : 2,530
Bakker[13]	2018	Netherlands	Prenatal with postnatal confirmation	47	119,297	1 : 2,538
Li[15]	2019	China	Prenatal with postnatal confirmation	529	1,748,038	1 : 3,304
Syngelaki[16,a]	2019	UK	Prenatal with postnatal confirmation	124	100,997	1 : 814
Zmora[18]	2019	Israel	Prenatal with postnatal confirmation	49	59,382	1 : 1,211
Total				**926**	**2,475,599**	**1 : 2,673**
Westland[17]	2013	Meta-analysis		2,094	4,253,483	1 : 2,031
Total including previous meta-analysis				**3,020**	**6,729,082**	**1 : 2,228**

URA unilateral renal agenesis, UK United Kingdom. [a] Included patients empty renal fossa without differentiation between unilateral renal agenesis and ectopic kidney.

Supplementary Table 1b Prevalence of multicystic dysplastic kidney in studies published after the previous meta-analysis was performed

Author	Year	Country	Age at diagnosis	Patients with MCDK	Size screened population	Prevalence
Saha[28]	2008	India	Prenatal with postnatal confirmation	3	6,682	1 : 2,227
Cordero[24]	2009	USA	Prenatal with postnatal confirmation	19	15,000	1 : 789
Halek[26]	2010	Czech Republic	Neonatal screening	4	6,088	1 : 1,522
Melo[27]	2012	Brazil	Neonatal screening	35	29,653	1 : 847
Al Naimi[19]	2013	Germany	Prenatal	33	16,000	1 : 485
Beke[21]	2014	Hungary	Pre- and early postnatal	17	19,602	1 : 1,153
Bondagji[22]	2014	Saudi Arabia	Prenatal with postnatal confirmation	13	43,209	1 : 3,324
Winding[30]	2014	Europe	Pre- and early postnatal	391	1,458,552	1 : 3,730
Gong[25]	2018	China	Neonatal screening	1	8,827	1 : 8,827
Chen[23]	2019	Taiwan	Pre- and postnatal	298	2,033,004	1 : 6,822
Total				**814**	**3,636,617**	**1 : 4,467**
Schreuder[29]	2009	Meta-analysis		371	1,588,271	1 : 4,281
Total including previous meta-analysis[a]				**1,080**	**4,515,858**	**1 : 4,181**

MCDK multicystic dysplastic kidney, USA United States of America. [a]The results from Wiesel *et al*[247] (105 cases among 709,030 births) were removed from the original meta-analysis because of overlap with Winding *et al*.

Supplementary Table 2a Characteristics of patients with unilateral renal agenesis from published cohorts

Author	Year	Number of patients	Left-sided URA N (%)	Male N (%)
Castellano-Martinez[248]	2016	21	12 (57%)	9 (43%)
Clinton[249, a]	2016	36	18 (50%)	NR
Davidovits[250]	2017	20	NR	13 (65%)
Dogan[251]	2013	51	33 (65%)	31 (61%)
Kostadinova[252]	2016	10	NR	6 (60%)
Laurichesse Delmas[14]	2017	177	101 (57%)	NR
La Scola[78, a]	2016	42	24 (57%)	NR
La Scola[246, a]	2020	18	8 (44%)	NR
Li[15]	2019	529	NR	241 (46%)
Marzuillo[80, a]	2017	199	99 (50%)	NR
Perlman[253]	2016	74	30 (41%)	NR
Sarhan[254]	2016	46	12 (26%)	24 (52%)
Stefanowicz[255]	2012	17	NR	8 (47%)
Xu[83]	2019	118	59 (50%)	62 (53%)
Zmora[18]	2019	49	23 (47%)	37 (76%)
Total[b]		**1,407**	**419 (51%)**	**431 (52%)**
Westland[17]	2013	2,684	1,080 (52%)	1,542 (63%)
Updated total[b]		**4,091**	**1,499 (52%)**	**1,973 (60%)**

URA unilateral renal agenesis, NR not reported. [a]Additional information obtained through personal communication with the authors. [b]Percentage reported as the number of patients with the characteristic divided by the number of patients for which the characteristic was reported.

Supplementary Table 2b Characteristics of patients with multicystic dysplastic kidney from published cohorts

Author	Year	Number of patients	Left-sided MCDK N (%)	Male N (%)
Akil[256]	2012	33	NR	17 (52%)
Al Naimi[19]	2013	33	14 (42%)	22 (67%)
Alsaif[257]	2019	44	22 (50%)	25 (57%)
Ayaz[258]	2017	21	10 (48%)	NR
Aytac[259]	2011	20	12 (60%)	18 (90%)
Balasundaram[260]	2018	38	20 (52%)	22 (58%)
Bartoli[261]	2018	14	NR	4 (29%)
Blachman-Braun[262]	2020	156	83 (53%)	98 (63%)
Brown[263]	2019	3,792	NR	2,072 (55%)
Brown[206]	2019	165	93 (56%)	82 (50%)
Carazo-Palacias[188]	2016	56	33 (59%)	38 (68%)
Chijioke[264]	2010	6	1 (17%)	NR
Clinton[249, a]	2016	28	15 (54%)	NR
Davidovits[250]	2017	12	NR	8 (67%)
Dogan[265]	2014	59	26 (44%)	31 (53%)
Eickmeyer[266]	2014	301	141 (47%)	165 (55%)
Faruque[267]	2020	106	55 (52%)	63 (59%)
Gaither[268]	2018	443	238 (54%)	235 (53%)
Gokce[269]	2012	25	12 (48%)	NR
Halek[26]	2010	4	NR	1 (25%)
Hayes[270]	2012	323	166 (51%)	182 (56%)
Hsu[271]	2012	34	15 (44%)	NR
Kara[272]	2018	128	66 (52%)	82 (64%)
Kashiwagi[273]	2018	4	2 (50%)	1 (25%)
Kiyak[274]	2009	90	46 (51%)	53 (59%)
Kumar[275]	2019	51	17 (33%)	29 (57%)
La Scola[78, a]	2016	55	33 (60%)	NR
La Scola[246, a]	2020	19	11 (58%)	NR
Mansoor[276]	2011	121	65 (54%)	60 (50%)
Marzuillo[80, a]	2017	185	107 (58%)	NR
Mashat[277]	2015	16	NR	12 (75%)

Supplementary Table 2b Characteristics of patients with multicystic dysplastic kidney from published cohorts (continued)

Author	Year	Number of patients	Left-sided MCDK N (%)	Male N (%)
Matsumura[278]	2018	17	7 (41%)	7 (41%)
Mattioli[279]	2010	12	NR	8 (67%)
Moralioglu[280]	2014	68	35 (51%)	40 (59%)
Poggiali[210]	2019	132	NR	74 (56%)
Sanna-Cherchi[75]	2009	40	NR	19 (48%)
Sarhan[281]	2014	63	32 (51%)	35 (56%)
Scala[282b]	2017	94	70 (50%)	NR
Sharada[283]	2014	47	22 (47%)	27 (57%)
Singh[284]	2009	22	12 (55%)	18 (82%)
Soliman[285]	2015	17	NR	10 (59%)
Tiryaki[286,b]	2013	102	18 (39%)	61 (60%)
van Vuuren[287]	2012	60	27 (45%)	NR
Weinstein[288]	2008	80	46 (58%)	42 (53%)
Westland[82]	2013	124	61 (49%)	81 (65%)
Whittam[289]	2014	91	54 (59%)	57 (63%)
Yamamoto[290]	2019	75	35 (47%)	29 (39%)
Zambaiti[245]	2019	40	19 (48%)	27 (68%)
Total[b]		**7,466**	**1,741 (51%)**	**3,855 (56%)**
Schreuder[29]	2009	3,557	1,663 (53%)	1,791 (59%)
Updated total[b]		**11,023**	**3,404 (52%)**	**5,646 (57%)**

MCDK multicystic dysplastic kidney, NR not reported. [a]Additional information obtained through personal communication with the authors. [b]Percentage reported as the number of patients with the characteristic divided by the number of patients for which the characteristic was reported.

Supplementary Table 3a Prevalence of associated urinary tract anomalies in children with unilateral renal agenesis from published cohorts

Author	Year	Number of patients	Patients with VCUG N (%[a])	Patients with VUR N (%[b])	Mild VUR (grade I-II) N (%[c])	Severe VUR (grade III-V) N (%[c])	Patients with UPJO N (%[b])
Dogan[251]	2013	51	15 (29%)	3 (20%)	NR	NR	2 (13%)
Laurichesse Delmas[14]	2017	177	NR	5 (NR)	NR	NR	6 (NR)
Sarhan[254]	2016	46	20 (43%)	4 (20%)	NR	NR	NR
Total		**274**	**35 (36%)**	**7 (20%)**	**NR**	**NR**	**8 (13%)**
Westland[17]	2013	2,684	770 (29%)	184 (24%)	NR	NR	38 (4.9%)
Updated total		**2,958**	**805 (29%)**	**191 (24%)**	**NR**	**NR**	**40 (5.0%)**

VCUG voiding cystourethrogram, VUR vesicoureteral reflux, UPJO ureteropelvic junction obstruction, NR not reported. [a]Calculated as percentage of total number of patients in the studies for which the number of patients who underwent VCUG was reported. [b]Calculated as percentage of number of patients with VCUG. [c]Calculated as percentage of patients with VUR.

Supplementary Table 3b Prevalence of associated urinary tract anomalies in children with multicystic dysplastic kidney from published cohorts

Author	Year	Number of patients	Patients with VCUG N (%[a])	Patients with VUR N (%[b])	Mild VUR (grade I-II) N (%[c])	Severe VUR (grade III-V) N (%[c])	Patients with UPJO N (%[b])
Akil[256]	2012	33	29 (88%)	10 (34%)	5 (50%)	5 (50%)	1 (3.4%)
Alsaif[257]	2019	44	43 (98%)	7 (16%)	6 (86%)	1 (14%)	0 (0%)
Aytac[259]	2011	20	20 (100%)	3 (15%)	NR	NR	3 (15%)
Balasundaram[260]	2018	38	35 (92%)	2 (5.7%)	1 (50%)	1 (50%)	0 (0%)
Bartoli[261]	2018	14	14 (100%)	2 (14%)	NR	NR	2 (14%)
Blachman-Braun[262]	2020	156	156 (100%)	34 (22%)	18 (72%)	7 (28%)	NR
Brown[206]	2019	165	77 (47%)	18 (23%)	7 (39%)	11 (61%)	NR
Calaway[291]	2014	133	133 (100%)	23 (17%)	12 (52%)	11 (48%)	NR
Calisti[292]	2008	26	26 (100%)	2 (7.7%)	NR	NR	2 (7.7%)
Carazo-Palacias[188]	2016	56	NR	8 (NR)	5 (63%)	3 (37%)	3 (NR)
Cordero[24]	2009	19	5 (26%)	2 (40%)	NR	NR	NR
Dogan[265]	2014	59	30 (51%)	8 (27%)	2 (25%)	6 (75%)	3 (10%)
Eickmeyer[266]	2014	301	239 (79%)	53 (22%)	NR	NR	10 (4.2%)
Faruque[267]	2020	106	106 (100%)	13 (12%)	0 (0%)	4 (100%)	6 (5.7%)

Supplementary Table 3b Prevalence of associated urinary tract anomalies in children with multicystic dysplastic kidney from published cohorts (continued)

Author	Year	Number of patients	Patients with VCUG N (%[a])	Patients with VUR N (%[b])	Mild VUR (grade I-II) N (%[c])	Severe VUR (grade III-V) N (%[c])	Patients with UPJO N (%[b])
Gaither[268]	2018	443	183 (41%)	20 (11%)	NR	NR	NR
Gokce[269]	2012	25	25 (100%)	6 (24%)	NR	NR	1 (4.4%)
Hayes[270]	2012	323	NR	37 (NR)	NR	NR	9 (NR)
Kara[272]	2018	128	74 (58%)	15 (20%)	6 (67%)	3 (33%)	6 (8.1%)
Kiyak[274]	2009	90	90 (100%)	7 (7.8%)	5 (71%)	2 (29%)	1 (1.1%)
Mansoor[276]	2011	121	101 (83%)	17 (17%)	11 (65%)	6 (35%)	5 (5.0%)
Marzuillo[80]	2017	185	NR	8 (NR)	3 (38%)	5 (62%)	1 (NR)
Matsumura[278]	2018	17	NR	1 (NR)	NR	NR	2 (NR)
Mattioli[279]	2010	12	NR	2 (NR)	NR	NR	NR
Moralioglu[280]	2014	68	68 (100%)	5 (7.4%)	1 (20%)	4 (80%)	1 (1.5%)
Sarhan[281, d]	2014	63	63 (100%)	20 (32%)	14 (88%)	2 (12%)	NR
Shankar[293]	2018	24	18 (75%)	3 (17%)	1 (33%)	2 (67%)	NR
Sharada[283]	2014	47	47 (100%)	13 (28%)	6 (50%)	6 (50%)	NR
Singh[284]	2009	22	NR	3 (NR)	NR	NR	3 (NR)
Tiryaki[286]	2013	102	84 (82%)	14 (17%)	NR	NR	8 (9.5%)
Yamamoto[290]	2019	75	75 (100%)	8 (11%)	4 (67%)	2 (33%)	NR
Total		**2,915**	**1,741 (60%)**	**364 (21%)**	**107 (57%)**	**81 (43%)**	**71 (4.1%)**
Schreuder[29]	2009	3,557	2,104 (59%)	415 (20%)	100 (60%)	68 (40%)	103 (4.8%)
Updated total		**6,472**	**3,845 (59%)**	**779 (20%)**	**207 (58%)**	**149 (42%)**	**174 (4.5%)**

VCUG voiding cystourethrogram; VUR vesicoureteral reflux; UPJO ureteropelvic junction obstruction; NR not reported. [a]Calculated as percentage of total number of patients in the studies for which the number of patients who underwent VCUG was reported. [b]Calculated as percentage of number of patients with VCUG. [c]Calculated as percentage of patients with VUR for whom grade was reported. [d]Mild was classified as grade I-III, severe as grade IV or V.

CHAPTER 7

Kidney injury rates after unilateral nephrectomy in childhood – a systematic review and meta-analysis

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ABSTRACT

Background

Unilateral nephrectomy is a relatively common procedure in children which results in a solitary functioning kidney (SFK). Living with an SFK predisposes to kidney injury, but it remains unknown which children are most at risk. We aimed to investigate kidney injury rates in patients who underwent unilateral nephrectomy in childhood and to investigate differences among nephrectomies performed for a congenital anomaly, malignancy, or other condition.

Methods

MEDLINE and EMBASE were searched for studies reporting kidney injury rates (*i.e.* proteinuria, hypertension, and/or a decreased GFR) of patients who underwent unilateral nephrectomy during childhood. Studies including five or more patients with at least 12 months of follow-up were eligible. Analyses were performed using random effects models and stratified by indication for nephrectomy. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) and meta-analysis of observational studies in epidemiology (MOOSE) guidelines were used for reporting.

Results

Over 5,000 unique articles were screened, of which 53 studies reporting on >4,000 patients were included in the analyses. Proteinuria, hypertension and a decreased GFR were present in 15.3%, 14.5%, and 11.9% of patients, respectively. Heterogeneity among the studies was large in several subgroups, impairing quantitative meta-analyses. However, none of our analyses indicated differences in injury rates between a congenital anomaly or malignancy as indication for nephrectomy.

Conclusions

Unilateral nephrectomy during childhood results in signs of kidney injury in >10% of patients, with no clear difference between the indications for nephrectomy. Therefore, structured follow-up is necessary in all children who underwent nephrectomy, regardless of the indication.

INTRODUCTION

Each year, over 2,000 children undergo a nephrectomy in the US.[32] Approximately 75% of these procedures are performed for benign disease, with indications such as multicystic dysplastic kidney (MCDK) or non-functioning kidney due to vesicoureteral reflux (VUR), duplex kidney, or ureteropelvic junction obstruction (UPJO).[32,294] A Wilms tumour is by far the most common indication in the remaining 25% with malignancies.[32,294]

After a unilateral nephrectomy, a solitary functioning kidney (SFK) remains. As living with an SFK predisposes to kidney injury due to glomerular hyperfiltration, lifelong follow-up is warranted, although the estimated risk of kidney injury varies greatly.[295] Studies report signs of kidney injury in up to 50% of children with an SFK, with some reporting a higher risk for an acquired SFK (*i.e.* after nephrectomy) than for an SFK from birth (*e.g.* after unilateral renal agenesis).[82,219] In addition, children undergoing nephrectomy for a malignancy could be at higher risk for kidney injury than children undergoing a nephrectomy for other reasons, especially if they were additionally treated with nephrotoxic chemotherapy or radiotherapy on the remaining kidney.

Although the high rate of kidney injury led to recommendations highlighting the need for follow-up of children with an SFK, the frequency of follow-up is largely opinion-based.[295] Follow-up could be individualized if more information on risk factors for kidney injury would be available.[296] Therefore, we addressed the following research questions in a systematic review and meta-analysis of the literature: 1) what is the prevalence of kidney injury in children who underwent a unilateral nephrectomy, and 2) does the indication for nephrectomy influence the rate of kidney injury?

MATERIALS AND METHODS

Review methods

Prior to the initiation of the systematic review, a protocol was created with detailed information on the research question, search strategy, inclusion and exclusion criteria and risk of bias assessment. Furthermore, we defined how to investigate heterogeneity and when meta-analysis would be performed, and registered the protocol on the PROSPERO website (registration number CRD42019129501). The PRISMA and MOOSE checklists were used to aid transparent reporting of the review.[297,298]

Search Strategy

Our search strategy was formulated with assistance of a professional librarian and aimed at identifying all relevant articles on paediatric nephrectomy (complete search strategy is included as supplementary material). We first searched the MEDLINE and EMBASE libraries in March 2019 and used the snowballing technique to identify relevant studies from the reference list of selected articles. Since no randomized clinical trials on the subject of our review were expected, trial registers and the Cochrane library were not searched. The identified studies were imported into EndNote and duplicates were removed using the methods of Bramer *et al.*[299] In September 2020, both MEDLINE and EMBASE were searched again to update our database with the most recent articles.

Selection of Eligible Studies

After deduplication, the articles were imported into Rayyan for title/abstract screening by two independent reviewers (SGW and AG).[300] In case of disagreement, a third reviewer (MS) was consulted. Articles were considered eligible if they: 1) reported on children (0-18y) who underwent a unilateral simple or radical nephrectomy, 2) reported at least one of the outcomes related to kidney injury (defined as proteinuria, hypertension, a decreased eGFR, and/or use of antihypertensive or antiproteinuric medication), 3) measured the outcomes at least 12 months after nephrectomy, 4) included at least five patients, and 5) were available in English. Studies including only children undergoing nephrectomy for hypertension were excluded, since it would not be possible to determine whether hypertension during follow-up was pre-existing or a consequence of living with SFK in these children. To avoid misclassification of tumour-associated hypertension as hypertension caused by living with SFK, only outcomes measured after at least one year of follow-up were taken into account.

Data Extraction

Full text screening and data extraction were performed in duplicate by the same two reviewers. We extracted information on study characteristics (*e.g.* study type, in- and exclusion criteria, duration of follow-up), the indications for nephrectomy, and the measurement of the outcomes using a predefined form. We grouped the indications for nephrectomy into congenital anomalies (*i.e.* MCDK, hydronephrosis, obstructive uropathy, VUR, and congenital not otherwise specified), malignancies, and mixed/other (*e.g.* trauma or calculi). If a study included nephrectomies for both congenital anomalies and malignancies, subgroups by indication were created within individual studies. If this was not possible, study outcomes were reported under the mixed/other indication group. Data on children who did not undergo nephrectomy were not extracted. Authors were contacted to provide more information when ambiguity about the patients who underwent nephrectomy was present. If the available data

permitted, kidney injury was defined as the presence of albuminuria or proteinuria (in our manuscript combined and referred to as proteinuria), hypertension, an eGFR <60ml/min/1.73m^2, or the use of antihypertensive and/or antiproteinuric medication. Generally, the outcome definitions from the studies were applied. When individual eGFR values were reported, the numbers of patients with an eGFR <60 and an eGFR <90ml/min/1.73m^2 were extracted. A selection of items from the Newcastle-Ottawa Scale was used to assess the quality of the studies. [301]

Data Analysis

For three outcome measures (proteinuria, hypertension, and eGFR), sufficient studies were available to perform quantitative analyses. The number of participants with the specific outcome were divided by the number of participants at risk in the particular study to calculate the proportion of affected patients. The proportions of affected patients from the individual studies were combined in meta-analyses using random effect models for all three outcomes separately. Between-study heterogeneity was assessed using the I^2 statistic and we established a maximum heterogeneity of 75% in our protocol to avoid overinterpreting meta-analyses results on incomparable studies leading to spurious conclusions. [302,303] The Paule-Mandel method was used to calculate the heterogeneity τ^2 and to assign weights to the studies. [304] The method by Hartung, Knapp, Sidik and Jonkman was used to adjust test statistics and confidence intervals, since this method reduces type 1 errors in case of substantial heterogeneity and/or a small number of studies. [305] Subgroup analyses were performed to compare the different indication groups (*i.e.* congenital, malignancy, and mixed/other) and to investigate other potential sources of heterogeneity, including follow-up duration (<7.5 year, 7.5-15 year, and ≥15 year), way of reporting (mean or median), study design (cohort or cross-sectional), size of the study (≤10, 10-49, or ≥50 participants), and year of publication (before or after 2010). Meta-regression analyses were used to evaluate the effect of these factors on estimated proportions and heterogeneity in multivariate analyses. All analyses were performed using R software (version 3.5.1).

RESULTS

Study selection

Our initial search yielded 7,237 results, of which 2,087 were duplicates. The title and abstract of 5,150 articles were screened for eligibility and 4,832 articles were excluded. From the remaining 318 articles, 26 had to be excluded because no full-text article was available (*e.g.* conference presentations), while 227 were excluded after reading the full text because they did not fulfil the inclusion criteria. At this stage, 65 studies were selected for data extraction, during which four additional studies[306-309] had to be

excluded because of an insufficient number of patients with nephrectomy from whom data were available, four studies[239,310-312] were excluded because of insufficient reporting of outcomes, and seven studies[313-319] were removed because a more recent article about the same cohort was available. This resulted in 50 articles for the initial analyses. Reference searching (n=1) and the updated search in September 2020 (n=2) yielded three more suitable articles, leading to a total of 53 articles included for analyses (Figure 1).

Study characteristics

The selected studies were published between 1969 and 2019 and consisted of cohort (n=31) and cross-sectional (n=22) studies (Table 1). Five studies reported on patients with a congenital indication for nephrectomy only, 34 reported on patients with a malignancy only, and 14 on both types of patients or patients with other indications. For seven of the studies reporting on both types of patients, we were able to create subgroups by indication, resulting in a total of 69 studies and subgroups for analyses. In total, 4,045 patients were included of whom 648 had congenital anomalies, 3,293 had malignancies, and 104 had other indications for nephrectomy (Table 2).

Large differences in the in- and exclusion criteria, outcome assessment, and definitions of kidney injury were present among the included studies (Table 1). Twenty-one studies excluded patients with a form of contralateral (*i.e.* in the SFK) disease and six used some form of kidney injury as exclusion criterion (*e.g.* patients with GFR <90 ml/min/1.73m^2 were excluded). Two studies did not report how proteinuria was measured and 13 had missing information on the method of blood pressure measurement used. Of studies that did report their measurement details, some used office blood pressure measurements (n=17) whereas others used ambulatory blood pressure monitoring (ABPM) (n=4). Urinalysis was reported for a single random sample (n=11) and for 24h urine collection (n=11). To estimate or measure GFR, some studies used creatinine based GFR estimations (n=15) while others used clearances based GFR measurements (n=33). Only five studies reported on antihypertensive medication use.

Outcomes

All outcomes were reported in more than 10% of the included patients: proteinuria was reported in 16.1% (248 of 1,537 patients, 42 studies), hypertension in 14.1% (427 of 3,039 patients, 43 studies), and a decreased eGFR in 13.2% (262 of 1,983 patients, 55 studies) (Table 3). Antihypertensive or antiproteinuric medication use was reported for 13.7% of patients (60 of 437), but in only five studies. Therefore, this outcome was not considered for further separate analyses.

Meta-analyses confirmed that signs of kidney injury were present in a considerable proportion of patients. The estimated proportions of patients with proteinuria, hypertension and a decreased eGFR were 15.3%, 14.5% and 11.9%, respectively (Table 3, Figures 2-4). Because the heterogeneity was larger than 75% in two out of the nine indication subgroups, results of our meta-analyses should be interpreted with care. Nevertheless, no differences in kidney injury rates across indication subgroups were visible and confidence intervals were largely overlapping.

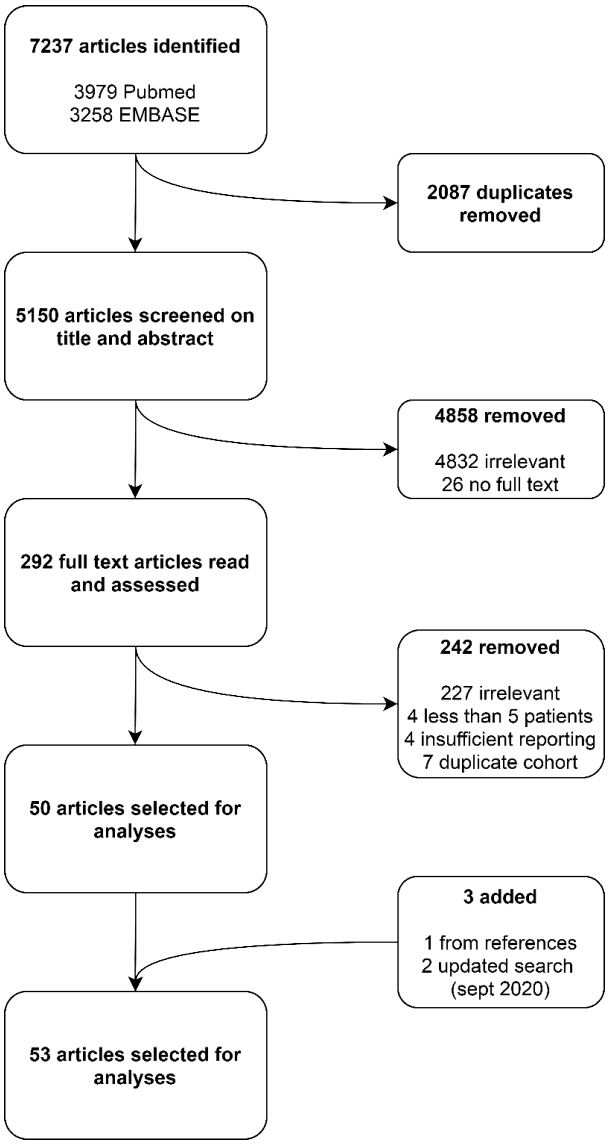


Figure 1 Flowchart of article selection

Table 1 Study design and characteristics

Author	Year	Study type	Cases: Inclusion criteria	Cases: Exclusion criteria	Outcome assessment		
					Proteinuria	Blood pressure	Kidney function
Abou Jaoude[219]	2011	Cohort	SFK	Contr. structural anomalies	single	avg 3 readings	Inulin Cl
Aperia[325]	1977	Cross sectional	Nx	?	-	?	24h Cr Cl
Argueso[326]	1992	Cross sectional	Nx at age <15y	Contr. structural anomalies	24h	?	24h Cr Cl
Barrera[327]	1989	Cross sectional	Nx for WT	-	single	?	Cr
Basto Catalina[328]	2019	Cohort	Nx	Stage 5 CKD	-	-	Cr
Baudoin[329]	1993	Cross sectional	Nx at age <16y	Contr. structural anomalies	24h	avg 4 readings	Polyfr Cl
Bhisitkul[330]	1991	Cross sectional	Nx for WT	RT	Single	?	Cr Cl
Cost[331]	2014	Cohort	Nx for WT	Syndromic WT	-	-	Inulin Cl
Cozzi[332]	2013	Cohort	Nx for WT	Bilateral disease	-	-	Cr
Cuckow[333]	1997	Cohort	VURD	-	-	-	EDTA Cl
Daw[334]	2009	Cohort	Nx for WT at age < 21y	Life expectancy <6wks	?	?	24h Cr Cl
de Graaf[335]	1996	Cohort	Nx for WT	-	-	-	Iothalamaat Cl
de Lucas[336]	2006	Cohort	SFK	-	Single	?	Cr Cl
Dekkers[337]	2013	Cross sectional	Nx for cancer	Bilateral cancer, no Cr available, <18y at study measurement	Single	Osc	Cr
Donckerwolcke[338]	2001	Cross sectional	Nx for cancer	-	?	?	Inulin Cl
Dursun[241]	2007	Cohort	Nx	Contr. structural anomalies or GFR<90	Single	ABPM	-

Table 1 Study design and characteristics (continued)

Author	Year	Study type	Cases: Inclusion criteria	Cases: Exclusion criteria	Outcome assessment		
					Proteinuria	Blood pressure	Kidney function
Elli[321]	2013	Cohort	Nx for WT at age < 16y	-	-	ABPM	-
Finklestein[339]	1993	Cohort	Nx for WT	Relapse within 5y, bilateral WT, cardiac disease	-	?	-
Gibson[340]	2017	Cohort	Nx for cancer	<18y old or <10y after diagnosis at study measurement	-	Avg 3 readings	-
Godbole[341]	2004	Cohort	Nx for benign disease	Contr. structural anomalies	-	-	EDTA/inulin Cl
Indolfi[342]	2001	Cohort	Nx for WT	-			-
Interiano[343]	2015	Cohort	Nx for WT at age < 15y	RT, bilateral or syndromic WT	Single	Avg 3 readings	Cr
Janeczko[344]	2015	Cohort	WT (incl. 9 with NSS)	-	Single	Osc	Cr
Jereb[345]	1973	Cross sectional	Nx for WT	Bilateral WT, <6y old at study measurement	-	?	Inulin Cl
Kazama[346]	2018	Cohort	Nx for WT	Bilateral or syndromic WT, genitourinary anomalies	Single	-	Cr
Kern[347]	2014	Cohort	Nx for WT <18	Syndromic or metastatic WT, NSS, no GFR	-	-	Cr
Kishore[348]	2014	Cross sectional	Nx for WT at age < 12y, >1y FU	Bilateral, relapsed or syndromic WT, nephrotoxic drugs	24h	Avg 3 readings	Cr Cl
Knijnenburg[349]	2012	Cohort	Nx for WT at age < 18y, >5y FU	-	Single	?	Cr

Table 1 Study design and characteristics (continued)

Author	Year	Study type	Cases: Inclusion criteria	Cases: Exclusion criteria	Outcome assessment		
					Proteinuria	Blood pressure	Kidney function
Kolvek[350]	2014	Cohort	SFK	Nx for malignancy	24h	Avg 3 ausc readings	Cr
Kosiak[351]	2018	Cross sectional	Nx for WT	Heminephrectomy, CAKUT, UTI, relapse, sec cancer	Single	Avg 3 osc readings	Cr
Lubrano[244]	2017	Cohort	SFK at age <18y	non-traumatic cause for Nx, nephrotoxic drugs, CAKUT, orthostatic proteinuria	24h	Ausc (<120cm)/ ABPM (>120cm)	DTPA Cl
Makipernaa[352]	1991	Cross sectional	Nx for WT	Survival <10y	24h	?	EDTA Cl
Mavinkurve-Groothuis[353]	2016	Cohort	Nx for WT	Bilateral or syndromic WT	-		Cr
Mitus[354]	1969	Cross sectional	Nx for cancer	-	-	-	Picrate Cl
Mpofu[355]	1992	Cross sectional	Nx for WT	-	-	Ascultatory	Cr Cl
Neu[356]	2017	Cross sectional	Nx for WT	-	-	Oscillometric	24h Cr Cl
Regazzoni[357]	1998	Cohort	Nx	Neoplastic syndrome	-	-	Cr + In Cl
Robitaille[358]	1985	Cross sectional	Nx	-	24h	?	24h Cr Cl
Sanpakit[359]	2013	Cohort	Tumour at age <15y	-	-	-	Cr
Schell[360]	1995	Cohort	Nx for NB or WT	-	1st	1x	Inulin Cl
Schiavetti[361]	2015	Cross sectional	Nx for WT	Bilateral disease, NSS	24h	Ascultatory	24h Cr Cl
Schmidt[362]	1992	Cross sectional	Nx	Contralateral kidney damage	-	-	Cr Cl
Seminara[363]	2019	Cohort	Nx for WT	-	-	-	Cr
Simon[364]	1982	Cohort	Nx	-	-	-	Inulin Cl (4x)

Table 1 Study design and characteristics (continued)

Author	Year	Study type	Cases: Inclusion criteria	Cases: Exclusion criteria	Outcome assessment		
					Proteinuria	Blood pressure	Kidney function
Spira[365]	2009	Cross sectional	SFK at age < 1 y	Kidney transplant	-	-	Cr Cl
Spreafico[322]	2014	Cohort	Nx for WT	Nephrotoxicity	24h	APBM	24h Cr Cl
Srinivas[366]	1998	Cross sectional	Nx for WT	-	24h	-	24h Cr Cl
Stefanowicz[255]	2011	Cohort	SFK	Bilateral disease	-	Ascultatory	Cr Cl
Taranta-Janusz[367]	2012	Cohort	SFK	other urinary tract abnormalities, HT, MA, cardiovascular or secondary kidney disease.	-	-	Cr Cl
Warshaw[368]	1985	Cross sectional	PUV	-	-	-	Cr Cl
Westland*[82]	2013	Cohort	SFK	GFR<30, dead by age of 1	24h	Oscillometric	Cr
Wikstad[369]	1986	Cross sectional	Nx	Contralateral abnormalities, RT or chemotherapy	-	1x	24h polyfr Cl
Zambaiti[245]	2019	Cross sectional	SFK	No antihypertensive medication, systemic disease or contr. kidney pathology	-	Osc, Avg 2 measurements	-

? investigated/measured but not reported, - none/not measured, * additional data were provided by the authors. 24h 24 hours, ABPM ambulatory blood pressure measurement, ausc auscultatory, Avg average, CAKUT congenital anomalies of the kidney and urinary tract, CKD chronic kidney disease, Cl clearance, Contr contralateral, Cr creatinine, DTPA diethylenetriaminepentaacetic acid, EDTA Ethylenediaminetetraacetic acid, FU follow-up, GFR glomerular filtration rate, HT Hypertension, In inulin, incl including, MA microalbuminuria, NB nephroblastoma, NSS nephron sparing surgery, Nx Nephrectomy, Osc oscillometric, PAH Para-aminohippurate, Polyfr polyfructosan, PUV posterior urethral valves, RT radiotherapy, SFK solitary functioning kidney, UTI urinary tract infection, VURD posterior urethral valves unilateral vesicoureteral reflux and renal dysplasia, wks weeks, WT Wilms tumour, y year.

Differences in follow-up duration, way of reporting (*i.e.* mean or median), study design, size of the study, and year of publication were all regarded as potential confounding factors and contributors to the large heterogeneity. However, sensitivity analyses in which subgroups were made for these variables did not substantially change point estimates for the affected proportions or decrease heterogeneity in most subgroups (Supplementary Tables 1-3). The only statistically significant difference was seen between the proportions of patients with hypertension in studies published before or after 2010, which were 10% and 20%, respectively ($p = 0.02$, Supplementary Table 2). Meta-regression including all potential confounders explained 0-21% of the heterogeneity and did not result in statistically significant differences between patients with congenital or malignant indications for nephrectomy for any outcome (Supplementary Tables 1-3). As can be expected, the cut-off used to define an abnormal GFR was strongly associated with the affected proportion of patients in both univariate analyses and meta-regression (Supplementary Table 3).

We hypothesized that the effect of follow-up duration on kidney injury rates could be substantial and was not sufficiently corrected for by creating tertiles of follow-up duration. Therefore, we plotted the proportion of patients affected with the different outcomes against the median (or mean, if median was not available) duration of follow-up for each study, as well as the proportion of patients with any outcome (Figure 5). No specific trends were visible in these scatterplots and separating studies reporting mean or median follow-up duration did not change these results.

DISCUSSION

In this systematic review, we found that many studies were performed on the prevalence of signs of kidney injury in patients who have undergone a nephrectomy during childhood. The estimated proportions of patients with proteinuria, high blood pressure and a decreased eGFR were 15.3%, 14.5% and 11.9% of patients, respectively, which clearly indicates that children undergoing a nephrectomy need long term follow up. Our results indicated no differences between children with a congenital anomaly or malignancy as indication for their nephrectomy. However, these results cannot be used to rule out the possibility of subgroups and should be interpreted with care due to the large heterogeneity among the studies included.

Heterogeneity is a common problem in meta-analyses of observational studies and is an issue that requires careful examination.[303] In our study, several underlying reasons could be identified, including the study in- and exclusion criteria, methods of outcome assessment, criteria used to determine the presence of kidney injury, and duration of follow-up.

Table 2 Results from included studies.

Author	Year	Subgroup	N	N (%) proteinuria	N (%) hypertension	N (%) decreased GFR	FU duration (years)	Kidney injury before nephrectomy	Lost during follow-up	Gender (% male)
Abou Jaoude[219]	2011	Total	53	9 (17%)	-	-	9.1	no	0%	45%
		Congenital	41	-	1 (2%)	7 (17%)	9.1			
		Malignancy	12	-	0 (0%)	0 (0%)	2.9			
Aperia[325]	1977	Congenital	6	-	0 (0%)	0 (0%)	4	-	-	38%
		Mixed	2	-	0 (0%)	0 (0%)	10.6			
Argueso[326]	1992	Total	50^	-	5 (10%)	-	28	no	-	57%
		Congenital	26	7 (27%)	-	8 (31%)				
		Malignancy	2	1 (50%)	-	1 (50%)				
		Mixed	2	0 (0%)	-	0 (0%)				
Barrera[327]	1989	Malignancy	16^	2 (14%)	6 (38%)	0 (0%)	17	-	-	50%
Basto Catalina[328]	2019	Congenital	32	12 (38%)	6 (19%)	7 (22%)	5.6	yes, 45%	2	45%
		Malignancy	6	1 (17%)	3 (50%)	1 (17%)	7.3			
Baudoin[329]	1993	Mixed	111	23 (21%)	25 (23%)	3 (3%)	26	-	23%	50%
Bhisitkul[330]	1991	Malignancy	12	0 (0%)	0 (0%)	1 (8%)	15	-	69%	50%
Cost[331]	2014	Malignancy	15	-	-	0 (0%)	2.1	-	0%	47%
Cozzi[332]	2013	Malignancy	60	-	-	32 (53%)	38.4	yes	-	42%
Cuckow[333]	1997	Congenital	12	-	-	10 (83%)	8.3	-	0%	100%
Daw[334]	2009	Malignancy	11	2 (18%)	-	6 (55%)	11.2	no	8%	25%
de Graaf[335]	1996	Malignancy	41	-	-	7 (17%)	13		0%	39%

Table 2 Results from included studies. (continued)

Author	Year	Subgroup	N	N (%) proteinuria	N (%) hypertension	N (%) decreased GFR	FU duration (years)	Kidney injury before nephrectomy	Lost during follow-up	Gender (% male)
de Lucas[336]	2006	Mixed	39	-	1 (3%)	3 (8%)	9.5	-	0%	62%
Dekkers[337]	2013	Malignancy	85^	15 (25%)	22 (31%)	7 (8%)	24.4	-	-	53%
Donckerwolcke[338]	2001	Malignancy	11	0 (0%)	0 (0%)	0 (0%)	5.5	-	-	73%
Dursun[241]	2007	Total	22	-	7 (32%)	-	4.1	-	0%	45%
		Congenital	14	0 (0%)	-	-				
		Malignancy	6	0 (0%)	-	-				
		Mixed	2	0 (0%)	-	-				
Elli[321]	2013	Malignancy	15	-	3 (20%)	-	9.9	-	40%	36%
Finklestein[339]	1993	Malignancy	1,171	-	83 (7%)	-	5	-	48%	48%
Gibson[340]	2017	Malignancy	226	-	68 (30%)	-	32	-	35%	52%
Godbole[341]	2004	Congenital	44	-	-	0 (0%)	1	-	0%	52%
Indolfi[342]	2001	Malignancy	27	10 (37%)	0 (0%)	0 (0%)	14.5	-	21%	33%
Interiano[343]	2015	Malignancy	75^	5 (7%)	5 (7%)	0 (0%)	19.6	-	0%	41%
Janeczko[344]	2015	Malignancy	50	2 (4%)	4 (8%)	12 (24%)	2	15/50	0%	44%
Jereb[345]	1973	Malignancy	16	-	0 (0%)	1 (6%)	6	-	-	-
Kazama[346]	2018	Malignancy	9^	1 (13%)	-	0 (0%)	20	-	-	22%
Kern[347]	2014	Malignancy	55	-	-	2 (4%)	6.3	-	-	40%
Kishore[348]	2014	Malignancy	29	1 (3%)	2 (7%)	1 (3%)	4.8	-	-	66%

Table 2 Results from included studies. (continued)

Author	Year	Subgroup	N	N (%) proteinuria	N (%) hypertension	N (%) decreased GFR	FU duration (years)	Kidney injury before nephrectomy	Lost during follow-up	Gender (% male)
Knijnenburg[349]	2012	Malignancy	206^	33 (18%)	43 (22%)	23 (11%)	9.9	-	Different per measurement	56%
Kolvek[350]	2014	Mixed	15	1 (7%)	5 (33%)	2 (13%)	11.6	-	0%	55%
Kosiak[351]	2018	Malignancy	53	7 (13%)	12 (23%)	0 (0%)	8.5	-	-	49%
Lubrano[244]	2017	Mixed	17	-	9 (53%)	-	14	2/17	0%	73%
Makipernaa[352]	1991	Malignancy	30^	3 (10%)	5 (17%)	0 (0%)	19.2	-	12%	50%
Mavinkurve-Groothuis[353]	2016	Malignancy	23	-	6 (26%)	1 (4%)	9.1	-	0%	50%
Mitus[354]	1969	Malignancy	108	-	-	54 (50%)	6.5	2/85	-	40%
Mpofu[355]	1992	Malignancy	71^	7 (10%)	0 (0%)	1 (2%)	9	no	-	47%
Neu[356]	2017	Malignancy	37^	5 (14%)	15 (41%)	6 (21%)	24.8	no	60%	43%
Regazzoni[357]	1998	Congenital	37	0 (0%)	0 (0%)	0 (0%)	15.2	no	-	62%
Robitaille[358]	1985	Total	27	3 (11%)	3 (11%)	-	23.3	-	-	67%
		Congenital	21	-	-	2 (10%)				
		Malignancy	4	-	-	0 (0%)				
		Mixed	2	-	-	0 (0%)				
Sanpakit[359]	2013	Malignancy	29	0 (0%)	9 (31%)	2 (7%)	4.8	yes	-	63%
Schell[360]	1995	Malignancy	40	-	0 (0%)	16 (40%)	1.9	no	-	-
Schiavetti[361]	2015	Malignancy	35	3 (9%)	1 (3%)	8 (23%)	19	no	14%	40%

Table 2 Results from included studies. (continued)

Author	Year	Subgroup	N	N (%) proteinuria	N (%) hypertension	N (%) decreased GFR	FU duration (years)	Kidney injury before nephrectomy	Lost during follow-up	Gender (% male)
Schmidt[362]	1992	Mixed	34^	-	1 (3%)	2 (11%)	11.9	no	-	34%
Seminara[363]	2019	Malignancy	46^	8 (23%)	-	5 (11%)	5	no	-	48%
Simon[364]	1982	Malignancy	17	-	-	0 (0%)		-	-	-
Spira[365]	2009	Congenital	21	1 (5%)	-	1 (5%)	1.8	no	-	57%
		Malignancy	28	1 (4%)	-	1 (4%)				
		Mixed	4	0 (0%)	-	0 (0%)				
Spreafico[322]	2014	Malignancy	15	1 (7%)	0 (0%)	0 (0%)	13.3	no	-	-
Srinivas[366]	1998	Malignancy	25	21 (84%)	0 (0%)	0 (0%)	4.9	no	30%	56%
Stefanowicz[255]	2011	Malignancy	30	7 (23%)	4 (13%)	9 (30%)	9.4	-	0	50%
Taranta-Janusz[367]	2012	Mixed	21	-	-	6 (29%)	-	-	-	33%
Warshaw[368]	1985	Congenital	6	1 (17%)	2 (33%)	1 (17%)	5.8	yes	16%	100%
Westland*[82]	2013	Mixed	227	43 (19%)	67 (30%)	13 (6%)	5.8	-	-	64%
Wikstad[369]	1986	Congenital	22	0 (0%)	-	-	13.2	-	-	50%
		Malignancy	15	4 (27%)	-	-	17.1	-	-	40%
Zambaiti[245]	2019	Congenital	11	-	2 (18%)	-	6.7	-	-	64%

^Not all measurements were performed on the entire cohort, * Additional data were provided by the authors, - No data was available.

Table 3 Proportions of affected patients and estimated heterogeneity.

Outcome	Subgroup	Number of studies	Total number of patients	Number of patients with outcome	Calculated affected proportion	Estimated affected proportion*	95% Confidence interval	I^2
Proteinuria	Overall	42	1,537	248	16.1%	15.3%	(11.6% - 20.0%)	59%
	Congenital	7	151	25	16.6%	15.9%	(5.3% - 39.0%)	57%
	Malignancy	26	906	136	15.0%	13.9%	(9.1% - 20.6%)	69%
	Mixed	9	480	87	18.1%	18.6%	(16.2% - 21.3%)	0%
Hypertension	Overall	43	3,039	427	14.1%	14.5%	(10.5% - 19.8%)	83%
	Congenital	6	133	11	8.3%	11.2%	(3.1% - 33.4%)	46%
	Malignancy	27	2,362	293	12.5%	13.3%	(8.6% - 20.2%)	86%
	Mixed	10	544	123	22.6%	19.2%	(9.6% - 34.8%)	70%
GFR	Overall	55	1,983	262	13.2%	11.9%	(8.6% - 16.2%)	80%
	Congenital	10	217	29	13.4%	12.8%	(3.9% - 34.7%)	72%
	Malignancy	35	1,324	204	15.4%	11.6%	(7.6% - 17.4%)	81%
	Mixed	10	442	29	6.6%	9.3%	(5.3% - 16.1%)	47%

* Estimated using random-effect meta-analyses.[302] The I^2 statistic was used to quantify between study heterogeneity.[370]

GFR, glomerular filtration rate

The detailed in- and exclusion criteria of most studies likely resulted in a selected study population: whereas data from a US registry showed that 74% of paediatric nephrectomies was for a benign indication, >80% of the patients in our systematic review had a malignant indication. [32] Since studies often excluded patients with contralateral anomalies or bilateral disease, the included patients likely represent a relatively healthy subset. We do not expect that this was a result of a narrow search strategy, since we identified over 5,000 unique articles and reference searching identified only one additional article.

The assessment of the study outcomes is also an important factor explaining heterogeneity. Three main indicators of kidney injury were selected for this study. Proteinuria is an early marker of kidney damage and can be measured using 24-hour urine collection, which is considered to be the most reliable method, or spot urines, which is more convenient and has been shown to yield reliable results as well. [217] Hypertension is a key component of glomerular hyperfiltration and consequence of kidney injury. Although blood pressure measurement using ambulatory blood pressure monitoring is more accurate than office blood pressure, [213,320] it was used in four studies only. [241,244,321,322] This may have led to measurement errors in the studies included in this systematic review. The probability of a measurement error is probably even larger when determining GFR, for which many different measurement techniques and estimations were used. [323] Furthermore, different cut-offs for GFR were used, with a much lower proportion of affected patients in studies using a cut-off of 60 ml/min/1.73m^2 than in studies using 90ml/min/1.73m^2 (6.0% and 26.6%, respectively). Given these issues, it is not surprising that heterogeneity was larger for hypertension (83%) and a decreased GFR (80%), than for proteinuria (59%).

We attempted to correct for follow-up duration and several additional sources of variation by creating subgroups and performing meta-regression analyses. We expected that the follow-up duration would be a main determinant of the number of patients with kidney injury, since hyperfiltration is considered to be the main mechanism behind kidney injury in patients who are living with an SFK. However, creating subgroups based on follow-up duration did not substantially reduce heterogeneity, follow-up duration was not statistically significantly associated with estimated proportions in meta-regression analyses, and no clear associations between follow-up duration and the proportion of affected patients was visible in our scatterplots including all studies (Figure 5). Since this was an unexpected finding, we investigated whether an effect of follow-up was visible when focussing on only the larger studies with over 50 included patients. The variance explained by follow-up duration was much larger, especially when focussing only on patients with a tumour as indication for nephrectomy (Figure 6). As such, the effect of follow-up duration in our overall analyses is probably diluted and confounded by the many small studies with widely varying results that were given an equal weight as the larger studies. One of these factors could be the definition of follow-up, which

we defined as age at outcome assessment minus age at nephrectomy. Children with congenital indications for nephrectomy may have lived with a unilateral malfunctioning or non-functioning kidney before nephrectomy, however, and might be prone to kidney injury at an earlier age as a result.

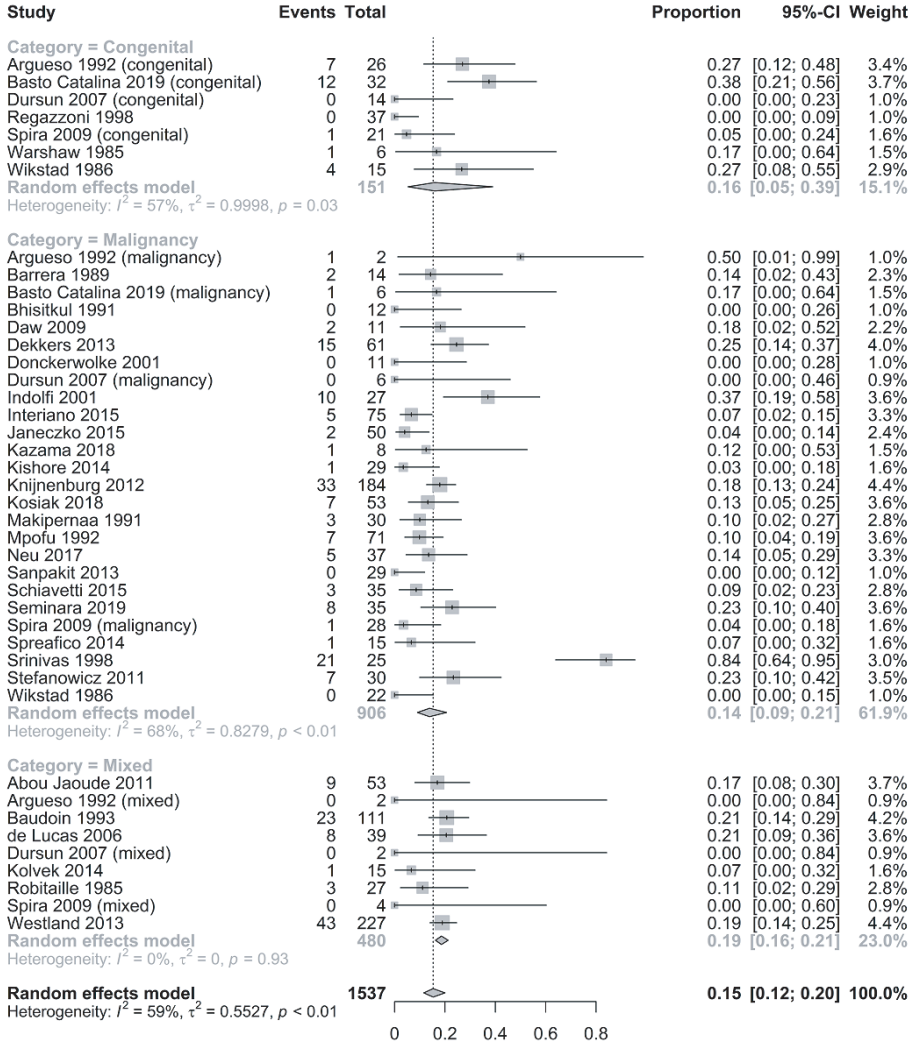


Figure 2 Forest plot with proportions of patients with proteinuria for different indications for nephrectomy.

Differences in study type could also have affected our results. Some studies reported on a cohort of patients that had systematically been followed after nephrectomy, whereas others called back individuals for a one-time assessment of kidney injury. However,

limiting our analyses to cohort studies did not change our results substantially. Also, several smaller studies were included, in which greater variation due to chance can be expected. Nonetheless, study size was not statistically associated with affected proportion of patients in meta-regression analyses.

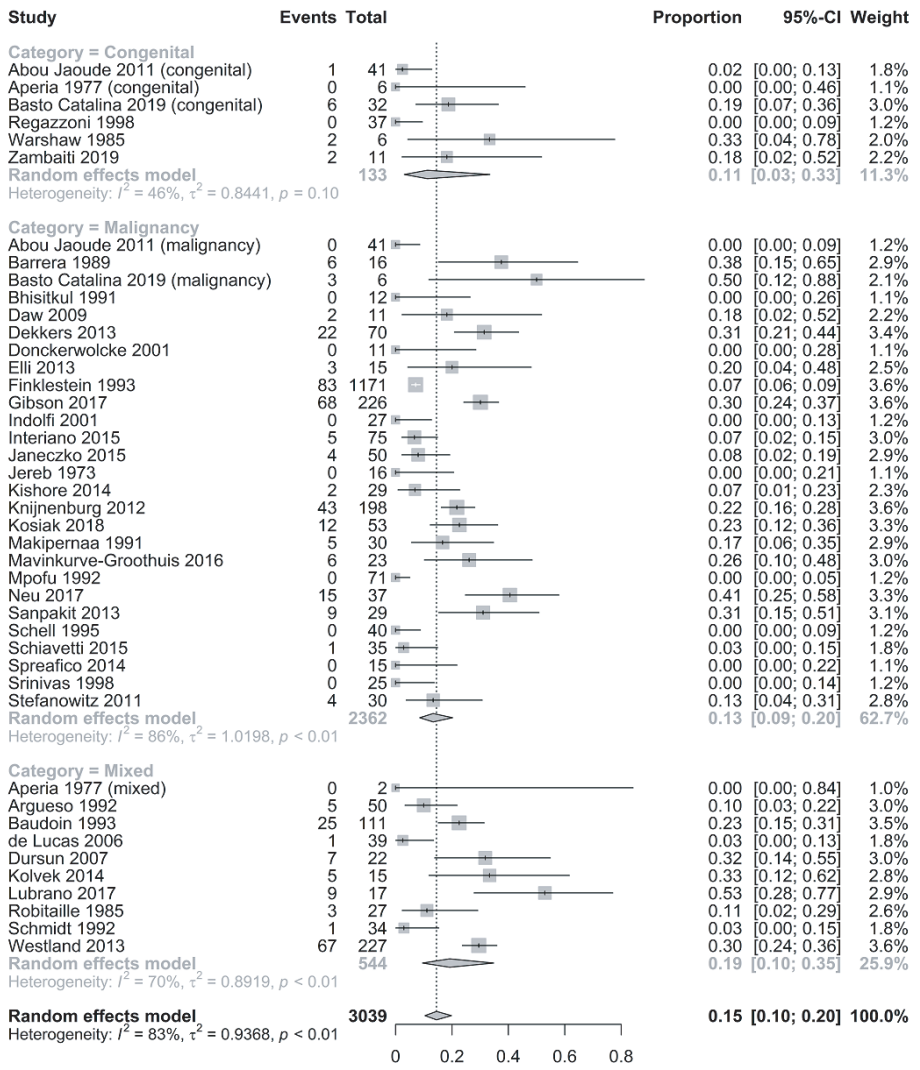


Figure 3 Forest plot with proportions of patients with hypertension for different indications for nephrectomy.

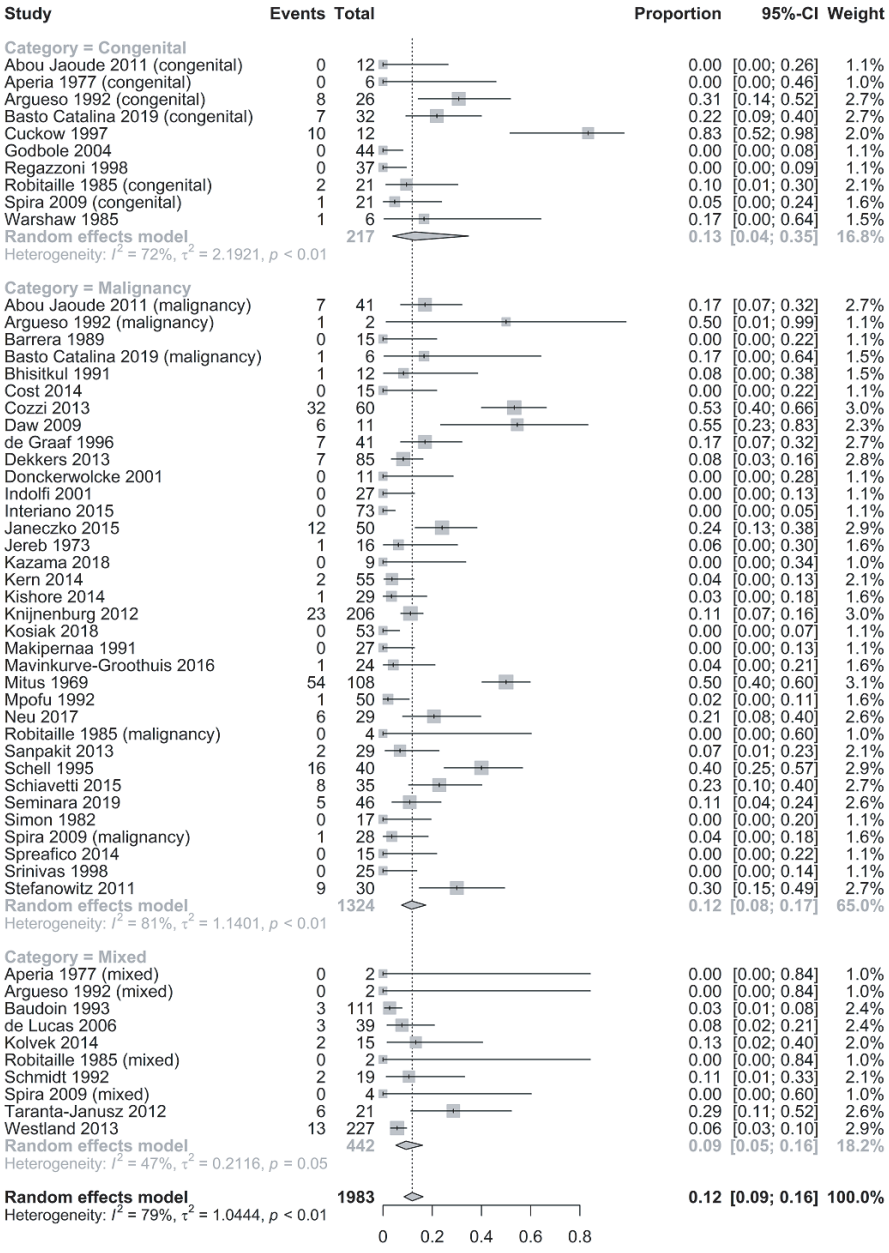


Figure 4 Forest plot with proportions of patients with a decreased estimated glomerular filtration rate for different indications for nephrectomy

A common approach to deal with heterogeneity in meta-analyses of observational studies is to create subgroups. [303] Although we planned several subgroup analyses *a priori*, these did not decrease heterogeneity substantially in most cases. Another possible approach is to perform meta-regression analyses, in which several possible mediators can be taken into account. Despite the inclusion of six potential contributors to heterogeneity, only a minor part (0-21%) of heterogeneity could be explained. Lastly, individual patient data meta-analyses could be a solution, but we refrained from this option because of the large number of cohorts that were published >10 years ago. Despite the limitations in interpretation of the meta-analyses that come with the large between-study heterogeneity, this systematic review benefits from its large number of included studies and considerable sample size. We showed that more that 10% of patients exhibits some form of kidney injury after undergoing a nephrectomy in childhood, which stresses the importance of a standardized follow-up protocol for these patients and high-quality research into the topic.

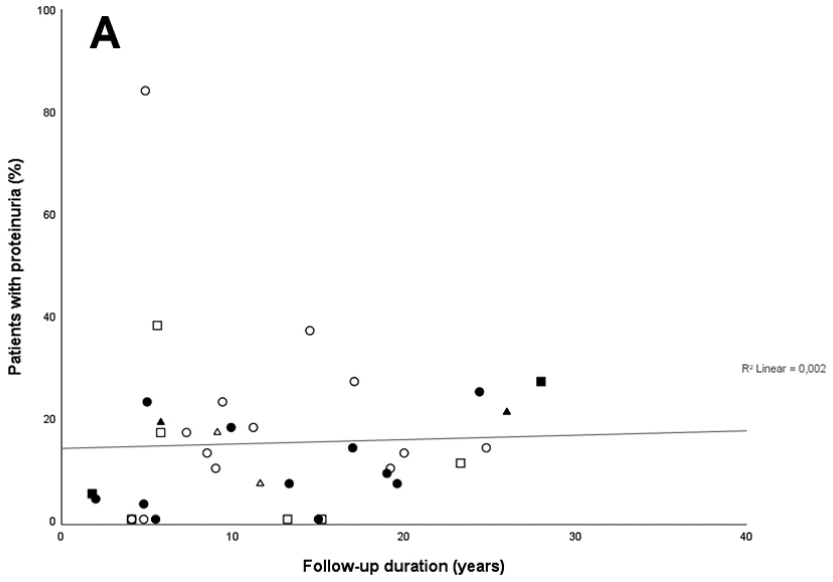
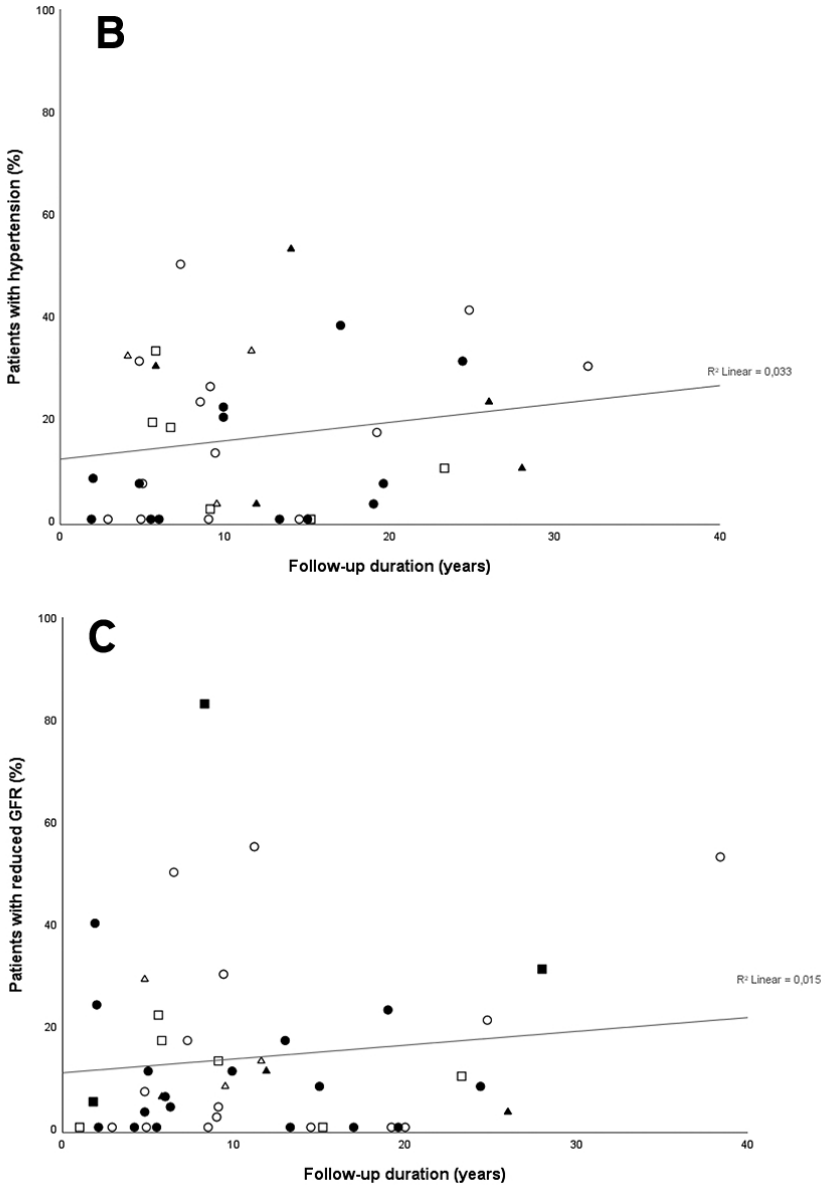


Figure 5 Scatterplots of study follow-up duration and percentage of affected individuals for the main outcomes: (**A**) proteinuria (B) hypertension (C) decreased estimated glomerular filtration rate (D) any sign of kidney injury, separated by indication for nephrectomy. Squares represent studies with congenital indication for nephrectomy only, circles represent studies with malignant indications, triangles represent studies with mixed indications. Filled symbols represent studies reporting median follow-up duration, empty symbols represent mean follow-up duration. Only studies with at least 1 affected participant were included.



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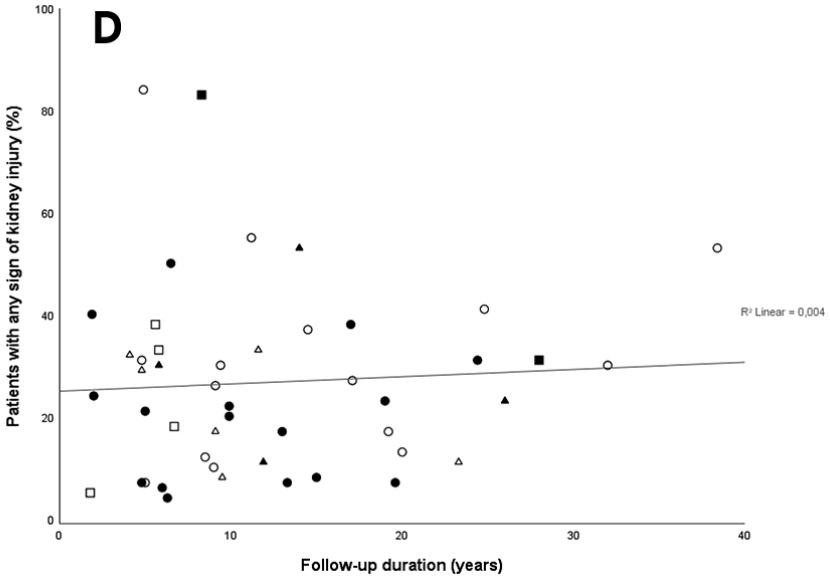

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Our results show that no indication subgroup remains sufficiently free from kidney injury to justify discharge from follow-up. Although not all patients will develop kidney injury after nephrectomy, stratification is not yet possible, which is similar to patients living with a congenital SFK.[296] Therefore, we recommend to perform screening for kidney injury in line with that performed in patients with congenital SFK, consisting of yearly measurement of blood pressure and proteinuria and 5-yearly estimation of the GFR.[324] More frequent GFR estimation may be needed in circumstances that increase the demand on the kidney, such as during rapid pubertal growth or pregnancy, and in individuals with obesity. Local practice patterns can guide whether follow-up is performed by a paediatric urologist or nephrologist, or a general care physician.

For future studies, we highly recommend reporting outcomes according to existing guidelines for the measurement and classification of hypertension and kidney function to overcome the limitations we encountered while summarizing the available evidence.[213,226] Furthermore, structured follow-up of all patients undergoing unilateral nephrectomy in childhood as recommended above will likely lead to more available data on a less selected population. Ideally, these data should be registered in a centralized

facility such as the registries of the European Reference Networks (ERNs) (*e.g.* ERKReg or the ERN eUROGEN registry) and made available for research, since availability of these data would bring tailored follow-up based on individual risk factors a step closer to clinical practice.

In conclusion, this systematic review and meta-analysis showed that signs of kidney injury were common in a large and heterogeneous population of patients that underwent nephrectomy in childhood. No difference was observed between nephrectomy for benign or malignant conditions. These results indicate that standardized follow-up is needed in all patients who underwent nephrectomy during childhood. More widespread and uniform reporting of data is needed to facilitate estimation of the risk of kidney injury in specific subpopulations and tailor clinical care accordingly.

SUPPLEMENTARY MATERIAL

Full search strategy

MEDLINE

("Nephrectomy"[Mesh] OR Nephroureterectom*[tiab] OR Nephrectom*[tiab] OR Heminephrectom*[tiab] OR solitary kidney*[Tiab] OR solitary functioning kidney*[Tiab] OR single kidney*[Tiab] OR remnant kidney*[tiab])

AND

(infan* OR newborn* OR new-born* OR neonat* OR baby OR baby* OR babies OR toddler* OR minors OR minors* OR boy[tiab] OR boys[tiab] OR boyhood OR girl* OR kid OR kids OR child[MeSH] OR child*[tiab] OR children[tiab] OR school child[tiab] OR school child*[tiab] OR adolescen*[tiab] OR juvenil* OR youth* OR teen* OR under*age* OR pubescen* OR pediatrics[mh] OR pediatric*[tiab] OR paediatric*[tiab] OR peadiatric*[tiab] OR school[tiab] OR school*[tiab])

AND

(hypertension [tiab] OR high blood pressure* [tiab] OR glomerular filtration rate* [tiab] OR GFR [tiab] OR Proteinuria [tiab] OR Albuminuria [tiab] OR renal insufficiency [tiab] OR kidney insufficiency [tiab] OR kidney failure [tiab] OR renal failure [tiab] OR kidney injury [tiab] OR renal injury [tiab] OR renal impairment [tiab] OR chronic kidney disease [tiab] OR chronic renal disease [tiab] OR kidney failure [tiab] OR renal failure [tiab] OR end stage kidney disease [tiab] OR end stage renal disease [tiab] OR outcome* [tiab] OR prognosis [tiab] OR renal damage [tiab] OR ("Kidney Diseases"[Mesh:NoExp] AND ("1965/01/01"[PDat] : "1966/12/31"[PDat])) OR "Proteinuria"[Mesh:NoExp] OR "Hypertension"[MeSH Terms] OR "Glomerular Filtration Rate"[Mesh] OR "Renal Insufficiency"[Mesh:NoExp] OR "Renal Insufficiency, Chronic"[Mesh] OR "Case-Control Studies"[Mesh] OR "Cohort Studies"[MeSH Terms] OR "Urologic Diseases/complications"[MeSH] OR "Albuminuria"[MeSH Terms])

NOT

(Animals[Mesh] NOT Humans[Mesh])

EMBASE

1	((Renal or kidney) adj2 (insufficien* or failure* or injur* or impair* or damage* or dysfunction*)).ti,ab,kw.
2	kidney failure/ or exp chronic kidney failure/ or end stage renal disease/ or mild renal impairment/ or moderate renal impairment/ or renal replacement therapy-dependent renal disease/ or severe renal impairment/ or subclinical renal impairment/
3	exp proteinuria/
4	hypertension/ or borderline hypertension/ or diabetic hypertension/ or essential hypertension/ or malignant hypertension/ or masked hypertension/ or prehypertension/ or exp renovascular hypertension/ or resistant hypertension/ or systolic hypertension/ or white coat hypertension/
5	exp glomerulus filtration rate/
6	exp case control study/
7	cohort analysis/
8	urinary tract disease/co [Complication]
9	prognosis/
10	(outcome* or prognos*).ti,ab,kw.
11	(glomerulus filtration rate or gfr).ti,ab,kw.
12	((Renal or kidney) adj2 (chronic or endstage or end stage) adj2 disease*).ti,ab,kw.
13	(proteinuria or albuminuria).ti,ab,kw.
14	(Hypertension or high blood pressure*).ti,ab,kw.
15	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14
16	nephrectomy/ or nephroureterectomy/ or radical nephrectomy/ or uninephrectomy/
17	(Nephroureterectom* or Nephrectom* or Heminephrectom* or (solitary adj2 kidney*) or solitary functioning kidney* or (single adj2 kidney*) or remnant kidney*).ti,ab,kw.
18	16 or 17
19	child/ or boy/ or girl/ or hospitalized child/ or exp infant/ or preschool child/ or school child/ or toddler/
20	pediatrics/ or child urology/ or pediatric emergency medicine/
21	(infan* or newborn* or new-born* or neonat* or baby or baby* or babies or toddler* or minors or minors* or boy or boys or boyhood or girl* or kid or kids or child* or children or school child or school child* or adolescen* or juvenil* or youth* or teen* or under age* or pubescen* or pediatric* or paediatric* or peadiatric* or school or school*).ti,ab,kw.
22	19 or 20 or 21
23	15 and 18 and 22
24	limit 23 to conference abstract
25	23 not 24

Supplementary Tables

Supplementary Table 1 Subgroup- and meta-regression analyses investigating the effect of potential confounders on the proportions of patients with proteinuria

Group	Subgroup analyses				Meta-regression analysis		
	Estimated affected proportion	95% Confidence interval	I^2	p-value	Regression coefficient	Standard error	p-value
Overall	15.3%	(11.6% - 20.0%)	59%				
Indication				0.34			
Congenital	15.9%	(5.3% - 39.0%)	57%		ref	ref	ref
Malignancy	13.9%	(9.1% - 20.6%)	69%		-0.0956	0.5818	0.87
Mixed	18.6%	(16.2% - 21.3%)	0%		-0.0538	0.6591	0.94
Follow-up duration				0.76			
<7.5 year	12.8%	(5.8% - 26.0%)	77%		ref	ref	ref
7.5-15 year	16.9%	(12.2% - 22.8%)	30%		-0.0944	0.5346	0.86
>15 year	15.9%	(11.2% - 22.1%)	36%		-0.0123	0.4873	0.98
Follow-up reporting				0.35			
Mean	17.2%	(11.4% - 25.2%)	66%		ref	ref	ref
Median	13.5%	(9.4% - 19.1%)	44%		-0.4366	0.4253	0.31
Year of publication				0.85			
≤2010	15.9%	(9.9% - 24.4%)	65%		0.0536	0.4727	0.91
>2010	15.1%	(10.9% - 20.5%)	50%		ref	ref	ref
Study size				0.92			
≤10 patients	16.1%	(9.8% - 25.4%)	0%		0.0001	0.7076	1.00
11-49 patients	14.2%	(8.8% - 22.2%)	69%		-0.0632	0.4691	0.89
≥50 patients	15.1%	(10.3% - 21.6%)	54%		ref	ref	ref
Study design				0.83			
Cohort	15.9%	(11.1% - 22.3%)	50%		ref	ref	ref
Cross-sectional	15.0%	(9.5% - 23.0%)	66%		0.0257	0.4810	0.96

The I^2 statistic was used to quantify between study heterogeneity.[302] Meta-regression analyses were carried out using the meta package in R and p-values were calculated using the Knapp-Hartung method.[370]

Supplementary Table 2 Subgroup- and meta-regression analyses investigating the effect of potential confounders on the proportions of patients with hypertension

Group	Subgroup analyses				Meta regression analysis		
	Estimated affected proportion	95% Confidence interval	I^2	p-value	Regression coefficient	Standard error	p-value
Overall	14.5%	(10.5% - 19.8%)	82%				
Indication				0.51			
Congenital	11.2%	(3.1% - 33.4%)	46%		ref	ref	ref
Malignancy	13.3%	(8.6% - 20.2%)	86%		0.7285	0.6108	0.24
Mixed	19.2%	(9.6% - 34.8%)	70%		1.5182	0.6738	0.03
Follow-up duration				0.68			
<7.5 year	13.9%	(7.6% - 23.8%)	89%		ref	ref	ref
7.5-15 year	17.5%	(9.3% - 30.3%)	76%		-0.3833	0.4215	0.37
>15 year	12.5%	(6.6% - 22.4%)	63%		0.4004	0.4642	0.39
Follow-up reporting				0.42			
Mean	16.3%	(10.1% - 25.1%)	87%		ref	ref	ref
Median	12.6%	(7.6% - 20.1%)	72%		-0.4046	0.3338	0.23
Year of publication				0.02			
≤2010	9.7%	(5.7% - 16.0%)	72%		-1.2884	0.3838	<0.01
>2010	19.6%	(13.2% - 27.9%)	69%		ref	ref	ref
Study size				0.09			
≤10 patients	32.1%	(9.0% - 69.3%)	0%		1.9110	0.8514	0.03
11-49 patients	12.4%	(7.7% - 19.3%)	66%		0.1731	0.3700	0.64
≥50 patients	15.9%	(9.0% - 26.5%)	94%		ref	ref	ref
Study design				0.87			
Cohort	14.9%	(9.1% - 23.3%)	88%		ref	ref	ref
Cross-sectional	14.1%	(8.7% - 22.0%)	62%		-0.0246	0.4004	0.95

The I^2 statistic was used to quantify between study heterogeneity.[302] Meta-regression analyses were carried out using the meta package in R and p-values were calculated using the Knapp-Hartung method.[370]

Supplementary Table 3 Subgroup- and meta-regression analyses investigating the effect of potential confounders on the proportions of patients with a decreased estimated glomerular filtration rate

Group	Subgroup analyses				Meta regression analysis		
	Estimated affected proportion	95% Confidence interval	I^2	p-value	Regression coefficient	Standard error	p-value
Overall	11.9%	(8.6% - 16.2%)	80%				
Indication				0.75			
Congenital	12.8%	(3.9% - 34.7%)	72%		ref	ref	ref
Malignancy	11.6%	(7.6% - 17.4%)	81%		-0.1958	0.4567	0.67
Mixed	9.3%	(5.3% - 16.1%)	47%		-0.3691	0.5486	0.50
Follow-up duration				0.99			
<7.5 year	12.1%	(7.4% - 19.1%)	82%		ref	ref	ref
7.5-15 year	11.5%	(5.5% - 22.3%)	67%		0.3028	0.9322	0.77
>15 year	11.8%	(5.8% - 22.5%)	80%		0.0643	0.4168	0.15
Follow-up reporting				0.40			
Mean	10.2%	(6.1% - 16.5%)	76%		ref	ref	ref
Median	13.4%	(8.5% - 20.3%)	82%		0.1754	0.3647	0.63
Year of publication				0.84			
≤2010	11.5%	(6.9% - 18.7%)	78%		0.6965	0.4063	0.09
>2010	12.3%	(8.0% - 18.4%)	79%		ref	ref	ref
Study size				0.71			
≤10 patients	13.3%	(8.1% - 21.1%)	0%		0.1841	0.5730	0.75
11-49 patients	12.3%	(8.0% - 18.4%)	65%		-0.3151	0.3938	0.43
≥50 patients	8.8%	(3.0% - 22.8%)	94%		ref	ref	ref
Study design				0.50			
Cohort	13.0%	(7.8% - 21.0%)	82%		ref	ref	ref
Cross-sectional	10.5%	(6.7% - 16.0%)	77%		-0.2780	0.4126	0.50
GFR cut-off				<0.01			
<60	6.9%	(4.4% - 10.6%)	83%		ref	ref	ref
<80	8.4%	(2.6% - 23.6%)	33%		0.1306	0.6840	0.85
<90	26.6%	(18.3% - 36.9%)	78%		1.8146	0.3665	<0.01
Other	6.5%	(1.8% - 20.8%)	48%		-0.4904	0.6357	0.44

The I^2 statistic was used to quantify between study heterogeneity.[302] Meta-regression analyses were carried out using the meta package in R and p-values were calculated using the Knapp-Hartung method.[370] GFR glomerular filtration rate.

CHAPTER 8

Risk stratification for children with a solitary function kidney

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In the last decade, it has become widely accepted that living with just one kidney may be harmful for that solitary functioning kidney (SFK).[295] This deterioration is explained by the hyperfiltration hypothesis from prof. Brenner,[183] which states that a reduction in the number of nephrons will result in glomerular hyperfiltration in the remaining nephrons. This hyperfiltration starts a vicious cycle of glomerular hypertension, hypertrophy and injury, which results in systemic hypertension, albuminuria and glomerulosclerosis with a further reduction in nephron numbers.

Kidney donation is the most common cause of living with an SFK and even though the absolute risk after kidney donation is limited, the risk for kidney failure is indeed increased.[371] However, not every kidney donor has the same risk for kidney failure. To assist in estimating an individual's risk for kidney failure after kidney donation, online calculators are available. These calculators take into account risk factors for kidney failure such as age, sex, race, obesity, and diabetes.

Children may present with an SFK as a consequence of a congenital non-functioning kidney or after nephrectomy, for instance due to malignancy. Being born with an SFK exposes glomeruli to a much longer period of hyperfiltration, and has been associated with kidney injury starting in childhood[82] and a higher risk of kidney failure later in life than after kidney donation.[75] Unfortunately, risk stratification within the group of SFK patients is not yet possible, as factors that differentiate between high and low risk groups need to be better established and the prediction model that was developed in a single study should be improved and confirmed externally.[210] With the increasing interest in the subject, however, and the increase in size of the cohorts described, it is to be expected that such a good prediction model and a risk calculator may be developed in the coming years.

Risk stratification

In recent years, the issue of risk stratification for kidney injury in the follow-up of children with an SFK has been addressed by several authors. Similar to risk factors for chronic kidney disease (CKD),[372] such risk factors can be grouped into four categories: genetic factors (risk alleles), perinatal factors (*e.g.*, low birth weight and premature birth), additional kidney or urinary tract abnormalities (such as vesicoureteral reflux or the absence of kidney hypertrophy), and additional hits to the kidney (*e.g.* urinary tract infections or obesity). In addition to these risk factors, the degree and period of hyperfiltration, and thereby the age of the patient, plays an important role. Patient characteristics such as sex and ethnicity could be involved. Most studies showed no association between sex and risk for kidney injury in SFK patients.[76,78,82,178,210,246,373] One notable exception is the study by Alfandary *et al.* in which male adolescents with SFK had a threefold higher risk of kidney injury compared to female patients.[76]

In the current edition of this journal, Matsell et al. have identified that the subtype of congenital SFK may also be a risk factor for kidney injury, with a higher risk in patients with unilateral renal agenesis (URA) compared to children with a multicystic dysplastic kidney (MCDK). [81] This is in line with the previous report from Sanna-Cherchi et al., who showed that the chance of needing dialysis at the age of 30 years was higher for patients with URA (~40%) compared with MCDK (~20%).[75] Previous studies have also suggested that the risk for kidney injury is higher in patients with acquired than congenital SFK. [219] Both findings strengthen the hypothesis that the cause of SFK is an important determinant of the risk for kidney injury.

Extent of glomerular hyperfiltration

Both the duration and timing of hyperfiltration play an important role in the risk of kidney injury. A longer period of hyperfiltration increases the chance of developing kidney injury in diabetic nephropathy, a well-known cause of hyperfiltration injury, where the duration of diabetes is associated with the risk of developing albuminuria. [374] In addition, older age has been shown to increase the proportion of SFK patients that develop hypertension[82], albuminuria, [82,375-377] and/or abnormal kidney function. [77,378] The impact of timing has been shown by the fact that the degree of glomerular hyperfiltration was twice as high after early kidney mass reduction when compared to nephrectomy in adulthood. [295,379] Thus, early kidney mass reduction may increase the risk of kidney injury. During adolescence, kidney function is well known to decline at an increased speed, as has been described in patients with kidney hypo/dysplasia[220] or with an SFK, [79,82] further increasing the effect of timing (kidney mass reduction before or after puberty) on the risk of kidney injury. The kidney function decline during puberty may be explained by the rapid growth with the subsequent metabolic demand on the kidney, which the SFK is not able to deliver without a relevant change in glomerular hemodynamics. This increase in glomerular hyperfiltration, and potentially in glomerular hypertension, will lead to more glomerular injury with subsequent albuminuria. Another explanation may be that the increased demand on the kidney unveils the influence of dysplastic nephrons, in line with the findings in patients with hypo/dysplasia. [220] The latter may also be an explanation for the differences found between URA and MCDK by Matsell *et al*. [81] Cause, timing and duration of the renal mass reduction with subsequent hyperfiltration are all factors that should therefore be considered in the risk stratification.

Genetic factors

Genetic factors contributing to kidney injury in patients with SFK are likely to be risk alleles that, in contrast to highly penetrant disease-causing mutations, are associated with a relatively small increased risk of disease only. In a large meta-analysis of genome-wide association studies of estimated glomerular filtration rate (eGFR) in the general population, 264 loci associated with kidney function were identified. [380] When a selection of 147 of these single nucleotide polymorphisms (SNPs) was combined to calculate

genetic risk scores, these scores proved to be associated with an increased risk of CKD and hypertension.[380] Although it is unlikely that similar sized cohorts of SFK patients will be composed, smaller studies may also identify variants with prognostic value, as illustrated by the results of cohorts of patients with obstructive uropathies suggesting that variants in angiotensin converting enzyme (*ACE2*), angiotensin II type 2 receptor (*AGTR2*) and cadherin 12 (*CDH12*) are associated with the development of kidney injury.[381,382] Interestingly, the variants in *ACE2, AGTR2* and *CDH12* were not among the 147 SNPs used to calculate genetic risk scores for signs of kidney injury in the general population. This indicates that risk alleles for kidney injury may differ between patients with congenital anomalies of the kidney and urinary tract (CAKUT) and healthy individuals, and that the search for additional risk alleles in CAKUT patients may benefit from a hypothesis-free approach.

Perinatal factors

In humans, nephrons are formed until approximately 36 weeks of pregnancy.[85] As a consequence, nephron formation may not be complete in infants born prematurely, and continued nephron development with signs of abnormal morphology has been found in preterm neonates.[383] The interindividual differences in kidney development that were observed in this study, suggested that early (during active nephrogenesis) postnatal exposure to an inflammatory environment[152] or nephrotoxins may play an additional role. Despite the knowledge that nephrotoxic drugs such as gentamicin or indomethacin can have disrupting effects on kidney development, they are frequently used in neonatal intensive care units.[384,385] Use of nephrotoxic drugs in premature infants with SFK may have even more detrimental effects and could pose a risk factor for kidney injury in these patients. Low birth weight (<2,500g) is associated with low nephron numbers,[1] and was associated with a risk of kidney injury twice that of children with a normal birth weight in patients with SFK.[82,181] Prematurity and low birth weight could explain some of the differences in kidney function in children with SFK, and should be considered in risk stratification.

Additional congenital anomalies of the kidney and urinary tract

In a normal CSFK, compensatory growth is expected to start before birth and continue throughout childhood.[17,29,78,181] Before birth, this growth can comprise of both hyperplasia (increase in cell number) and hypertrophy (increase in cell size).[130] The evidence that the absence of compensatory growth is associated with the risk of kidney injury is convincing. Several studies have found that larger SFK size, especially when present at birth, was associated with a lower risk of kidney injury[76-78,82,181,210,241] suggesting that a responsive mechanism triggering hyperplasia is very important in dealing with kidney mass reduction. The evidence supporting an association between additional CAKUT of the SFK and kidney injury is also strong, with hazard ratios varying between 1.7 and 13, which is in line with the results reported by dr. Matsell and colleagues.[75-79,81,82,210,240,386,387] The association of additional CAKUT in the SFK with kidney injury may be explained

by the fact that these patients are more prone to recurrent urinary tract infections or other events that further decrease the number of nephrons.

Table 1 Potential risk factors for kidney injury in children with SFK

Risk factor	Magnitude	References
Patient characteristics		
Male sex	0.8 – 4.4	76,78,82,178,210,246,373
Ethnicity	n/a	n/a
Duration of hyperfiltration		
Older age (per year)	1.1	82,178,375-378
Cause of SFK		
Acquired vs congenital SFK	n/a	219
URA vs MCDK	2.1 - 2.7	75,81,210
Genetic factors		
Risk alleles	n/a	n/a
Perinatal factors		
Premature birth	2.0	210
Low birth weight	2.1 – 2.7	82,210
Additional kidney or urinary tract abnormalities		
Smaller SFK size (per SDS)	1.1	76-78,82,181,210,241
Additional CAKUT	1.7 – 13	75-79,81,82,210,240,386,387
Additional hits to the kidney		
Nephrotoxic drug use	n/a	n/a
Urinary tract infections	1.6 – 6.7	78,82,210,240
Obesity	1.4 - 15	76,78,79,229,246,387
Smoking	1.5	76,79,229
Diabetes	4.4	388-390
Hypertension	n/a	77,388-390
Cardiovascular disease	5.6	390
Pregnancy	2.4	227,228

n/a not available.

Additional hits

In the situation of a reduced nephron number, other exposures that hurt the kidney or increase the demand on the kidney are likely to increase the risk of kidney injury. Such an increased risk has been identified repeatedly for recurrent urinary tract

infections (UTIs).[78,82,210] Modifiable risk factors, such as obesity, smoking or diabetes, should be avoided as much as possible because of their associations with hypertension, albuminuria and kidney function deterioration.[76,181,229,387] For diabetes, hypertension and cardiovascular disease, a direct relation with faster development of CKD was shown in patients with SFK.[388-390] Pregnancy is a period of additional demand on the SFK, which results in a risk for gestational hypertension and preeclampsia that is more than twice that of women with two kidneys.[227,228] Therefore, careful monitoring of women with a SFK is necessary during pregnancy.

Previously, participation in contact sport has been advised against because of potential kidney trauma to the SFK with potential devastating consequences. The extremely low incidence of kidney injury caused by sports participation, however, has led to a revision of these guidelines. The potential benefits of sports participation are highly likely to surpass the risks.[235]

Clinical implications

The interesting results reported by Matsell et al. provide another step towards further personalizing the care for patients with a SFK.[81] Large cohorts of SFK patients will allow for the development of prediction models that can be used to stratify patients into risk categories. The recently published prediction model developed by Poggiali *et al*.[210] is an excellent example of what such a model could bring. In its current form, however, the additive value of this prediction model is limited, since the high risk group is mainly determined by occurrence of multiple UTIs. This information is not yet available in early life and would only allow for adjustment of the follow-up strategy after a second or third UTI has taken place. The same is likely to be done in regular care, since most clinicians would increase follow-up frequency and/or consider treatment in a child with SFK and recurrent UTIs. A prediction model would be especially helpful if it would contain only predictive factors occurring early in life, for instance within the first year, so a tailored follow-up strategy can be initiated from then on. Furthermore, the current model was based on only 18 events in 162 patients. In prediction modelling, a minimum of 10 events per variable (EPV) is usually advised and an EPV ≥20 has been advocated to obtain more reliable predictions.[391] Collaborative efforts of research groups will be needed to create and validate prediction models that are applicable in different centres and healthcare settings. If such a prediction model is established, it will likely improve the current recommendations for the follow-up of SFK patients,[295,324] allowing for better and more cost-efficient clinical management. In addition, identification of SFK patients with a high risk of kidney injury facilitates targeted research in these groups, for example regarding innovative biomarkers of kidney injury or future therapies slowing down or even preventing the progression of kidney injury.

CHAPTER 9

Uncovering risk factors for kidney injury in children with a solitary functioning kidney

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ABSTRACT

Children with a solitary functioning kidney (SFK) have an increased risk of kidney injury. The exact risk of and risk factors for kidney injury remain unknown, however, which impedes personalized care. We recruited a nationwide multicentre cohort of 944 SFK patients to get more insight into this. Patients were born in 1993-2020 and diagnosed with congenital or acquired SFK before adulthood. The median follow-up duration was 12.8 years and four indications of kidney injury were studied: urine protein-creatinine ratios, blood pressure, estimated glomerular filtration rate (eGFR), and use of anti-hypertensive/proteinuric medication. For each indicator except medication use, separate cut-off values for any injury and severe injury were used. Survival analyses indicated that at 18 years of age, any or severe kidney injury were present in 75% and 39% of patients with congenital SFK, respectively. Risk factors for kidney injury included kidney agenesis as cause of the SFK, anomalies in the SFK, and high BMI at last follow-up. Kidney agenesis and being overweight were specifically associated with proteinuria and high blood pressure, whereas anomalies in the SFK were associated with reduced eGFR. The high prevalence of kidney injury in SFK patients stresses the need for long-term follow-up, in which lifestyle is an important topic to address. More research into the etiological role of the risk factors identified will help to translate our findings into individualized care strategies.

INTRODUCTION

A solitary functioning kidney (SFK) is a common condition caused by congenital absence of one of the kidneys, a major malformation resulting in one non-functioning kidney, or unilateral nephrectomy. Previous work showed that an SFK in childhood predisposes to kidney injury (*i.e.* high blood pressure or chronic kidney disease (CKD)) later in life. The exact prevalence of kidney injury is uncertain, however, with estimates ranging from 6 to 60% at 15 years of age.[76,80,82,210] Long-term studies are scarce, but the few studies with follow-up into adulthood showed considerable proportions of patients with kidney injury, ranging from 25% of patients with an estimated glomerular filtration rate (eGFR) <60ml/min in one study[83] to 30% of patients reaching kidney failure in another.[75] Although these studies likely included selected subgroups of more severely affected patients and may have overestimated the consequences of having an SFK from early in life, even mild to moderate kidney injury in childhood can lead to long-term complications such as cardiovascular disease.[5,392] Therefore, kidney injury in children with SFK should be identified and treated as early as possible.

Although potential roles for genetic, perinatal, and lifestyle-related factors have been highlighted, it is still unknown why some SFK patients develop kidney injury whereas others do not.[75,78-80,82,83,181,210,231,296] Therefore, identification of risk factors for kidney injury in this population remains needed. We created the Solitary Functioning Kidney: Aetiology and Prognosis (SOFIA) study, a large nationwide cohort of SFK patients, to provide an improved estimate of the prevalence of kidney injury and to identify risk factors in children with an SFK.

METHODS

Patients

Patients were eligible for this study when diagnosed with an SFK (defined as <20% differential function on MAG-3 or DMSA scans or unilateral absence of kidney tissue on kidney ultrasound) before their 18th birthday and born between January 1st 1993 and December 31st 2020. Patients were derived from the AGORA (Aetiologic research into Genetic and Occupational/environmental Risk factors for Anomalies in children) data- and biobank[87,393] or informed about the study during clinical practice and/or using written information materials by paediatricians, paediatric nephrologists, and urologists from 36 hospitals in The Netherlands. After obtaining informed consent, electronic health records were searched for information regarding the cause of the SFK, exposure to potential risk factors for kidney injury, and follow-up data on the presence of kidney injury. Parents were asked to provide additional information on these topics using online questionnaires (available upon request). Some patients (112/990, 11%) had previously

been included in the KIMONO study.[82] The study protocol was approved by the Regional Committee on Research Involving Human Subjects [registration number 2018-4524].

Outcome classification

Four indicators of kidney injury were considered: proteinuria, high blood pressure, reduced eGFR, and use of antihypertensive and/or antiproteinuric medication. For all indicators except medication use, two cut-off values were used, reflecting any and severe kidney injury (Box 1). The last available record for each indicator of kidney injury was used to score the presence of the indicator and if applicable, previous records were investigated to determine the starting age. Reference values for blood pressure were adopted from the American Academy of Pediatrics (AAP) and the American Heart Association (AHA), with elevated blood pressure classified as any kidney injury and stage 1 or 2 hypertension classified as severe kidney injury (box 1).[213,394,395] Serum creatinine measurements were used to calculate eGFR using the recent CKiD U25 age and sex dependent equation,[396] which showed high accuracy in children and young adults. When creatinine was measured before 2010, the original Schwartz formula was used, to take into account the transition from Jaffe to isotope dilution mass spectrometry (IDMS) traceable creatinine measurements in the Netherlands around 2010.[397]

Box 1. Definitions of kidney injury.

	Cut-off value for any injury	Cut-off value for severe injury
Proteinuria	uPCR >20 mg/mmol and/or uACR >3 mg/mmol (≥2 years); uACR >10 mg/mmol (<2 years)	uPCR >50 mg/mmol and/or uACR >30 mg/mmol
High blood pressure	Office BP: SBP and/or DBP ≥p90 for age and sex or ≥120/80 mmHg (whichever is lower) ABPM: 24h SBP and/or DBP ≥p90 for age and sex	Office BP: SBP and/or DBP ≥p95 for age and sex or ≥130/80 mmHg (whichever is lower) ABPM: 24h SBP and/or DBP ≥p95 for age and sex
Reduced eGFR	<90 ml/min/1.73m^2 (≥2 years)	<60 ml/min/1.73m^2 (≥1 year)
Medication	Prescription of any of the following medication classes: ACE inhibitors, ARBs, calcium antagonists, or thiazide diuretics	

uPCR urine protein-creatinine ratio, uACR urine albumin-creatinine ratio, BP blood pressure, SBP systolic blood pressure, DBP diastolic blood pressure, ABPM ambulatory blood pressure measurement, eGFR estimated glomerular filtration rate, ACE angiotensin converting enzyme, ARB angiotensin receptor blocker.

Risk factors

Potential risk factors for kidney injury were predefined based on existing literature and knowledge of the pathophysiological mechanisms leading to kidney injury.[296] These included female sex, birth weight percentile, preterm birth (gestational age <37 weeks), cause of the SFK, presence of extrarenal congenital anomalies, right-sided SFK, CAKUT in the SFK, urinary tract infections (UTIs) in the first year of life, SFK length within 90 days and at one year of age, and high BMI at last follow-up.

Cause of the SFK was classified as congenital when a result of CAKUT, acquired when a clear non-congenital cause was present (*e.g.* Wilms tumour or thrombosis of the renal vein), and other/unknown when the cause could not be determined (e.g. dysplasia with recurrent UTIs). Length of the SFK was categorized using reference values based on Akhavan *et al.* and age- and sex-specific references were used to classify BMI as normal or high.[398,399] Inclusion via an academic hospital was regarded as potential confounder. Details on outcome and risk factor classifications as well as multiple imputation strategies are described in the Supplementary Methods.

Statistical analyses

Kaplan-Meier analysis was used to calculate survival without any kidney injury and without severe kidney injury. We assumed that all patients received follow-up until study closure, to account for shorter or less frequent follow-up of patients with favourable characteristics. Furthermore, follow-up after the age of 18 years was not used for the survival analyses, because data from follow-up at adult nephrologists or primary care physicians could not always be obtained. Patients were either censored at study closure or at 18 years of age, whichever came first, to account for selective loss to follow-up in patients with a low risk of kidney injury. Sensitivity analyses were performed in which patients were censored on the actual date of last follow-up or at 18 year of age.

Cox proportional hazards models were used to estimate hazards ratios (HR) with 95% confidence intervals (CI) for each potential risk factor. Four outcomes were studied: any kidney injury (also including severe injury), severe kidney injury, eGFR <90 ml/min/1.73m^2, and hyperfiltration injury (any proteinuria or high blood pressure and/or medication use). Crude HRs were derived from the original database and adjusted HRs were estimated using a model containing the factor of interest and all other potential risk factors after multiple imputation of missing values. The cause of SFK, presence of CAKUT in the SFK, and SFK length were only considered as potential risk factors in children with congenital SFK. To avoid multicollinearity, separate models were created for SFK length within 90 days and at 1 year of age. We created log-log plots to check proportional hazards assumptions and investigated time-varying effects of sex and

BMI by including interaction terms with time in our model. Analyses were stratified by cause of the SFK (congenital or acquired) and performed using SPSS version 25.0.

RESULTS

In total, 990 SFK patients provided informed consent. Clinical information could be obtained for 944 patients (95%) and questionnaires were completed by parents of 883 SFK patients (89%). A flowchart describing patient recruitment and number of patients lost to follow-up is included as Supplementary Figure 1. Sixty percent of patients with clinical information were male and 76% had a congenital SFK. Among the congenital causes (n=715), an antenatal diagnosis was recorded in 76% and multicystic dysplastic kidney (MCDK, n=308) and unilateral kidney agenesis (UKA, n=150) were most common. Detailed clinical information is provided in Table 1.

Table 1 Clinical characteristics of the 944 patients with solitary functioning kidney.

Factor	Congenital (n=715)	Acquired (n=103)	Unknown cause (n=126)
Academic centre	463 (65%)	89 (86%)	79 (63%)
Female sex	254 (36%)	54 (52%)	65 (52%)
Birthweight			
<p20	126 (24%)	21 (23%)	26 (24%)
p20-p40	112 (17%)	13 (14%)	15 (14%)
p40-p60	109 (16%)	12 (13%)	22 (20%)
p60-p80	135 (20%)	18 (20%)	18 (16%)
>p80	161 (24%)	27 (30%)	29 (26%)
Preterm birth	102 (15%)	12 (13%)	11 (9.9%)
Extrarenal congenital anomaly[a]	158 (22%)	10 (10%)	23 (18%)
Genital anomaly (boys)	55 (12%)	1 (1%)	6 (9.8%)
Genital anomaly (girls)	25 (9.8%)	0 (0%)	3 (4.6%)
Congenital heart defect	42 (5.9%)	2 (1.9%)	7 (5.6%)
Anorectal malformation	32 (4.5%)	0 (0%)	2 (1.6%)
Syndrome or association	30 (4.2%)	4 (3.9%)	3 (2.4%)
Other congenital anomaly	65 (9.1%)	4 (3.9%)	7 (5.6%)
Right-sided SFK	367 (51%)	59 (57%)	70 (56%)
Any CAKUT in SFK	284 (46%)	6 (21%)	9 (60%)
Severe CAKUT in SFK[b]	133 (21%)	2 (7.1%)	6 (40%)
UTI in first year of life	189 (28%)	15 (16%)	17 (15%)

Table 1 Clinical characteristics of the 944 patients with solitary functioning kidney. (continued)

Factor	Congenital (n=715)	Acquired (n=103)	Unknown cause (n=126)
BMI at last follow-up			
Normal weight	378 (86%)	77 (83%)	91 (87%)
Overweight/obese	61 (14%)	16 (17%)	14 (13%)
Antenatal diagnosis	542 (76%)	n/a	n/a
Cause of cSFK			
UKA	150 (21%)	n/a	n/a
MCDK	308 (43%)	n/a	n/a
Hypo/dysplasia	68 (10%)	n/a	n/a
Unilateral obstruction	52 (7.3%)	n/a	n/a
PUV	39 (5.5%)	n/a	n/a
VUR	56 (7.8%)	n/a	n/a
Other/unknown	42 (5.9%)	n/a	n/a
Length of SFK (first 90 days)			
<p50	46 (14%)	n/a	n/a
p50-p75	36 (11%)	n/a	n/a
p75-p95	109 (33%)	n/a	n/a
>p95	137 (42%)	n/a	n/a
Length of SFK (first year)			
<p50	48 (12%)	n/a	n/a
p50-p75	37 (9.0%)	n/a	n/a
p75-p95	94 (23%)	n/a	n/a
>p95	234 (57%)	n/a	n/a

n (%) or median (interquartile range). Percentages calculated for subjects with non-missing values.

SFK solitary functioning kidney, CAKUT congenital anomalies of the kidney and urinary tract, BMI body mass index, cSFK congenital solitary functioning kidney, UKA unilateral kidney agenesis, MCDK multicystic dysplastic kidney, PUV posterior urethral valves, VUR vesicoureteral reflux, n/a not applicable. [a]Numbers do not add up to 100% because some patients had more than one extrarenal anomaly. [b]Severe CAKUT was defined as grade 3 or 4 hydronephrosis, grade 3-5 VUR, parenchymal abnormalities or defects and/or dysplasia on any ultrasound, voiding cystourethrography, or nuclear scan.

Patients had been followed for a median duration of 12.8 years. The last age at follow-up was higher for patients with acquired or unknown cause of SFK compared to a congenital cause (median ages 18.0, 17.1, and 11.3 years, respectively). At the end of follow-up, 553 patients (59%) showed one or more indicators of kidney injury and 255 patients (27%) had at least one indicator of severe injury. High blood pressure was most common, with 323 patients (34%) having their last blood pressure measurement above the threshold for any kidney injury and 172 patients (18%) above the threshold for severe

injury. Another 50 patients (5%) had normal blood pressures at last measurement, but used antihypertensive or antiproteinuric medication. An eGFR <90 ml/min/1.73m^2 was present in 290 patients (31%) of whom 26 (3%) had an eGFR <60 ml/min/1.73m^2 and 10 had received a kidney transplant (1%). In 152 (16%) and 15 (2%) patients, the reduced eGFR was the only indicator of any and severe kidney injury, respectively. Any and severe proteinuria were diagnosed in 68 (7%) and 20 (2%) patients, respectively. An overview of all combinations of injury is provided in Supplementary Table S1 and data regarding the missingness of outcomes is provided in Supplementary Table S2.

The proportion of patients without kidney injury decreased steadily with age (Figures 1 and 2). At 18 years of age, 75% of patients with congenital SFK and 80% of patients with acquired SFK had one or more indicators of kidney injury. When restricting to severe injury, these percentages were 39% and 37%, respectively. Similar patterns were observed when including patients with UKA or MCDK only, or patients diagnosed antenatally only (Supplementary Figures 2-3). When censoring patients at the date of last follow-up instead of at the end of the study, estimated proportions of patients with kidney injury were 3-12% larger (Supplementary Figures 4-5).

Analysis of the potential risk factors in patients with congenital SFK revealed that female sex, severe CAKUT in the SFK, and high BMI at last follow-up were associated with an increased risk of any kidney injury (Table 2). For severe kidney injury, both MCDK and hypo/dysplasia decreased the risk compared to patients with UKA, while severe CAKUT in the SFK, SFK length <p50 at one year of age, and especially high BMI were associated with increased risks. We found no indications for time-varying effects of sex and BMI. None of the potential risk factors were associated with kidney injury in the smaller population of patients with acquired SFK (Supplementary Table 3).

In Table 3, risk factors for specific forms of injury (*i.e.* eGFR <90 ml/min/1.73m^2 or hyperfiltration injury (proteinuria, high blood pressure, and/or medication use)) are reported. Female sex, severe CAKUT in the SFK, and smaller SFK length at one year were associated with reduced eGFR, whereas birthweight >p80, MCDK as cause of SFK, and high BMI were associated with hyperfiltration injury only. None of the potential risk factors were clearly associated with reduced eGFR or hyperfiltration injury in the smaller population of patients with acquired SFK (Supplementary Table 4).

The percentage of missing values in our potential risk factors ranged from 0-54% (Supplementary Table 5). To assess the potential impact of selective missingness, sensitivity analyses were performed in which missing values were included in the reference category, assuming that abnormal values would have been recorded instead of left out. These analyses indicated that selective missingness did not influence the conclusions (Supplementary Table 6). Restricting analyses to patients with UKA or MCDK revealed similar results (Supplementary Tables 7-8).

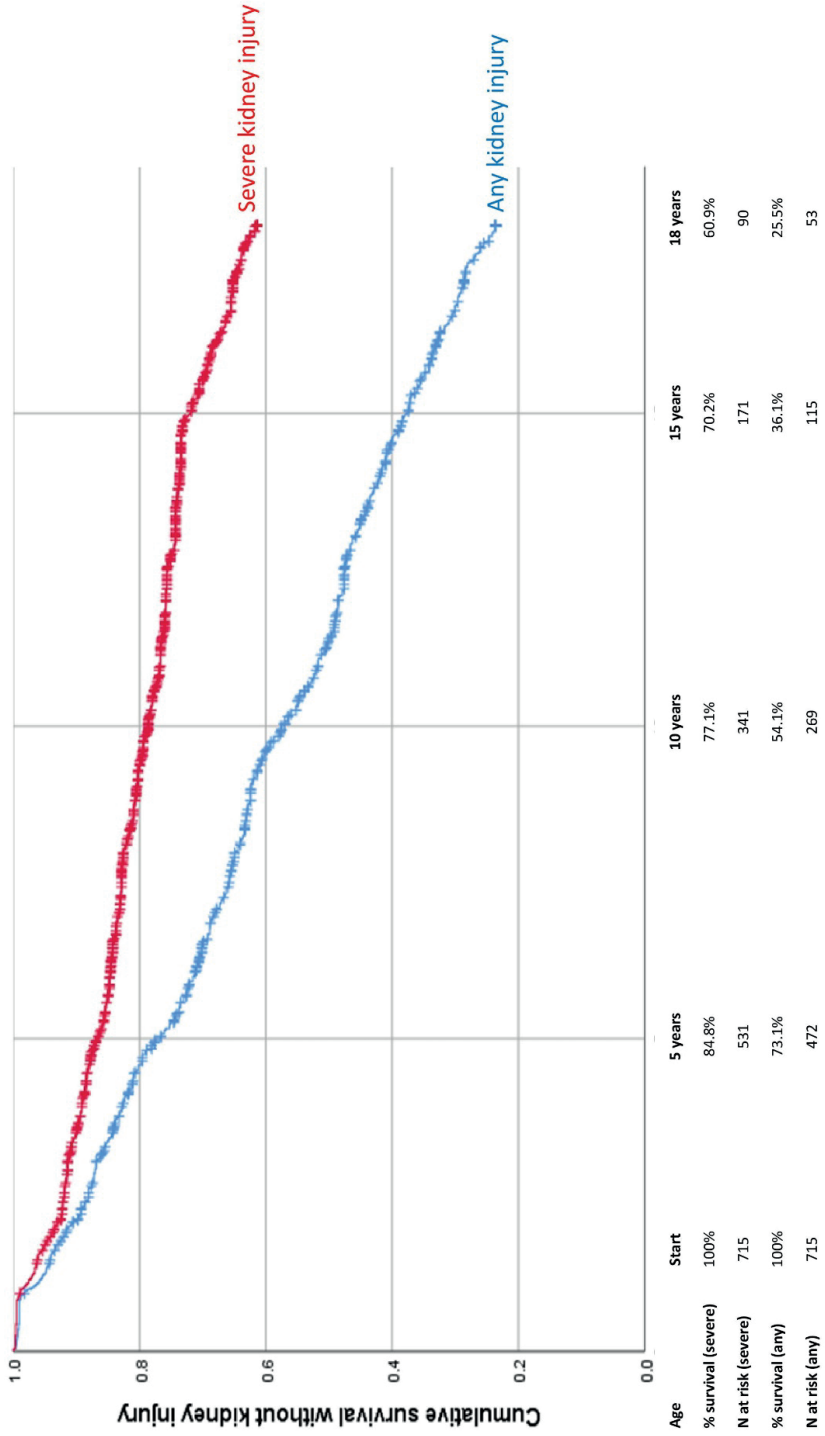
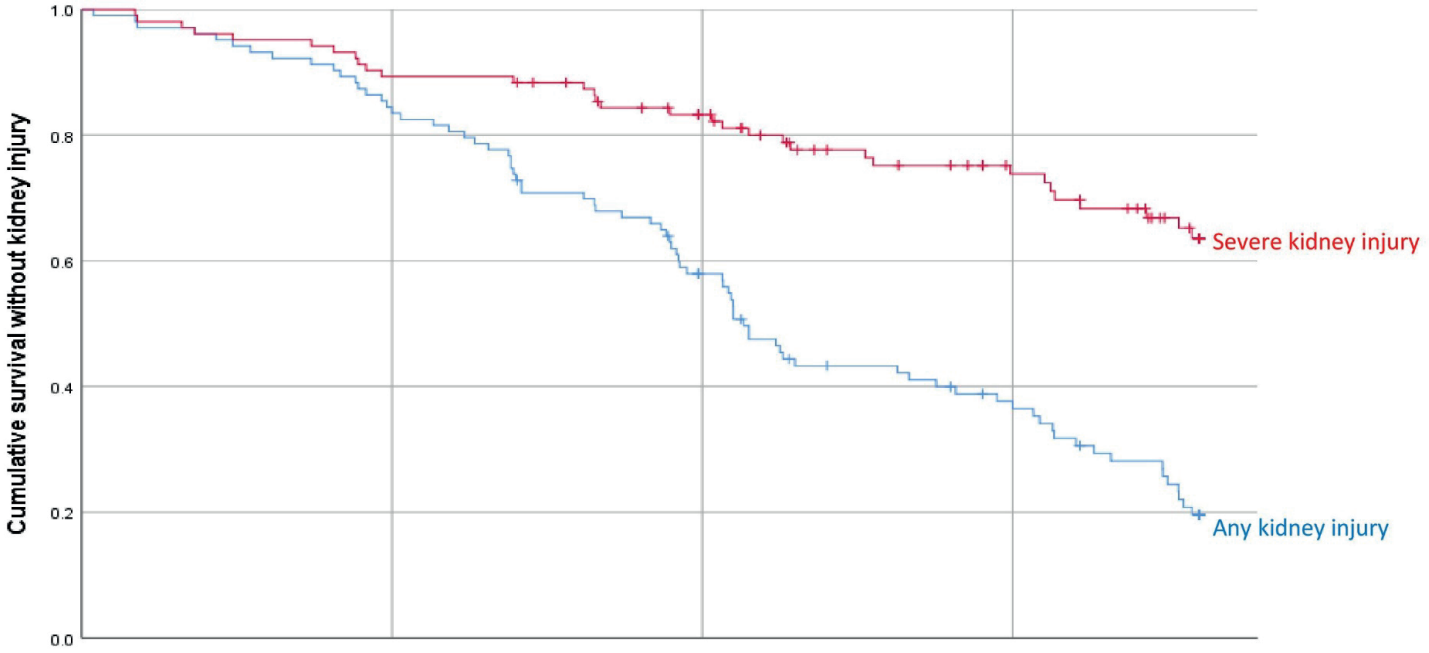


Figure 1 Cumulative survival without any kidney injury (blue line) or without severe kidney injury (red line) among children with congenital solitary functioning kidney.



Age	Start	5 years	10 years	15 years	18 years
% survival (severe)	100%	89.3%	83.3%	73.8%	63.5%
N at risk (severe)	103	92	78	54	38
% survival (any)	100%	83.5%	58.0%	37.5%	19.6%
N at risk (any)	103	86	56	31	16

Figure 2 Cumulative survival without any kidney injury (blue line) or without severe kidney injury (red line) among children with acquired solitary functioning kidney.

Table 2 Crude and adjusted hazard ratios for potential risk factors for any and severe kidney injury in children with congenital solitary functioning kidney.

Factor	No kidney injury (n = 320)	Any kidney injury (n = 395)	cHR	aHR[a]	95% CI[a]	Severe kidney injury (n = 187)	cHR	aHR[a]	95% CI[a]
Academic centre	189 (59%)	274 (69%)	1.0	1.0	0.8-1.3	133 (71%)	1.2	1.2	0.8-1.7
Female sex	103 (32%)	151 (38%)	**1.3**	**1.3**	**1.1-1.7**	62 (33%)	1.1	1.2	0.8-1.6
Birthweight percentile									
<p20	71 (23%)	91 (24%)	1.1	1.1	0.7-1.5	44 (24%)	1.3	1.2	0.7-2.0
p20-p40	51 (17%)	61 (16%)	1.2	1.1	0.8-1.7	30 (17%)	1.2	1.2	0.7-2.1
p40-p60	55 (18%)	54 (14%)	1.0	1.0	ref	23 (13%)	1.0	1.0	ref
p60-p80	63 (21%)	72 (19%)	1.2	1.2	0.8-1.7	37 (20%)	1.3	1.3	0.7-2.2
>p80	64 (21%)	97 (26%)	1.3	1.3	0.9-1.8	48 (26%)	1.6	1.5	0.9-2.6
Prematurity	43 (14%)	59 (16%)	1.0	0.9	0.6-1.2	32 (18%)	1.1	0.9	0.6-1.3
Cause of CSFK									
UKA	62 (19%)	88 (22%)	1.0	1.0	ref	49 (26%)	1.0	1.0	ref
MCDK	149 (47%)	159 (40%)	0.8	0.8	0.6-1.1	67 (36%)	0.7	**0.6**	**0.4-0.9**
Hypo/dysplasia	34 (11%)	34 (8.6%)	0.8	0.7	0.4-1.0	16 (8.6%)	0.7	**0.5**	**0.3-1.0**
Unilateral obstruction	21 (6.6%)	31 (7.8%)	0.9	0.8	0.5-1.2	14 (7.5%)	0.8	0.6	0.3-1.2
PUV	32 (3.8%)	27 (6.8%)	0.9	0.8	0.5-1.4	15 (8.0%)	1.1	0.9	0.4-1.9
VUR	22 (6.9%)	34 (8.6%)	0.9	0.8	0.5-1.2	15 (8.0%)	0.8	0.7	0.3-1.3
Other/unknown	20 (6.3%)	22 (5.6%)	0.7	0.6	0.4-1.1	11 (5.9%)	0.7	0.7	0.3-1.6
Any extrarenal anomaly	59 (18%)	99 (25%)	1.0	0.9	0.7-1.2	41 (22%)	0.9	0.8	0.5-1.2
Right-sided SFK	160 (50%)	207 (52%)	1.0	1.0	0.8-1.2	93 (50%)	1.0	1.0	0.7-1.4

Table 2 Crude and adjusted hazard ratios for potential risk factors for any and severe kidney injury in children with congenital solitary functioning kidney. (continued)

Factor	No kidney injury (n = 320)	Any kidney injury (n = 395)	cHR	aHR[a]	95% CI[a]	Severe kidney injury (n = 187)	cHR	aHR[a]	95% CI[a]
Severe CAKUT in SFK[b]	40 (14%)	93 (27%)	**1.3**	**1.3**	**1.0-1.7**	50 (31%)	**1.6**	**1.5**	**1.0-2.3**
UTI in first year	71 (23%)	118 (32%)	1.1	1.1	0.8-1.4	60 (34%)	1.3	1.0	0.7-1.5
SFK length (90 days)[c]									
<p50	15 (8.5%)	31 (20%)	1.5	1.3	0.6-2.9	14 (23%)	**2.0**	2.0	0.5-8.0
p50-p75	16 (9.1%)	20 (13%)	0.9	0.9	0.6-1.5	6 (9.7%)	0.8	0.9	0.3-2.2
p75-p95	67 (38%)	42 (28%)	0.7	0.8	0.5-1.3	16 (26%)	0.7	0.8	0.4-1.7
>p95	78 (44%)	59 (39%)	1.0	1.0	ref	26 (42%)	1.0	1.0	ref
SFK length (1 year)[c]									
<p50	17 (8.1%)	31 (15%)	1.3	1.4	0.9-2.3	18 (21%)	**1.8**	2.3	1.0-5.3
p50-p75	16 (7.7%)	21 (10%)	1.1	1.1	0.7-1.7	4 (4.6%)	0.6	0.7	0.3-1.9
p75-p95	52 (25%)	42 (21%)	0.9	1.0	0.7-1.5	14 (16%)	0.7	0.9	0.5-1.6
>p95	124 (59%)	110 (54%)	1.0	1.0	ref	51 (59%)	1.0	1.0	ref
High BMI at last follow-up	10 (6.3%)	51 (18%)	**1.8**	**1.6**	**1.0-2.6**	31 (25%)	**2.9**	**2.4**	**1.2-4.8**

cHR crude hazards ratio, aHR adjusted hazards ratio, CI confidence interval, ref reference, CSFK congenital solitary functioning kidney, UKA unilateral kidney agenesis, MCDK multicystic dysplastic kidney, PUV posterior urethral valves, VUR vesicoureteral reflux, SFK solitary functioning kidney, CAKUT congenital anomalies of the kidney and urinary tract, UTI urinary tract infection, BMI body mass index. Bold values indicate associations with a 95% confidence interval not including 1.0. [a] Based on multivariable model including SFK length at 1 year of age, except for hazards ratio of SFK length (90 days). [b] Severe CAKUT was defined as grade 3 or 4 hydronephrosis, grade 3-5 VUR, parenchymal abnormalities or defects and/or dysplasia on any ultrasound, voiding cystourethrography or nuclear scan. [c]The length of the SFK was compared to reference values based on Akhavan *et al*[398]

Table 3 Crude and adjusted hazard ratios for potential risk factors for impaired eGFR (<90 ml/min/1.73m2) and hyperfiltration injury (proteinuria, high blood pressure, or antihypertensive medication use) in children with congenital solitary functioning kidney.

Factor	eGFR ≥90 ml/min/1.73m² (n = 341)	eGFR <90 ml/min/1.73m² (n = 194)	cHR	aHR[a]	95% CI[a]	No hyper-filtration injury (n = 414)	Hyperfiltration injury (n = 286)	cHR	aHR[a]	95% CI[a]
Academic centre	241 (71%)	144 (74%)	1.0	1.0	0.7-1.4	260 (63%)	196 (69%)	0.9	1.0	0.7-1.3
Female sex	104 (31%)	92 (47%)	**2.0**	**2.3**	**1.6-3.1**	152 (37%)	97 (34%)	1.0	1.0	0.7-1.3
Birthweight percentile										
<p20	75 (23%)	52 (28%)	1.3	1.4	0.8-2.3	94 (24%)	63 (23%)	1.1	1.0	0.7-1.6
p20-p40	51 (16%)	32 (17%)	1.3	1.4	0.8-2.5	66 (17%)	44 (16%)	1.2	1.1	0.7-1.7
p40-p60	55 (17%)	28 (15%)	1.0	1.0	ref	70 (18%)	37 (14%)	1.0	1.0	ref
p60-p80	61 (19%)	34 (19%)	1.1	1.2	0.7-2.1	80 (20%)	54 (20%)	1.2	1.2	0.8-1.9
>p80	81 (25%)	38 (21%)	1.0	1.2	0.7-2.0	82 (21%)	77 (28%)	1.5	1.5	1.0-2.2
Prematurity	51 (16%)	33 (18%)	1.0	0.9	0.6-1.3	60 (15%)	40 (14%)	0.8	0.8	0.6-1.2
Cause of CSFK										
UKA	75 (22%)	34 (18%)	1.0	1.0	ref	76 (18%)	70 (25%)	1.0	1.0	ref
MCDK	148 (43%)	72 (37%)	1.1	1.2	0.8-1.9	191 (46%)	110 (39%)	**0.7**	**0.6**	**0.5-0.9**
Hypo/dysplasia	40 (12%)	14 (7.2%)	0.9	0.8	0.4-1.5	39 (9.4%)	28 (9.8%)	0.8	0.7	0.4-1.2
Unilateral obstruction	21 (6.2%)	16 (8.2%)	1.4	1.2	0.6-2.3	30 (7.2%)	22 (7.7%)	0.7	0.6	0.4-1.0
PUV	18 (5.3%)	20 (10%)	1.5	1.6	0.8-3.4	20 (4.8%)	19 (6.6%)	0.7	0.7	0.4-1.4
VUR	22 (6.5%)	24 (12%)	1.5	1.4	0.8-2.7	32 (7.7%)	23 (8.0%)	0.7	0.7	0.4-1.1
Other/unknown	17 (5.0%)	14 (7.2%)	1.5	1.2	0.6-2.4	26 (6.3%)	14 (4.9%)	**0.5**	0.5	0.3-1.0
Any extrarenal anomaly	81 (24%)	56 (29%)	1.0	1.0	0.7-1.5	86 (21%)	67 (23%)	0.9	0.8	0.6-1.1

Table 3 Crude and adjusted hazard ratios for potential risk factors for impaired eGFR (<90 ml/min/1.73m2) and hyperfiltration injury (proteinuria, high blood pressure, or antihypertensive medication use) in children with congenital solitary functioning kidney. (continued)

Factor	eGFR ≥90 ml/min/1.73m² (n = 341)	eGFR <90 ml/min/1.73m² (n = 194)	cHR	aHR[a]	95% CI[a]	No hyper-filtration injury (n = 414)	Hyperfiltration injury (n = 286)	cHR	aHR[a]	95% CI[a]
Right-sided SFK	164 (48%)	109 (56%)	1.2	1.1	0.8-1.4	220 (53%)	142 (50%)	0.9	0.8	0.7-1.1
Severe CAKUT in SFK[b]	55 (19%)	62 (38%)	**1.8**	**1.6**	**1.1-2.3**	66 (18%)	65 (26%)	1.1	1.1	0.8-1.6
UTI in first year	89 (28%)	78 (43%)	**1.5**	1.3	0.9-1.9	108 (28%)	78 (29%)	0.9	0.9	0.6-1.2
SFK length (90 days)[c]										
<p50	20 (13%)	18 (32%)	**3.8**	2.0	0.8-5.1	25 (12%)	20 (17%)	0.9	1.1	0.4-2.9
p50-p75	15 (9.4%)	11 (19%)	**2.6**	1.7	0.7-4.0	22 (11%)	14 (12%)	0.6	0.7	0.4-1.4
p75-p95	59 (37%)	17 (30%)	1.6	1.3	0.6-3.1	76 (37%)	31 (27%)	0.6	0.7	0.4-1.1
>p95	66 (41%)	11 (19%)	1.0	1.0	ref	85 (41%)	50 (44%)	1.0	1.0	ref
SFK length (1 year)[c]										
<p50	19 (9.3%)	18 (22%)	**2.9**	**2.7**	**1.3-5.6**	24 (9.5%)	24 (16%)	1.1	1.3	0.7-2.3
p50-p75	16 (7.8%)	13 (16%)	**2.6**	**2.5**	**1.2-5.5**	24 (9.5%)	13 (8.4%)	0.7	0.7	0.3-1.4
p75-p95	43 (21%)	22 (27%)	**2.0**	**2.1**	**1.1-4.0**	65 (26%)	27 (17%)	0.7	0.8	0.5-1.2
>p95	126 (62%)	29 (35%)	1.0	1.0	ref	140 (55%)	91 (59%)	1.0	1.0	ref
High BMI at last follow-up	27 (12%)	29 (17%)	1.4	1.2	0.8-2.0	26 (10%)	35 (19%)	**1.5**	**1.6**	**1.0-2.7**

eGFR estimated glomerular filtration rate, cHR crude hazards ratio, aHR adjusted hazards ratio, CI confidence interval, ref reference, CSFK congenital solitary functioning kidney, UKA unilateral kidney agenesis, MCDK multicystic dysplastic kidney, PUV posterior urethral valves, VUR vesicoureteral reflux, SFK solitary functioning kidney, CAKUT congenital anomalies of the kidney and urinary tract, UTI urinary tract infection, BMI body mass index. Bold values indicate associations with a 95% confidence interval not including 1.0. [a]Based on multivariable model including SFK length at 1 year of age, except for hazards ratio of SFK length (90 days). [b]Severe CAKUT was defined as grade 3 or 4 hydronephrosis, grade 3-5 VUR, parenchymal abnormalities or defects and/or dysplasia on any ultrasound, voiding cystourethrography or nuclear scan. [c]The length of the SFK was compared to reference values based on Akhavan *et al*[398]

DISCUSSION

In this cohort of 944 patients with SFK, we found that indicators of severe kidney injury were present in 39% of patients with congenital SFK and 37% of patients with acquired SFK at the age of 18 years. Any indicators of injury were present in 75% and 80% of patients, respectively. These findings clearly indicate that SFK is a condition that warrants long-term follow-up.[324] Kidney agenesis, CAKUT in the SFK, and high BMI were associated with increased hazard ratios for kidney injury in children with congenital SFK. We observed that CAKUT in the SFK and smaller SFK length showed strong associations with eGFR <90 ml/min/1.73m^2, whereas UKA and high BMI were associated with hyperfiltration injury.

In 2009, Sanna-Cherchi *et al.* were the first to report severe kidney injury in patients with SFK, with up to 30% of SFK patients showing kidney failure by the age of 30 years.[75] Others confirmed the possibility of severe outcomes and reported milder forms of kidney injury in 6-60% of patients.[17,78-80,82,83,181,210,231,270,276] These results were highly dependent on inclusion criteria, follow-up duration, and definition of kidney injury. The heterogeneity in these studies and results led to ongoing discussions on the implications of SFK.[295,400] Here, we confirm that having an SFK from childhood results in kidney injury in the large majority of patients.

The most common outcome in our cohort was high blood pressure. The prevalence of severe high blood pressure (18%) was similar to that observed previously. In the KIMONO study, 26% of children with SFK had hypertension, diagnosed at a mean age of 5 years.[82] Alfandary *et al.* found that high blood pressure was more common in 17-year old army recruits with SFK as compared to controls (32% vs 23%), while Xu *et al* found hypertension in 32% of adult patients with UKA at a median age of 32 years.[76,83] In contrast, Marzuillo *et al.* identified only two patients with hypertension in their well-characterized cohort of 322 children with antenatally diagnosed SFK, who were followed for a median duration of 7 years.[80] Similar definitions of hypertension were used and none of these studies, including our own, used ambulatory blood pressure measurements systematically. Therefore, the low proportion of patients with hypertension in the Italian cohort is most likely caused by differences in study population and follow-up duration. Similar patterns were visible in the rates of proteinuria and reduced eGFR, which were also higher in our study than in the Italian cohort, but lower than in the KIMONO study and the Israeli cohort.[76,80,82]

Others previously identified low birth weight, preterm birth, kidney agenesis, CAKUT in the SFK, recurrent UTI, smaller SFK size, and elevated BMI as risk factors for kidney injury.[75,76,78-83,181,210,231] In this study, we confirmed most associations, but not those with UTI or preterm birth. Both low and high birth weight may increase the risk of kidney injury in SFK patients, but this should be interpreted with caution since effect sizes are

relatively small and CIs include one. We identified an almost two-fold lower risk of severe kidney injury in patients with MCDK or hypo/dysplasia compared to UKA (adjusted HRs 0.6 and 0.5, respectively), which is in line with earlier findings by Matsell *et al*.[81] Since our multivariable model corrects for CAKUT in the SFK and extrarenal anomalies, these factors cannot explain the higher risk for patients with UKA. More likely, etiological differences play a role; agenesis is thought to result from a failed interaction between the ureteric bud and metanephric mesenchyme in early kidney development, whereas MCDK may arise later in development as a consequence of obstruction or abnormal branching of the ureteric bud.[9,11,401] As such, the SFK remaining after UKA is more likely to have developed suboptimally as well, resulting in a higher risk of injury. It is unclear why hypo/dysplasia also results in a lower risk; timing during nephrogenesis may play a role, although this warrants further investigation.

Whereas CAKUT in the SFK and smaller SFK size increased the HRs for reduced eGFR, UKA and elevated BMI increased the risk of hyperfiltration injury. We hypothesize that CAKUT in the SFK and smaller SFK size reflect a lower nephron number, leading to lower GFR from birth onwards. Being overweight might immediately increase glomerular filtration pressure, leading to proteinuria and hypertension, but may reduce the GFR after prolonged exposure only. Animal models proved that SFKs are capable of nephron hyperplasia prenatally, with nephron numbers ~70% of those in individuals born with two kidneys.[130] Therefore, increased SFK length soon after birth is considered to reflect increased nephron number, whereas absence of compensatory enlargement indicates failed compensatory mechanisms. This does not explain, however, why the HRs for SFK size after one year are larger compared to those for SFK size within 90 days. Perhaps children with an SFK failing to show compensatory enlargement between 90 days and one year of age more often have a structurally abnormal SFK than children whose SFK size increases to >p95 in this period. The association between reduced eGFR and female sex was not observed when using the revised Schwartz formula for eGFR (aHRs 2.3 and 1.1 for CKiD U25 and revised Schwartz formulas, respectively). This indicates that the increased HR was likely caused by underestimation of eGFR in girls 5-14 years old in the CKiD U25 equation.[396] To our knowledge, our study is the first that indicates differences in risk factors for a reduction in GFR and signs of hyperfiltration injury. Although we grouped hypertension and proteinuria as hyperfiltration injury, we cannot exclude other causes of these outcomes and some misclassification will have occurred. Nonetheless, our findings illustrate that different pathophysiological mechanisms may play a role and that presence of different combinations of risks factors could lead to different kidney injury trajectories.

As we performed a retrospective study, several limitations should be considered. First, this design could have led to information bias, because patients without kidney injury may have been discharged from follow-up earlier. To take this into account, we assumed that all patients received follow-up until study closure. Although this may

have resulted in an underestimation of the prevalence of kidney injury, using the actual date of last follow-up likely results in an overestimation of the risk of kidney injury. Furthermore, the multicentre study may have resulted in variation among centres in the execution of measurements, especially for blood pressure and creatinine. Data regarding the indication for antihypertensive or antiproteinuric medication was not available, but excluding patients with congenital heart disease or patients with medication use as only outcome did not change results substantially (Supplementary Table S9). Missing information on risk factors, such as preterm birth, low birth weight, and occurrence of UTI was a potential limitation, but our study benefitted from the parental questionnaires that were used to complete this information for most patients (~95%). We also performed multiple imputation for missing information to keep all patients in the multivariable analyses and repeated our analyses in a database in which all missing values were included in the reference category. Reassuringly, our results proved robust even in this scenario.

A major strength of our study is its large cohort size, which is more than twice the size of the largest study on SFK reported so far.[82] This facilitated several secondary analyses and allowed more robust estimation of the long-term prevalence of kidney injury. Lastly, our broad inclusion criteria and multicentre design, with 7 academic and 29 non-academic centres participating, prevented inclusion of severely affected patients only and improved generalizability, which was a substantial problem in previous studies.

Our results may have several implications for clinical practice. The high number of patients with kidney injury indicates that all children with an SFK require long-term follow-up.[324] If not already present, kidney injury may very well become manifest in early adulthood, warranting adequate transition and continued follow-up of which patients, parents, and healthcare providers should be aware. Follow-up of SFK patients without severe injury may best be delivered by primary care physicians and should focus on early identification of proteinuria, hypertension, or a reduced eGFR. If kidney injury is discovered, local referral practices should ensure that patients are seen by the most appropriate medical specialist.

The identification of high BMI as factor that is strongly associated with kidney injury highlights the importance of lifestyle management in patients with SFK and can be implemented in clinical practice directly. Improvements in lifestyle, such as following a healthy diet and adhering to physical activity guidelines, have been shown effective for the prevention and treatment of hypertension in children.[213] Furthermore, extensive guidelines on prevention and treatment of childhood overweight and obesity are available, such as the staged treatment approach recommended by the AAP Expert Committee on Obesity.[402,403] Lastly, further research into the underlying mechanisms of

kidney injury in children with SFK may help to understand the pathophysiology, develop preventative strategies, and ultimately reduce the rates of kidney injury.

In conclusion, this study shows that a large proportion of children with SFK will develop kidney injury, for which we identified distinct risk factors that can be used in clinical management. Patients with SFK should be followed into adulthood and special attention should be given to overweight as preventable risk factor. Further studies should validate our findings regarding different risk factor patterns for different outcomes and help translation of our findings into individualized care strategies.

SUPPLEMENTARY MATERIAL

Supplementary Methods

Outcome classification

Proteinuria was defined using the urine protein-creatinine ratio (uPCR) or albumin-creatinine ratio (uACR) in first morning or random spot urine samples. When both were available, uPCR was used, since this was the predominant method during the study period. Office and ambulatory blood pressure measurements (ABPM) were used, with a preference for 24h mean ABPM results when both were available (n=10). When multiple BPs were available, the lowest value was used for the study to prevent over classification of high BP. Patients who had a kidney transplantation in the past were categorized as having a severely reduced eGFR, regardless of their current creatinine levels. Since data were gathered from medical files, we rely on the individual physicians' adherence to guidelines for measurement of all outcomes.

Risk factor classification

Birth weight and gestational age were retrieved from parental questionnaires when unavailable in health records. Birth weight percentiles were calculated using Dutch reference values.[404] The cause of SFK was classified as acquired when a clear non-congenital cause, such as a Wilms tumour, was present. Congenital causes were further subclassified into unilateral kidney agenesis (UKA), multicystic dysplastic kidney (MCDK), kidney hypo/dysplasia, unilateral obstruction (*e.g.* ureteropelvic junction obstruction), vesicoureteral reflux (VUR), posterior urethral valves, and other/unknown congenital causes. Descriptions of ultrasounds, voiding cystourethrography's, and nuclear scans were used to determine the presence of CAKUT in the SFK. Severe CAKUT was defined as grade 3-4 hydronephrosis, grade 3-5 VUR, parenchymal abnormalities or defects, and/or kidney dysplasia. Kidney dysplasia was considered present when reduced corticomedullar differentiation, parenchymal abnormalities or defects, persistent foetal lobulation, small kidney size and/or other evidence of structural abnormalities were reported by the radiologist involved. The occurrence of UTIs during the first year of life was assessed using health records and parental questionnaires.

Reference values for length of the SFK were calculated based on the reference values from Akhavan *et al*.[398] The reported values were smoothed using an exponential function (linear regression with age, age^2 and age ^3) in SPSS to calculate unstandardized predicted values, which led to plausible expected value for all ages and percentiles (Figure S6). Health records were searched for ultrasounds with measurements of the length of the SFK. We included the measurement of SFK length from the last ultrasound within 90 days or 1 year after birth if no dilation of the SFK was observed. If the last ultrasound within the respective timeframe could not be used because of dilation, previous ultrasounds were assessed in search for length measurements in absence of

dilation. Patients were assigned to a predefined category of SFK length (<P5, P5-P25, P25-P50, P50-P75, P75-P95, or >P95) based on the age at measurement and SFK length (Table S10). At a later stage, categories were combined to create larger numbers for the analyses.

Multiple imputation

To account for missing values, the variables birthweight, gestational age, BMI at last follow-up, urinary tract infection, and CAKUT of the SFK were imputed using the information of all variables in the multivariable analyses plus BMI at 1 year, GFR at 1 year, date of birth, date of last follow-up, and presence of any injury at last follow-up. For the analyses on length of the SFK, only patients without CAKUT of the SFK were taken into account. A separate database with only these patients was created and missing values were imputed using the information of the same variables mentioned previously, except for the variable CAKUT of the SFK. All imputation procedures were performed using multiple imputation with 10 imputation datasets in SPSS version 25.0.

Supplementary Tables

Supplementary Table 1 Combinations of signs of any and severe kidney injury in 944 patients with solitary functioning kidney

Type of injury	Any injury (n=553)	Severe injury (n=255)
Proteinuria only	21 (2.2%)	4 (0.4%)
High blood pressure only	195 (21%)	143 (15%)
Reduced eGFR only	153 (16%)	15 (1.6%)
Medication use only	18 (1.9%)	49 (5.2%)
Proteinuria + high blood pressure	10 (1.1%)	2 (0.2%)
Proteinuria + reduced eGFR	7 (0.7%)	1 (0.1%)
Proteinuria + medication use	5 (0.5%)	4 (0.4%)
High blood pressure + reduced eGFR	77 (8.2%)	4 (0.4%)
High blood pressure + medication use	13 (1.4%)	17 (1.8%)
Reduced eGFR + medication use	15 (1.6%)	4 (0.4%)
Proteinuria + high blood pressure + reduced eGFR	5 (0.5%)	1 (0.1%)
Proteinuria + high blood pressure + medication use	1 (0.1%)	-
Proteinuria + reduced eGFR + medication use	11 (1.2%)	6 (0.6%)
High blood pressure + reduced eGFR + medication use	14 (1.5%)	3 (0.3%)
Proteinuria + high blood pressure + reduced eGFR + medication use	8 (0.8%)	2 (0.2%)

Values are depicted as number (%). – none.

Supplementary Table 2 Number of missing values for signs of kidney injury, stratified by cause of the solitary functioning kidney

Sign of kidney injury	Congenital (n=715)	Acquired (n=103)
Proteinuria	72 (10%)	9 (9%)
High blood pressure	62 (9%)	1 (1%)
eGFR	180 (25%)	9 (9%)
Medication use	11 (2%)	0 (0%)

Values are depicted as number (%). SFK solitary functioning kidney, eGFR estimated glomerular filtration rate.

Supplementary Table 3 Crude and adjusted hazard ratios for potential risk factors for any and severe kidney injury in children with acquired solitary functioning kidney

Factor	No kidney injury (n = 26)	Any kidney injury (n = 77)	cHR	aHR	95% CI	Severe kidney injury (n = 32)	cHR	aHR	95% CI
Academic centre	18 (90%)	71 (86%)	0.7	0.8	0.4-1.5	33 (92%)	0.9	0.9	0.2-3.2
Female sex	9 (45%)	45 (54%)	1.2	1.2	0.8-2.0	20 (56%)	1.5	1.7	0.8-3.7
Birthweight percentile									
<p20	5 (21%)	16 (24%)	1.3	1.2	0.5-2.9	4 (16%)	1.3	1.2	0.3-5.9
p20-p40	5 (21%)	8 (12%)	1.2	1.2	0.4-3.1	4 (16%)	1.6	1.8	0.4-7.8
p40-p60	5 (21%)	7 (10%)	1.0	1.0	ref	3 (12%)	1.0	1.0	ref
p60-p80	2 (8.3%)	16 (24%)	1.7	1.5	0.6-3.7	8 (32%)	3.3	2.0	0.5-7.9
>p80	7 (29%)	20 (30%)	1.8	1.8	0.8-4.4	6 (24%)	1.8	1.5	0.4-5.9
Prematurity	4 (17%)	8 (11%)	0.8	0.9	0.4-2.1	2 (7.4%)	0.6	0.8	0.2-3.7
Any extrarenal anomaly	2 (10%)	8 (9.6%)	0.7	0.6	0.2-1.5	4 (11%)	0.6	0.6	0.1-3.3
Right-sided SFK	11 (55%)	48 (58%)	1.3	1.3	0.8-2.0	21 (58%)	1.0	1.0	0.5-2.1
UTI in first year	2 (12%)	13 (17%)	1.2	1.2	0.6-2.3	3 (9.1%)	0.7	0.7	0.1-3.3
High BMI at last follow-up	1 (5.9%)	15 (20%)	1.1	1.2	0.6-2.4	10 (31%)	1.5	1.9	0.6-5.4

cHR crude hazards ratio, aHR adjusted hazards ratio, CI confidence interval, ref reference, SFK solitary functioning kidney, UTI urinary tract infection, BMI body mass index.

Supplementary Table 4 Crude and adjusted hazard ratios for potential risk factors for impaired eGFR (<90 ml/min/1.73m²) and hyperfiltration injury (proteinuria, high blood pressure, or antihypertensive medication use) in children with acquired solitary functioning kidney

Factor	eGFR ≥90 ml/min/1.73m² (n = 47)	eGFR <90 ml/min/1.73m² (n = 47)	cHR	aHR	95% CI	No hyperfiltration injury (n = 49)	Hyperfiltration injury (n = 53)	cHR	aHR	95% CI
Academic centre	43 (92%)	38 (81%)	**0.5**	0.5	0.2-1.3	39 (80%)	49 (93%)	1.9	1.8	0.6-5.2
Female sex	19 (40%)	29 (62%)	1.6	1.7	0.9-3.3	24 (49%)	30 (57%)	1.2	1.3	0.7-2.3
Birthweight percentile										
<p20	10 (24%)	8 (20%)	0.8	0.6	0.2-2.0	9 (20%)	11 (24%)	1.2	1.3	0.4-3.8
p20-p40	6 (15%)	5 (12%)	1.1	1.0	0.3-3.4	7 (16%)	6 (13%)	1.2	1.3	0.4-4.1
p40-p60	6 (15%)	6 (15%)	1.0	1.0	ref	7 (16%)	5 (11%)	1.0	1.0	ref
p60-p80	8 (20%)	8 (20%)	1.0	0.9	0.3-2.5	7 (16%)	11 (24%)	1.6	1.3	0.4-3.9
>p80	11 (27%)	14 (34%)	1.7	1.7	0.6-4.6	15 (33%)	12 (27%)	1.2	1.3	0.4-3.8
Prematurity	5 (12%)	5 (12%)	1.0	1.1	0.3-3.5	7 (15%)	4 (8.3%)	0.6	0.6	0.2-2.0
Any extrarenal anomaly	7 (15%)	3 (6.4%)	0.4	0.3	0.1-1.0	5 (10%)	5 (9.4%)	0.8	0.9	0.3-2.4
Right-sided SFK	25 (53%)	29 (62%)	1.3	1.5	0.8-2.9	28 (57%)	31 (59%)	1.1	1.2	0.6-2.1
UTI in first year	6 (13%)	8 (20%)	1.6	2.1	0.9-5.1	7 (16%)	8 (17%)	1.1	0.8	0.4-1.8
High BMI at last follow-up	7 (17%)	8 (17%)	0.9	0.9	0.3-2.6	4 (8.7%)	12 (26%)	1.7	1.8	0.8-3.8

eGFR estimated glomerular filtration rate, cHR crude hazards ratio, aHR adjusted hazards ratio, CI confidence interval, ref reference, SFK solitary functioning kidney, UTI urinary tract infection, BMI body mass index.

Supplementary Table 5 Number of missing values for potential risk factors for kidney injury, stratified by cause of the solitary functioning kidney

Factor	Congenital (n=715)	Acquired (n=103)
Centre of inclusion	0 (0%)	0 (0%)
Sex	0 (0%)	0 (0%)
Birthweight	31 (4%)	12 (12%)
Gestational age	28 (4%)	8 (8%)
Cause of SFK	0 (0%)	0 (0%)
Any extrarenal anomaly	0 (0%)	0 (0%)
Side of SFK	0 (0%)	0 (0%)
CAKUT in SFK	91 (13%)	n/a[a]
UTI in first year	41 (6%)	11 (11%)
SFK length 90 days	387 (54%)	n/a[a]
SFK length 1 year	302 (42%)	n/a[a]
BMI at last follow-up	276 (39%)	10 (10%)

[a]not included in the analyses for children with acquired solitary functioning kidney.

Supplementary Table 6 Adjusted hazard ratios for potential risk factors for all outcomes in children with congenital solitary functioning kidney, with missing values classified in the reference category

Factor	Any kidney injury (n = 395)		Severe kidney injury (n = 187)		eGFR <90 ml/min/1.73m² (n = 194)		Hyperfiltration injury (n = 286)	
	aHR[a]	95% CI[a]	aHR[a]	95% CI[a]	aHR[a]	95% CI[a]	aHR[a]	95% CI[a]
Academic centre	1.0	0.8-1.2	1.2	0.8-1.6	1.0	0.7-1.4	1.0	0.7-1.2
Female sex	**1.4**	**1.1-1.7**	1.2	0.9-1.7	**2.3**	**1.6-3.2**	1.0	0.8-1.3
Birthweight percentile								
<p20	1.1	0.8-1.5	1.2	0.8-2.1	1.4	0.9-2.2	1.1	0.8-1.7
p20-p40	1.2	0.8-1.7	1.3	0.8-2.3	1.5	0.9-2.5	1.2	0.8-1.8
p40-p60	1.0	ref	1.0	ref	1.0	ref	1.0	ref
p60-p80	1.2	0.8-1.6	1.3	0.8-2.2	1.2	0.7-1.9	1.3	0.8-1.9
>p80	1.3	1.0-1.8	**1.7**	**1.1-2.8**	1.2	0.7-1.9	**1.5**	**1.1-2.2**

Supplementary Table 6 Adjusted hazard ratios for potential risk factors for all outcomes in children with congenital solitary functioning kidney, with missing values classified in the reference category (continued)

Factor	Any kidney injury (n = 395)		Severe kidney injury (n = 187)		eGFR <90 ml/min/1.73m^2 (n = 194)		Hyperfiltration injury (n = 286)	
	aHR[a]	95% CI[a]	aHR[a]	95% CI[a]	aHR[a]	95% CI[a]	aHR[a]	95% CI[a]
Prematurity	0.8	0.6-1.1	0.9	0.6-1.3	0.8	0.6-1.3	0.8	0.6-1.1
Cause of CSFK								
UKA	1.0	ref	1.0	ref	1.0	ref	1.0	ref
MCDK	0.8	0.6-1.1	**0.6**	**0.4-1.0**	1.2	0.8-1.9	**0.6**	**0.5-0.9**
Hypo/dysplasia	0.7	0.5-1.0	**0.6**	**0.3-1.0**	0.8	0.4-1.5	0.7	0.5-1.2
Unilateral obstruction	0.8	0.5-1.2	0.7	0.3-1.3	1.1	0.6-2.2	0.6	0.4-1.1
PUV	0.8	0.5-1.3	0.9	0.5-1.8	1.6	0.8-3.1	0.7	0.4-1.3
VUR	0.8	0.5-1.2	0.7	0.4-1.3	1.4	0.8-2.6	0.7	0.4-1.1
Other/unknown	0.7	0.4-1.2	0.9	0.4-1.8	1.3	0.7-2.7	0.6	0.3-1.0
Any extrarenal anomaly	1.0	0.7-1.2	0.8	0.5-1.1	1.0	0.7-1.5	0.8	0.6-1.1
Right-sided SFK	1.0	0.8-1.2	1.0	0.8-1.4	1.0	0.8-1.4	0.9	0.7-1.1
Severe CAKUT in SFK[b]	**1.5**	**1.1-1.9**	**1.6**	**1.1-2.3**	**1.7**	**1.2-2.4**	1.2	0.9-1.7
UTI in first year	1.1	0.8-1.4	1.0	0.7-1.5	1.3	0.9-1.8	0.9	0.6-1.2
SFK length (90 days)[c]								
<p50	1.3	0.8-2.0	**2.1**	**1.1-4.3**	1.4	0.8-2.6	1.1	0.6-1.9
p50-p75	0.8	0.5-1.3	0.4	0.1-1.2	1.3	0.7-2.6	0.7	0.4-1.4
p75-p95	0.7	0.5-1.0	0.6	0.3-1.1	0.6	0.3-1.0	0.8	0.5-1.2
>p95	1.0	ref	1.0	ref	1.0	ref	1.0	ref
SFK length (1 year)[c]								
<p50	1.4	0.9-2.2	2.3	1.2-4.5	1.7	0.9-3.2	1.4	0.8-2.3
p50-p75	1.1	0.7-1.7	0.4	0.1-1.3	1.8	0.9-3.6	0.8	0.4-1.4
p75-p95	1.0	0.7-1.5	0.9	0.5-1.6	1.3	0.8-2.3	0.9	0.6-1.4
>p95	1.0	ref	1.0	ref	1.0	ref	1.0	ref
High BMI at last follow-up	**1.9**	**1.4-2.6**	**3.2**	**2.1-4.8**	**1.7**	**1.1-2.6**	**1.5**	**1.0-2.1**

eGFR estimated glomerular filtration rate, aHR adjusted hazards ratio, CI confidence interval, ref reference, CSFK congenital solitary functioning kidney, UKA unilateral kidney agenesis, MCDK multicystic dysplastic kidney, PUV posterior urethral valves, VUR vesicoureteral reflux, SFK solitary functioning kidney, CAKUT congenital anomalies of the kidney and urinary tract, UTI urinary tract infection, BMI body mass index. Bold values indicate associations with a 95% confidence interval not including 1.0. [a]Based on multivariable model including SFK length at 1 year of age, except for hazards ratio of SFK length (90 days). [b]Severe CAKUT was defined as grade 3 or 4 hydronephrosis, grade 3-5 VUR, parenchymal abnormalities or defects and/or dysplasia on any ultrasound, voiding cystourethrography or nuclear scan. [c]The length of the SFK was compared to reference values based on Akhavan *et al*[398].

Supplementary Table 7 Crude and adjusted hazard ratios for potential risk factors for any and severe kidney injury in children with unilateral kidney agenesis or multicystic kidney dysplasia

Factor	No kidney injury (n = 211)	Any kidney injury (n = 247)	cHR	aHR[a]	95% CI[a]	Severe kidney injury (n = 116)	cHR	aHR[a]	95% CI[a]
Academic centre	124 (59%)	158 (64%)	0.9	0.9	0.7-1.3	81 (70%)	1.2	1.2	0.8-2.0
Female sex	69 (33%)	97 (39%)	**1.3**	1.3	1.0-1.6	39 (34%)	1.1	1.1	0.7-1.6
Birthweight percentile									
<p20	48 (24%)	66 (28%)	1.2	1.1	0.7-1.7	34 (30%)	1.7	1.3	0.6-2.7
p20-p40	35 (18%)	40 (17%)	1.2	1.1	0.6-1.7	17 (15%)	1.3	1.1	0.5-2.5
p40-p60	36 (18%)	30 (13%)	1.0	1.0	ref	11 (9.6%)	1.0	1.0	ref
p60-p80	39 (20%)	41 (17%)	1.2	1.2	0.7-1.9	22 (19%)	1.6	1.6	0.7-3.4
>p80	41 (21%)	59 (25%)	1.4	1.4	0.9-2.2	30 (26%)	1.9	2.0	1.0-4.0
Prematurity	23 (11%)	42 (18%)	1.2	1.1	0.8-1.5	20 (17%)	1.4	1.2	0.6-2.1
Cause of CSFK									
UKA	62 (29%)	88 (36%)	1.0	1.0	ref	49 (42%)	1.0	1.0	ref
MCDK	149 (71%)	159 (64%)	0.8	0.8	0.6-1.1	67 (58%)	**0.7**	**0.6**	**0.4-1.0**
Any extrarenal anomaly	41 (19%)	74 (30%)	1.1	1.0	0.7-1.4	33 (28%)	1.1	0.8	0.5-1.4
Right-sided SFK	105 (50%)	130 (53%)	1.1	1.0	0.8-1.3	58 (50%)	1.0	1.0	0.7-1.5
Severe CAKUT in SFK[b]	22 (12%)	39 (18%)	1.0	1.0	0.7-1.5	22 (22%)	1.3	1.2	0.7-2.0
UTI in first year	29 (14%)	47 (20%)	1.1	1.1	0.8-1.5	22 (20%)	1.1	0.9	0.6-1.6

Supplementary Table 7 Crude and adjusted hazard ratios for potential risk factors for any and severe kidney injury in children with unilateral kidney agenesis or multicystic kidney dysplasia (continued)

Factor	No kidney injury (n = 211)	Any kidney injury (n = 247)	cHR	aHR[a]	95% CI[a]	Severe kidney injury (n = 116)	cHR	aHR[a]	95% CI[a]
SFK length (90 days)[c]									
<p50	11 (7.9%)	21 (19%)	1.6	1.4	0.6-3.0	11 (24%)	**2.5**	2.3	0.5-10.1
p50-p75	10 (7.2%)	13 (12%)	0.9	0.9	0.5-1.6	4 (8.7%)	1.0	0.8	0.2-2.9
p75-p95	53 (38%)	29 (27%)	0.7	0.8	0.5-1.4	14 (30%)	0.9	1.0	0.4-2.3
>p95	65 (47%)	46 (42%)	1.0	1.0	ref	17 (37%)	1.0	1.0	ref
SFK length (1 year)[c]									
<p50	13 (8.2%)	22 (15%)	1.4	1.3	0.8-2.1	13 (20%)	1.9	1.9	0.8-4.7
p50-p75	7 (4.4%)	16 (11%)	1.5	1.2	0.7-2.0	3 (4.5%)	0.9	0.8	0.3-2.5
p75-p95	39 (25%)	27 (19%)	0.9	0.9	0.6-1.4	12 (18%)	0.8	0.9	0.4-1.7
>p95	99 (63%)	81 (56%)	1.0	1.0	ref	38 (58%)	1.0	1.0	ref
High BMI at last follow-up	8 (7.8%)	27 (16%)	**1.6**	1.5	0.8-2.8	17 (24%)	**2.7**	2.3	1.0-5.5

cHR crude hazards ratio, aHR adjusted hazards ratio, CI confidence interval, ref reference, CSFK congenital solitary functioning kidney, UKA unilateral kidney agenesis, MCDK multicystic dysplastic kidney, SFK solitary functioning kidney, CAKUT congenital anomalies of the kidney and urinary tract, UTI urinary tract infection, BMI body mass index. Bold values indicate associations with a 95% confidence interval not including 1.0. [a]Based on multivariable model including SFK length at 1 year of age, except for hazards ratio of SFK length (90 days). [b]Severe CAKUT was defined as grade 3 or 4 hydronephrosis, grade 3-5 VUR, parenchymal abnormalities or defects and/or dysplasia on any ultrasound, voiding cystourethrography or nuclear scan. [c]The length of the SFK was compared to reference values based on Akhavan *et al*[398].

Supplementary Table 8 Crude and adjusted hazard ratios for potential risk factors for impaired eGFR (<90 ml/min/1.73m2) and hyperfiltration injury (proteinuria, high blood pressure, or antihypertensive medication use) in children with unilateral kidney agenesis or multicystic kidney dysplasia

Factor	eGFR ≥90 ml/min/1.73m² (n = 223)	eGFR <90 ml/min/1.73m² (n = 106)	cHR	aHR[a]	95% CI[a]	No hyperfiltration injury (n = 267)	Hyperfiltration injury (n = 180)	cHR	aHR[a]	95% CI[a]
Academic centre	156 (70%)	70 (66%)	0.9	0.9	0.6-1.4	163 (61%)	115 (64%)	0.9	1.0	0.7-1.4
Female sex	69 (31%)	56 (53%)	**2.5**	**2.2**	**1.5-3.4**	102 (38%)	60 (33%)	0.9	0.9	0.7-1.3
Birthweight percentile										
<p20	57 (27%)	35 (34%)	1.4	1.2	0.6-2.4	65 (26%)	45 (26%)	1.2	1.1	0.6-1.9
p20-p40	31 (15%)	21 (21%)	1.7	1.4	0.7-3.1	49 (19%)	26 (15%)	1.0	1.0	0.5-1.8
p40-p60	33 (16%)	12 (12%)	1.0	1.0	ref	45 (18%)	20 (12%)	1.0	1.0	ref
p60-p80	36 (17%)	18 (18%)	1.2	1.3	0.6-2.8	48 (19%)	31 (18%)	1.3	1.3	0.7-2.2
>p80	53 (25%)	16 (16%)	0.9	0.9	0.4-2.0	47 (19%)	51 (30%)	1.8	**1.9**	**1.1-3.1**
Prematurity	31 (15%)	21 (21%)	1.2	0.8	0.5-1.5	35 (14%)	28 (16%)	1.0	1.1	0.7-1.7
Cause of CSFK										
UKA	75 (34%)	34 (32%)	1.0	1.0	ref	76 (29%)	70 (39%)	1.0	1.0	ref
MCDK	148 (66%)	72 (68%)	1.1	1.2	0.8-2.0	191 (72%)	110 (61%)	**0.7**	**0.7**	**0.5-0.9**
Any extrarenal anomaly	60 (27%)	38 (36%)	1.2	1.1	0.7-1.7	59 (22%)	52 (29%)	1.0	0.9	0.6-1.3
Right-sided SFK	107 (48%)	65 (61%)	1.5	1.2	0.8-1.9	142 (53%)	89 (49%)	0.9	0.9	0.6-1.2
Severe CAKUT in SFK[b]	32 (16%)	21 (23%)	1.2	1.2	0.7-2.0	32 (13%)	27 (17%)	0.9	0.9	0.6-1.4
UTI in first year	39 (18%)	28 (28%)	1.5	1.5	1.0-2.4	45 (18%)	29 (17%)	0.8	0.8	0.5-1.2

Supplementary Table 8 Crude and adjusted hazard ratios for potential risk factors for impaired eGFR (<90 ml/min/1.73m2) and hyperfiltration injury (proteinuria, high blood pressure, or antihypertensive medication use) in children with unilateral kidney agenesis or multicystic kidney dysplasia (continued)

Factor	eGFR ≥90 ml/min/1.73m² (n = 223)	eGFR <90 ml/min/1.73m² (n = 106)	cHR	aHR[a]	95% CI[a]	No hyperfiltration injury (n = 267)	Hyperfiltration injury (n = 180)	cHR	aHR[a]	95% CI[a]
SFK length (90 days)[c]										
<p50	12 (10%)	14 (37%)	**4.4**	2.1	0.8-5.3	18 (11%)	13 (16%)	1.0	1.1	0.4-2.9
p50-p75	11 (9.2%)	6 (16%)	2.2	1.4	0.5-4.0	14 (8.8%)	9 (11%)	0.7	0.7	0.3-1.5
p75-p95	46 (38%)	9 (24%)	1.1	1.1	0.4-3.5	57 (36%)	23 (27%)	0.7	0.7	0.4-1.3
>p95	51 (43%)	9 (24%)	1.0	1.0	ref	70 (44%)	39 (46%)	1.0	1.0	ref
SFK length (1 year)[c]										
<p50	13 (8.8%)	15 (27%)	**4.5**	**3.7**	**1.5-8.9**	19 (10%)	16 (14%)	1.0	1.0	0.5-1.9
p50-p75	8 (5.4%)	10 (18%)	**5.1**	**3.4**	**1.4-8.6**	14 (7.5%)	9 (8.0%)	0.8	0.7	0.3-1.6
p75-p95	30 (20%)	13 (24%)	**2.3**	**2.3**	**1.1-5.0**	46 (25%)	18 (16%)	0.7	0.7	0.4-1.2
>p95	97 (66%)	17 (31%)	1.0	1.0	ref	108 (58%)	69 (62%)	1.0	1.0	ref
High BMI at last follow-up	19 (12%)	13 (14%)	1.2	1.0	0.5-2.0	16 (10%)	19 (17%)	1.5	1.7	0.9-3.0

eGFR estimated glomerular filtration rate, cHR crude hazards ratio, aHR adjusted hazards ratio, CI confidence interval, ref reference, CSFK congenital solitary functioning kidney, UKA unilateral kidney agenesis, MCDK multicystic dysplastic kidney, PUV posterior urethral valves, VUR vesicoureteral reflux, SFK solitary functioning kidney, CAKUT congenital anomalies of the kidney and urinary tract, UTI urinary tract infection, BMI body mass index. Bold values indicate associations with a 95% confidence interval not including 1.0. [a]Based on multivariable model including SFK length at 1 year of age, except for hazards ratio of SFK length (90 days). [b]Severe CAKUT was defined as grade 3 or 4 hydronephrosis, grade 3-5 VUR, parenchymal abnormalities or defects and/or dysplasia on any ultrasound, voiding cystourethrography or nuclear scan. [c]The length of the SFK was compared to reference values based on Akhavan *et al*[398].

Supplementary Table 9 Cumulative survival without signs of kidney injury at the age of 18 years after exclusion of patients using medication other than renin-angiotensin-aldosterone system inhibitors, patients with congenital heart disease, and patients in whom medication use was the only sign of kidney injury.

	Original data	Sensitivity analyses excluding non-RAASi	Sensitivity analyses excluding CHD patients	Sensitivity analyses excluding medication only
Congenital SFK – severe injury	39%	38%	40%	34%
Congenital SFK – any injury	75%	74%	75%	74%
Acquired SFK – severe injury	37%	34%	37%	39%
Acquired SFK – any injury	80%	80%	80%	80%

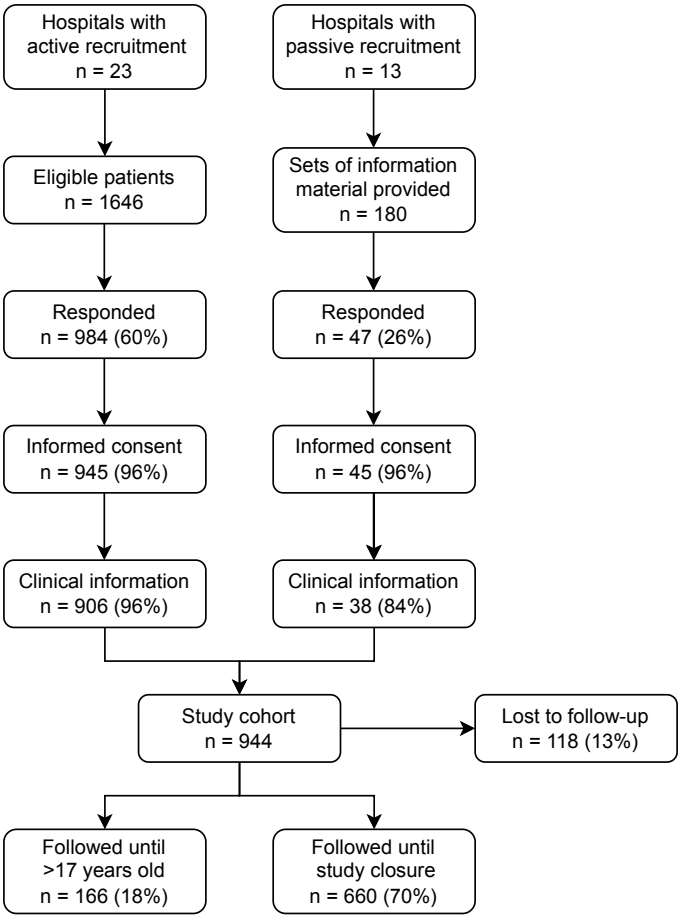
RAASi renin-angiotensin-aldosterone system inhibitors, CHD congenital heart disease.

Supplementary Table 10 Comparison of reference values for SFK length in the first year of life by Akhavan *et al*[398] and smoothed reference values for this study

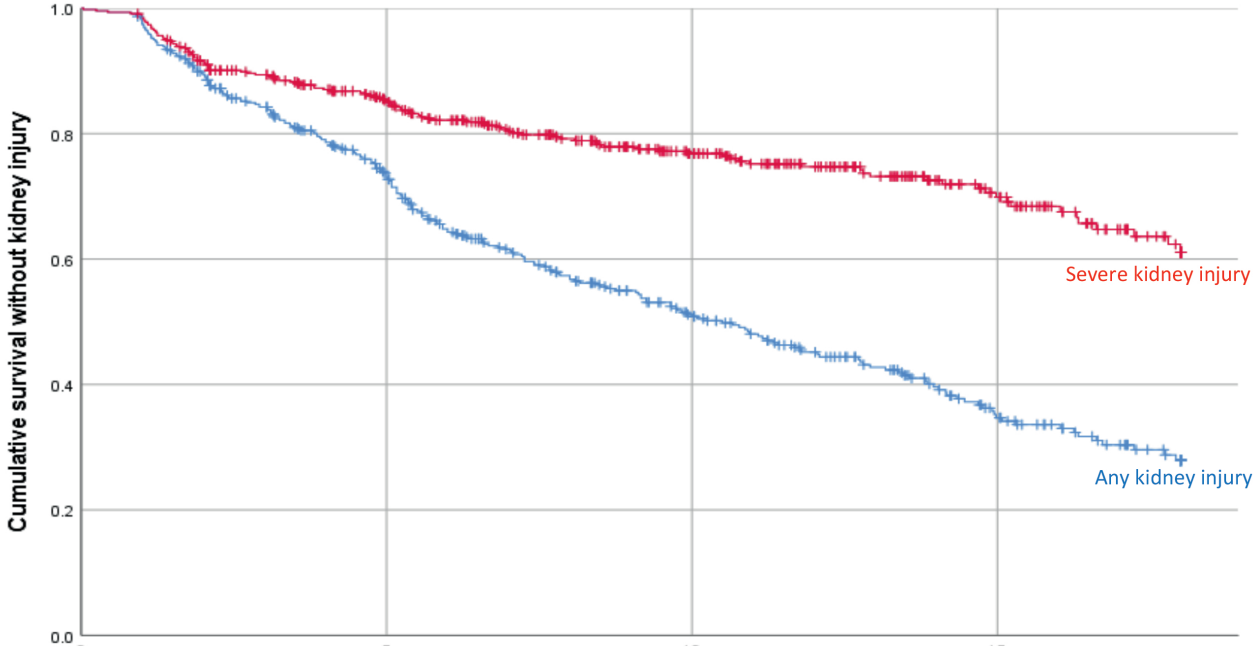
	P5		P25		P50		P75		P95	
Age in months	Original	Smoothed	Original	Smoothed	Original	Smoothed	Original	Smoothed	Original	Smoothed
0-1 mo	35.0	35.7	40.6	41.7	44.0	44.2	47.2	46.7	53.9	52.6
1-2 mo	41.0	38.7	46.0	43.9	48.2	47.4	50.0	50.8	54.0	56.6
2-3 mo	39.0	41.5	45.0	46.1	49.6	50.1	54.5	54.0	61.0	59.5
3-4 mo	44.0	44.2	47.0	48.2	51.1	52.3	54.5	56.3	60.4	61.5
4-5 mo	48.5	46.6	52.0	50.2	55.2	54.1	60.0	58.0	64.0	62.7
5-6 mo	48.6	48.7	52.0	52.0	56.4	55.6	59.8	59.2	62.5	63.3
6-7 mo	48.8	50.4	50.0	53.5	55.6	56.7	59.1	59.9	64.5	63.7
7-8 mo	52.0	51.6	54.0	54.6	57.5	57.5	59.3	60.5	63.0	63.9
8-9 mo	53.0	52.3	56.0	55.3	59.5	58.2	63.0	60.9	67.5	64.2
9-10 mo	52.1	52.4	56.0	55.4	57.4	58.6	59.8	61.5	60.7	64.9
10-11 mo	51.7	51.8	55.0	54.8	59.0	59.0	62.7	62.2	67.0	66.0
11-12 mo	50.4	50.4	53.0	53.6	59.6	59.3	63.3	63.3	68.3	67.9

Mo months.

Supplementary Figures

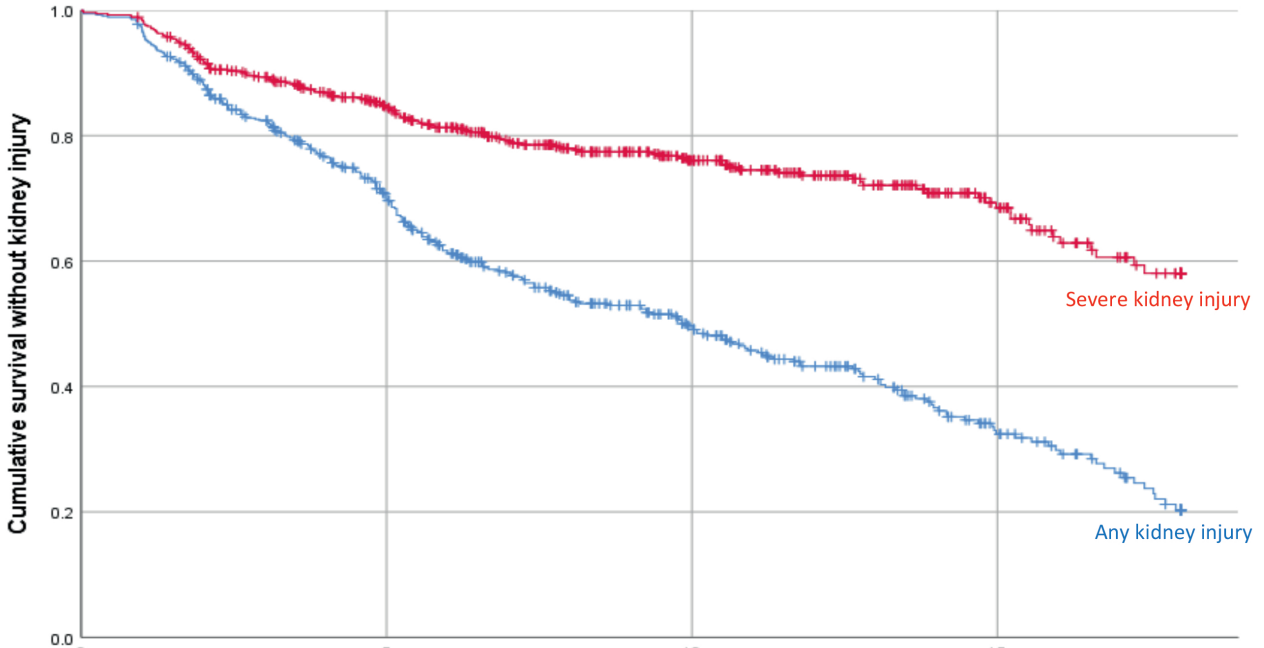


Supplementary Figure 1 Flowchart of patient recruitment and number of patients lost to follow-up. Hospitals with active recruitment were hospitals in which patient lists were available or patients with solitary functioning kidney could be searched in electronic patient records. In hospitals with passive recruitment, information materials were spread by healthcare providers during regular patient care, but no active case finding was possible.



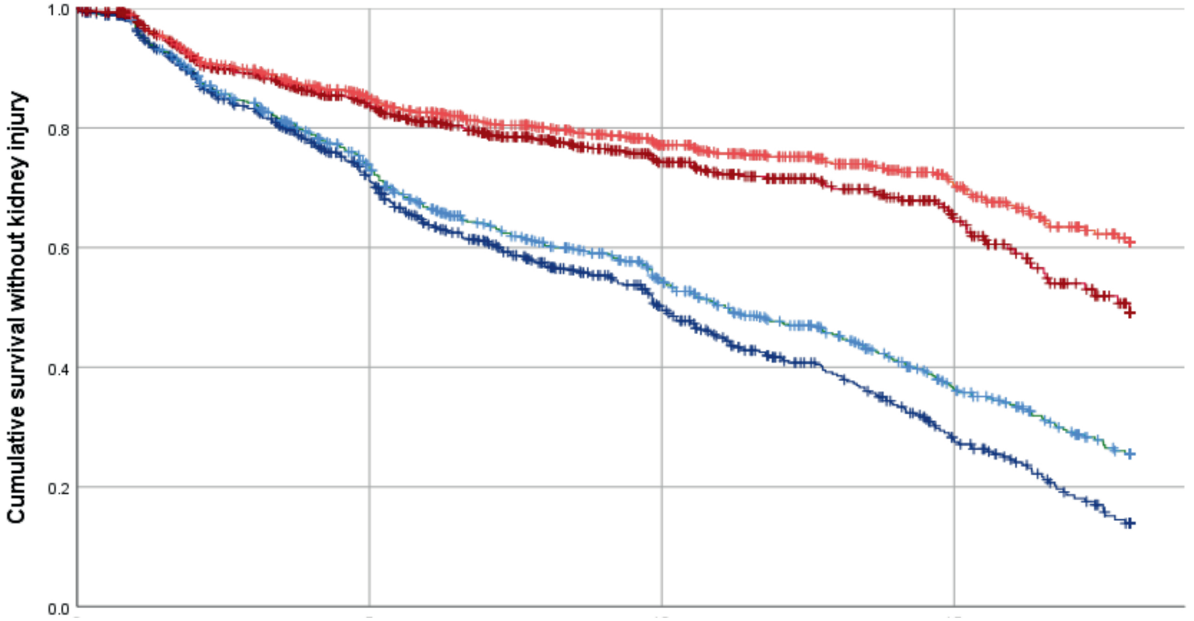
Age	Start	5 years	10 years	15 years	18 years
% survival (severe)	100%	85.0%	76.8%	69.1%	61.1%
N at risk (severe)	458	331	204	99	47
% survival (any)	100%	73.0%	50.8%	34.7%	27.9%
N at risk (any)	458	292	154	67	31

Supplementary Figure 2 Cumulative survival without any kidney injury (blue line) or without severe kidney injury (red line) among children with unilateral kidney agenesis or multicystic kidney dysplasia.



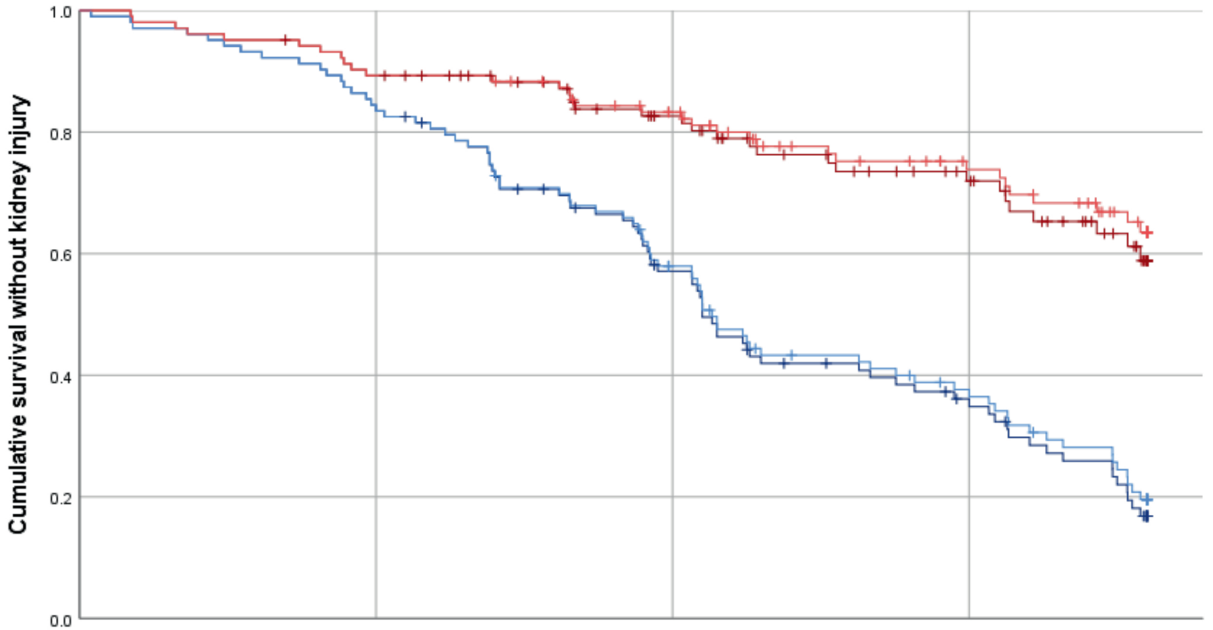
Age	Start	5 years	10 years	15 years	18 years
% survival (severe)	100%	84.4%	76.1%	68.5%	58.0%
N at risk (severe)	542	385	215	84	40
% survival (any)	100%	69.9%	49.1%	32.4%	20.3%
N at risk (any)	542	331	157	57	22

Supplementary Figure 3 Cumulative survival without any kidney injury (blue line) or without severe kidney injury (red line) among children with antenatally detected congenital solitary functioning kidney.



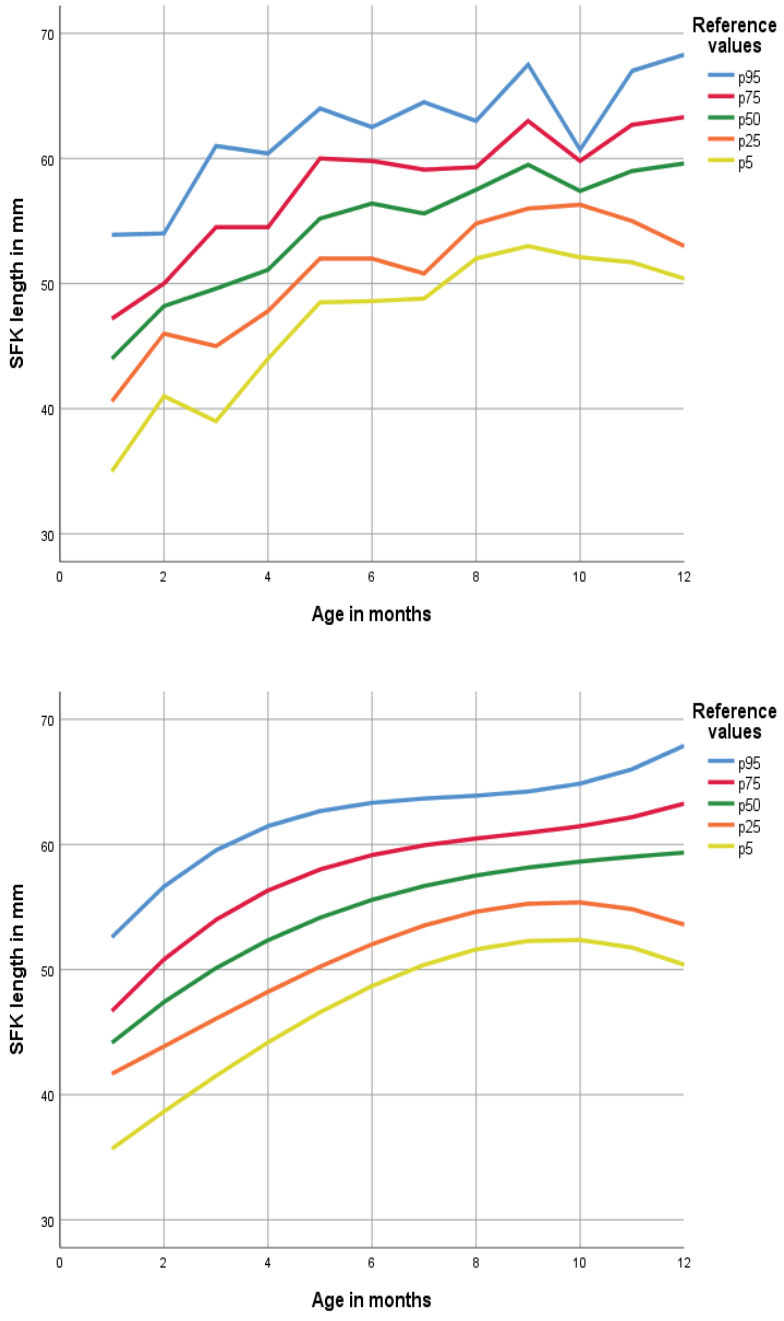
Age	5 years	10 years	15 years	18 years
% survival (original, severe)	84.8%	77.1%	70.2%	60.9%
% survival (alternative, severe)	83.6%	74.2%	64.4%	49.2%
% survival (original, any)	73.1%	54.1%	36.1%	25.5%
% survival (alternative, any)	71.1%	49.5%	27.5%	13.9%

Supplementary Figure 4 Sensitivity analysis comparing cumulative survival without kidney injury estimated using the original censoring approach (at study closure or 18th birthday, whichever came first) and an alternative censoring approach (date of last follow-up visit or 18th birthday, whichever came first) in patients with congenital solitary functioning kidney. Original data are depicted in light red (severe injury) and light blue (any injury), alternative data are depicted in dark red (severe injury) and dark blue (any injury).



Age	5 years	10 years	15 years	18 years
% survival (original, severe)	89.3%	83.3%	73.8%	63.5%
% survival (alternative, severe)	89.3%	82.6%	71.9%	58.8%
% survival (original, any)	83.5%	58.0%	37.5%	19.6%
% survival (alternative, any)	83.5%	57.1%	34.9%	16.8%

Supplementary Figure 5 Sensitivity analysis comparing cumulative survival without kidney injury estimated using the original censoring approach (at study closure or 18th birthday, whichever came first) and an alternative censoring approach (date of last follow-up visit or 18th birthday, whichever came first) in patients with acquired solitary functioning kidney. Original data are depicted in light red (severe injury) and light blue (any injury), alternative data are depicted in dark red (severe injury) and dark blue (any injury).



Supplementary Figure 6 Comparison of reference values for SFK length in the first year of life by Akhavan *et al*[398] (upper panel) and smoothed reference values for this study (lower panel).

CHAPTER 10

General discussion and future perspectives

GENERAL DISCUSSION

As described in the introduction, the research described in this thesis was initiated with two aims: 1) to get more insight into the genetic and environmental causes of congenital solitary functioning kidney (CSFK), and 2) to obtain more knowledge on the long-term consequences of living with SFK from childhood. In line with these two aims, this thesis was divided into two parts, with the first part focussed on the aetiology of CSFK and the second part dedicated to the long-term outcomes.

In part one, we performed a genome-wide association study (GWAS), which indicated that single nucleotide variants (SNVs) may play a role in the aetiology of CSFK, and we suggested that the *HGF* gene may be more important in the development of the kidney and urinary tract than previously thought. Furthermore, we used novel techniques to investigate two monozygotic twin pairs discordant for CSFK. Although we could not explain the discordant phenotypes, postzygotic mutations may play a role in CSFK aetiology and our study provides an example of how to investigate such mutations. We also newly identified maternal stress as environmental risk factors for CSFK and confirmed known associations with e.g. conception using in vitro fertilization/ intracytoplasmic sperm injection and maternal infections during pregnancy. Lastly, we showed that gene-environment interactions contribute to the aetiology of CSFK, with the interaction between maternal overweight or obesity and a SNV near the *ARSB* gene as most important finding.

In part two, we used the available literature to provide evidence-based recommendations for the management of children with CSFK, which should include an early postnatal evaluation, with a key role for ultrasound imaging, and structured follow-up based on the results of previous evaluations. Our systematic review and meta-analysis showed that the risk of kidney injury in children with acquired solitary functioning kidney (ASFK) is not dependent on the indication for nephrectomy and appears to be in line with the risk in children with CSFK. In addition, we argued that in order to personalize predictions of kidney injury risk in children with SFK, several potentially relevant risk factors, including genetic factors, should be further studied. Large, international collaborations should be initiated to facilitate such studies. Lastly, we showed that the rate of kidney injury was high in our cohort of almost 1,000 patients with SFK. We identified overweight as an important targetable risk factor for kidney injury in these children. We also hypothesized that risk factors, such as congenital anomalies of the kidney and urinary tract (CAKUT) in the SFK may cause a direct reduction of the estimated glomerular filtration rate (eGFR), whereas factors, such as overweight may first result in hyperfiltration injury before a reduction in eGFR is to be expected.

The current chapter will highlight some key methodological aspects of our studies, provide interpretations of our research findings in the context of other studies, discuss

the clinical implications of our findings, and provide recommendations for future research.

METHODOLOGICAL CONSIDERATIONS

Study designs

To answer the research questions posed in the beginning of this thesis, several study designs were used. Although these vary from detailed case description and evaluation in the twin study presented in **Chapter 3** to a systematic review and meta-analyses in **Chapter 7**, all designs used were observational. Since as early as the 1970s, study designs have been ranked hierarchically based on their presumed level of evidence, with case series rated as providing a low level of evidence, followed by case-control and cohort studies in the middle, randomized-controlled trials just below the top, and systematic reviews or meta-analyses of well conducted studies as providing the highest level of evidence (Figure 1).[405,406] In more recent years, many modifications to this so-called level-of-evidence pyramid have been proposed based on emerging insights. Especially Murad *et al.* propose two interesting modifications, by incorporating the quality of a study and viewing systematic reviews and meta-analyses as a tool to summarize evidence, rather than depicting them as the summum of evidence.[407]

Although randomized controlled trials and prospective cohort studies provide higher levels of evidence, these studies are in many cases not feasible or unethical to conduct. This was also the case for most studies in this thesis. For our aetiological studies in part I, case-control designs were the best option since CSFK is a relatively rare condition. Studying rare conditions using a cohort study would require very large sample sizes and a long time to acquire enough participants with the outcome, which would be very inefficient. Of utmost importance when conducting a case-control study is selecting cases and controls from the same source population, as failure to do so can result in selection bias. Other methodological challenges include the risk of recall bias and other forms of information bias, appropriate handling of missing data, and untangling causal effects from confounding. These challenges and our approaches to deal with them will be addressed later in this discussion.

For our studies on the follow-up and clinical management of SFK in part II, we used different designs. For the two research questions relating to the management of children with CSFK and the risk of kidney injury in children with ASFK, many published studies were available already. We used systematic approaches to summarise the existing literature for both research questions and translated the results into clinical practice guidelines and a systematic review with meta-analysis, respectively. For the clinical practice guidelines, we refrained from displaying the scoring of the available

evidence underlying our recommendations, since all available literature consisted of observational studies with a similar level of evidence. In the systematic review and meta-analysis, we used a selection of items from the Newcastle-Ottawa Scale to assess the quality of the studies included. [301] Lastly, our study on risk of and risk factors for kidney injury in children with SFK was designed as a retrospective cohort study. Although this design carries the advantage that the outcomes are already available, it also brings methodological challenges such as the selection of a representative patient group, varying data quality, and missingness of data, which will be discussed later. In order to report all of our studies in a transparent way, the STROBE criteria for reporting observational research were applied. [408]

Study populations

Selection of patients

The main source of patients in the studies included in this thesis is the AGORA data- and biobank. [87] As described in the introduction, clinical and questionnaire information and DNA samples from children with congenital anomalies and healthy controls are stored in the AGORA data- and biobank. When the SOFIA study started in 2018, 242 patients with SFK had been included in the AGORA data- and biobank from the Radboud university medical center and UMC Utrecht. We aimed to increase the number of participants to at least 800 to improve the power of the studies planned. To do so, we contacted paediatricians, paediatric nephrologists, and/or paediatric urologists from all hospitals in The Netherlands and asked them to contribute to patient recruitment. They could do so by either performing a search for SFK patients in their electronic medical records and inviting these patients via mail to participate, or by inviting patients with SFK during daily clinical care. The first method was our preferred option, since this likely would result in a higher number of eligible patients and a more representative sample of all SFK patients that were known in the participating centres, increasing the external validity of our studies. Ultimately, the first patient recruitment method was possible in 23 hospitals and led to the inclusion of 670 additional patients. The second recruitment option was used in 13 hospitals and resulted in 38 included patients. Together with the patients already included in AGORA, this led to a study cohort of 950 SFK patients. For 944 of these, clinical data were available.

Methodological considerations regarding the selection of patients

An important aim for our cohort was to have a representative sample of SFK patients. Many previous studies may have overestimated the prevalence of kidney injury, as patients were recruited from academic or specialized hospitals only. [74,75,319] By including patients from 7 academic and 29 general hospitals, we likely included a more representative sample of SFK patients. Nonetheless, the majority of patients (67%) was still recruited via an academic hospital and the median follow-up duration was longer in patients from academic hospitals than in those from general hospitals (14 vs

10 years, respectively). This could indicate that we were more successful in recruiting and/or obtaining follow-up in academic hospitals, which may have led to selection with overrepresentation of more severely affected patients despite our attempts to include a representative sample. However, another possible explanation of the longer follow-up duration in academic hospitals is that older patients, who are more likely to have kidney injury, were referred to academic hospitals because of kidney injury, whereas younger patients without injury remained under follow-up in a general hospital.

Selection of controls

Two control populations were used for the research in this thesis. For most studies, controls from the AGORA data- and biobank were used. These controls were children without major structural congenital anomalies as defined by EUROCAT,[138] recruited in collaboration with municipalities throughout The Netherlands. For our GWAS, we used a second control group derived from the Nijmegen Biomedical Study. This is a population-based study conducted by the departments for Health Evidence and Laboratory Medicine of the Radboud university medical center, for which 22,451 randomly selected age- and sex-stratified inhabitants of the municipality of Nijmegen received an invitation to fill out a survey. A subset of 6,468 participants donated blood samples and was available as control group for the GWAS. Using the Nijmegen Biomedical Study as control group increased our power to detect associations due to the larger sample size compared to the 750 AGORA controls from whom DNA was available. A limitation of this control group is that the participants were not assessed for CSFK and could be unjustly included as control. Because of the low prevalence of CSFK, however, it is unlikely that a large number of controls was affected, thus restricting the potential impact of this limitation.

Methodological considerations regarding the selection of controls

An important prerequisite to draw valid conclusions from case-controls studies is selecting cases and controls from the same source population. An important strength of the population-based AGORA control group is that we selected municipalities representing the catchment area of the cases, to minimize selection bias. Unfortunately, selection bias cannot be ruled out completely, due to selective non-response to the invitation to participate in the study.[409] In our studies, mothers of controls were slightly higher educated than mothers of patients, suggestive of some non-respondent bias, but this was adjusted for in our analyses. An advantage of our approach over a hospital-based control group, is that it rules out possible associations between the condition of the controls and the risk factors under study. An important disadvantage of using healthy controls is an increased risk of recall bias, which occurs when exposures are remembered or reported differently by cases and controls.[410] Although some recall bias may have been present, we expect that the types of exposure investigated (*e.g.* use of fertility treatment, smoking, maternal diseases) as well as the period of study (*i.e.* just before and during pregnancy) limited the possibility that recall bias occurred.

For the studies described in this thesis, selecting controls from the same birth years as the cases was of utmost importance, as some of the exposures assessed as environmental risk factors varied greatly over time. Folic acid use, for example, increased over the study years (1993-2021), whereas maternal smoking decreased. Although the AGORA controls were selected to cover the same years of childbirth as the patients in the AGORA data- and biobank, the exact distribution of year of childbirth differed between cases and controls. Two main methods are available to take this into account: adjustment or matching. In general, matching is only preferred when confounders are strongly associated with the outcome and unequally distributed over the categories of the exposure of interest. [411] Since this was true for year of childbirth, we chose to match cases and controls on this characteristic. Although this strongly reduced the number of controls, it probably resulted in only a minor decrease in statistical power because a 1:3 case-control ratio could be maintained. [412]

Definition of SFK phenotypes

In previous studies by our group[36] and others, [37,38] differences in risk factors for different CAKUT subtypes were identified. Therefore, we studied CSFK specifically and collected information on the exact cause of the SFK in all patients in our study population. This proved challenging, however, because not all CSFK were diagnosed antenatally making it hard to distinguish ASFK from CSFK. In some cases, it was difficult to differentiate between different causes of CSFK. In 87% of patients, the classification as CSFK or ASFK could be made reliably. Differentiation into the cause of CSFK (*e.g.* unilateral kidney agenesis (UKA) or MCDK) was possible in 94% of CSFK patients. The group that could not be classified reliably mainly consisted of children diagnosed antenatally with a dysplastic kidney (with or without cysts), which can have several causes, such as MCDK, ureteropelvic junction obstruction, or a lower urinary tract obstruction, such as posterior urethral valves. [187] Moreover, many studies showed that a MCDK is likely to involute, [266,268,276,284,288,413-417] which may lead to misclassification as UKA or kidney hypo/dysplasia (KHD). For the GWAS, we grouped UKA, MCDK, and KHD as CSFK, because these conditions are all congenital kidney abnormalities that are frequently combined in the literature and likely share aetiological risk factors. For our study into environmental risk factors, we used a stricter definition of CSFK and included only patients with UKA and MCDK to create an even more homogeneous group. Lastly, for our prognostic study, we stratified for congenital and acquired causes of SFK, since compensatory hyperplasia is only possible prenatally, and could have occurred in patients with all congenital subtypes of SFK.

Data quality

Exposure and outcome assessment

Our research into environmental risk factors was based on self-reported data from parents obtained using questionnaires that were completed during childhood. Ideally, these data would be gathered at the time of interest or as shortly afterwards as possible. In research on congenital anomalies, however, collecting data prospectively (*i.e.* during pregnancy) is infeasible, because millions of pregnant women would need to be included to obtain adequate sample sizes of patients with specific congenital anomalies. Therefore, our best option is to acquire data as soon as possible after birth. In the AGORA data- and biobank, we aim to include patients when they present with their condition in a participating hospital. Many patients included in the studies described in this thesis (~75%), however, were recruited at a later moment in time because they were diagnosed in other hospitals and/or were not asked or did not consent to participate in the AGORA data- and biobank before the start of the SOFIA study. This led to a long time between childbirth and filling out the questionnaires for many cases (median 7 years, interquartile range (IQR) 3-12 years), inevitably associated with reduced validity and precision of the answers. All AGORA controls were also included retrospectively, however, with a median time between childbirth and filling out the questionnaires of 6 years (IQR 4-9 years). The comparable lag time between childbirth and filling out the questionnaire for cases and controls reduced the risk of differential recall, thus limiting recall bias. As mentioned previously, the type and timing of the exposures under study further decreased the chances of recall bias, despite the long lag time. This was also seen in previous studies investigating the risk for VACTERL with two different sources of controls (one population-based, the other consisting of children with other congenital anomalies). These studies yielded, for instance, similar ORs for use of ART, indicating that this factor is not prone to recall bias. [418,419]

Another important aspect of reliable exposure assessment is consistent use of measurement instruments. In our study of environmental risk factors, however, we used both paper and online questionnaires. Most parents of controls received paper questionnaires, whereas most parents of cases filled out the online questionnaire. Although the questions in both forms of the questionnaire were identical, this carried a risk of differential assessment of exposure status, since the online questionnaire took prior answers into account. In case of smoking, for example, no further questions about smoking were asked in the digital questionnaire when mothers reported not to have smoked, whereas mothers completing the paper questionnaires could fill out all smoking questions whether or not applicable. Due to the strong association between questionnaire type and disease status, the potential bias could not be corrected for in the analyses.

For genome-wide associations studies, exposure assessment is done using genotyping, with subsequent quality controls steps to minimize the risk of false positive findings. The patients and controls included in the AGORA data- and biobank were genotyped on similar arrays, allowing for comparison with a low risk of batch effects. The number of controls (n = 750) available in AGORA was limited, however, which is why we also used the much larger (n = 6,468) control group from the Nijmegen Biomedical Study, although these controls were genotyped on a different array. To avoid type 1 errors caused by batch effects, we imputed this dataset based on markers shared between the two genotyping chips, but the number of shared markers was relatively low, which could impair imputation quality. Indeed, fewer markers passed the post-imputation quality threshold (Minimac imputation quality score) of 0.6 in the combined dataset of AGORA cases and NBS controls compared to the dataset of AGORA cases and AGORA controls (9.3 million versus 10 million, respectively). For the other quality control steps in GWASs, numerous guidelines are available.[420-422] Most guidelines contain the same items but use slightly different procedures or thresholds. We chose to adhere to the stricter quality control thresholds to ensure a low risk of false positive findings. Unfortunately, no comparable cohort with genotyping data could be found for replication, which is considered the last important quality control step in GWAS.[423]

Adequate assessment of clinical characteristics and outcomes of patients was an important aspect of our study into the risk of and risk factors for kidney injury in patients with SFK. Ideally, such measurements would be standardized using a study protocol. For our study, however, we used information from medical files which contained data collected during regular clinical care. This method enables data on a large cohort to be collected in a relatively short amount of time. Limitations of this approach are that measurements were not standardized, not always performed according to the gold standard (*e.g.* office blood pressure instead of ambulatory blood pressure measurement (ABPM)), and sometimes missing. Nevertheless, the reliability and validity of these measurements are expected to be relatively high since all measurements were taken by experienced healthcare providers according to clinical guidelines. For some outcomes, such as blood pressure, the choice of measurement instrument (*i.e.* office blood pressure instead of ABPM) could have caused misclassification, however. Previous studies showed that masked hypertension is frequent in children with SFK,[242-245] causing underestimation of the prevalence of high blood pressure, whereas white coat hypertension occurs in a smaller subgroup and may lead to overestimation.[240,246] Because we relied on the last blood pressure measurement only, we could not officially diagnose hypertension, for which elevated blood pressure reading at three consecutive visits are needed. For eGFR, debates are ongoing about the optimal equation to calculate it, although the Schwartz formula was shown to give accurate results in children with SFK.[221] Furthermore, a change of methods used to determine creatinine concentration during the long period of data included may have led to misclassification in some

patients. Given the low number of patients with an isolated reduced eGFR, however, the impact of this misclassification is likely to be low.

Handling missing data

To obtain reliable results, the data should be as complete as possible. The rates of missing data in our study on environmental risk factors varied from 1% to 11%. These missing values were mainly caused by parents not fully completing the questionnaires. In these situations, a complete case analysis provides results for a subset of participants with complete data only and likely results in bias. This was avoided by creating 10 imputed datasets under the assumption that data were missing at random. Although it is virtually impossible to prove such an assumption, sensitivity analyses showed that our results were robust to other types of missingness as well.

A similar approach was used for the data on risk factors for kidney injury. Data on risk factors were mainly missing when clinical information for a specific factor was not available or when parents did not fill out the follow-up questionnaire. When information on a clinical risk factor was missing from the medical file, this factor was most likely not present or had a normal value in the specific patient. When information was not available because of non-completion of the questionnaire, missingness was likely random. Missing data on risk factors from both medical files and questionnaires were imputed to allow for complete case analyses. Furthermore, sensitivity analyses were used to test robustness of our results against non-random missingness patterns in the clinical risk factors.

For the outcomes, we chose a conservative approach in which we used the date of study closure as censoring date in children without kidney injury. Due to standard practice in the Netherlands, children with SFK who did not show signs of kidney injury were often discharged from follow-up visits, unless there was a specific reason (*e.g.* recurrent UTIs) to continue follow-up. Therefore, children with a higher chance of developing signs of kidney injury will have had more follow-up visits and a longer follow-up duration than patients with less chance of developing the outcome. We anticipated this selective loss to follow-up by censoring all patients at study closure or at their 18th birthday, whichever came first, whether they actually had control visits during this entire period or not. This method likely resulted in underestimation of the proportion of children with kidney injury, since some children may have developed kidney injury between their actual last follow-up visit and the moment of censoring. However, calculating with the date of their actual last follow-up visit would likely have resulted in substantial overestimation of the proportion of kidney injury, given the selective loss to follow-up. We preferred slight underestimation over major overestimation in the proportion of patients that developed signs of kidney injury.

Adjustment for confounders

When attempting to identify causal relations, investigators performing observational studies have to deal with the possibility of confounding. Confounding by external factors can influence or introduce associations between the exposure and outcome of interest that deviate from the true association. An external factor can be a confounder when it is a cause of the outcome, is associated with and precedes the exposure of interest, and is not located in the causal pathway between exposure and outcome. Although it is common practice to control for a range of background variables as if they were confounders, unnecessary adjustment can be harmful and can even introduce confounding.[142] Therefore, careful selection of confounders is warranted, for which directed acyclic graphs (DAGs) are increasingly being used and recommended.[142,424] Creating a DAG does not prevent inadequate correction for confounders, but increases transparency because it requires specification of the assumptions made while selecting confounders. Furthermore, DAGs facilitate easy selection of a minimally sufficient set of confounders, which increases statistical power and reduces the risk of overcorrection. Therefore, we created DAGs for our study into environmental risk factors. For GWASs, population stratification is the most important source of confounding. To prevent confounding in our GWAS, we performed our analyses after filtering on ancestral background and corrected for the first four principal components to further reduce the possibility of population stratification. A similar procedure was followed for the gene-environment interaction analyses, in which we corrected for the principal components as well as for maternal education level, which we determined to be the only probable confounders of the interactions under study.

INTERPRETATION OF RESEARCH FINDINGS AND FUTURE PERSPECTIVES

Part I: Aetiology of congenital solitary functioning kidney

Although a large number of studies have been performed on CAKUT, the aetiology of CSFK is still largely unknown. This introduces uncertainty for parents of children with CSFK, impedes genetic counselling of families, and limits possibilities for prevention. In the first part of this thesis, we studied several aspects of the aetiology of CSFK to provide more insight into genetic and environmental causes of CSFK and identify promising topics for further research.

The aetiology of CSFK is multifactorial

In **Chapters 2-5**, we investigated several different aetiological mechanisms for CSFK. Our results indicate that all of these mechanisms may contribute to CSFK development. One of the two genome-wide significant SNVs (rs140804918) found in **Chapter 2** was particularly interesting given its relation with the *HGF* gene, which is known to be

involved in kidney development. Another high-ranking SNV, rs148413365, did not meet the threshold for genome-wide significance, but its close proximity to the *KCTD20* and *STK38* genes could indicate functional relevance. Since we were one of the first groups that collected DNA from a large number of CSFK patients, we had limited options for replication of our findings. Therefore, our results can be used to substantiate a role for SNVs in the aetiology of CSFK, but associations for the specific SNVs warrant confirmation. Our investigation of two monozygotic twin pairs discordant for CSFK in **Chapter 3** was one of the first attempts to identify postzygotic mutations in CSFK patients. Although we were unable to find such mutations, similar research in larger groups of patients may provide more insight into whether postzygotic mutations form a relevant aetiological mechanism for CSFK.

Additional support for a multifactorial aetiology of CSFK is presented in **Chapters 4 and 5**. In **Chapter 4**, we provided evidence that several parental and environmental factors were associated with an increased risk of CSFK, including conception using IVF/ICSI and maternal stress, infections, and smoking during pregnancy. Since maternal stress was newly identified as risk factor for CSFK, it is important that others incorporate it as potential risk factor in their studies to validate our findings. The associations with maternal infections and smoking added to existing evidence and highlight the importance of addressing these factors in further research as well as in preventive efforts. The same holds true for use of folic acid containing supplements and a younger maternal age, which were both factors associated with a reduced risk of CSFK. In **Chapter 5**, we studied interactions between genetic and environmental risk factors. Our results indicated that this mechanism may be relevant for the aetiology of CSFK and nominate the interaction between an intronic variant in the *ARSB* gene (rs3098698) and maternal BMI for future research.

Results from genetic and GxE studies should be substantiated by functional studies

Although the results presented in Chapters 2 and 5 are substantiated by *in silico* evidence of biological relevance, they have not been assessed in functional studies. GWASs have been performed for a wide range of traits and diseases and yielded thousands of statistically significant results, but the biological consequences of the variants found have typically not been assessed.[425] After replication in an independent cohort, a highly necessary first step to confirm our findings, animal models could be developed to get more insight into the role of *HGF* and specific *HGF* variants in kidney development. Early functional studies already showed HGF protein expression in the metanephric mesenchyme,[426] while adding an antiserum against HGF resulted in inhibition of branching morphogenesis in mice.[116,426] Although a complete loss of Hgf or Met expression in mice leads to embryonic death, studies on conditional knockout of Hgf in the kidney have not yet been performed. Creating such a model could further substantiate partial loss of *HGF* gene expression as a cause of CSFK. Similar studies

could be initiated for the *KCTD20* and *STK38* genes, if the SNV identified in our study would be replicated.

Demonstrating biological relevance would also substantiate the interaction between maternal BMI and the rs3098698 SNV. We hypothesized that high glucose and/or insulin levels transmitted by overweight or obese mothers could interact with this SNV, which results in lower *ARSB* expression and a reduced availability of the insulin receptor in the child. To study such effects, kidney organoids from patient-derived cells could be used as a first step, since these are more readily available at lower costs compared to mouse models and contribute to a reduction in the number of laboratory animals used.[427] After evaluating *ARSB* expression in kidney organoids derived from cells with the SNV, compared to those derived from wildtype cells, the availability of the insulin receptor and functional consequences of glucose and insulin administration could be assessed. Lastly, exposing kidney organoids with and without the SNV to different glucose and insulin levels and studying the effect on kidney development could provide a fascinating insight into the functional relevance of this interaction.

Aetiological knowledge may benefit patients and parents

Knowledge regarding the aetiology of SFK can have important implications when counselling patients or their parents, but also when deciding on whether testing for genetic causes is appropriate. Our findings indicate that SFK is for the most part a multifactorial disorder, in which several genetic mechanisms, environmental risk factors, and interactions between genetic and environmental factors play a role. This makes it difficult to pinpoint a specific cause in the individual patient, although one disease-causing variant or CNV may underlie the condition in some patients. Finding such monogenic causes can assist in disease classification and management,[33] and use of a structured diagnostic workflow including whole exome sequencing (WES) has proven to be cost-efficient for a broad range of chronic kidney disease (CKD) patients, including patients with CAKUT and CKD stage 2 or higher.[428] Therefore, the current practice of testing for pathogenic variants and CNVs in patients with CSFK and associated extrarenal congenital anomalies or a family history of CAKUT remains important, despite our findings of several other mechanisms involved in CSFK development.

These findings, supporting a multifactorial aetiology of CSFK, can also be used to reduce feelings of guilt in parents, since it is highly unlikely that a single environmental or parental factor is sufficient to have caused the CSFK. Parents may also benefit from aetiological knowledge when considering future pregnancies. Although CSFK specific numbers are lacking and no information was given on genetic testing, Glinianaia *et al.* reported a risk of 1 in 357 for giving birth to a child with a urinary tract defect in a first pregnancy and a recurrence risk of 1 in 47 in the pregnancy thereafter.[429] When no disease-causing variant or CNV is identified in a patient with CSFK, the risk of CSFK in a future pregnancy is probably still higher than in the general population,

given the possibility that parents carry risk-increasing SNVs, which are not detectable using conventional genetic tests, such as WES, or are repeatedly exposed to similar environmental risk factors. This is illustrated by the study of Wu *et al.*, who identified a pathogenic mutation using WES in only one of nine patients with UKA and a positive family history.[43] More research into the aetiology of CSFK and the risk of recurrence in a second pregnancy after negative genetic tests would further improve counselling.

Policymakers should support aetiological research and implementation of findings

Of all pregnancies in The Netherlands, 3% is complicated by a congenital anomaly.[430] Moreover, congenital anomalies were the second cause of death in children of 0-15 years old in The Netherlands, while annual costs were estimated at almost 300 million euros.[431,432] Research into the aetiology is difficult, however, due to challenges in ascertainment and classification of congenital anomalies, difficulties in data collection for relevant aetiological factors (*e.g.* exposures to environmental factors and material for genetic analyses), and limited numbers of participants because of the rarity of many anomalies.[66] Many of these difficulties could be overcome with better registration. Currently, two registries with data on congenital anomalies exist in The Netherlands: Perined registers all perinatal outcomes throughout The Netherlands, including diagnoses of anomalies, while Eurocat Netherlands registers congenital anomalies in the northern part of The Netherlands, including information on a number of potential risk factors obtained via parental questionnaires. Both of these registries have limitations, however. Whereas Perined has nationwide coverage, Eurocat is restricted to only a small part of The Netherlands, which limits generalizability and results in a smaller sample size. Perined, on the other hand, has limited ascertainment because of its focus on the perinatal period, which results in missed diagnoses, and relies on obstetric records for information on potential risk factors. The European Union supports European Reference Networks (ERNs), that are currently creating registries for rare disorders in order to improve patient care. Although the European-wide scale is a great asset of these registries, not all congenital anomalies can be included. An isolated non-familial CSFK, for instance, is not eligible based on the current criteria. Moreover, only centres that are part of an ERN are actively involved in the registries. In a policy document,[433] the National Institute for Public Health and the Environment concluded that a more comprehensive registry in the Netherlands is desirable and achievable if structural funding is provided by the government. In other countries, such as Denmark, Norway, and Sweden, or for other disease areas, such as cancer (The Netherlands comprehensive cancer organisation (IKNL)), excellent examples exist on how more focus on registration could improve and stimulate research.

Strict application of privacy laws, both within The Netherlands and concerning international collaborations, formed another barrier to our research ambitions. Collaborations are indispensable for successful research on congenital anomalies, to

create patient populations of sufficient size and generalizability. When trying to obtain data from other hospitals in The Netherlands, however, the approval of the Regional Committee on Research Involving Human Subjects Arnhem-Nijmegen was often not considered sufficient, and many hospitals performed their own additional evaluation before approval for data sharing. Surprisingly enough, even informed consent provided by the patient was not always enough to obtain data from medical records. This caused significant delays in our data collection and created a lot of extra work, while the Dutch law on research involving human subjects dictates that research should only be judged by a single medical ethics committee.[434] For international collaborations, the General Data Protection Regulation (GDPR) act, that came into effect in 2018, has resulted in important constraints, partly because of fear to violate the GDPR.[435] As an example, Radboudumc security experts translated article 32 of the GDPR, that describes security of processing, into a screening list of more than 300 questions to be answered by a data receiving party outside the EU. Such provisions make collaborations with new parties virtually impossible and prevented us from externally validating our study results, which limits the impact of our research. In order to balance data protection and the need for collaborative research efforts, compliance with the GDPR should be made much easier by translating it into a more basic set of requirements and offering more extensive support to researchers.

Implementation of findings from aetiological research is important and warrants attention from the relevant healthcare providers and government institutes. Our findings on environmental risk factors underline the importance of lifestyle in women who want to become pregnant and reaffirm the advice to take folic acid supplements and quit smoking. In addition, maternal stress was identified as a risk factor for CSFK. Combined with evidence of other negative effects of maternal stress during pregnancy and the availability of strategies to reduce these effects, this should lead to more focus on the identification, prevention and treatment of stress in pregnant women.

The future of aetiological research on congenital anomalies

Novel genetic techniques, such as long-read sequencing and whole-genome sequencing (WGS), as well as innovative use of existing techniques, such as deep-exome sequencing in search for postzygotic variants, will substantially enhance our ability to identify genetic causes of congenital anomalies. In contrast to current forms of sequencing, in which reads of up to 600 bases are generated, long-read sequencing can produce reads of more than 10 kb.[436] This may overcome limitations of traditional forms of sequencing, including sequencing complex regions and repetitive regions, and theoretically allows for identification of variation in RNA transcripts, for instance induced by alternative splicing.[436,437] Improving error rates, increasing throughput, and decreasing costs is necessary, however, before widespread application is possible. Whole-genome sequencing, on the other hand, mainly brings challenges related to the amount of data produced, for which tools to assist interpretation and prioritization have to be

developed and integrated. When that has happened, WGS brings many opportunities for studying noncoding and structural variants. An interesting example of the use of WGS in CAKUT is the study performed by Chan *et al.* on a group of 132 patients with posterior urethral valves (PUV), in which they successfully identified a common and a rare variant in loci not previously implicated in PUV.[100] Similar to PUV, CSFK is a disorder with a complex aetiology and limited numbers of patients, which suggests that WGS may also help clarify the aetiology of CSFK. The same goes for postzygotic mutations, which are increasingly recognized as cause of disease,[126] but are rarely investigated in patients with congenital anomalies. A positive exception was the recent publication of a somatic mosaicism in *PBX1* as cause of kidney hypoplasia in a 20-year old male patient.[438] More wide-spread integration of deep-sequencing techniques will help identify genetic causes of CSFK and increase our understanding of CSFK aetiology.

Epigenetics may provide a link between parental exposure to environmental factors and disorders in the offspring via mechanisms, such as DNA methylation, histone modification, ATP-dependent chromatin remodelling, and microRNAs.[439] Research into these mechanisms for CAKUT is limited to a case study in which 514 differently methylated regions were reported in a monozygotic twin pair discordant for UKA.[53] Much more is known for other disorders, such as congenital heart defects, where researchers investigated genome-wide methylation patterns, chromatin remodelling, and micro RNAs in different case-control studies.[440] Time has come to perform research on epigenetic differences in children with CAKUT as well to provide important new insights into its pathophysiology.

Novel techniques may not only change the way (genetic) data will be analysed in the future, but also provides opportunities for innovative data collection. Digitalization of society has led to an exponential increase in the use of wearable devices and other electronics capable of recording health-related data. As a large majority of users report to be willing to share health data from wearables with healthcare professionals,[441] these devices could generate enormous amounts of data for research, which are increasingly being used.[442] Applications for research on congenital anomalies, for instance to capture data on environmental exposures, are scarce, however. Because CSFK is a rare condition like most congenital anomalies, it is unlikely that future studies can solely be based on prospectively collected data on environmental exposures during the aetiologically relevant time window, unless devices capable of recording such exposures are adopted almost universally. For now, wearables can be used as a tool for validation of more conventional methods of data collection on some environmental risk factors, such as physical activity. Furthermore, it is not unlikely that exposures that are difficult to measure, such as stress, could be objectified better with help of wearables. Getting more insight into the validity of different methods of data collection as well as the timing of exposure, can greatly enhance future research into environmental risk factors.

From focussed to integrative approaches

In order to further advance knowledge on the aetiology of CSFK and other congenital anomalies, bigger cohorts with more data are urgently needed. The only way to achieve this is by creating international multicentre collaborations, consisting of professionals with different expertise. The ERNs can provide useful frameworks for many disorders but as yet, the data collected in ERN registries have a clinical focus, and additional registries or data will be needed for research. Biobanks can add great value to such registries, since they can ensure protocolized collection and storage of biological materials from participants, ideally using uniform procedures across all participating centres. In addition to clinical data and patient materials, structured data collection on environmental risk factors is important for future research. Data on exposures during pregnancy, for example, are ideally collected as early as possible to minimize recall bias. Therefore, gathering multiple types of data, as is done in the AGORA data- and biobank, should be more common, given the opportunities for research these data offer.

When such large data- and biobanks are established and a sufficient number of patients has been included, integrative research approaches should be used. Studies should no longer focus on a single aetiological mechanism, but rather try to investigate all mechanisms hypothesized to be relevant in a combined or sequential approach. For a complex disorder, such as CSFK, this could mean that exome sequencing using a gene panel, ideally to a sequencing depth sufficient to detect mosaicism variants, is first applied. Next, pathogenic CNVs can be filtered out, before looking at factors that are risk-increasing instead of causative by themselves (*e.g.* SNVs and environmental factors). Techniques such as WGS may facilitate many different analyses aimed at different genetic mechanisms, thus increasing efficiency and cost-effectiveness, while epigenetics and gene-environment interactions may reveal entirely different mechanism. Structural support for large, well-designed research projects is vital and can be of great value for healthcare, which is illustrated by examples, such as the UK Biobank. Congenital anomalies, which cause a large burden of disease for many young patients and their families, deserve such support as well.

Part II: Outcomes and management of children with solitary functioning kidney

Each year, over 5,000 new-borns are diagnosed with CSFK in the EU and USA alone and a comparable number of children undergoes a nephrectomy. The increased risk of kidney injury for these children necessitates guidelines for the optimal management of their condition. With the research described in this thesis, we aimed to summarize current knowledge, provide new insights for improving clinical management, and identify areas that need further research.

Predicting the future risk of kidney injury is important for tailored clinical management

In **Chapter 6**, we provided recommendations for the clinical management of children with CSFK and identified several knowledge gaps in the current literature. Since the introduction of structured ultrasound screening during pregnancy, most children with CSFK are identified antenatally, which allows for a structured evaluation soon after birth. This first evaluation serves three main goals: confirming the antenatally suspected diagnosis, identifying children that need intervention soon after birth, and predicting the future risk of kidney injury. In almost all patients, confirming a suspected diagnosis of CSFK can be done using ultrasound. High sensitivity and specificity, combined with the widespread availability and non-invasive nature, make ultrasound the preferred diagnostic modality. Scintigraphy should only be used when a hypoplastic kidney (rather than complete agenesis) is suspected or when differentiation between MCDK and severe hydronephrosis is difficult.

An intervention is mostly indicated for children with CSFK who have additional CAKUT in the CSFK. We and others showed that additional anomalies, such as VUR or UPJO, occur in approximately one in three children with CSFK.[17,29] In case of high grade VUR or a severe UPJO, prophylactic use of antibiotics and/or urological interventions may be needed to prevent kidney scarring. The most sensitive method for detecting VUR is a voiding cystourethrogram (VCUG), but the invasiveness of the procedure limits its suitability as screening tool. In addition, the approach to VUR has changed over the years, and now a more watchful waiting approach is used instead of endoscopic or surgical intervention. So, demonstrating VUR with a VCUG only irregularly changes clinical management. Therefore, we recommend ultrasound screening for dilatation of the kidney and urinary tract in all children with suspected CSFK. When high grade VUR is suspected, VCUG can be used as second line investigation, while mercapto acetyl tri glycine (MAG-3) scintigraphy is the preferred diagnostic modality when UPJO or another urinary tract obstruction is suspected. Although other guidelines give the same recommendation,[89] it remains based on expert opinion only. Future studies should compare the prevalence of kidney scars among children with CSFK in hospitals using this approach and in hospitals routinely performing VCUG and MAG-3 scintigraphy to substantiate our recommendation.

The third goal of the first evaluation of a new-born with CSFK is to predict the future risk of kidney injury, since a prediction can assist in tailoring further management to the individual patient. In **Chapters 6 and 8**, we aimed to provide insight into factors that may be predictive of future risk of kidney injury. Factors that are well substantiated and should already be used in clinical practice include the status of the CSFK (*i.e.* absence of compensatory enlargement and presence of additional CAKUT), the status of the urinary tract (*i.e.* occurrence of urinary tract infections early in life), and signs of suboptimal prenatal development (*i.e.* premature birth, low birth weight, or small for

gestational age. Many other factors, however, may potentially influence the long-term risk of kidney injury but have not yet been investigated or yielded conflicting results in previous studies. Several of those factors were included in our large cohort study reported in **Chapter 9**. In this study, we found that the most important risk factors for kidney injury were the cause of CSFK (*i.e.* a higher risk for children with UKA than MCDK), a kidney length <p50, CAKUT in the SFK, and overweight or obesity

For patients who acquire an SFK during childhood, predictive factors are also needed to allow for tailored follow-up. We hypothesized that the indication for nephrectomy could be one such factor and compared the reported kidney injury rates after unilateral nephrectomy in childhood. In our systematic review and meta-analysis, reported in **Chapter 7**, we did not find differences in the prevalence of kidney injury between children with ASFK after a congenital anomaly or children with ASFK after a malignancy. This indicates that the indication of nephrectomy is not a suitable indicator to use for stratified follow-up. We also found that the prevalence of all types of kidney injury was >10% and could not identify a group with a low risk of kidney injury. Therefore, children with ASFK should receive similar long-term follow-up as children with CSFK, regardless of the indication for nephrectomy. An important limitation we encountered is that outcomes after nephrectomy in childhood were not reported consequently and consistently among the studies we identified for our systematic review. This could indicate a lack of standardized clinical follow-up and/or lack of consensus on relevant outcome measures. As a result, the review contained mostly patients who had undergone nephrectomy because of a malignancy, whereas registries indicate that most nephrectomies are performed for benign indications.[32] This probably reflects more structured follow-up of patients with childhood malignancies, compared to other patients undergoing nephrectomies. Moreover, many different outcome definitions were used, which impeded combining the results of multiple studies. Centralized registries, such as those of the ERNs, should be used to collect these data and make them available for research.

The risk of kidney injury in children with SFK is an important but difficult research topic

In a landmark study by Sanna-Cherchi *et al.,*[75] up to 30% of patients with CSFK showed kidney failure at the age of 30 years. Many others found rates of kidney injury that varied between 6 and 60% of patients.[76,80-82,210,246] These numbers were influenced by critical aspects of the studies, such as patient population, follow-up duration, and thresholds used to define kidney injury. Most studies were performed in a selected population (often at a single institution or at academic hospitals only), were limited in follow-up duration, and used varying thresholds to define kidney injury. In **Chapter 9**, we described how we created the SOFIA study, in which we formed a study network encompassing as many hospitals in The Netherlands as possible. This led to a multicentre collaboration among 36 hospitals and resulted in a cohort of almost 1,000

patients with SFK. Because of our large dataset, we were able to estimate long-term outcomes based on a larger group of patients, identify risk factors for kidney injury from multivariable models, and distinguish different risk factor patterns involved.

We found signs of kidney injury in 75% and 80% of patients with congenital and acquired SFK, respectively. Signs of severe kidney injury were present in 39% and 37% of patients. These findings are in line with previous studies reporting severe outcomes, such as Sanna-Cherchi *et al.*[75] and Westland *et al.*,[82] but differ from those reported by Marzuillo *et al.*.[80] As touched upon earlier in this discussion, our population was mostly gathered in academic hospitals, which may have resulted in selection of patients with worse outcomes. Inclusion via an academic hospital turned out not to be an important risk factor for kidney injury in our study, however. Sensitivity analyses showed that our results were not driven by specific subgroups, such as patients diagnosed antenatally or with UKA or MCDK only. The retrospective nature of our data, however, introduced challenges to ensure validity of the data and to limit missing information as much as possible. Although prospective studies with uniform definitions and measurement of risk factors and outcomes could provide higher data quality, prospective studies of similar size and duration will be hard to finance and keep running.

Our data provided confirmation of the long-term risk of kidney injury, but additional questions remain. The median follow-up duration in our cohort, which was approximately 13 years, is one of the longest reported so far. Questions about prognosis into adulthood are harder to answer, however, but several steps could be taken to improve this prognosis. First, current cohorts should be followed for a longer period of time, since this will be an efficient way of acquiring extra data. Another quick improvement could be made with better registration, for instance by incorporating SFK as cause of kidney failure in relevant registries. In addition, more knowledge could be acquired by longitudinally investigating relevant clinical parameters, such as blood pressure, urinary protein excretion, and eGFR. In patients with autosomal dominant polycystic kidney disease, for example, longitudinal eGFR decline is one of the criteria to select rapidly progressing patients for treatment with Tolvaptan.[443] For SFK, however, studies mostly investigated the presence of signs of kidney injury at different ages and analysed these on group level using survival analyses. By incorporating repeated measurements of the relevant clinical parameters, we could determine which patients show a progressive decline in kidney function and improve the prediction of relevant outcomes, for instance using clustering techniques. This information could also be used for studies into the mechanisms of kidney injury, for instance by comparing patients with rapid and slow progression of CKD.

Towards personalized care for patients with SFK

In **Chapters 7 and 8**, we showed that the currently available data are of insufficient quality and amount for clinically relevant predictions of the risk of kidney injury, which are vital to personalize care for patients with SFK. Therefore, our cohort study in **Chapter 9** aimed to identify important risk factors for kidney injury and found the cause of CSFK (*i.e.* a higher risk for children with UKA than MCDK), a kidney length <p50, CAKUT in the SFK, and overweight or obesity. Our findings confirm results of most previous studies, although we did not find an association between low birthweight or preterm birth and kidney injury. Unfortunately, the effect sizes in our study were not large and consistent enough to build a prediction tool for kidney injury, and we could not identify subgroups who remained completely injury free.

When comparing our findings with published guidelines, questions can be raised concerning some of the current recommendations. In 2011, Corbani *et al.* recommended a stratified approach based on the presence or absence of CAKUT in the SFK, [88] with yearly evaluation of blood pressure, urine albumin, and serum creatinine when CAKUT is present, versus a two-yearly follow-up until puberty in absence of additional CAKUT. After puberty, asymptomatic patients can be followed with visits every 3-5 years. A similar stratification approach was advocated by Westland *et al.*, who advised blood pressure and urinary albumin measurements every six months for children with additional CAKUT and every year for children without, as well as a GFR estimation every five years, as long as it remains above 60ml/min/1.73m^2 and no proteinuria or high blood pressure develops. [90] More recently, La Scola *et al.* proposed a stratification into three risk categories, based on kidney length, presence of additional CAKUT, and signs of kidney injury. [89] Children classified as having a low risk (those with compensatory hypertrophy, no CAKUT, and no signs of kidney injury), are advised to have blood pressure measured every year, urinary protein every five years (after the age of three), and no creatinine measurements. Children with medium risk (defined as CSFK without compensatory hypertrophy and/or with additional CAKUT) are advised to have annual measurements of blood pressure, urine protein levels, and serum creatinine, whereas individualized follow-up is recommended for children with a high risk. In our study, however, additional CAKUT was associated with a 50% increased risk of kidney injury, whereas compensatory hypertrophy (SFK length >p95) was only associated with a decreased risk of kidney injury when compared to children with an SFK length <p50. When selecting patients from our cohort according to the guidelines by La Scola *et al.*, 10% of the children in the low risk category would develop an eGFR <90ml/min/1.73m^2, but no creatinine measurements would be performed. In our entire cohort, 16% of the children with an eGFR <90ml/min/1.73m^2 did not have proteinuria or hypertension alongside their reduced eGFR. As such, we believe that the discriminative ability of the current risk factors is insufficient for stratification in clinical practice. Until better risk prediction is possible, all children with SFK should be checked annually on proteinuria and hypertension and at least once every five years for a reduced eGFR until puberty, with intervals of two years from that time onwards.

Next steps in risk prediction

A novel finding in our study was that risk factors differed between the types of kidney injury: the cause of CSFK and presence of overweight or obesity were associated with hyperfiltration injury, whereas CAKUT in the SFK and smaller SFK size were associated with a lower eGFR, but not hyperfiltration injury. When referring back to the Brenner hypothesis, CAKUT in the SFK and smaller SFK size may result in or reflect a further decrease in the filtration surface area in the short term. Overweight, on the other hand, may be an additional factor leading to an increase in single nephron glomerular filtration and/or blood pressure, which will subsequently add to the stress on the remaining nephrons and lead to extra hyperfiltration injury. The latter will most likely be reflected in proteinuria and/or hypertension before a reduction in eGFR is visible. If further studies confirm our observation, it should be used in clinical practice by increasing the frequency of creatinine measurements in patients with additional CAKUT or a small SFK size, whereas the frequency of urinary protein and blood pressure measurements should be increased in patients with overweight.

As the initial insult to the kidney is a reduced nephron endowment (in CSFK) or removal of kidney tissue (in ASFK), resulting in a lower nephron number, the number of remaining nephrons is likely to be an important determinant of the degree of filtration pressure and subsequent risk of injury to these nephrons. *In vivo* measurement of nephron numbers would thus be of great value to estimate future risk of kidney injury. Recent advances have made the introduction of such measurements more realistic, with cationized ferritin as promising contrast agent for use in MRI or PET scans. [444] An estimated nephron number, when available without invasive procedures and at a reasonable cost, could replace less accurate proxy measurements, such as size of the SFK, [445] and greatly improve both our understanding and management of SFK.

In addition, it may be expected that genetic susceptibility to kidney injury plays a role. Using a meta-GWAS of approximately one million participants, Wuttke *et al.* were able to compose a genetic risk score (GRS) associated with CKD based on 147 SNVs likely relevant for eGFR. [380] As a first step, this risk score could be applied to children with SFK as well, to determine its predictive ability for a reduced eGFR in this specific CKD population. If large enough cohorts of SFK patients become available, attempts could be made to discover SFK-specific eGFR-related SNVs, which could ultimately lead to the development of an SFK-specific GRS and use of this score in risk-based clinical management of SFK patients.

The ultimate goal in risk prediction would be the development of an all-inclusive prediction model. Such a model should provide a risk estimate that is accurate enough to serve as the foundation for tailored clinical management. Several clinical factors, such as the type of CSFK (*i.e.* UKA or MCDK), SFK length, and the occurrence of febrile UTI, have been suggested to be useful for risk prediction and some have even been combined in prediction models already. [181,210,446] None of these models, however, has

been externally validated, and clinical implementation is not possible as a result. To develop better prediction models, we will not only need additional data on clinical factors, but also on genetic factors and possibly on other determinants of the risk of kidney injury. In addition, any potentially useful prediction model should be deferred to a rigorous external validation procedure before implementation in daily clinical practice.

From risk prediction to risk reduction

Although accurate risk prediction and personalized care for children with SFK would already be a substantial improvement, reducing the risk of kidney injury would have an even bigger impact on patients' lives. The lower number of nephrons in these patients likely triggers an increase in single nephron GFR (SNGFR), which may be beneficial in the short term, but contributes to fluid flow shear stress to podocytes with subsequent podocyte injury. Substances reducing the SNGFR are therefore promising candidates for risk reduction. Currently, the first line therapy in children with CSFK who have hypertension or proteinuria are ACE inhibitors. These drugs reduce the intra-glomerular pressure and promote natriuresis, resulting in a lower blood pressure, reduction of urinary protein excretion, and protection of the kidney from hyperfiltration injury.[223,447,448] Moreover, ACE inhibitors were shown to have long-lasting protective effects on the kidney in animal models of CSFK, when administered for a restricted period shortly after birth.[449,450] The lower kidney volume and lower eGFR observed in one of these studies indicate that compensatory effects, such as kidney hypertrophy and increased intra-glomerular pressure, may be prevented by early ACE inhibition, providing a potentially long-lasting protection against kidney injury.[449] Additional studies are warranted, however, to get insight into long-term effects before trials in children with CSFK are opportune. When appropriate, careful selection of CSFK patients with high risk of kidney injury for such trials, for instance using prediction models, greatly increases the potential to detect meaningful benefits.

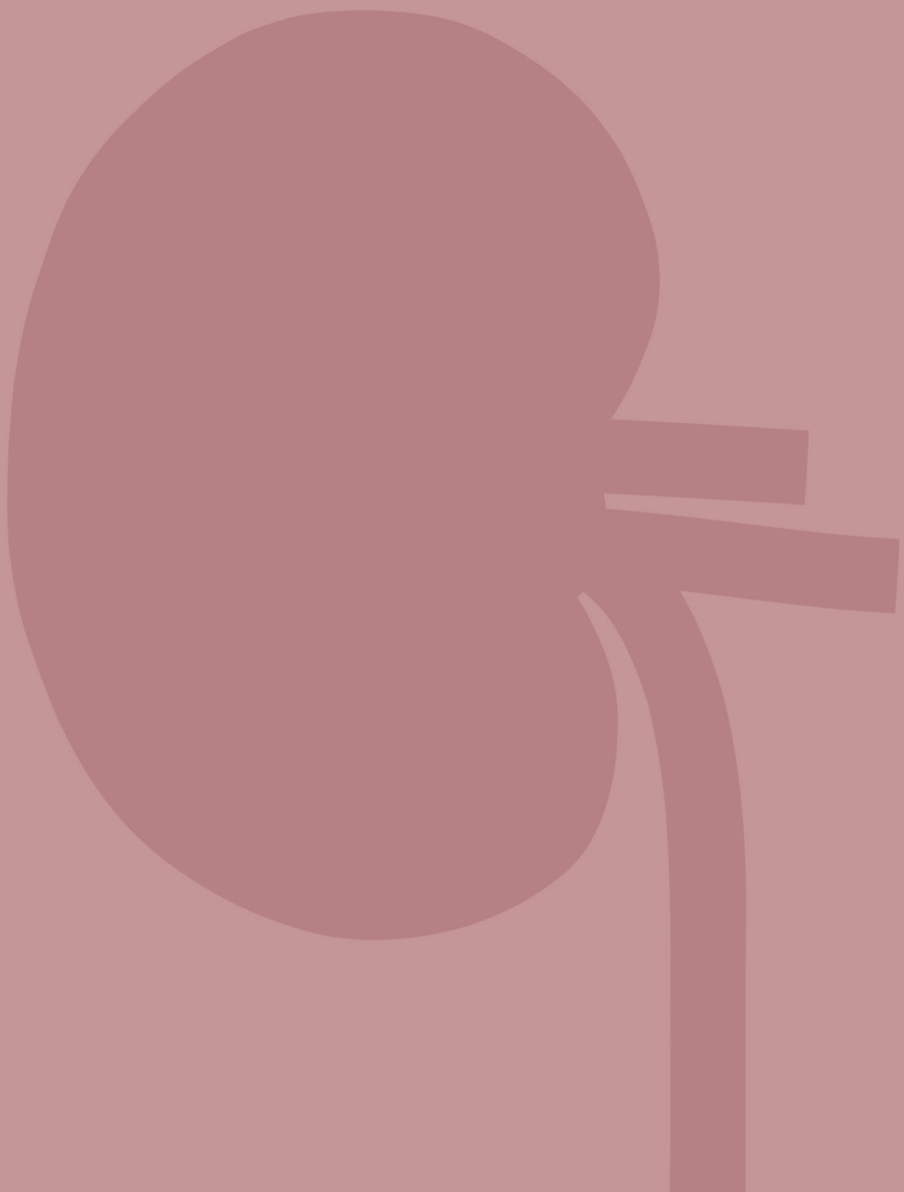
In addition, the tubular response to a reduced nephron number or altered tubuloglomerular feedback may play a role. As tubulointerstitial fibrosis correlates better with CKD progression than glomerulosclerosis,[451] tubules may be more than passive bystanders, and tubuloglomerular feedback mechanisms are known to be vital for maintaining the GFR.[2] Cells of the macula densa in the juxtaglomerular apparatus sense sodium levels, followed by arteriolar vasoconstriction or -dilatation and RAAS activation or inactivation to maintain a stable GFR.[2] In individuals with hyperfiltration, this feedback mechanism is disturbed by upregulation of the tubular sodium-glucose cotransporter 2 (SGLT2) in response to increased glomerular filtration of glucose.[452] This results in enhanced tubular reabsorption of glucose and sodium, which exposes the macula densa to a low sodium concentration despite the GFR. Tubuloglomerular feedback then leads to inappropriate dilation of the afferent arteriole and stimulation of the RAAS-system causing vasoconstriction of the efferent arteriole. The successful introduction of SGLT2 inhibitors in adult populations with diabetic nephropathy[453] and

CKD without diabetes[454] confirms the important role of the SGLT2 transporter and highlights the potential value of these drugs for other patients with hyperfiltration-mediated kidney injury. To assess whether these drugs can be beneficial for children with SFK, mechanistic studies should first investigate whether SGLT2 is upregulated in SFK as well. If so, successful application of SGLT2 inhibitors in animal models of SFK may pave the way for a subsequent introduction of these drugs in children with SFK.

Enhancing our understanding of the mechanisms behind kidney injury in children with SFK may also lead to successful development of other new drugs. Besides studying compensatory adaptations in animal models of SFK, research into epigenetic responses to reduced nephron endowment could provide insight into the mechanisms underlying long-term outcomes. Over the past years, next-generation sequencing and array-based platforms for studying epigenetic alternations have become available and the roles of several epigenetic pathways in kidney disease become clearer. In an epigenome-wide association study (EWAS) of almost 5,000 adults, differential methylation at 19 sites was associated with eGFR, and five of these sites were also associated with renal fibrosis.[455] Epigenetic therapies are already available for certain types of cancer, but are currently limited by low response rates and severe side effects.[456] In patients with Alport syndrome, a hereditary kidney disease, a phase 2 trial with an anti-MiR21 molecule (lademirsen) was underway but stopped because of lack of benefit.[457] Other drugs targeting epigenetic modifications are in development as well, but mostly focus on treatment of cancer.[458] When epigenetic mechanisms behind kidney injury in SFK become known and the specificity of epigenetic therapies is substantially improved, compensatory adaptations to an SFK may be steered towards favourable physiological responses, preventing injury before it occurs.

CONCLUDING REMARKS

Our studies indicate that the aetiology of CSFK is multifactorial and requires further research that integrates multiple aetiological mechanisms. Creating large, international collaborations with standardized data collection is vital for solving the complex aetiology of CSFK. Such studies could also provide a basis for research into the pathophysiology of kidney injury, as enhancing our understanding of the pathophysiology in children with CSFK and ASFK will ultimately allow for personalized risk prediction and – hopefully – risk reduction. Until then, knowledge on associations between clinical factors and outcomes should be used to stratify care and reduce the risk of kidney injury as much as possible.



APPENDICES

SUMMARY

A solitary functioning kidney (SFK) is a condition in which only one of the kidneys is functioning. An SFK can be congenital or the result of an acquired condition, such as a Wilms tumour or trauma. Each year, approximately 100 children in The Netherlands are born with a congenital SFK (CSFK), and a similar number of children undergoes a nephrectomy. Although this means that SFK is not a rare condition in accordance with the EU definitions for rare diseases, much is still unknown about the causes of congenital SFK. The outcomes of children living with an SFK are also debated. Several studies have been performed on this topic, and almost all showed that signs of kidney injury, such as high blood pressure, proteinuria, and a lower estimated glomerular filtration rate (eGFR), are more common in children with an SFK. It remains uncertain, however, how often these signs of kidney injury occur, and what the risk factors for kidney injury are. Therefore, this thesis addresses two major aims in two distinct parts clearly related to each other:

1) To get more insight into the genetic and environmental causes of CSFK
2) To obtain more knowledge on the long-term consequences of living with SFK from childhood

The first chapter of this thesis (**Chapter 1**) provides an overview of the current knowledge on the aetiology of CSFK and the long-term outcomes of SFK in children. This chapter also provides the research questions that were formulated to achieve the aims of this thesis and introduces the data sources that were used while conducting the studies.

Part I: Aetiology of congenital solitary functioning kidney

Several mechanisms may play a role in the aetiology of CSFK. In **Chapter 2**, we investigated the role of single nucleotide variants (SNVs) using a genome-wide association study (GWAS). In this study, 452 patients with CSFK were compared to two control groups, consisting of 669 healthy children from the AGORA data- and biobank and 5,363 adults from the Nijmegen Biomedical Study. Two variants reached genome-wide statistical significance, of which one indicated a role for *HGF* in the aetiology of CSFK. Another 30 SNVs showed suggestive significance, with an interesting variant located close to the *KCTD20* and *STK38* genes. Based on these results, we concluded that common variants likely play a role in the aetiology of CSFK, but additional studies are needed to replicate our findings because a large external validation cohort was not available during the study.

Postzygotic mutations are increasingly recognized as causes of disease, but were not yet investigated in children with CSFK. Therefore, we searched for postzygotic

mutations in two monozygotic twin pairs discordant for CSFK in **Chapter 3**. Using deep-exome sequencing, we identified several variants present in the affected but not the unaffected sibling. Validation using long-read sequencing for the three most interesting variants indicated that the variants considered to be post-zygotic in the initial sequencing run were more likely to be technical artefacts. Although we were unable to identify mosaicism in these two monozygotic twin pairs, studies in larger groups may provide a more definitive answer to the question whether postzygotic mutations are a relevant contributor to CSFK development.

Non-genetic factors also contribute to the development of congenital anomalies. We investigated the role of environmental and parental risk factors for CSFK in **Chapter 4**. To do so, we performed a large case-control study including 434 CSFK patients and 1,302 healthy controls, for whom data on exposure to risk factors during pregnancy were collected using parental questionnaires. This study was the first to identify maternal stress as a risk factor for CSFK, highlighting the potential health benefits of reducing stress in pregnant women. In addition, we confirmed previous associations with conception using IVF or ICSI, maternal smoking, and maternal infections during pregnancy. Use of folic acid supplements and a younger maternal age were associated with a reduced risk of CSFK. These results highlight the multifactorial aetiology of CSFK and should be used to substantiate efforts to perform more research into environmental risk factors for CSFK and other congenital anomalies. Furthermore, knowledge on the risk factors for CSFK should be used to optimize advice given to women who are or want to become pregnant.

The last chapter of part I, **Chapter 5**, focusses on interactions between genetic and environmental risk factors. Although gene-environment interactions have been identified for other congenital anomalies, their role in the aetiology of CSFK was still unknown. We combined data from the GWAS reported in Chapter 2 and the study on environmental risk factors in Chapter 4 to fill this knowledge gap. In the case-control study, 381 CSFK patients and 598 healthy controls could be included. We first selected the SNVs that were statistically significantly associated with one of the six selected environmental risk factors independently. Gene-environment interaction analyses were performed for these SNVs in a second step. One SNV (rs3098698), which results in lower *ARSB* expression, showed a statistically significant interaction with maternal overweight. Two other interactions (between rs11264047 and maternal smoking and between rs367873445 and maternal overweight) were not statistically significant, but could be explained biologically as well. Our results indicate that gene-environment interactions may contribute to CSFK aetiology. They also show the importance of studies combining data on genetic and environmental risk factors for an integral understanding of the aetiology.

When combining the results of all studies described in part I, it becomes clear that CSFK is a multifactorial disorder, for which large, collaborative research efforts are indispensable.

Part II: Outcomes and management of children with solitary functioning kidney

In part II, we focussed on the consequences of living with SFK and optimizing the clinical management of these patients. For **Chapter 6**, the available literature was scrutinized to formulate recommendations on the clinical management of children with CSFK. In an initial clinical evaluation soon after birth, it is important to verify the antenatally suspected diagnosis, to identify children who may need an intervention shortly afterwards, and to predict the future risk of kidney injury. We concluded that the available evidence is sufficient to recommend ultrasound as the tool to confirm the diagnosis. To identify children who may need an intervention, no evidenced-based approach could be extracted from the literature. Until more research is available, we recommend watchful waiting in most children with CSFK, unless severe dilatation is seen on ultrasound. The risk of kidney injury is also difficult to predict. Although several studies have been conducted on this topic, the rates of injury varied widely between studies and risk factors remained insufficiently clear. Therefore, we recommended structural long-term follow-up in children with CSFK until more knowledge on the risk of and risk factors for kidney injury in these children is available.

The long-term consequences of living with an acquired SFK are also largely unknown and warrant further study. We took up this gauntlet ourselves by performing a systematic review and meta-analysis on kidney injury in children with acquired SFK, which is described in **Chapter 7**. With a systematic search of MEDLINE and EMBASE, we identified 53 studies that met the inclusion criteria. Meta-analysis of these studies showed that high blood pressure, proteinuria and a lower eGFR were present in 15%, 15% and 12% of included patients, respectively. No difference was observed between children that underwent nephrectomy because of a congenital anomaly or because of a malignancy, which indicates that this factor cannot be used to stratify follow-up. Our review indicates, however, that outcomes of children after nephrectomy are not systematically reported in the literature, which impedes drawing definitive conclusions. Improving the registration and reporting of outcomes is vital to improve care for these patients based on evidence-based recommendations.

In **Chapter 8**, we summarize factors that can be used for risk stratification of children with SFK, and provide our vision on how to further tailor the clinical management of these patients based on the individuals patient's needs. Factors that have already been investigated thoroughly and are readily usable in clinical practice include the size of the SFK, the presence of additional congenital anomalies of the kidney and urinary

tract, the occurrence of urinary tract infections, and factors impeding optimal kidney development such as premature birth or dysmaturity. It may be expected that other factors, such as the cause of the SFK, genetic risk alleles, and additional hits during childhood will be added to this list in the future. Furthermore, risk factors should be combined in a prediction model to allow for quantification of an individual patients' risk of kidney injury. Development and validation of such a model will only be possible using information on large numbers of patients through collaborative efforts of research groups.

Chapter 9 describes how we created a large multicentre cohort to contribute to the knowledge on the risk of and risk factors for kidney injury in children with SFK. We managed to include 944 patients from 36 hospitals in The Netherlands, and acquired data about their follow-up for a median of 13 years. Survival analyses showed that signs of kidney injury were present in 75% of children with congenital SFK and in 80% of children with acquired SFK at 18 years of age. More severe signs of injury were present in 39% and 37% of children with congenital and acquired SFK, respectively. Using Cox proportional hazards models, we found that kidney agenesis as cause of the SFK, anomalies in the SFK, and high BMI at last follow-up were associated with a higher risk of kidney injury. These results substantiate our recommendation of a thorough and long-term follow-up. Unfortunately, the associations between risk factors and outcomes were not strong enough to construct a prediction model, for which more data remain needed. Future research should also further investigate the differences between risk factors for proteinuria and high blood pressure on one hand versus those for a lower eGFR on the other hand.

In the general discussion presented in **Chapter 10**, the results from our studies are put into a broader perspective. First, a critical appraisal of the methods of the studies is provided, in which choices on study design, study populations, and definitions are explained. Furthermore, several aspects of data quality are discussed, including their potential implications for our findings. Based on our results in part I, we argue that the aetiology of CSFK is multifactorial. We also provide guidance on how knowledge on the aetiology of CSFK can be increased and how this may benefit patients and their parents. We also make the case for additional support of aetiological research and envision how novel techniques and integrative approaches can be incorporated to advance our understanding of CSFK development. The results in part II of this thesis can be used to tailor clinical management of all patients with SFK. We increased the knowledge on the risk of kidney injury in SFK patients by reporting on the largest cohort in the world so far, and provided guidance on how to work towards better risk estimates. Furthermore, we emphasize the importance of accurate risk prediction and offer tools to work towards that goal. Lastly, we outlined how additional insight into the pathophysiology of kidney injury may be used to reduce the risk thereof for children with SFK.

NEDERLANDSE SAMENVATTING

De meeste kinderen worden geboren met twee functionerende nieren, die van belang zijn voor onder andere het uitscheiden van afvalstoffen en het op peil houden van de bloeddruk. Jaarlijks worden echter ongeveer 100 kinderen in Nederland geboren met maar één nier: een mononier. Daarnaast moeten ongeveer net zoveel kinderen een operatie ondergaan waarbij een nier wordt verwijderd (zogeheten verworven mononier), bijvoorbeeld vanwege een aanlegstoornis van die nier of vanwege een tumor. Hoewel er dus een aanzienlijk aantal kinderen leeft met een mononier, weten we nog maar weinig over de oorzaken en gevolgen hiervan. De studies die gekeken hebben naar lange termijn gevolgen laten zien dat tekenen van nierschade zoals een hoge bloeddruk, eiwitverlies in de urine (proteïnurie), en een verlaagde nierfunctie (eGFR) vaker voorkomen dan bij kinderen die twee werkende nieren hebben. Het is echter niet precies duidelijk hoeveel vaker en wat de risicofactoren voor nierschade zijn. Daarom richt dit proefschrift zich op twee belangrijke doelen:

1) Het verkrijgen van inzicht in de verschillende oorzaken van een aangeboren mononier
2) Meer kennis verwerven over de lange termijn gevolgen van het leven met een mononier vanaf de kindertijd

In **hoofdstuk 1** van dit proefschrift wordt een overzicht van de huidige kennis over de oorzaken en gevolgen van een mononier gegeven. Daarnaast worden de onderzoeksvragen genoemd die zijn geformuleerd om bovenstaande doelen te behalen. Tot slot worden de bronnen van data geïntroduceerd die gebruikt werden voor de uitgevoerde studies.

Deel 1: Het ontstaan van een aangeboren mononier

Verschillende mechanismes kunnen bijdragen aan het ontstaan van een aangeboren mononier. In **hoofdstuk 2** onderzochten we de rol van kleine genetische veranderingen (SNVs). Hiervoor gebruikten we een genoom-brede associatie studie, waarin we 452 patiënten met een aangeboren mononier vergeleken met 669 gezonde kinderen uit de AGORA data- en biobank en 5363 volwassenen die meededen aan de Nijmegen Biomedical Study. In onze studie vonden we twee kleine genetische veranderingen die statistisch significant waren. Eén daarvan kon gelinkt worden aan het *HGF* gen, dat mogelijk een rol speelt in de ontwikkeling van de nier. Daarnaast vonden we dertig SNVs die mogelijk een rol spelen maar niet duidelijk genoeg naar voren kwamen. De meest interessante hiervan wijst op een mogelijke rol voor de genen *KCTD20* of *STK38*. Uit de resultaten van dit onderzoek concludeerden we dat kleine genetische veranderingen waarschijnlijk kunnen bijdragen aan het ontstaan van een mononier, maar andere studies zijn nodig om onze resultaten te bevestigen.

Er wordt steeds meer bekend over de rol die veranderingen in het erfelijk materiaal die zijn ontstaan na het samensmelten van de eicel en de zaadcel (zogeheten postzygote mutaties) kunnen spelen bij het ontstaan van ziektes. Deze zouden ook kunnen bijdragen aan het ontstaan van een aangeboren mononier, hoewel dit nog niet goed is onderzocht. Daarom probeerden we in **hoofdstuk 3** postyzgote mutaties aan te tonen bij twee eeneiige tweelingen waarvan een van de kinderen een mononier heeft. Door het erfelijk materiaal een groot aantal keer af te lezen ("deep exome sequencing") konden we verschillende mutaties vinden die voorkwamen bij het kind met de mononier maar niet bij de gezonde tweelingbroer of -zus. Toen we deze bevindingen probeerden te valideren via een andere techniek ("long read sequencing") bleken ze echter op technische artefacten te berusten. Vergelijkbare studies in grotere groepen patiënten met een mononier zouden kunnen uitwijzen of postzygote mutaties toch een rol spelen bij het ontstaan van een mononier.

Aangeboren aandoeningen kunnen niet alleen het gevolg zijn veranderingen in het erfelijk materiaal, maar ook van blootstellingen van buiten het kind zelf, zoals medicatiegebruik of leefstijl van de moeder. In **hoofdstuk 4** keken we naar dit soort blootstellingen in een studie waarbij we door ouders ingevulde vragenlijsten van 434 patiënten met een mononier vergeleken met die van 1302 gezonde kinderen. Hierbij vonden we dat stress van de moeder was geassocieerd met het ontstaan van een mononier, wat een extra reden zou moeten zijn om stress bij zwangere vrouwen zoveel mogelijk te beperken. Daarnaast vonden we dat conceptie via IVF of ICSI, roken van de moeder en het optreden van infecties tijdens de zwangerschap mogelijk een hoger risico geven op het ontstaan van een mononier. Het gebruik van foliumzuur en een jongere leeftijd van de moeder waren geassocieerd met een lager risico. Onze resultaten laten zien dat er ook niet-erfelijke factoren een rol spelen bij het ontstaan van een mononier. Daarom moeten voldoende middelen vrijgemaakt worden voor onderzoek naar de rol van omgevingsfactoren in het ontstaan van aangeboren aandoeningen zoals een mononier. De resultaten van dit soort onderzoek zouden vervolgens gebruikt moeten worden om adviezen aan vrouwen die zwanger zijn of willen worden te verbeteren.

Hoofdstuk 5 richt zich op interacties tussen erfelijke en omgevingsfactoren. Voor deze studie werden de data uit de genoom-brede associatie studie beschreven in hoofdstuk 2 en de studie naar omgevingsfactoren in hoofdstuk 4 gecombineerd. Van 381 patiënten met een mononier en 598 gezonde controles waren gegevens over erfelijke en omgevingsfactoren bekend. In onze studie testten we eerst welke SNVs geassocieerd waren met één van de zes geselecteerde omgevingsfactoren. Vervolgens werd voor deze SNVs gekeken of er sprake was van een statistisch significante interactie met de betreffende omgevingsfactor, wat bij één SNV zo bleek te zijn. Bij kinderen zonder deze SNV, zorgt overgewicht van de moeder voor een hoger risico op een mononier. Kinderen met deze SNV hadden geen hogere risico op een mononier bij overgewicht van de moeder, wat kan komen doordat deze SNV zorgt voor lagere expressie van het

ARSB gen. Twee andere interacties leken wel biologisch verklaarbaar maar kwamen niet sterk genoeg naar voren om hun rol definitief vast te kunnen stellen.

Samen laten de resultaten in deel 1 van dit proefschrift zien dat het belangrijk is om zowel genetische data als gegevens over omgevingsfactoren te verzamelen, om zo alle aspecten van het ontstaan van een mononier te kunnen onderzoeken. Hiervoor zijn grote onderzoekssamenwerkingen onmisbaar.

Deel 2: De gevolgen van het leven met een mononier

In het tweede deel van dit proefschrift wordt gekeken naar de gevolgen van het leven met een mononier en hoe deze kinderen zo goed mogelijk te begeleiden. In **hoofdstuk 6** geven we op basis van bestaande literatuur adviezen voor de controles bij kinderen met een mononier. Bij de eerste controle, die binnen een maand na de geboorte moet plaatsvinden, zijn drie dingen van belang. Allereerst moet de diagnose bevestigd worden, waarvoor een echo van de nieren een geschikte methode is. Daarnaast is het belangrijk om te achterhalen bij welke kinderen op korte termijn (chirurgisch) ingegrepen moet worden. Helaas waren er geen goede studies beschikbaar waarop we konden baseren hoe dit het beste gedaan kan worden. Ons advies is om behalve de echo geen aanvullend onderzoek te verrichten, tenzij er echografisch uitzetting van de nier of urinewegen gezien wordt of er meerdere urineweginfecties optreden. Tot slot is het ook van belang om het risico op nierschade op de lange termijn te voorspellen. Dit is echter moeilijk, omdat de resultaten van reeds gepubliceerde studies niet goed overeen komen. Daarom dienen alle kinderen met een mononier regelmatig gecontroleerd te worden op tekenen van nierschade, totdat we beter kunnen inschatten welke kinderen een hoog of laag risico op nierschade hebben.

Hoofdstuk 7 richt zich op de gevolgen van een verworven mononier. We identificeerden 53 bestaande studies en voegden de resultaten hiervan samen, om er zo achter te komen hoe vaak tekenen van nierschade voorkomen. Daarnaast keken we of de reden voor het verwijderen van een nier uitmaakte voor de lange termijn gevolgen. Een hoge bloeddruk (15%), proteïnurie (15%) en een verlaagde eGFR (12%) kwamen allemaal regelmatig voor en de reden voor het verwijderen van een nier leek hierin geen verschil te maken. Onze studie liet daarnaast zien dat niet in alle studies gerapporteerd werd hoe vaak er nierschade optreedt nadat een nier verwijderd is, waardoor het moeilijk was een definitieve conclusie te trekken. Om de zorg voor kinderen met een verworven mononier te verbeteren dient deze rapportage verbeterd te worden. Tot die tijd is het nodig om alle kinderen met een verworven mononier te blijven controleren.

We bekeken ook in de literatuur van welke factoren bekend is dat zij het risico op nierschade bij kinderen met een mononier beïnvloeden, en gaven onze visie op meer persoonsgerichte zorg voor kinderen met een mononier in **hoofdstuk 8**. De grootte van de mononier, aanwezigheid van bijkomende aangeboren aandoeningen van de

nier of urinewegen, het optreden van urineweginfecties en factoren die de nieraanleg beïnvloeden (zoals vroeggeboorte of laag geboortegewicht) kunnen nu al gebruikt worden voor een eerste risicoschatting. Waarschijnlijk kunnen ook andere factoren, zoals de oorzaak van de mononier, het genetische risicoprofiel en factoren die nierschade geven na de geboorte (zoals nefrotoxische medicatie) in de toekomst gebruikt worden. Idealiter zouden al deze factoren in een voorspellend model gecombineerd worden, zodat voor iedere patiënt een persoonlijke risicoschatting gemaakt kan worden. Het ontwikkelen en valideren van een dergelijk model is echter alleen mogelijk als informatie beschikbaar is over grote aantallen patiënten door samenwerking tussen verschillende onderzoeksgroepen.

In **hoofdstuk 9** beschrijven we de SOFIA studie: een cohort van 944 kinderen met een mononier verzameld in 36 Nederlandse ziekenhuizen. Zij werden gemiddeld 13 jaar gevolgd waaruit geschat kon worden dat 75% van de kinderen met een aangeboren mononier en 80% van de kinderen met een verworven mononier minstens één teken van nierschade vertoont op een leeftijd van 18 jaar. Bij bijna 40% van deze patiënten zal sprake zijn van ernstige tekenen van nierschade. Risicofactoren voor nierschade waren agenesie als oorzaak van de mononier, bijkomende aangeboren aandoeningen in de mononier en een hoge BMI tijdens de kindertijd. Deze getallen onderbouwen ons advies om alle kinderen met een mononier te blijven controleren. Meer gegevens blijven nodig om te voorspellen wie een hoog risico loopt en wie een laag risico, omdat de huidige risicofactoren niet onderscheidend genoeg waren. Vervolgstudies zouden ook het verschil in risicofactoren voor hoge bloeddruk en proteïnurie in vergelijking met een verlaagde eGFR verder moeten onderzoeken.

Tot slot wordt in de discussie in **hoofdstuk 10** besproken hoe de resultaten van onze studies in breder perspectief gezien moeten worden. Allereerst hebben we kritisch de methodologie van onze studies beschouwd en onderbouwen we onze keuzes op het gebied van de studieopzet, studiepopulaties en de gebruikte definities. Daarnaast gaan we in op de kwaliteit van de gebruikte data en bespreken we de mogelijke gevolgen hiervan voor onze bevindingen. Op basis van de resultaten in deel 1 van dit proefschrift betogen we vervolgens dat het ontstaan van een aangeboren mononier een multifactorieel proces is. We schetsen hoe kennis over dit proces verhoogd kan worden en waarom dat van belang is voor patiënten en hun ouders. We pleiten verder voor meer steun voor onderzoek naar de oorzaken van aangeboren aandoeningen en geven onze visie op het integreren van bestaande en nieuwe technieken om ons begrip van het ontstaan van een aangeboren mononier verder te verhogen. De bevindingen in deel 2 van dit proefschrift kunnen gebruikt worden om patiënten met een mononier zo goed mogelijk te behandelen. De resultaten van de SOFIA studie dragen bij aan de kennis over het risico op nierschade en wat de risicofactoren hierop zijn. Bovendien beschrijven we hoe belangrijk individuele risicoschattingen zijn en hoe deze vormgegeven kunnen worden. Tot slot geven we aan hoe begrip van het ontstaan van nierschade bij kinderen met een mononier kan helpen om het risico hierop zo veel mogelijk te beperken.

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RESEARCH DATA MANAGEMENT

The studies in the current thesis were conducted in accordance with the principles of the Declaration of Helsinki. The data were collected during regular clinical care and via procedures specifically for research purposes, which the Regional Committee on Research Involving Human Subjects (CMO Radboudumc) approved before the studies were initiated. Eligible individuals and/or their legal caregivers were informed about our studies using patient information folders. They were given ample time to consider their participation and had the opportunity to ask questions about the study to their own physician, members of the study team, and independent physicians. If they agreed to participate, written informed consent was obtained prior to inclusion. The informed consent forms are securely archived at the Radboud university medical center.

Several sources of data were used for the studies in this thesis. For chapter 2, DNA was isolated from blood or saliva samples stored in the AGORA data-and biobank, which is part of the Radboud Biobank. Use of the Radboud Biobank ensures safe and protocolized storage, facilitating reuse of data in line with the FAIR principles. Genotyping data were stored on a server of the Radboud university medical center. For chapter 3, DNA was isolated and analysed at the Department of Genetics of the Radboud university medical center, and results were stored on a server of that department. The data for chapter 4 were obtained using parental questionnaires, which were available on paper and online. Paper questionnaires were labelled with a study code and entered in the online data management system CastorEDC. The pseudonymized paper questionnaires were then stored in the archive of the Department for Health Evidence at the Radboud university medical center. Digital questionnaires were coded upon creation and send to participants directly from CastorEDC. After completion, they were automatically locked to prevent changes and/or illegitimate access. For chapter 9, clinical information was collected from electronic medical records in the participating hospitals using an electronic case record form in CastorEDC. All CastorEDC databases are accessible for members of the research team only.

The remaining chapters were based on either a combination of the data sources described above (chapter 5) or on literature. All databases and scripts used for data cleaning and analysis are stored on internal servers of the Department for Health Evidence (H:/REPRO/AGORA/AGORA-kindernefrologie_Sander). The privacy of study participants is ensured by using pseudonymized study codes, with a key file stored in a separate location to which only selected members of the research team have access. Data in the AGORA data- and biobank are stored for 25 years or indefinitely, depending on the permission given by the participant. These data are available for researchers after approval by the AGORA project board and, if necessary, the relevant committee on research involving human subjects. To increase findability for external researchers, the AGORA data- and biobank is registered in ERDRI.dor, Orphanet, and BBMRI-NL. Other data will be stored for 15 years after conclusion of the studies and are available from the corresponding author upon reasonable request.

LIST OF PUBLICATIONS

In this thesis

Groen in 't Woud S, van Gelder MMHJ, van Rooij IALM, Feitz WFJ, Roeleveld N, Schreuder MF*, and van der Zanden LFM* for the SOFIA study group. The role of gene-environment interactions in the aetiology of congenital solitary functioning kidney. Nephrol Dial Transplant. 2023 (in press).

Groen in 't Woud S, Roeleveld N, Westland R, Renkema KY, Steffens MG, Gracchi V, Lilien MR, van Wijk JAE, Feitz WFJ, Schreuder MF*, and van der Zanden LFM* for the SOFIA study group. Uncovering risk factors for kidney injury in children with a solitary functioning kidney. Kidney Int. 2023;103(1):156-65.

Groen in 't Woud S, Roeleveld N, van Rooij IALM, Feitz WFJ, Schreuder MF*, and van der Zanden LFM* for the SOFIA study group. Environmental and parental risk factors for congenital solitary functioning kidney - a case-control study. Pediatr Nephrol. 2023 *(in press)*.

Groen in 't Woud S, Maj C, Renkema KY, Westland R, Galesloot T, van Rooij IALM, Vermeulen SH, Feitz WFJ, Roeleveld, N, Schreuder MF, and van der Zanden LFM for the SOFIA study group. A Genome-Wide Association Study into the Aetiology of Congenital Solitary Functioning Kidney. Biomedicines. 2022;10(12).

Groen in 't Woud S*, Gobino A*, Roeleveld N, van den Heuvel LPWJ, Feitz WFJ, van der Zanden LFM*, and Schreuder, MF*. Kidney injury rates after unilateral nephrectomy in childhood - a systematic review and meta-analysis. Nephrol Dial Transplant. 2022;37(12):2457-73.

Groen in 't Woud S, van der Zanden LFM, and Schreuder MF. Risk stratification for children with a solitary functioning kidney. Pediatr Nephrol. 2021;36(11):3499-503.

Groen in 't Woud S, Westland R, Feitz WFJ, Roeleveld N, van Wijk JAE, van der Zanden LFM, and Schreuder, MF. Clinical Management of Children with a Congenital Solitary Functioning Kidney: Overview and Recommendations. Eur Urol Open Sci. 2021;25:11-20.

Other publications

van der Zanden LFM, **Groen in 't Woud S**, van Rooij IALM, Quaedackers JSLT, Steffens M, de Wall LLL, Schreuder, MF Feitz WFJ, and Roeleveld N. Maternal risk factors for posterior urethral valves. Front Pediatr. 2023; 11:1110117

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Groen in 't Woud S, Reuver N, Feitz WFJ, Quaedackers JSLT, Nijman R, Steffens M, de Wall LLL, Roeleveld N, Schreuder MF, and van der Zanden LFM. Compensatory Hypertrophy in Paediatric Patients with a Unilateral Ureteropelvic Junction Obstruction. Eur Urol Open Sci. 2021;34:10-6.

Groen in 't Woud S, Renkema K, Schreuder M, Wijers C, van der Zanden L, Knoers N, Feitz WFJ, Bongers EMHF, Roeleveld N, and van Rooij IALM. Maternal risk factors involved in specific congenital anomalies of the kidney and urinary tract: A case-control study. Birth Defects Res A Clin Mol Teratol. 2016;106(7):596-603.

Groen in 't Woud S, van Rooij IA, van Gelder MMHJ, Olney RS, Carmichael SL, Roeleveld N, and Reefhuis J for the National Birth Defects Prevention Study. Differences in risk factors for second and third degree hypospadias in the national birth defects prevention study. Birth Defects Res A Clin Mol Teratol. 2014;100(9):703-11.

*Authors contributed equally

PHD PORTFOLIO

Department	Department for Health Evidence
PhD period	01/06/2018 – 28/02/2023
PhD Supervisor(s)	prof. dr. W.F.J. Feitz, prof. dr. M.F. Schreuder
PhD Co-supervisor(s)	dr. L.F.M. van der Zanden, dr. ir. C.J.A. Roeleveld

Training activities	**Hours**
Courses	
Introduction day - Radboudumc (2018)	6.00
Introduction course for PhD candidates - RIHS (2018)	15.00
Hands on; genome association analyses – master Biomedical Sciences (2018)	84.00
Projectmanagement voor Promovendi - Radboud University (2019)	45.00
eBROK course - Radboudumc (2019)	26.00
Modern Methods of Data Analysis – postmaster University Utrecht (2019)	126.00
Causal Inference - master Biomedical Sciences (2019)	84.00
Scientific integrity – Radboudumc (2019)	20.00
Master for Junior Course – IPNA-ESPN (2020)	14.00
Reproduction Epidemiology and Toxicology - master Biomedical Sciences (2020)	84.00
Presentation Skills – Radboud University (2021)	28.00
R Course - Radboudumc (2021)	14.00
Grant Writing and Presenting for Funding Committees – Radboud University (2021)	18.00
Career Workshop – Radboud University (2022)	28.00
"Modern Methods of Data Collection" - master Biomedical Sciences (2022)	84.00
Seminars	
Radboudumc workshops (2018)	8.00
Radboudumc Grand rounds (2018)	2.00
aHUS and complement - Radboudumc dept. Paediatric Nephrology (2019)	1.00
Radboud Research Rounds (2019)	3.00
WEON preconference course (2019)	4.00
Genetics of Birth Defects (2020)	1.00
Seminars Radboudumc dept. for Health Evidence (2020)	6.00
Big Data in Renal Genetics (2020)	1.00
Radboud Integrity Round (2021)	1.00
Renal Disorders Theme Lunch Meetings (2021)#	10.00
RIHS seminars/webinars (2022)	4.00
Radboud Integrity Round (2022)	1.00

Conferences	
New Kids on the Block symposium - Nierstichting (2018)	8.00
New Frontiers symposium - Radboudumc (2018)	16.00
RIHS PhD retreat (2018)#	21.00
Winterschool - Nierstichting (2019)	28.00
Netherlands Epidemiology (WEON) congress (2019)	21.00
Dutch Nephrology Days (2020)#	7.00
Annual meeting of the European Society for Paediatric Nephrology (ESPN) (2021)#	35.00
Dutch Nephrology Days (2021)#	7.00
Kidney Week - American Society of Nephrology (ASN) (2021)#	21.00
RIHS PhD retreat (2022)	14.00
Nederlandse Vereniging voor Kindergeneeskunde (NVK) congress (2022)#	14.00
Netherlands Epidemiology (WEON) congress (2022)^^#	28.00
Annual meeting of the European Society for Paediatric Urology (ESPU) (2022)#	21.00
Annual meeting of the European Society for Paediatric Nephrology (ESPN) (2022)#	35.00
International Pediatric Nephrology Association (IPNA) congress (2022)#	35.00
Other	
Journal club dept. for Health Evidence - Measurement in Medicine (2018)	28.00
Journal club dept. of Paediatric Nephrology (2018)	14.00
Journal club dept. for Health Evidence – History of Epidemiology: past – present – state of the art (2019)	28.00
Journal club dept. for Health Evidence – Systematic Reviews (2019)	28.00
Journal club dept. of Paediatric Nephrology (2019)	28.00
Journal club dept. for Health Evidence – Clinical Prediction Models (2020)	28.00
Journal club dept. for Health Evidence – Risk Assessment (2020)	28.00
Journal club dept. for Health Evidence – Innovative Study Designs (2021)	28.00
Journal club dept. for Health Evidence – Genetic Epidemiology (2021)	28.00
Journal club dept. for Health Evidence – Pharmacoepidemiology (2022)	28.00

Teaching activities	**Hours**
Lecturing	
Human embryology in perspective	24.00
Preparatory course for research internship - master Medicine (2019-2020)	80.00
Coordinator & teacher "*Meten is weten*" - bachelor Medicine/Biomedical Sciences (2021)	40.00
Supervision of internships / other	
Supervision of Grant proposal - bachelor Medicine/Biomedical Sciences (2018-2022)	135.00
Daily supervision of internship - bachelor Biomedical Sciences (2020)	32.00
Daily supervision of research internship - master Medicine (2 students) (2020-2021)	52.00
Total	**1,658.00**

^Poster presentation ^^Awarded with best poster prize #Oral presentation.

CURRICULUM VITAE

Sander Groen in 't Woud was born on May 13, 1991 in Zevenbergen. After obtaining his pre-university degree at the Erasmus College in Zoetermeer in 2009, he travelled through Australia for six months before starting his medical studies at the Radboud University in Nijmegen. During his bachelor, he participated in the *Honours Programme Medical Sciences*, which consisted of several courses and small internships at the Radboud university medical center, as well as a longer internship at the Centers of Disease Control and Prevention in Atlanta, GA, USA. His research in the USA focussed on risk factors for hypospadias and sparked his interest in epidemiology and research into congenital anomalies.

After returning to Nijmegen, he continued doing research at the Department for Health Evidence, which led to an article on risk factors for congenital anomalies of the kidney and urinary tract (CAKUT). An important finding was that risk factors differed among CAKUT subtypes, which led to the recommendation to focus on specific CAKUT subtypes in future research. After obtaining his medical degree in 2017, Sander worked as resident internal medicine at Bernhoven hospital for six months, before starting his PhD project on solitary functioning kidneys in children.

During his PhD project, Sander also specialized in Epidemiology with a tailored trajectory consisting of several courses in order to qualify for an Epidemiology B registration. His newly obtained knowledge proved very useful for the studies in his PhD project, which focussed on the aetiology and prognosis of a solitary functioning kidney in children. For his research, he initiated collaboration with 36 hospitals throughout The Netherlands to recruit almost 1,000 patients with a solitary functioning kidney (the SOFIA study). Supported by an Erasmus grant, he made two visits to Bonn to work together with experts on a genome-wide association study, and he obtained additional funding from Stichting Urologie 1973 for a novel study into discordant monozygotic twins. He also gave lectures and supervised students during projects and internship at the Medical Faculty of the Radboud University.

After completion of his PhD project, Sander started as resident in Clinical Genetics at the Radboud university medical center and continued doing research on solitary functioning kidney by creating an international collaboration. He lives in Malden with his wife Milou and their son Teije.

DANKWOORD

Allereerst wil ik hier alle deelnemers aan de SOFIA studie bedanken. Het was geweldig om te zien hoe betrokken vele van jullie waren bij de studie en hoe graag jullie wilden helpen om deze tot een succes te maken. Zonder jullie medewerking was het nooit gelukt om dit proefschrift te schrijven, waarvoor ik jullie erg dankbaar ben.

Als promotor is het bij uitstek je taak om de grote lijn te bewaken en te zorgen dat er uiteindelijk een boekje komt. **Wout**, ik kan niet anders zeggen dan dat die taak jou op het lijf is geschreven. Al vanaf het eerste jaar stimuleerde je mij om te denken aan het eindproduct en mijn verdere carrière, wat mij zeker heeft geholpen om mijzelf niet te verliezen in de acute vragen. Daarnaast had je altijd een simpele (en meestal ook doeltreffende) oplossing voor wat mij grote uitdagingen leken. Bedankt voor je nuchtere advies en het is een groot verlies voor AGORA dat je volgend jaar met emiraat gaat.

Al sinds ik begon met onderzoek heb ik mij verbaasd over de veelzijdigheid van **Michiel**. Je hebt mij niet alleen erg geholpen met al mijn klinische vragen, maar kon ook vaak precies de goede vragen stellen bij genetische, epidemiologische en ethische dillema's. Gelukkig betrapten Loes en ik je er soms nog op dat je iets verkondigde wat net niet helemaal klopte en konden we concluderen dat zelfs jij uiteindelijk maar een mens bent. Maar niet alleen inhoudelijk was je een hele fijn begeleider: je nam ook graag een mentorrol op je en was je er altijd als eerste bij om te benadrukken dat het allerbelangrijkste een goede werk-privé balans is. Inmiddels ben je meer dan verdiend benoemd tot hoogleraar en het voelt als een groot voorrecht om jou als promotor te hebben.

Het werk in mijn proefschrift was zo veel minder goed geweest zonder het scherpe oog voor structuur en detail van **Loes**. Hoewel ik soms wel kon balen als ik weer een manuscript vol met suggesties terug kreeg van jou, werd het altijd veel beter leesbaar als ik ze verwerkt had. Je was daarnaast een hele fijne en laagdrempelige begeleider bij wie ik voor alles terecht kon en met wie het ook op de afdelingsuitjes en andere activiteiten altijd gezellig was. De laatste jaren vormden we als AGORA-coördinatie duo een mooi team waarin we elkaar uitstekend aanvulden en ik hoop dat we dat nog lang kunnen voortzetten.

Nel, jij bent misschien wel de aanstichter van dit hele proefschrift, want als ik via het Honours programma niet bij jou en Iris terecht was gekomen had ik de reproductie epidemiologie en klinische genetica waarschijnlijk nooit ontdekt. Daarnaast ben je met recht mijn epidemiologische geweten; het leek wel of je op al mijn vragen over studieopzet, analyses en interpretatie een antwoord had. Het is mooi om jouw passie voor onderwijs en het opleiden van jonge collega's te zien en ik voel me bevoorrecht om een van de onderzoekers onder jouw vleugels te zijn geweest.

Geachte leden van de manuscriptcommissie **professor van Zelst-Stams, professor Nijenhuis** en **professor Cornel**, ik wil u hartelijk bedanken voor het kritisch lezen van mijn proefschrift en ik kijk er naar uit om het tijdens de verdediging met u te bediscussiëren. Voor een kritische blik op mijn plannen en voortgang kon ik ook altijd terecht bij mijn mentor **professor Jack Wetzels**. Jouw vragen hebben mij erg geholpen om te komen tot scherp geformuleerde onderzoeksvragen en gestimuleerd om mijn eigen resultaten kritisch te blijven beschouwen.

Een van de hoogtepunten van mijn promotietraject was de samenwerking met alle kinderartsen, kindernefrologen en (kinder)urologen in de verschillende ziekenhuizen die meededen aan de SOFIA studie. Ik ben jullie enorm dankbaar voor de hoeveelheid tijd en energie die jullie geïnvesteerd hebben in de studie en het was nooit zo'n succes geworden zonder jullie. Daarnaast heb ik ervan genoten om met jullie te sparren over het beleid bij kinderen met een mononier en ik hoop dat de resultaten van de studie kunnen bijdragen aan het verbeteren van de zorg voor deze patiënten.

De grote kracht van AGORA en ArtDECO is de collegiale groep waarin we samen het onderzoek naar en de zorg voor kinderen met een aangeboren aandoening vooruit proberen te brengen. Dank aan alle betrokkenen voor jullie inzet en de fijne sfeer waarin we samen gewerkt hebben. Het liefst zou ik jullie allemaal bij naam benoemen. **Rik,** ik hoop net zo'n goede arts en onderzoeker te worden als jij en hoop dat ik je dan over een paar jaar ook nog op de racefiets kan verslaan. We moeten nog steeds een keer de klimmetjes rondom Nijmegen opzoeken. **Kirsten,** de tripjes naar Utrecht voor dateverzameling in het UMCU waren altijd een productief en gezellig uitje, dank daarvoor!

Met het grote aantal deelnemers was er een heleboel data die verzameld, ingevoerd, gecleand en geanalyseerd moest worden, wat mij nooit alleen gelukt was. **Birgit**, je hebt Loes en mij ontzettend geholpen binnen AGORA en was ook nog eens heel gezellig om in het team te hebben. Succes met het afronden van je master en je verdere carrière. Al jarenlang is **Ursula** de vaste kracht op wie we binnen AGORA kunnen bouwen en jouw bijdrage aan dit en vele andere AGORA projecten mag niet onderschat worden. Verder wil ik ook graag stagiaires **Benthe, Michelle, Nieke** en **Maaike** bedanken voor hun hulp en kritische vragen, het was een groot plezier om jullie te begeleiden. **Benthe**, erg leuk dat je terug komt om je PhD binnen onze groep te doen, ik kijk er naar uit om dit onderzoek samen voort te zetten. Veel dank ook aan student-assistenten **Vera** en **Tess** voor al het invoerwerk dat jullie gedaan hebben.

Dear **Carlo**, you have taught me many things about GWAS and introduced me to a world of programming and data science that was fascinating to discover. I had a wonderful and productive time during my visits to Bonn and I am very grateful that you were willing to host me at your institution. My decision to pursue a career in clinical genetics was

definitely supported by the enthusiastic ideas of **Alex Hoischen**. Thank you for your help and inspiration for our twin studies.

Het was een groot genoegen om op zo'n collegiale en gezellige afdeling als Health Evidence aan mijn proefschrift te kunnen werken. Helaas zorgde COVID voor een stuk minder onderling contact, maar ook daar wisten we als SOA wel iets op te verzinnen. **Iris**, jij was mijn eerste onderzoeksbegeleider en hebt je enthousiasme voor de epidemiologie volledig op mij overgebracht. Het is bewonderenswaardig met hoeveel energie jij je inzet voor het onderwijs, wat je ook nog weet te combineren met onderzoek en de nodige sportiviteit! **Marleen**, in jouw streven naar excellent onderzoek en het toepassen van nieuwe epidemiologische technieken ben je een goed voorbeeld voor mij en vele studenten. Met de PRIDE studie heb je een prachtige studie neergezet die hopelijk nog vele mooie publicaties gaat opleveren. En hoewel geen HEV-collega wil ik ook graag **Jennita** in dit rijtje noemen. Ik vond en vind het nog steeds heel bijzonder hoe gastvrij je mij hebt opgevangen in Atlanta. Niet alleen heb ik tijdens mijn stage veel van je geleerd op wetenschappelijk gebied, maar dankzij jou en je gezin heb ik ook nog eens een hele leuke tijd in de VS gehad. Ook jij hebt er met je passie voor onderzoek naar aangeboren aandoeningen aan bijgedragen dat ik dit veld in ben gegaan. Tot slot was mijn PhD nooit zo leuk geweest zonder alle huidige en voormalige mede-PhD's bij Health Evidence, in het bijzonder **Scott, Tamara, Romy, Liesbeth, Rana, Elsemieke, Jim, Ivy,** en **Jasper**. **Scott**, jij was als kamergenoot degene met wie ik al mijn hoogtepunten en frustraties kon delen. Daarnaast bleek het uitermate handig om altijd een statisticus in de buurt te hebben. Fijn dat je mijn paranimf wilt zijn en ik hoop intussen genoeg statistische kennis van je opgedaan te hebben om het tijdens mijn verdediging op eigen kracht te redden.

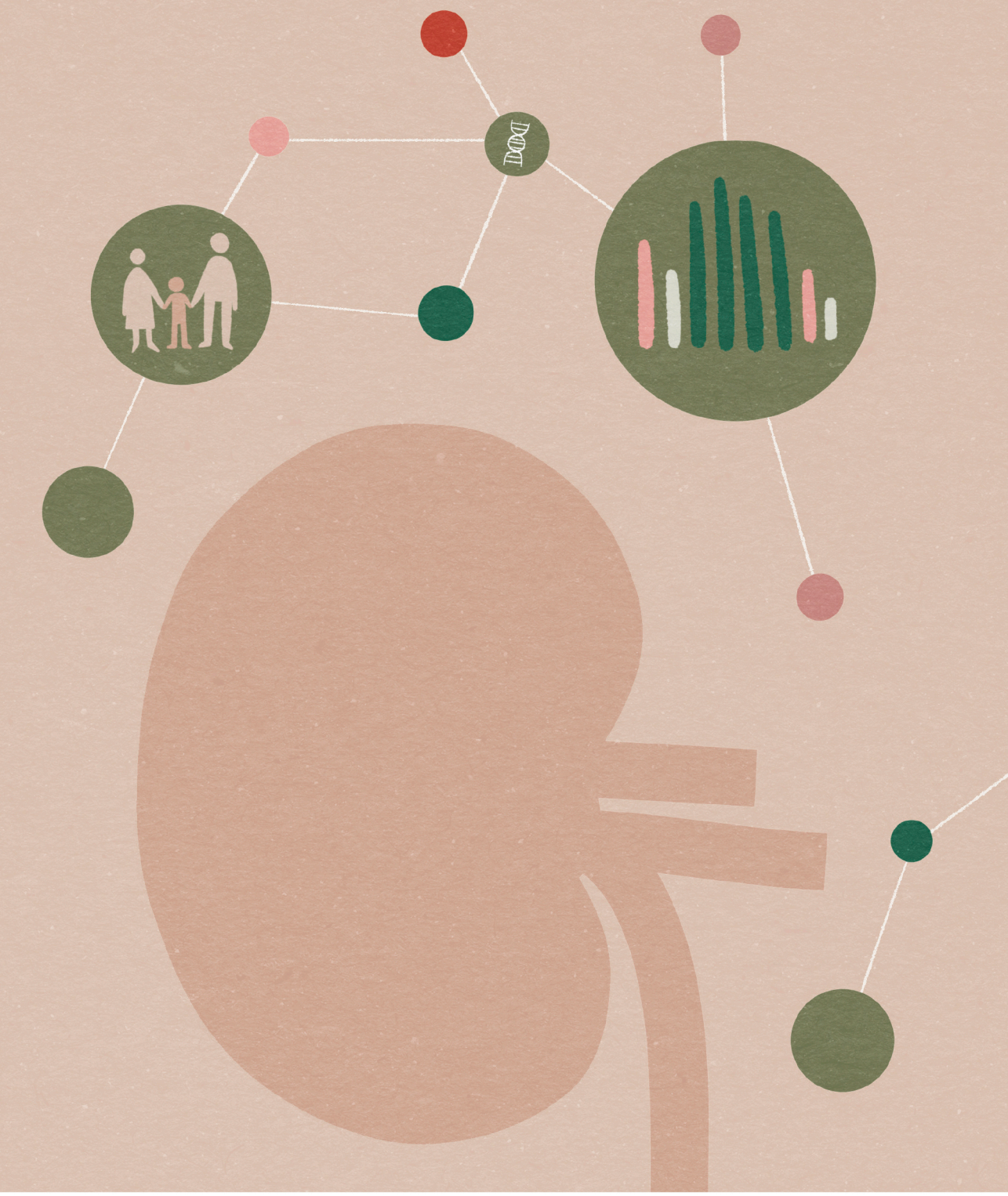
Ook alle collega's van de afdeling Kindernefrologie wil ik graag bedanken voor zowel hun hulp bij de studie als de goede vragen bij de research meetings. **Linda, Nicole, Marlies, Jacqueline, Femke, Alessandro, Anne, Bert, Kioa, Susan, Romy, Marloes, Jitske** en de anderen die ik nu ongetwijfeld vergeet: het was ontzettend fijn jullie collega te zijn. Inmiddels ben ik op de afdeling Klinische Genetica in minstens net zo'n warm bad terecht gekomen. Bedankt allemaal voor de gezelligheid, collegialiteit en de steun bij het afronden van mijn proefschrift. **Carlo**, ondanks dat je niet inhoudelijk bij mijn proefschrift betrokken was, was je gelijk bereidt mij te helpen om patiënten hiervoor te counselen. Als begeleider van de basisartsen bij de Klinische Genetica heb jij diezelfde behulpzaamheid en meedenkende houding . Dank daarvoor! **Ernie**, jij was degene die mij voor het eerst kennis liet maken met de Klinische Genetica en dit leidde niet alleen tot een leuke en leerzame onderzoeksstage, maar ook tot mijn stap om te solliciteren bij de Klinische Genetica. Je bent een ontzettend fijne collega die altijd oog heeft voor de mens achter de patiënt/student/collega.

Hoewel het gelukkig zelden echt nodig was, kon ik met **Nori**, **Gerben** en **Dorien** heerlijk klagen over (onderzoeks)frustraties onder het mom van intervisie. Dank jullie wel voor jullie frisse blikken vanuit verschillende invalshoeken, die altijd weer hielpen om op een andere manier naar dingen te kijken. Jullie verdedigingen waren prachtige voorbeelden en ik hoop mijn tijd als PhD kandidaat op net zo'n mooie manier af te kunnen sluiten.

Hoewel een promotietraject soms niet alleen werk maar ook je levensdoel lijkt te worden, waren er gelukkig genoeg mensen om mij heen om mij te wijzen op de andere leuke dingen in het leven. Al sinds het Erasmus ken ik niemand met zulke wilde ideeën als **Kennard**. Gelukkig konden we meestal een middenweg vinden die nog steeds leuk maar iets minder gek was. Ook met **Merle** en **Sabrina** heb ik in de afgelopen 10 jaar al heel wat meegemaakt. Hoewel we nu goed over het land verspreid zijn, hoop ik dat we in de toekomst nog minstens net zoveel leuke dingen gaan doen! Voor ontspanning kon ik ook altijd terecht op de atletiekbaan, niet alleen om hard te lopen maar ook voor veel gezelligheid bij alle Haasjes. **Mark**, nu we allebei niet meer zoveel hardlopen kunnen we gelukkig bijpraten op de fiets of zonder sportieve activiteit. Hopelijk kunnen we in de toekomst nog samen naar wat sportieve doelen toewerken en ik ben blij dat je mij als paranimf wilt ondersteunen tijdens mijn verdediging.

Tot slot wil ik graag mijn (schoon)familie bedanken voor alle steun en interesse de afgelopen jaren. Ik prijs mijzelf gelukkig nog steeds te kunnen genieten van een **opa** en **oma** met wie je heerlijk kunt kletsen en tompoezen eten. **Jasper** en **Loreen**, hoewel we een heel ander leven hadden zal dat met de komst van onze Teije vast veranderen. Ik hoop dat onze kinderen net zo leuk worden als die van jullie. **Merlijn** en **Suus,** het is altijd super leuk om met jullie te kletsen over werk, politiek, reizen en alle andere interessante dingen in het leven. Mooi om te zien welk pad jullie kiezen. **Theo** en **Marly,** ik had mij nooit voor kunnen stellen dat zulke leuke en betrokken schoonouders bestonden. Ik heb veel van jullie geleerd en geniet altijd weer van onze bezoekjes aan jullie. Lieve **mam** en **pap**, hoewel jullie niet altijd begrepen welke ideeën ik mij nu weer op de hals haalde, hebben jullie altijd achter mij gestaan en waren (en zijn) jullie er voor mij als ik dat nodig heb. Dankjewel voor hoe jullie mij gemaakt hebben tot wie ik nu ben.

Lieve **Milou**, wat ben ik blij mijn leven met jou te mogen delen. Het is heerlijk om samen met jou te kunnen genieten van alle kleine en grote dingen in het leven. En waar ik jou soms moet afremmen heb je mij vaak gestimuleerd om net dat stapje extra te zetten, wat mij bij dit proefschrift en daarbuiten veel heeft geholpen. Wij zijn met recht een superteam en dat is met de komst van onze zoon **Teije** alleen nog maar meer zo geworden. Ik hou van jullie, ben trots op jullie en kijk uit naar alle mooie momenten die we samen nog gaan beleven.



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