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Refining kidney survival in 383 genetically characterized patients with nephronophthisis

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Refining kidney survival in 383 genetically characterized patients with nephronophthisis

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Abstract

Background

Nephronophthisis (NPH) comprises a group of rare disorders accounting for up to 10% of end-stage kidney disease (ESKD) in children. Prediction of kidney prognosis poses a major challenge. We assessed differences in kidney survival, impact of variant type and the association of clinical characteristics with declining kidney function.

Methods

Data was obtained from three independent sources: the network for early onset cystic kidney diseases clinical registry (n=105), an online survey sent out to the European Reference Network for Rare Kidney Diseases (n=60) and a literature search (n=218).

Results

383 individuals were available for analysis: 116 *NPHP1*, 101 *NPHP3*, 81 *NPHP4* and 85 *NPHP11/TMEM67* patients. Kidney survival differed between the four cohorts with a highly variable median age at onset of ESKD: *NPHP3,* 4.0 years (IQR 0.3-12.0); *NPHP1*, 13.5 years (IQR 10.5-16.5); *NPHP4,* 16.0 years (IQR 11.0-25.0) and *NPHP11/TMEM67,* 19.0 years (IQR 8.7-28.0). Kidney survival was significantly associated with the underlying variant type for *NPHP1*, *NPHP3* and *NPHP4*. Multivariate analysis for the *NPHP1* cohort revealed growth retardation (HR 3.5) and ACEI treatment (HR 2.8) as two independent factors associated with an earlier onset of ESKD, while arterial hypertension was linked to an accelerated GFR decline.

Conclusions

The presented data will enable clinicians to better estimate kidney prognosis of distinct NPH patients and thereby allow personalized counselling.

Keywords: nephronophthisis, kidney survival, prognostic factors, end-stage kidney disease, genetic variant severity, genotype-phenotype correlations.

Introduction

Nephronophthisis (NPH) comprises a clinically and genetically heterogeneous group of autosomal recessive tubulointerstitial cystic kidney disorders representing the most frequent monogenic cause of end-stage kidney disease (ESKD) in children and adolescents[1,2]. Incidence ranges from 1:1,000,000 to 1:50,000 depending on ethnicity and underlying disease-causing variant[3,4]. Variants in 25 genes have been identified to be associated with NPH, still leaving about 40% of patients genetically unsolved[4-7]. A large homozygous deletion on chromosome 2q12-q13 including the entire *NPHP1* gene is the most frequent disease-causing variant accounting for 20-40% of all cases. Variants in the other known *NPHP* genes make up for 3% or less each[5, 8-10]. Three clinical subtypes have been established based on age at onset of ESKD: Infantile, juvenile, and adolescent NPH[11,12]. The so called “classical juvenile NPH” is the most common entity associated with ESKD at a median age of 13 years[13]. Conversely, in infantile NPH, ESKD is reached before the age of 5 years (mean age 8 months) whereas in adolescent forms kidney function is usually preserved until adulthood (mean age 19 years)[11,14,15]. Variants in *NPHP2* and *NPHP3* rather predispose for infantile NPH, whereas other genes are linked to late onset forms (e.g. *NPHP4*)[5,16]. However, the more widespread use of modern sequencing techniques in combination with clinical information gained from large-scale databases opened up new perspectives to define more precisely the age spectrum for the onset of ESKD associated with distinct gene variants. This particularly applies to the homozygous *NPHP1* deletion[17,18]. Likewise, distinctive *NPHP3* variants have been reported to cause late onset NPH in contrast to the majority of patients displaying an infantile disease course[19-21].

Despite the large number of identified *NPHP* genes, only a few of them are of relevance in routine clinical practice: *NPHP1, NPHP3, NPHP4* and *NPHP11/TMEM67.* These four genes account for 75% of all identified disease-causing variants[9]. Additionally, *NPHP5/IQCB1 and NPHP6/CEP290* represent the two main causes for the clinical spectra of Senior-Løken and Joubert syndrome[16,22,23].

NPH-related diagnostic approaches have shifted from a predominantly clinical assessment to the early use of multigene panels and exome sequencing as a result of technical progress[24-26]. This allows for a detailed molecular diagnosis early in life, sometimes even before the onset of kidney symptoms. However, due to the lack of clear-cut genotype-phenotype correlations, phenotypic variability and the consequential unpredictability of individual disease progression, clinical management and personalized counselling remain major challenges. The prognostic uncertainty poses an enormous psychological burden on pediatric patients and their families facing a potentially life-threatening disease of unclear onset and extent.

Here, we analyzed differences in gene-related kidney survival based on large patient cohorts of >80 each. Furthermore, we assessed potential prognostic factors for the *NPHP1* cohort, representing the clinically most relevant group. This is one of the largest studies addressing kidney survival for NPH in genetically characterized individuals.

Methods

Patient recruitment

Phenotypic data of 383 genetically characterized individuals was obtained from three independent sources: the Network of Early Onset Cystic Kidney Diseases (NEOCYST) clinical registry (n=105)[27], an online survey sent out to the members of the European Reference Network for Rare Kidney Diseases (ERKNet; n=60) and a complementary literature search (n=218) (Figure 1). Additionally, clinical information of 44 genetically unsolved individuals was available from the NEOCYST registry. Patients were enrolled in the registry from February 1, 2010, through December 31, 2020. The ERKNet-based online-survey asked for a limited dataset containing information on gender, age, genetic background, GFR decline, hypertension, proteinuria and a medical history of ACE inhibitor treatment. All patients and/or their parents gave written informed consent. The study was approved by the Ethics Committee of the University of Münster (2016-284-f-S) in accordance with the Declaration of Helsinki.

For the literature search, the MEDLINE library was screened for original articles including case reports up to March 2020 providing data on kidney survival/ onset of ESKD for the following genotypes: *NPHP3, NPHP4 and NPHP11/TMEM67.* In total, data from 218 individuals (86 *NPHP3; 64 NPHP4* and 68 *NPHP11/TMEM67)* were identified and extracted from the literature containing information on the age at ESKD and the underlying disease-causing variants (Table S1). No literature-based data was generated for the *NPHP1* cohort. Here solely information from the NEOCYST registry (n=80) or reported via questionnaire (n=36) were used for analysis (Figure 1). For the *NPHP1* cohort duplicate inclusions were thoroughly excluded by crosschecking identifying information (initials, month and year of birth, gender). In case of all other genotypes combined information on individual genetic variants, gender and age were used to avoid duplicate inclusions from different data sources. This way overall 3 duplicates were identified and withdrawn before analysis, resulting in phenotypic data of a total of 383 genetically characterized individuals (Figure1).

Genetic testing

Due to the study design, various methods for genetic testing were used, including PCR-based gel electrophoresis or MLPA for detection of homozygous *NPHP1* deletions (n=97/165), targeted Sanger sequencing on the basis of the patient's phenotype (n=11/165) as well as massively parallel sequencing based approaches such as multigene panel sequencing and exome sequencing (n=57/165)[28,29].

Variables

Estimated GFR (eGFR) was assessed by the FAS equation for the full age spectrum[30]. Annual eGFR decline was determined in patients with at least two subsequent eGFR values in intervals of at least 3 months and displayed as absolute values (ml/min/1.73m^2 BSA per year).

ESKD was defined as either the start of renal replacement therapy (chronic dialysis treatment or kidney transplantation) or an eGFR value below 10 ml/min/1.73 m^2 BSA. Proteinuria was defined as >300 mg/24h (>0.2 g/g). Growth retardation was defined by a baseline height below the 3rd percentile or height development crossing the 3rd percentile.

Variant classification

Interpretation of the clinical significance of variants was completed following the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) guidelines[31] using the VarSome[32] online tool (VarSome The Human Genomics Community). Additionally, to the automatically generated criteria manual adjustment for the following two criteria was made: PM3 (recessive disorders, detected in trans with a pathogenic variant) and PP4 (patient's phenotype or family history is highly specific for a disease with a single genetic etiology). Further interpretation of functional effects of human nsSNPs was conducted using the PolyPhen-2[33] (http://genetics.bwh.harvard.edu/pph2/), SIFT[34] (https://sift.bii.a-star.edu.sg/), PROVEAN[35] (http://provean.jcvi.org/about.php), varSEAK Online (https://varseak.bio, developed by JSI medical systems GmbH, Ettenheim, Germany) and ClinVar (release 13./31.07.2021)[36] (https://www.ncbi.nlm.nih.gov/clinvar/) online tools.

Statistical analysis

Based on the detailed phenotypic data provided by the NEOCYST registry and complemented by information gained from the online survey, cross-sectional and longitudinal statistical analyses were performed on the *NPHP1* cohort (n=116). Kidney survival was defined as the time from birth to the age at onset of ESKD. Patients who did not reach ESKD were censored at their age of last follow-up. Kidney survival was depicted using Kaplan-Meier estimator and the association of potential prognostic factors was estimated by Cox regression analyses in a univariate and multivariate fashion. P-values of p≤0.05 were considered noticeable ("significant"). Results are considered exploratory, not confirmatory. No adjustment for multiple testing was performed. An overall significance level was not determined and cannot be calculated. Statistical analyses were performed using R software and SAS (SAS Institute Inc., Cary, NC, USA).

Results

Kidney survival

Using the data of all 383 genetically determined and 44 genetically unsolved patients, Kaplan Meier analysis was performed and revealed remarkable differences between the genetically defined subgroups (Figure 2): A rapid decline of kidney survival

between the age of 8 and 16 years was observed in the *NPHP1* subgroup (Figure 2a). By 16.5 years of age, 75% of affected teenagers received kidney replacement therapy (Figure 2b). 19% of patients retained residual kidney function beyond the age of 20 years – 5 individuals with a homozygous *NPHP1* deletion and 3 carrying other biallelic *NPHP1* variants (Table S1). Kidney survival for patients carrying *NPHP4* variants was characterized by a later onset and a slower decline compared to *NPHP1* (Figure 2a). Main differences between both groups were illustrated by the age when 50% (13.5 vs. 16 years) and 75% (16.5 vs. 25 years) of patients reached ESKD. Almost no age difference was observed for the onset of 25% receiving RRT (Figure 2b). Interestingly, kidney survival of so far genetically unsolved individuals was comparable to survival curves observed for *NPHP1* and *NPHP4* (Figure 2a).
NPHP11/TMEM67 related kidney survival was characterized by an overall lower percentage of ESKD as well as a flatter decline, particularly in the second decade. Two distinct groups could be identified: those with severe kidney involvement represented by 25% having reached ESKD as early as 8.7 years and those with mild or no obvious kidney involvement with another 25% of patients not requiring kidney replacement therapy at the age of 28 (Figure 2a). An even more pronounced picture emerged for the *NPHP3* group: While approximately half of the patients experienced infantile NPH with an early onset of ESKD before the age of 4 years, a minority of 15% retained residual kidney function beyond the age of 20 (Figure 2a). Gene-related differences in mean age, median age, and interquartile age ranges for the onset of ESKD are summarized in Table 1.

Impact of variant type

We sub-classified the genetic variants into three different categories: truncating-truncating (trunc/trunc), truncating-missense (trunc/mis) and missense-missense (mis/mis). Truncating variants were defined as predicted loss of function (Table S1 and Table S5). Due to the nature of the presentation of variants in the *NPHP1* group a different classification was applied to this cohort: biallelic truncating variants including a homozygous deletion (n=105) and other genetic *NPHP1* variants (n=11).
For *NPHP1*, *NPHP3* and *NPHP4*, the type of underlying disease-causing variant was significantly associated with kidney survival. In the *NPHP3* cohort the presence of at least one truncating variant (trunc/trunc or trunc/mis) lead to an early onset of ESKD before the age of two years in >50% (Figure 3a). In contrast, the presence of two predicted missense variants was associated with a significantly better kidney survival ($p<0.0001$). For *NPHP4*, only the presence of two truncating variants was associated with a significantly ($p=0.0021$) worse kidney outcome compared with patients carrying at least one missense variant (Figure 3b). In individuals carrying biallelic truncating *NPHP1* variants including those with a homozygous deletion a significantly worse kidney survival was observed compared to individuals with at least one missense variant ($p=0.03$) (Figure 3c). However, when only analyzing individuals with a homozygous deletion (n=97) and comparing them to the rest of the cohort (n=19), no significant difference in kidney survival was observed ($p=0.20$; Figure S1). Also, no association between variant type and kidney survival was apparent for *NPHP11/TMEM67* (Figure 3d).

Impact of non-genetic factors

A cross-sectional multivariate analysis was applied to the *NPHP1* cohort, representing the only group providing sufficient information on the clinical variables used for analysis (Figure 4). In total, 116 *NPHP1* patients (54 female) with a median age of 10.5 years (0.6-31.5) at study entry were included comprising a phenotypic spectrum of isolated

kidney disease in 101 individuals, while the remaining 15 children displayed characteristic features for Senior-Løken syndrome (n=7), Joubert syndrome (n=3), congenital oculomotor apraxia type Cogan II (n=4) or Bardet-Biedl syndrome (n=1). Details of the extrarenal phenotype are outlined in table S6. In 62 individuals (52%) arterial hypertension was reported, accompanied by significant proteinuria in 26% - either of both conditions leading to ACE inhibitor treatment in 27 individuals. Patients were characterized 'hypertensive' based on the start of antihypertensive treatment (Table2).

Cross-sectional multivariate analysis revealed growth retardation (HR 3.52; CI 1.67-7.41) and ACEI treatment (HR 2.80; CI 1.13-6.91) as two significant ($p<0.05$) and independent factors correlated with an earlier onset of ESKD (Figure 4A).
Furthermore, univariate analysis on the GFR trajectory revealed arterial hypertension and again ACEI treatment to be significantly ($p<0.01$) associated with an accelerated annual GFR decline (Figure 4B;Table S3). Based on 47 deltaGFR values from 23 individuals treated with ACEIs against 125 values from 62 individuals without treatment, we observed a difference in annual GFR loss of 7.2+/-0.4 vs. 5.2+/-0.6 ml/min/1.73m^2 BSA. Patients with and without ACEI treatment were comparable regarding sex, age and CKD-stage (Table 3). In single cases with only temporary ACEI treatment, intra-individual GFR decline was also higher under the influence of ACEIs compared to no treatment (Figure 5). However, in a subsequent multivariate approach adjusted for each individual factor only the impact of arterial hypertension remained significant with an annual GFR loss of 7.0+/-0.5 vs. 4.6+/-0.4 ml/min/1.73m^2 BSA ($p<0.01$).

Discussion

Since the identification of a large homozygous 290 kb deletion covering the entire *NPHP1* gene in the 1990ies[7], tremendous progress has been made in the molecular understanding of nephronophthisis and related ciliopathies[1,37-41]. However, the focus of most scientific efforts has been on the discovery of the genetic background and molecular pathways[5,7,9,42]. Consequently, a lack of clear-cut genotype-phenotype correlations and prognostic factors is preventing reliable prognoses regarding kidney survival and individual counselling. Here, we present one of the largest studies addressing kidney survival in genetically characterized NPH cohorts.
Back in 1997, *Hildebrandt et al.* established creatinine based, age–dependent quartiles for the *NPHP1*-related progression of chronic kidney failure. The survey was based on a large number of 308 serial serum creatinine values – however, originating from 19 individuals only. The present study covered a significantly higher number of patients including 97 individuals with a homozygous *NPHP1* deletion. Still, results were consistent with those of the historical study: Median age at ESKD was 13.5 years versus 13.1 years in the historical cohort and 12.9 years in a recent survey on 20 Chinese children[13,43]. Quartiles of 25 and 75% reaching ESKD were similar with 10.5 and 16.5 years compared to 11.3 and 17.3 years[13]. In a series of 20 Egyptian children with *NPHP1* variants including 5 with a homozygous deletion, *Soliman et al* reported a significantly earlier onset of ESKD (7.9 years)[44]. Surprisingly, in that survey children without *NPHP1* deletion showed a worse kidney outcome compared to those with a homozygous deletion while in our study it was the other way around ($p=0.03$). Eight *NPHP1* patients (five with a homozygous deletion) retained residual kidney function beyond the age of 20 years. This is in line with previous case reports and the recent awareness of *NPHP1* variants being responsible for a remarkable number of adult-onset ESKD[12,13,16-18].

Biallelic variants in *NPHP3* were originally described in a Venezuelan pedigree presenting with late onset ESKD[15,19]. More recent reports identified *NPHP3* variants as one of the most relevant genetic causes for infantile NPH[9,20,45,46]. In a worldwide cohort of 1056 patients displaying a NPH phenotype, 17 were identified with either a homozygous or compound heterozygous variant in *NPHP3,* of whom 9 presented an onset of ESKD before the age of 2 and only 3 individuals reached ESKD beyond 5 years of age[9]. Similarly, ESKD in our *NPHP3* cohort (n=15) occurred at a median age of 3.3 years. No association between the *NPHP3* genotype and the age at ESKD has been shown so far[15,42]. Here we found a significant association between the time of ESKD and variant type: individuals carrying two missense variants presented a significantly better kidney survival compared to those with at least one truncating variant. However, it needs to be mentioned that 24/27 patients with two missense variants belonged to the same Venezuelan pedigree[19].
For *NPHP4,* there was significant phenotypic overlap with the *NPHP1* cohort, particularly when presenting as isolated kidney disease and reaching ESKD in the early teens. However, the overall kidney survival was much better as compared with other NPHP groups. Even the `trunc/trunc´ scenario was characterized by juvenile NPH mimicking the situation of most *NPHP1* patients. Comparable survival rates were observed among large international NPH cohorts comprising 45 patients with *NPHP4* variants[42,47]. In contrast, *Halbritter et al.* described 22 *NPHP4* patients with 19 experiencing rather early ESKD (mean 12.5 years)[9]. None of the studies revealed a correlation between variant type and the onset of ESKD. We observed an earlier onset of ESKD in individuals with two truncating *NPHP4* alleles. Supporting this observation, a case series on a consanguineous Filipino family carrying two *NPHP4* missense variants reported a consistently late onset kidney failure (median age 36.2 years)[48].
NPHP11/TMEM67 related kidney survival was characterized by an overall lower percentage of ESKD accounting for 35% of NEOCYST patients and 45% when including published cases. This is in line with a large genotype-phenotype study including 22 individuals with Joubert syndrome caused by biallelic *NPHP11/TMEM67* variants, of whom only 50% showed kidney involvement[49]. Although the overall onset of ESKD in our cohort was 11.9 years, two groups could be distinguished: those with severe and early kidney involvement and those with mild or no obvious kidney phenotype. This reflects the wide phenotypic spectrum covered by *NPHP11/TMEM67* variants comprising isolated liver disease, Joubert syndrome with and without kidney involvement, COACH syndrome, RHYNS syndrome and lethal Meckel-Gruber syndrome (MKS)[50-53]. Previous genotype-phenotype correlation studies identified the association of variant and phenotypic severity, observing mostly truncating variants or missense variants clustering within exons 8 to 15 to be causative for lethal MKS phenotypes[54]. However, this did not apply to kidney survival in the current study population. The presence of disease-causing variants in exons 8-15 was not associated with age at ESKD: in patients with such variants in one allele, onset of ESKD was 11.8 years (n=19) compared with 11.3 years in patients without a disease-causing variant in exons 8-15 (n=18). Biallelic exon 8-15 variants (n=4) were associated with ESKD at a median age of 9.2 years (n=3) (Table S4). This observation underpins the idea of the organ-specific impact of distinct genotypes – an important point to keep in mind for the clinical management of affected patients.

So far, neither molecular markers nor clinical factors associated with kidney prognosis have been unravelled for NPH. Here, applying an in-depth phenotyping registry-based approach, we were able to identify two independent factors associated with an earlier onset of ESKD in *NPHP1* patients: 1) growth retardation and 2) ACEI treatment. While

growth retardation was observed in 34% consistent with previous data (11-40%)[47,55], information on the effects of ACEI treatment in the setting of NPH is extremely sparse. The association of ACEIs with a negative effect on kidney survival observed in our study appeared peculiar at first glance considering the nephroprotective benefits of ACEI treatment in the setting of chronic kidney disease[56,57]. However, a detailed look at the original pediatric data reveals that these benefits were mainly limited to glomerulopathies and CAKUT disorders, but did not apply to cystic kidney diseases[58,59]. Regarding individuals with autosomal dominant polycystic kidney disease (ADPKD), clear benefits of ACEI treatment have been described in the literature. Yet, many of these benefits rather applied to blood pressure control and cardiac outcome while evidence for a direct benefit on the decline of kidney function remains sparse[60]. In fact, there is only one prospective observational study directly showing a slower GFR decline in ADPKD patients treated with ACEIs compared to a matched group treated with diuretics[61]. Further evidence is either based on indirect conclusions drawn from an epidemiological study or on the presence of increased plasma renin activity levels[62, 63]. In contrast there have also been studies on ADPKD cohorts that failed to show beneficial effects of ACEI treatment on the decline of kidney function[64]. The limited amount of data included in our study (n=27) does not allow us to draw final conclusions at this stage. Yet, the univariate analysis of individual GFR trajectories revealed that the use of ACEIs was also significantly associated with a faster GFR decline (Table S3; Figure 5). Further investigation beyond the scope of this manuscript is necessary, including more data and prospective studies to confirm the current impression as it would have a major impact on clinical practice recommendations.

In addition to ACEI treatment, we observed a significant negative impact of arterial hypertension on the annual GFR decline - both in a univariate and multivariate approach. This appears contradictory at first since both, arterial hypertension and antihypertensive ACEI treatment had the same negative effect on the GFR trajectory. A potential explanation for this phenomenon might be the content overlap expressed by 27/62 hypertensive individuals receiving ACEI treatment. Noteworthy, after adjusting for each individual factor in the multivariate analysis, only the impact of hypertension remained significant. The high number of hypertensive patients in our cohort appeared striking considering the absence of elevated blood pressure levels being referred to as a typical feature of NPH[5,11,12,65]. In fact, different cohort studies reported pre-dialysis arterial hypertension in up to 30%, exceeded by the ratio in our cohort of 62/116[47,55]. Against this background it must be mentioned that in most cases arterial hypertension was closely related to ESKD reflected by an average interval between the diagnosis of hypertension and the onset of ESKD of 1.5 years (Table 2). This again raises the question whether the arterial hypertension is a consequence of advanced chronic kidney disease rather than an independent risk factor for the early onset of ESKD. Supporting this assumption, pre-dialysis antihypertensive treatment was started in 48 cases only. The remaining 14 individuals were diagnosed hypertensive concomitant with ESKD at their first clinical presentation (Table2). Neither concomitant extrarenal organ involvement nor the sonographic detection of kidney cysts did have an impact on kidney prognosis.

The current study was limited by the fact, that different genetic methodology was included. Unfortunately, the registry-based approach did not allow a universal use of state-of-the-art genetic analysis since many of the patients were no longer in pediatric care and there was no access to current blood samples. Also, the classification of genetic missense variants as `predicted mild´ without proof of functional data may be vulnerable. However, we tried to address these concerns as best as possible by the

application of ACMG-criteria on all variants to (re-)assess the validity of all included and partly formerly published variants (Table S5).
Here, we provide evidence that NPH-related kidney survival is not only determined by the underlying affected gene but may also vary depending on variant type. Growth retardation and the use of ACEIs were identified as independent factors associated with an earlier onset of ESKD in the setting of an underlying *NPHP1* genotype. Furthermore, arterial hypertension was linked to an accelerated GFR decline regardless of the kind of antihypertensive treatment. The presented data enable clinicians to better estimate individual kidney prognosis and thereby facilitate personalized counselling.

Disclosure statement
Nothing to declare.

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Author contributions
J.C.K. and M.K. designed the study; J.C.K., R.K., K.-P.S., M.D.-H., S.K. and A.-K.T. collected data; C.B. performed most of the genetic analyses; J.C.K., J.G., R.K., K.-P.S., M.D.-H., A.-K.T. and M.K. analysed data, J.G. carried out statistical analysis, J.C.K. and M.D.-H. made the figures and drafted the manuscript; all authors contributed their patients, gave valuable intellectual input and critically revised and approved the final version of the manuscript.

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Supplementary Material
Table S1: Genotype, variant type and data source of all individuals carrying biallelic variants in *NPHP1*, *NPHP3*, *NPHP4* or *NPHP11/TMEM67* included in the study.
Table S2: Patient baseline characteristics at study entry (NEOCYST registry cohort and ERKNet survey cohort).
Table S3: Univariate analysis of patient characteristics on the annual eGFR slope.
Table S4: Impact of the genetic involvement of exon 8-15 in *NPHP11/TMEM67* on the onset of ESKD.
Table S5: Characterization of disease-causing variants of all included 383 patients following ACMG criteria.
Table S6: Clinical characterization of 15 individuals with a homozygous *NPHP1* deletion presenting an extrarenal phenotype.
Figure S1: Kidney survival of individuals with a homozygous *NPHP1* deletion compared to other *NPHP1* genotypes.
Supplementary information is available at KI Report´s website.

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Table 1: Kidney survival of genetically defined NPH cohorts:

	NPHP1	***NPHP3***	***NPHP4***	***NPHP11***	***unsolved***
Total number of participants	116	101	81	85	44
Participants with ESKD (%)	70%	81%	100%	45%	57%
Mean age at onset of ESKD (years/ range)	12.5 (5.3 – 27.6)	7.7 (0-47)	17.1 (6-54)	11.9 (0-32)	11.1 (2.1-18.6)
Median age and interquartile range (IQR) for the onset of ESKD (years)	13.5 (10.5-16.5)	4.0 (0.25-12.0)	16.0 (11.0-25.0)	19.0 (8.7-28.0)	15.4 (9.7-n.a.)

Table 2: Clinical characteristics of *NPHP1* patients at the timepoint when classified "hypertensive". Arterial hypertension was either defined by the start of antihypertensive treatment before the onset of ESKD (n=48) or simultaneously with the start of renal replacement therapy (RRT) (n=14*).

characteristics	patients
sex	
• female	27
• male	35
age at diagnosis (yrs)	11.2 (2.4-33.4)
CKD stage	
• CKD I	2
• CKD II	1
• CKD III	16
• CKD IV	17

• CKD V	26
average **time from** diagnosis **hypertension to ESKD** (yrs)	1.52 (0 – 7.1)
Pre-RRT antihypertensive treatment	
• no	14*
• mono	32
• dual	8
• triple	8
• calcium antagonist	23
• betablocker	18
• ACEIs	27
• other	4

Table 3: Clinical characteristics of 85 *NPHP1* patients with subsequent eGFR values on and off ACEI treatment.

DeltaGFR values were determined for individuals with at least two subsequent eGFR values in intervals of minimum 3 months. This way we were able to generate 125 deltaGFR values from 62 patients without and 47 deltaGFR values from 23 patients with the influence of ACEI treatment.

	ACEI -	**ACEI +**
number of patients (n)	62	23
deltaGFR values (n)	125	47
male/female	33/29	12/11
age at study entry (years)	10,2 +/- 3,7 (2,4-20,8)	13,9+/-7,9 (4,4-33,4)
CKD-stage		
CKD1	3 (4%)	2 (9%)
CKD2	7 (10%)	1 (4%)
CKD3	26 (38%)	11 (48%)

CKD4	23 (34%)	10 (43%)
CKD5	9 (13%)	3 (13%)
absolute GFR-loss/year (ml/min/1,73m^2 BSA)	5.2 +/-0.6	7.2 +/-0.4

Figure 1: Patient recruitment

Phenotypic data of 383 genetically characterized individuals was obtained from three independent sources: the Network of Early Onset Cystic Kidney Diseases (NEOCYST) clinical registry (n=105), an online survey sent out to the members of the European Reference Network for Rare Kidney Diseases (ERKNet; n=60) and a complementary literature search (n=218). Homogeneous data from 116 *NPHP1* patients obtained from the NEOCYST registry (n=80) and the online survey (n=36) was fused for analyses of impact of clinical factors. Gene-specific and variant-related kidney survival was analysed including all 383 genetically characterized individuals (*NPHP1*: n= 116; *NPHP3*: n= 101; *NPHP4*: n= 81; *NPHP11/TMEM67*: n= 85) originating from the

NEOCYST registry (n=105), the online survey (n=60) and a comprehensive literature search (n=218).

Figure 2: Gene-related kidney survival

Differences in gene-related kidney survival displayed as Kaplan-Meier survival curve (a) and median age (black line)/interquartile range (IQR) for the onset of ESKD in 50%, 25% and 75% of participants (b). Significant statements: *NPHP1* vs. *NPHP3*:

p<0.0001; *NPHP1* vs. *NPHP4*: p<0.003; *NPHP1* vs. *NPHP11*: p=0.057; *NPHP3* vs. *NPHP4*: p<0.0001; *NPHP3* vs. *NPHP11*: p<0.0001; *NPHP4* vs. *NPHP11*: p=0.539; *NPHP1* vs. *NPHP3* vs. *NPHP4* vs. *NPHP11*: p<0.0001.

Figure 3: Variant-related kidney survival

Genetic variants for *NPHP3*, *NPHP4* and *NPHP11/TMEM67* were sub-classified into truncating/truncating, truncating/missense or missense/missense, defined by the presence of either two loss of function, one loss of function and one missense mutation

or two missense mutations. The *NPHP1* group was divided into biallelic truncating including a homozygous deletion and others. a) *NPHP3* (n=98); b) *NPHP4* (n=81); c) *NPHP1* (n=116); d) *NPHP11* (n=85).

Figure 4: Cross-sectional analysis of *NPHP1* patients identifying clinical factors
a: Multivariate cross-sectional analysis identifying clinical factors for an early onset of ESKD in patients with *NPHP1* gene variations; HR= hazard ratio.

b: Univariate cross-sectional analysis of *NPHP1* patients displaying the differences in annual deltaGFR (e.g.: eGFR slope= deltaGFR male - deltaGFR female) for multiple clinical characteristics: ACEI treatment and most strikingly arterial hypertension were associated with an accelerated GFR decline; however, in a multivariate approach adjusted for each characteristic, only the influence of arterial hypertension remained significant.

Figure 5: Representative eGFR trajectories from 4 patients with biallelic *NPHP1* variants and temporary ACEI treatment.

In all four cases, eGFR decline was more pronounced under the influence of ACEIs compared to no treatment - irrespective of CKD stage and age. However, no statistical significance was reached.

Figure 1

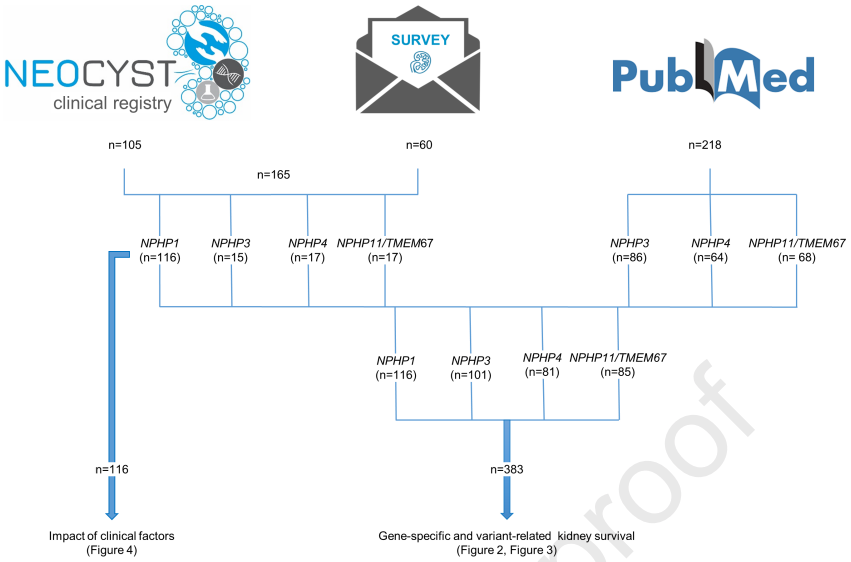


Figure 1

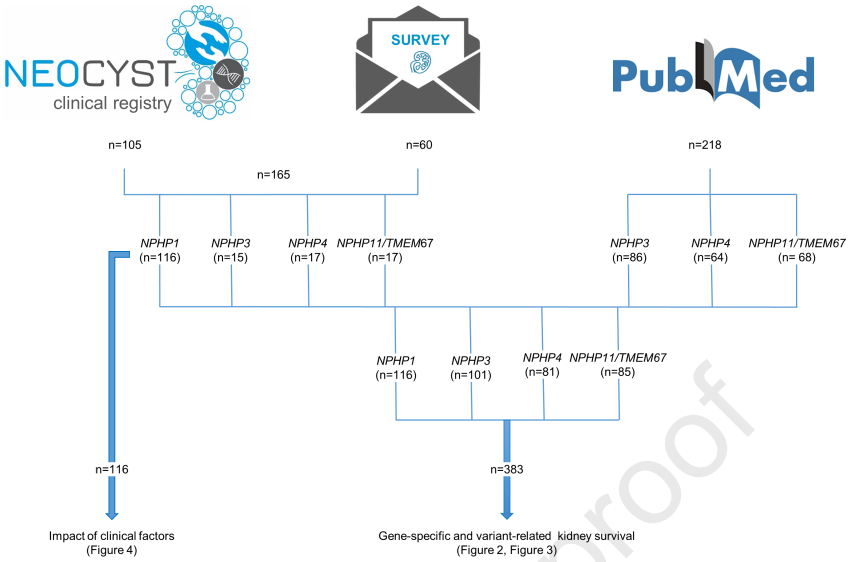


Figure 2

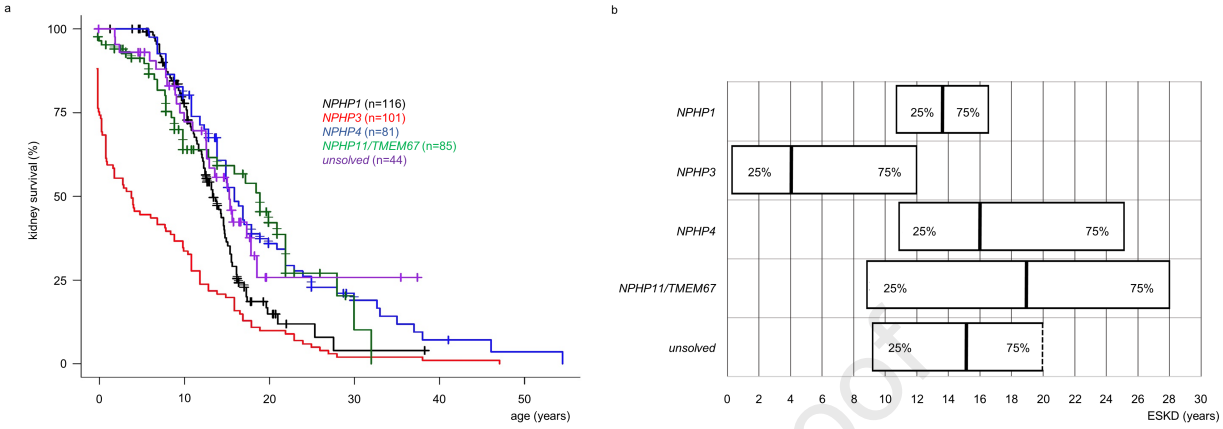


Figure 3

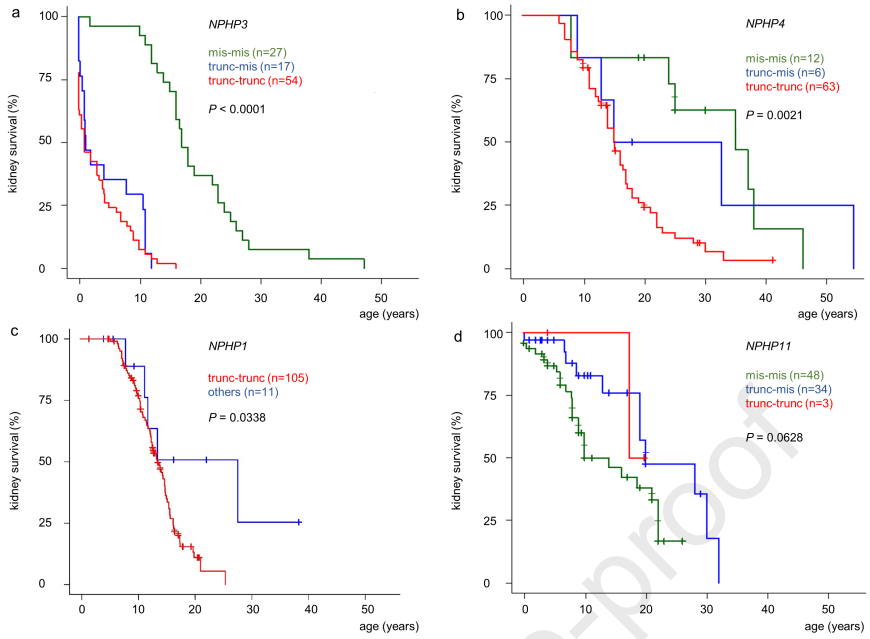
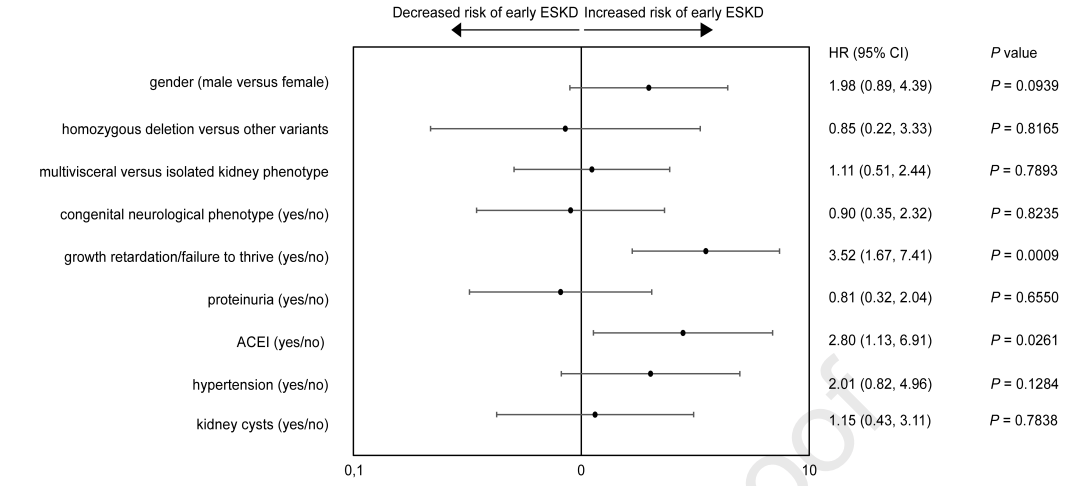


Figure 4

a



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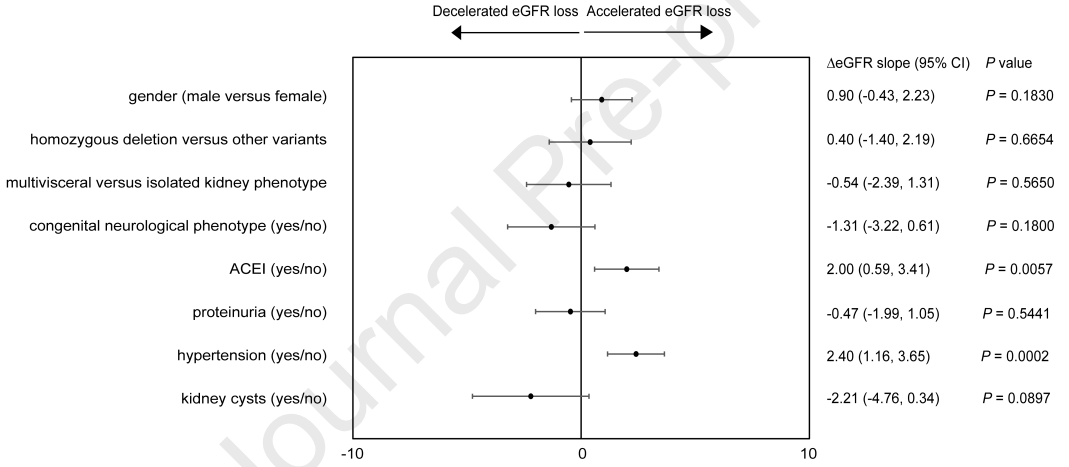

Figure 5