Value of renal gene panel diagnostics in adults waiting for kidney transplantation due to undetermined end-stage renal disease

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End-stage renal disease (ESRD) of undetermined etiology is highly prevalent and constitutes a significant clinical challenge, particularly in the context of kidney transplantation (KT). Despite the identification of numerous rare hereditary nephropathies over the last few decades, patients with undetermined ESRD are not being systematically investigated for rare genetic causes in clinical practice. To address this, we utilized mutation analysis in patients on the kidney transplant waitlist and scrutinized underlying renal diagnoses of 142 patients in a single center KT-waitlist. This cohort was stratified into 85 cases of determined and 57 cases of undetermined ESRD. The latter patients were analyzed by a renal gene panel for mutations in 209 genes associated with ESRD. The most likely genetic diagnoses in 12% of the tested individuals with undetermined ESRD were established. All of these patients showed mutations in genes encoding components of the glomerular filtration barrier. Taken together, hereditary nephropathies, including autosomal dominant polycystic kidney disease, were identified in 35 of the 142 patients of the waitlist cohort. By significantly increasing the proportion of hereditary diagnoses from 29 to 35 patients, the rate of undetermined ESRD significantly decreased from 57 to 51 patients. This study demonstrates the beneficial use of genetic diagnostics in significantly unraveling undetermined ESRD cases prior to KT. Thus, in the absence of renal histology or the presence of unspecific histological conditions, such as hypertensive nephrosclerosis, focal segmental glomerulosclerosis or thrombotic microangiopathy, genetic analysis may provide a robust and specific renal diagnosis and allow for optimizing pre- and post-KT management.

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ESRD represents an increasingly prevalent health burden and is associated with high mortality and morbidity worldwide.[1] Underlying renal conditions leading to ESRD are extremely heterogeneous. Little is known about the prevalence of hereditary nephropathies among adults with ESRD. To date, less than 10% of adult ESRD is thought to be genetic, mainly attributed to clinically diagnosed autosomal dominant polycystic kidney disease (ADPKD). Other hereditary nephropathies are more difficult to diagnose clinically and necessitate at least specific histologic abnormalities, such as the distinct glomerular basement membrane alterations in Alport syndrome (AS).[2] Although renal histology obtained through kidney biopsy represents the mainstay of diagnostics that define various kidney disorders, this tool often proves inapplicable in patients with ESRD due to atrophic kidneys and the added risk of postinterventional hemorrhage. Because of absent or unspecific renal histology in the late stages of chronic kidney disease (CKD), a significant proportion of patients waiting for a kidney transplant (KT) do not exhibit a definite diagnosis, leading them to be classified under CKD of undetermined etiology.[3] Data from large registries indicate that in 15% and 20% of adult ESRD cases, the primary cause remains unresolved,[3–5] a percentage that is likely underestimated considering that nonspecific diagnoses of hypertensive and vascular-related nephropathies represent another 20% to 25%.[3–5] Knowledge of the underlying kidney disease is crucial for ESRD management in the context of transplantation, as the primary etiology may affect graft survival by recurrence and/or rejection. In addition, adequate living kidney donor evaluation requires prior exclusion of genetic risk variants in potential donors from the same family to minimize future donor CKD. Because of advancements in genetic diagnostics, the genetic basis of a multitude of rare kidney disorders has been discovered over the last 2 decades.[6]

Despite recent progress,[7–9] comprehensive genetic testing is not conducted on a regular basis in CKD. However, hereditary nephropathies without syndromal appearance, other than ADPKD, are often impossible to diagnose solely on a clinical basis. In this single-center study, we aimed to systematically assess patients on the KT waitlist for their presumed etiologies. After stratification into the groups of determined and undetermined ESRD, we investigated patients with undetermined ESRD through gene panel diagnostics for underlying Mendelian kidney conditions. This genetic approach (Figure 1, Supplementary Table S1) seeks to address the high proportion of unknown etiology before KT.

RESULTS

On thorough evaluation of primary ESRD causes, 40% of patients (57 of 142) on the KT waitlist were classified as *undetermined* and 60% (85 of 142) as *determined*. Defined subgroups (*determined* vs. *undetermined*) did not differ significantly in terms of age, sex, or age at first renal replacement therapy/ESRD (Supplementary Table S2). Renal biopsy rates were significantly higher for patients with *determined* ESRD (30.6%; 26 of 85) than for patients with *undetermined* ESRD (24.6%; 14 of 57) (Supplementary Table S2).

In 60% of the waitlist (85 of 142), patients with determined ESRD were subcategorized by their primary cause as *hereditary* or *nonhereditary*. The majority of these patients were classified as *nonhereditary* (n = 56), as there was no clinical indication for an underlying Mendelian disorder. Among those classified as nonhereditary, biopsy-proven IgA nephropathy was the most frequent diagnosis (n = 20), followed by diabetic nephropathy (n = 7), drug toxicity (n = 7), chronic pyelonephritis (n = 7), anti-neutrophil cytoplasmic antibody–associated vasculitis, anti–glomerular basement membrane disease, lupus nephritis, and malignancy (Figure 1, Supplementary Table S2). Conversely, 29 patients were

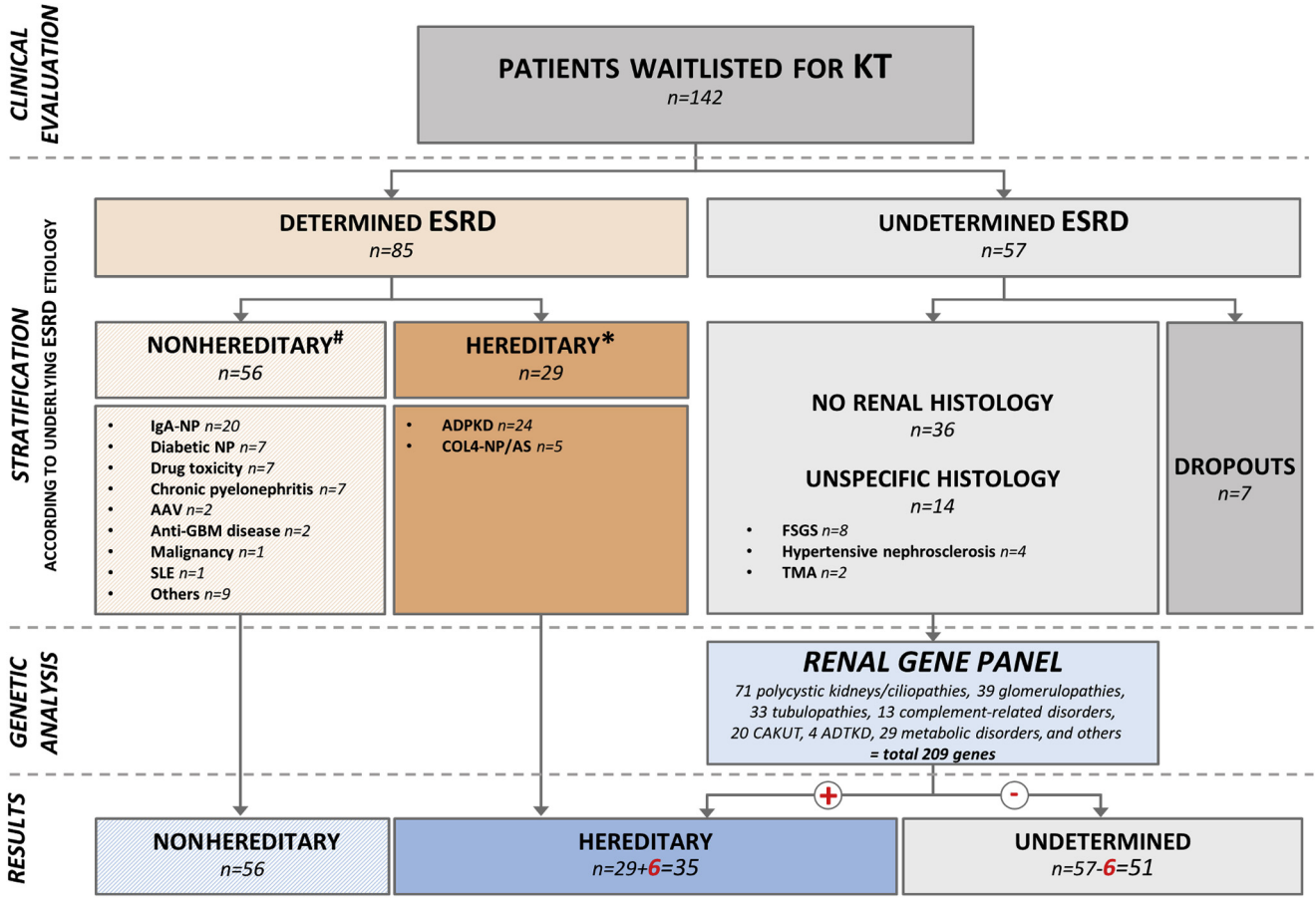


Figure 1 | Study design and stratification. By thorough clinical evaluation, patients on the waitlist were divided into 2 groups, *determined* and *undetermined*, according to the plausibility of their presumed renal condition. Determined end-stage renal disease (ESRD) was further stratified into *nonhereditary* (#non-Mendelian disorders) and *hereditary* (*Mendelian disorders). All recruited patients (n = 50) with an undetermined etiology (n = 57 − 7 dropouts) were analyzed by a renal gene panel, containing 209 Mendelian kidney disease genes (Supplementary Table S2). The aim was to identify the hitherto undetermined ESRD patients with an underlying genetic condition. Genetic analysis detected 6 patients with pathogenic or likely pathogenic mutations, constituting the primary cause of ESRD in these patients. AAV, anti-neutrophil cytoplasmic antibody–associated vasculitis; ADPKD, autosomal dominant polycystic kidney disease; anti-GBM, antiglomerular basement membrane antibody; AS, Alport syndrome; CAKUT, congenital anomalies of the kidney and urinary tract; COL4, collagen 4; FSGS, focal segmental glomerulosclerosis; KT, kidney transplantation; NP, nephropathy; SLE, systemic lupus erythematosus; TMA, thrombotic microangiopathy.

categorized as *hereditary* for their clinically or genetically diagnosed Mendelian disorder. These patients mainly had clinically diagnosed ADPKD (n = 24),[10] 16 of which had been genetically confirmed (Supplementary Table S3). In another 5 cases, we found histologically and genetically diagnosed COL4-nephropathy/AS before study initiation (Figure 1, Supplementary Tables S2 and S3).

In 40% (57 of 142) of the waitlist, however, we did not observe a definite renal diagnosis, hence called *undetermined* ESRD. In these patients, biopsy data were either missing (n = 43) or unspecific (n = 14), such as in 8 patients with biopsy-proven focal segmental glomerulosclerosis (FSGS) and 6 patients with a histologic diagnosis of hypertensive nephrosclerosis, 2 of whom displayed additional preglomerular thrombotic microangiopathy (Figure 1).

Among 50 genetically analyzed patients with undetermined ESRD (57 minus 7 dropouts), 12% (n = 6) were found to carry pathogenic or likely pathogenic variants (Tables 1 and 2, Figure 2). In another 8% (n = 4), we identified variants of unknown significance (VUS) according to the classification of the American College of Medical Genetics and Genomics (Supplementary Table S4).[11]

Among the 6 patients with pathogenic or likely pathogenic variants, one-third (2 of 6) were found to carry *COL4A5* mutations. Variants in *COL4A5* are known to be associated with a broad clinical spectrum, ranging from early-onset AS and FSGS to milder forms of COL4-nephropathies with renal manifestation later in life, particularly in females.[18] In this study, patients with a pathogenic *COL4A5* finding (P9, P26) presented with adult-onset ESRD (range, 43–56 years) in the absence of renal biopsy data (P9) or unspecific histologic diagnoses of nephrosclerosis without ultrastructural glomerular basement membrane assessment (P26). Hearing loss, the most common extrarenal manifestation, was only reported in the male patient P26 and his mother (Table 2). Both subjects carried previously reported pathogenic heterozygous (P9, female)[12] and hemizygous variants (P26, male) (Table 2).[13] In addition to the known pathogenic *COL4A5* change in P26, we also identified a previously reported heterozygous *COL4A3* missense variant[14] with relevant frequency in the general population (0.5%, European Non-Finnish) (Table 2).[18]

In a patient (P11) with proteinuric kidney disease and ESRD at age 28, we identified a novel heterozygous missense variant in *PAX2*, a gene associated with papillorenal syndrome and isolated genetic FSGS in adults.[19] The detected nucleotide change, c.263C>A (p.Pro88His), is highly conserved and affects the homeodomain-like and paired domain of PAX2, not present in publicly available SNP databases (gnomAD/ExAc). On segregation analysis with confirmed paternity, both parents were tested wild type, indicating a *de novo* variant. Interestingly, this individual, who had never undergone kidney biopsy and did not show any ocular involvement suggestive of papillorenal syndrome, had reported a history of renal disease in his father. However, this was later determined to be due to nephrectomy after diagnosis of renal clear cell carcinoma (Table 2, Supplementary Figure S1).

Table 1 | Renal gene panel—gene group composition

Entity	No. of genes	Inheritance
Polycystic kidneys/ciliopathies	71	AD, AR, XL
Glomerulopathies	39	AD, AR, XL
Tubulopathies	33	AD, AR, XL
Complement-related disorders	13	AD, AR
CAKUT	20	AD, AR
ADTKD	4	AD
Metabolic disorders and others	29	AD, AR, XL
Total	209	

AD, autosomal dominant; ADTKD, autosomal dominant tubulointerstitial kidney disease; AR, autosomal recessive; CAKUT, congenital anomalies of the kidney and urinary tract; XL, X-linked.
For detailed gene composition see Supplementary Table S1.

Among cases with biopsy-proven FSGS (n = 8), we were able to (newly) diagnose genetic forms in 3 patients (P45, P48, P49). Two families showed dominant inheritance, and in one instance with a negative family history (P49), recessive FSGS resulting from compound heterozygous *NPHS2* mutations was identified and confirmed by segregation analysis of both parents (Table 2, Supplementary Figure S1).[16,17] In P48, a patient whose deceased father also depended on dialysis for chronic renal failure of unknown etiology, we identified a previously reported heterozygous missense mutation in *INF2* (c.550G>A; p.Glu184Lys).[15] In accordance with other disease-causing *INF2* variants, this variant affects the functionally relevant diaphanous inhibitory domain of its respective protein.[15] This patient reported a history of proteinuric kidney disease from adolescence and progressed to ESRD by age 18. Concomitant neurological signs and symptoms of Charcot-Marie-Tooth disease were not reported in P48 nor in his father.

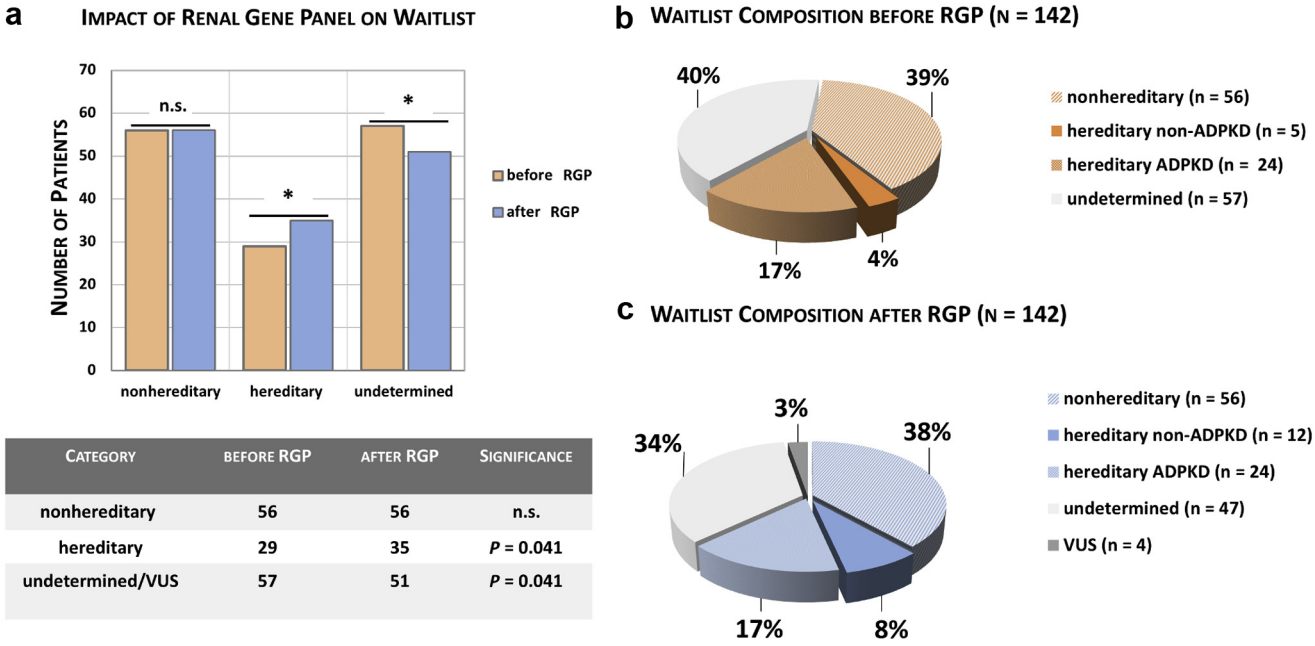
In a patient with ESRD at 61 years (P45), we detected a novel, likely pathogenic missense variant in *WT1* (Table 2). The patient had 2 healthy daughters in their early 40s and a 61-year-old brother with stage 4 CKD and long-term nephrotic kidney disease who refused diagnostic kidney biopsy. Therefore, we performed segregation analysis for genetic confirmation. Although both unaffected daughters tested wild type, the affected brother was found to harbor the *WT1*-c.1165C>T (p.Arg389Cys) variant, located in the beginning of the nucleotide-binding zinc finger domain of the encoded protein.[20] This finding suggests isolated genetic FSGS and is most likely causal for CKD and ESRD in the patient and his brother despite the unusual age of onset and the absence of extrarenal manifestations typically associated with mutations in *WT1* (Table 2, Supplementary Figure S1).[21]

By establishing a likely molecular diagnosis in 6 of 57 patients with previously undetermined ESRD, we significantly reduced the proportion of patients with undetermined ESRD from n = 57 to n = 51 ($P = 0.041$), thus increasing the rate of hereditary nephropathies on the KT waitlist significantly from n = 29 to n = 35 ($P = 0.041$) (Figure 2a and b). Furthermore, the proportion of ADPKD in the total group of hereditary kidney diseases decreased from 83% (24 of 29) to 69% (24 of

Table 2 | Identified variants in 6 patients with undetermined ESRD according to ACMG classification[11]

Patient no./sex	Renal biopsy/ extrarenal involvement	Age at ESRD (yr)	Family history	Gene	Genetic variant	Genetic diagnosis/ inheritance/ segregation	Prediction (SIFT MutTaster PolyPhen2)	ACMG Manual	MAF (gnomAD)	HGMD (2018.4)	Clinical implications
P9/F	None/none	56	Sister with ESRD	*COL4A5*	c.3427G>A p.Gly1143Ser het	AS/COL4-NP/XL/n.a.	del. d.-c. prob. dam.	Pathogenic PS1, PS4, PM2, PP2, PP3, PP5	–	[12]	No recurrence, audiometry, family counseling
P26/M	Nephrosclerosis/ deafness	43	Mother with deafness	*COL4A5*	c.5030G>A p.Arg1677Gln hem	AS/COL4-NP/XL/n.a.	del. d.-c. prob. dam.	Likely pathogenic PP2, PP3, PP4, BS1	–	[13]	No recurrence, eye examination, family counseling
				COL4A3	c.4421T>C p.Leu1474Pro het	AS/COL4-NP/AD/n.a.	del. d.-c. prob. dam.	Uncertain PS1, PM2, PP2, PP3, PP5	0.49%	[14]	
P48/M	FSGS/none	18	Father with ESRD	*INF2*	c.550G>A p.Glu184Lys het	Fam. FSGS/AD/n.a.	del. d.-c. prob. dam.	Pathogenic PS1, PS4, PM1, PM2, PP3, PP4	–	[15]	No recurrence, family counseling
P49/M	FSGS/none	39	neg.	*NPHS2*	c.871C>T p.Arg291Trp het	Fam. FSGS/AR/yes	del. d.-c. prob. dam.	Pathogenic PS1, PM2, PP2, PP3, PP4, PP5	–	[16]	No recurrence, family counseling
					c.686G>A p.Arg229Gln het		tol. polym poss. dam.	–	3.6%	[17]	
P11/M	None/none	28	Father with CKD (RCC)	*PAX2*	c.263C>A p.Pro88His het	Fam. FSGS/AD/*de novo*	del. – prob. dam.	Likely pathogenic PS2, PM2, PP2, PP3	–	nov.	No recurrence, family counseling
P45/M	FSGS/none	61	Brother with NS/CKD4	*WT1*	c.1165C>T p.Arg389Cys het	Fam. FSGS/AD/yes	del. – prob. dam.	Likely pathogenic PM2, PP1, PP2, PP3, PP4	–	nov.	No recurrence, family counseling

ACMG, American College of Medical Genetics and Genomics; AD, autosomal dominant; AR, autosomal recessive, b.-p., biopsy-proven; dam., damaging; d.-c., disease-causing; ESRD, end-stage renal disease; F, female; fam., familial; FSGS, focal segmental glomerulosclerosis; gen, genetic; GN, glomerulonephritis; GP, glomerulopathy; hem, hemizygous; het, heterozygous; hom, homozygous; M, male; MAF, minor allele frequency; n.a., not available; neg., negative; nov., novel; NP, nephropathy; NS, nephrotic syndrome; polym., polymorphism; pos., positive; poss., possibly; prob., probably; RCC, renal cell carcinoma; tol., tolerated.
Patients with pathogenic or likely pathogenic variants. All variants were considered likely pathogenic on the basis of the ACMG manual in synopsis with expert evaluation of the clinical phenotype and family history. HGMD professional, Version 2018.4 (https://portal.biobase-international.com/hgmd/pro/all.php); gnomAD (http://gnomad.broadinstitute.org/).



Category	Before RGP	After RGP	Significance
nonhereditary	56	56	n.s.
hereditary	29	35	$P = 0.041$
undetermined/VUS	57	51	$P = 0.041$

Figure 2 | Impact of genetic analysis on kidney transplantation (KT)–waitlist composition. (**a**) By identification of 6 patients with pathogenic or likely pathogenic mutations (Table 2), genetic analysis (renal gene panel [RGP]) significantly reduced the number of undetermined end-stage renal disease (from n = 57 to n = 51) and increased the number of hereditary disorders on the waitlist (from n = 29 to n = 35). The McNemar chi-squared test was used for statistical analysis. (**b**) Impact of genetic analysis (RGP) on waitlist composition with newly classified cases as displayed in Table 2. Although autosomal dominant polycystic kidney disease (ADPKD) accounted for 83% (24 of 29) and non-ADPKD accounted for 17% (5 of 29) of hereditary cases before genetic analysis, the ratio shifted to 69% (24 of 35) versus 31% (11 of 35) after genetic analysis. n.s., not significant; VUS, variants of unknown significance.

35), increasing the proportion of non-ADPKD conditions after renal gene panel analysis (Figure 2b).

Furthermore, an additional 4 VUS and 1 risk allele (*APOL1*-G1) were detected in 4 patients. In these cases, variant pathogenicity was either uncertain, or of insufficient genetic effect size to account for chronic renal failure on a Mendelian basis (Supplementary Table S4).

In a 62-year-old woman (P6) with ERSD at age 54 without kidney biopsy and an extensive family history of undetermined adult ESRD, we identified a possibly pathogenic variant in *UMOD*. This variant was also found in the affected son who suffered from ESRD at age 30 (Supplementary Table S4, Supplementary Figure S1). Alteration of this amino acid residue has not been previously reported. However, substitutions of both neighboring amino acid residues (p.Cys50 and p.Cys52) are known mutations in patients with autosomal dominant tubulointerstitial kidney disease caused by *UMOD*.[22,23] Despite this, inconclusive *in silico* prediction and incomplete segregation analysis result in this variant being classified as VUS. Unfortunately, additional DNA samples could not be obtained given that several affected family members were deceased.

In a male patient of East-African descent (P50) with biopsy-proven FSGS and ESRD at age 38, we identified the common homozygous *APOL1* G1-risk-allele and a variant of unknown significance, a rare heterozygous *COL4A3* non-glycine missense mutation affecting the triple helical region that has not previously been reported in association with AS or FSGS (c.221C>T; p.Pro74Leu) (Supplementary Table S4). Because of the patient's history as an unaccompanied refugee, the reported family history was incomplete and DNA samples for segregation analysis were not available for further genetic confirmation.

Lastly, in 2 additional patients with biopsy-proven FSGS, heterozygous missense variants in 2 known FSGS genes, *CD2AP* (P46) and *ACTN4* (P43), were detected. However, because of the relative allele frequency in the general population (0.02% and 0.05%, respectively) and inconclusive *in silico* prediction, particularly in case of the recently reported *ACTN4* variant (p.Ala427Thr),[24] both changes were classified as VUS (Supplementary Table S4). In P43, parental segregation showed paternal transmission in the absence of a significant renal phenotype in the father at 60 years of age. However, incomplete penetrance has been widely reported in *ACTN4*[25] and therefore did not allow for a definite variant classification.

The mean coverage of the targeted sequences was 119 and the mean number of identified variants was 17,140. A total of 98.2% of the targeted sequences were covered 10 times or more and 96.2% were covered 20 times or more. On average, less than 5 targets were not covered at all, less than 10 targets were partially covered, and less than 50 targets were covered with less than 10 reads. Next generation sequencing (NGS)-based analysis for copy number variations yielded a larger *PAX2* deletion (P43) that was not confirmed by consecutive multiplex ligation-dependent probe amplification.

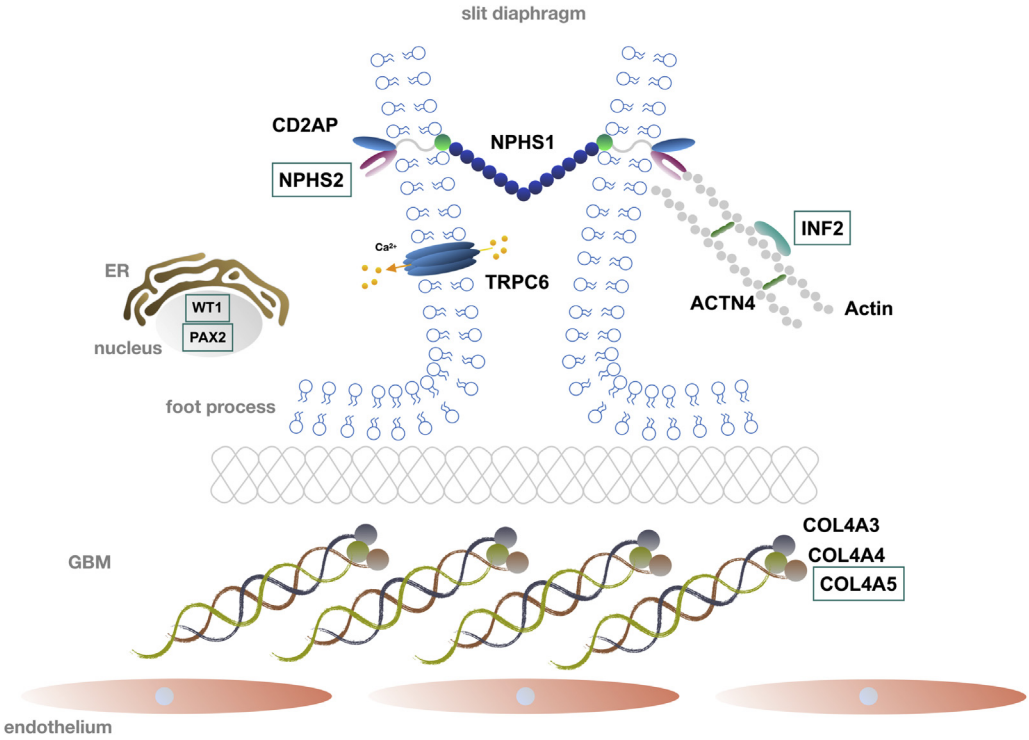


Figure 3 | Glomerular filtration barrier with identified hereditary glomerulopathies. Illustration of molecular genetic findings and their respective protein localization as part of the glomerular filtration barrier. Five distinct glomerular proteins with pathogenic and likely pathogenic findings (Table 2) are marked by green frames. ACTN4, alpha-actinin 4; CD2AP, CD2-associated protein; COL4A3/4/5, collagen type 4 alpha 3/4/5 chains; ER, endoplasmatic reticulum; GBM, glomerular basement membrane; INF2, inverted formin 2; NPHS1, nephrin; NPHS2, podocin; PAX2, paired box 2; TRPC6, transient receptor potential cation channel subfamily C member 6; WT1, Wilms tumor protein.

DISCUSSION

With 40% of undetermined ESRD cases on the waitlist, the prevalence of patients with unresolved etiology in our cohort was twice as high as in large clinical ESRD and CKD-registries.[3–5] Considering the individual scrutiny of each patient during the stratification process, this result is reasonable, given the scarcity of kidney biopsy data in our cohort.

The aim of this study was to reduce the high proportion of undetermined ESRD by genetic analysis with a kidney-specific gene panel for Mendelian nephropathies. Of 50 tested individuals with undetermined ESRD, we found pathogenic or likely pathogenic variants in 6 patients (Table 2), thereby establishing a specific renal diagnosis in 12% of the analyzed cohort before transplantation. Together with the group of primarily diagnosed patients with ADPKD and AS (Supplementary Table S3), hereditary nephropathies thereby accounted for as much as 25% of CKD etiologies on our waitlist. In addition, in another 4 patients, VUS were found (Supplementary Table S4). In the latter cases though, the etiology remains unknown without further functional evidence or extensive segregation analysis. Especially in certain CKD genes, putatively pathogenic variants were recently shown to be found in "self-declared healthy adults," underlining the importance of thorough phenotyping for variant interpretation.[26]

Because of its characteristic phenotype and a positive family history in 90% of all cases, diagnosis of ADPKD can be largely based on clinical criteria.[10] However, other hereditary nephropathies do not display such specific presentation and are therefore missed without genetic diagnostics. Clinical diagnosis remains a significant challenge, particularly in recessive diseases and genetic disorders with incomplete penetrance, such as in genetic FSGS due to mutated *ACTN4* (P43). In addition, many hereditary kidney disorders are considered to be pediatric conditions and may be misdiagnosed when age of onset is in early adulthood. This was recently demonstrated in a study on nephronophthisis, where 0.5% of the adult ESRD population showed homozygous *NPHP1* deletions, but only 12% were clinically diagnosed correctly.[27] The increased contribution of non-ADPKD conditions after genetic analysis in our study (Figure 2b) corroborates the need of genetic diagnostics to adequately detect these heterogeneous disorders.

Interestingly, our study only yielded mutations in genes encoding glomerular, notably podocytic, structures (Figure 3). Despite these genes typically being associated with syndromic diseases such as Charcot-Marie-Tooth in *INF2*, papillorenal syndrome in *PAX2*, Denys-Drash syndrome in *WT1*, and AS in *COL4A5*, all our patients presented with isolated renal manifestations. Nonsyndromic appearance, however, may be more frequent in the adult population but implies diagnostic hurdles, as clinical phenotypes are unspecific and hard to distinguish in the absence of extrarenal manifestations.

In this study, mutations of collagen type 4 encoding genes, particularly *COL4A5*, were found most frequently. Together with the 5 patients who were already diagnosed with COL4 nephropathies before study initiation, this group accounts for 5% (7 of 142) of the total waitlist cohort. This aligns with the estimated prevalence of AS and COL4 nephropathies, constituting the second most frequent hereditary kidney disease after ADPKD.[28] A similar diagnostic contribution was recently found in the largest CKD-cohort analysis by Groopman *et al.*,[9] where pathogenic *COL4A3/4/5* variants together accounted for 30% of all genetic diagnoses, only 1% less than those due to *PKD1/2* variants. In addition, variant detection was remarkably high in adult patients with FSGS in our study. Of 8 patients enrolled, 3 harbored pathogenic or likely pathogenic variants and an additional 3 showed VUS, highlighting the value of genetic testing in adult FSGS in the transplantation setting. Diagnosis of genetic/familial FSGS is relevant for prognosis when assessing the risk of recurrence after kidney transplantation, as ESRD caused by mutations in genes encoding structural proteins of the glomerular filter (e.g., *INF2*, *NPHS2*) is extremely unlikely to recur after KT (Figure 3).[29] Hence, transplantation of donor organs without the respective defect may constitute a curative treatment. Mutational analysis is also essential in evaluating related living donors. This applies particularly to X-linked conditions, such as *COL4A5* nephropathy, where females typically have a milder clinical presentation with later onset of disease.[30] This is also demonstrated by a female patient (P9) in this study.

Underlying genetic factors are not only of prognostic value (with regard to the outcome of renal transplantation), but they may also help to address unforeseen complications in the course of graft monitoring. Although no case of genetic thrombotic microangiopathy was found in this study, knowledge of pathogenic complement gene variants is essential in atypical hemolytic-uremic syndrome/thrombotic microangiopathy, as this condition is known to frequently recur after transplantation, making immediate diagnosis and therapy crucial for graft survival and outcome.[31]

Limitations of our study include the single-center approach with a modest cohort size of relative ethnic homogeneity. Secondly, even though our panel comprised a large number of CKD-associated genes, various genes were not covered because of technical limitations of the applied panel (e.g., *ANKS6*, *DGKE*, *FAN1*, *GANAB*, *IFT172*, *NOTCH2*, *NUP93*, *SEC61A1*). Notably, recently identified candidate genes, such as *DNAJB11*,[32] *DZIP1L*,[33] *PARN*,[8] and *VTN*,[34] were not part of the analysis. Complete coverage of a growing number of disease genes can only be warranted by whole exome or genome sequencing instead of targeted NGS approaches. The utility of whole exome sequencing in differential diagnosis of CKD etiologies has recently been demonstrated in several large cohorts of both pediatric and adult patients with advanced CKD and ESRD.[8,9,35] Furthermore, deep intronic variants, mutations within variable number tandem repeats, such as the *MUC1*-dupC mutation,[36] and changes in regulatory regions, as shown by the recently identified promoter mutation in *PMM2*,[37] would have been missed by our approach. In addition, as NGS data were analyzed for copy number variations, this tool may not be as sensitive as the gold standard of multiplex ligation-dependent probe amplification in regions of lower coverage. Lastly, some discretion is applied to defining undetermined ESRD, and the classification of some conditions remains controversial. Although cases of chronic pyelonephritis were considered determined ESRD in this study (Figure 1), instead, they could be undiagnosed cases of congenital anomalies of the kidney and urinary tract or autosomal dominant tubulointerstitial kidney disease, supporting their classification as undetermined ESRD and the role for genetic diagnostics. This was also underlined by identification of 4.5% genetic disorders in clinically diagnosed tubulointerstitial disease by Groopman *et al.*[9] However, similar to the present study, the highest diagnostic yield was reported among patients with congenital or cystic kidney disease (23.9%), followed by nephropathies of unknown origin (17.1%).[9]

CONCLUSION

In conclusion, our study demonstrates that genetic testing through a kidney-specific gene panel is able to reveal the underlying condition in a significant proportion of patients with formerly undetermined ESRD. These findings allow for improved pre- and posttransplant management, help in assessing the risk of kidney disease in relatives, and may lead to enhanced graft survival in the future. Multicenter longitudinal studies are required to replicate these findings in larger waitlist cohorts and evaluate the benefit of genetic analysis before kidney transplantation in the long term.

METHODS

The cohort comprises 142 adult patients (141 families), 45 females and 97 males, with a median age of 51.7 years (range, 23.9–73.9 years) waiting for KT on September 1, 2016, at the University Hospital Leipzig, Germany. Total median age at first renal replacement therapy/ESRD was 46.1 years (range, 15.4–67.7 years). The vast majority of patients were Caucasian (n = 136), followed by Asian (n = 3) and African ethnicity (n = 3). Kidney biopsy data were available in 40 patients (28%). First, 2 trained nephrologists individually revisited and scrutinized patients' medical histories (clinical evaluation) to subsequently divide the cohort into cases of *determined* and *undetermined* ESRD, respectively (Figure 1). Undetermined ESRD was defined as exclusion of the following 4 criteria: (i) specific histological renal diagnosis (e.g., IgA nephropathy, pauci-immune vasculitis); (ii) specific morphological renal diagnosis (e.g., ADPKD); (iii) specific molecular genetic renal diagnosis; and (iv) specific and plausible clinical diagnosis (e.g., history of long-term (>10 years) insulin-dependent diabetes mellitus before ESRD). Patients with unspecific histological conditions suggesting an underlying genetic cause, such as biopsy-proven FSGS, hypertensive nephrosclerosis, thrombotic microangiopathy, and/or chronic interstitial nephritis, were included in the *undetermined* group. Second, the *determined* group was stratified by the nature of their underlying condition into: (i) *nonhereditary*, for renal disease of non-Mendelian origin, and (ii) *hereditary*, for renal disease of Mendelian origin. Mendelian disease was defined clinically in

ADPKD, whenever patients displayed typical bilateral polycystic kidney enlargement on imaging,[10] or genetically in non-ADPKD conditions, whenever a molecular genetic diagnosis had been established before study enrolment (Figure 1, Supplementary Table S1). After approval by the local ethics committee (Institutional Review Board at the University of Leipzig), we recruited patients from the *undetermined* group for enrollment in genetic analysis. Blood samples and updated clinical information were obtained after written informed consent. Mutation analysis was collaboratively conducted at the Institutes of Human Genetics at the University of Leipzig and in Ingelheim (Bioscientia, Germany). Our renal gene panel comprising 209 OMIM-listed genes associated with various hereditary kidney diseases was applied to patients with undetermined ESRD (Figure 1, Table 1, Supplementary Table S2). The analyzed genes were part of a larger OMIM-gene panel including 4813 genes (TruSight One; Illumina, USA). Library preparation was followed by NGS on an Illumina HiSeq platform. For variant and copy number variation analysis, we used an automated bioinformatic pipeline (Varvis Software, Germany). Variant interpretation was executed in accordance with the American College of Medical Genetics and Genomics[11] and subsequently evaluated for possible pathogenicity by a team of experienced geneticists and nephrologists after complete genotype-phenotype comparison. Segregation analysis by Sanger sequencing was performed for confirmation of identified genetic variants whenever available. Copy number variations detected by NGS had to be confirmed by multiplex ligation-dependent probe amplification.

DISCLOSURE

All the authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

IO, JM, AB, AW, DS, THL, and JaH stratified the cohort, recruited patients, and gathered detailed clinical information for the study. IO, JuH, TW, CB, and RAJ performed NGS and mutation analysis. IO, JM, and JaH performed genotype-phenotype correlation. JM and JaH conceived of and directed the study with RAJ. JaH with JM, RS, and IO wrote the manuscript. All authors approved the final version.

SUPPLEMENTARY MATERIAL

Table S1. Detailed list of covered genes with gene code, associated OMIM number, and mean coverage per gene.

Table S2. Composition of waitlist at University Hospital Leipzig before genetic testing with a renal gene panel. Of note, there was no significant difference between subgroups of determined ESRD (*nonhereditary* + *hereditary*) and undetermined ESRD in sex, median age ($P = 0.12$), and median age at first RRT/ESRD ($P = 0.07$) (2-tailed *t*-test).

Table S3. Genetic findings among patients with determined ESRD due to clinically diagnosed hereditary kidney disease before study initiation.

Table S4. Patients with variants of unknown significance (VUS) on the basis of the ACMG manual in synopsis with expert evaluation of the clinical phenotype and family history. HGMD professional, Version 2018.4 (https://portal.biobase-international.com/hgmd/pro/all.php); gnomAD (http://gnomad.broadinstitute.org/).

Figure S1. Pedigrees of family members with (likely) pathogenic findings according to Table 2 (main text) and pedigree of a family member with VUS in *UMOD*.

Supplementary References.

Supplementary material is linked to the online version of the paper at www.kidney-international.org.

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