Whole-Exome Sequencing Enables a Precision Medicine Approach for Kidney Transplant Recipients

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ABSTRACT

Background Whole-exome sequencing (WES) finds a CKD-related mutation in approximately 20% of patients presenting with CKD before 25 years of age. Although provision of a molecular diagnosis could have important implications for clinical management, evidence is lacking on the diagnostic yield and clinical utility of WES for pediatric renal transplant recipients.

Methods To determine the diagnostic yield of WES in pediatric kidney transplant recipients, we recruited 104 patients who had received a transplant at Boston Children's Hospital from 2007 through 2017, performed WES, and analyzed results for likely deleterious variants in approximately 400 genes known to cause CKD.

Results By WES, we identified a genetic cause of CKD in 34 out of 104 (32.7%) transplant recipients. The likelihood of detecting a molecular genetic diagnosis was highest for patients with urinary stone disease (three out of three individuals), followed by renal cystic ciliopathies (seven out of nine individuals), steroid-resistant nephrotic syndrome (nine out of 21 individuals), congenital anomalies of the kidney and urinary tract (ten out of 55 individuals), and chronic glomerulonephritis (one out of seven individuals). WES also yielded a molecular diagnosis for four out of nine individuals with ESRD of unknown etiology. The WES-related molecular genetic diagnosis had implications for clinical care for five patients.

Conclusions Nearly one third of pediatric renal transplant recipients had a genetic cause of their kidney disease identified by WES. Knowledge of this genetic information can help guide management of both transplant patients and potential living related donors.

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CKD is an important cause of morbidity and mortality, often progressing to ESRD and necessitating dialysis and renal transplantation. Causes of CKD in children and young adults differ greatly from those in older individuals. Data from the 2014 North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) show that the most common causes of ESRD in the pediatric population include congenital anomalies of the kidney and urinary tract

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(CAKUT) (39%), chronic glomerulonephritis (GN) (16%), steroid-resistant nephrotic syndrome (SRNS) (15%), renal cystic ciliopathies (6%), and nephrolithiasis/nephrocalcinosis (2.5%). In 6% of patients, the cause of kidney disease is unknown.[1]

In recent years, we have gained a better understanding of the genetic landscape of CKD in children. In fact, it has been shown that a monogenic disease-causing mutation in one of approximately 220 genes can be identified in up to 20% of patients who develop CKD before 25 years of age.[2] Specifically, a monogenic cause can be identified in 5%–14% of patients with CAKUT,[3–5] 11%–30% of patients with SRNS,[6–10] 14% of patients with chronic GN,[11] 33%–63% of patients with a renal cystic ciliopathy,[12–15] and 15%–29% of patients with urinary stone disease.[16–19] This has important implications for the clinical management of children and young adults with CKD. For example, patients with SRNS who harbor mutations in *COQ2*, *COQ6*, or *ADCK4* may respond to administration of coenzyme Q_{10}, which provides a therapeutic option in an otherwise untreatable disease.[20–23]

Establishing a molecular genetic diagnosis can be of particular importance in the renal transplant population. There are case reports describing situations in which, during the work-up for allograft dysfunction, an underlying genetic diagnosis is identified in a transplant recipient, their living donor, or the deceased donor kidney.[24,25] M'dimegh *et al.* describe a 23-year-old man who developed early allograft dysfunction after renal transplantation. It was not until calcium oxalate deposits were seen on allograft biopsy that a diagnosis of primary hyperoxaluria type 1 (PH1) was suspected.[24] In another example, several case reports describe patients with allograft dysfunction in whom subsequent work-up demonstrated that their donors had clinically occult Fabry disease.[25–27] In all scenarios, identification of a precise molecular diagnosis before transplantation would have helped to guide peri-transplant management and donor ascertainment, thereby avoiding early allograft failure.

To our knowledge, there is currently no systematic study on the prevalence of monogenic disease in pediatric renal transplant recipients, nor on the utility of whole-exome sequencing (WES) in guiding the management of these patients. We therefore hypothesized that by WES, we can identify a causative mutation in at least 20% of renal transplant recipients and that important consequences for clinical management may result. Here, we report on the results from WES in 104 renal transplant recipients who manifested with CKD before 25 years of age and who received a renal transplant at Boston Children's Hospital within a continuous 10-year period from 2007 to 2017.

Significance Statement

Case reports describe scenarios in which previously undiagnosed genetic disorders, such as primary hyperoxaluria type 1, caused early allograft failure in kidney transplant recipients. Whole-exome sequencing (WES) has found that approximately 20% of pediatric patients with CKD have a relevant mutation, but the diagnostic yield of WES in kidney transplant recipients is not known. In this study of 104 kidney transplant recipients at a single center, use of WES provided a molecular genetic diagnosis for 34 out of 104 (32.7%) patients. Such diagnoses enabled identification of potential prospective consequences for many patients; in others, receiving the diagnosis earlier in the course of their disease might have mitigated negative consequences. The authors propose considering WES for any child or young adult with CKD.

METHODS

Human Patients

This study was approved by the Institutional Review Board of Boston Children's Hospital. Patients who developed CKD before 25 years of age and who received a renal transplant between 2007 and 2017 at Boston Children's Hospital were enrolled after obtaining informed consent. Our recruitment process is summarized in Supplemental Figure 1.

A total of 104 of 272 probands who met our inclusion criteria were enrolled for WES. Of the 168 probands who were not enrolled, 41 had transitioned care to a different hospital, 18 were unable to provide consent due to guardianship issues, and two were deceased. We excluded 23 probands who developed ESRD secondary to nonintrinsic renal disease (*e.g.*, septic shock). 45 probands declined to participate and 39 remain to be approached.

A small subset of patients had been referred for clinical genetic testing before initiation of this study because of a family history of renal disease or the presence of clinical features suggestive of an underlying genetic disorder. These patients were included in our study as our goal was to determine the diagnostic utility of WES for all kidney transplant recipients with a primary renal disease.

The primary clinical diagnosis of each patient was determined *via* chart review, and categorized as either CAKUT, SRNS, chronic GN, renal cystic ciliopathy, nephrolithiasis/nephrocalcinosis, or ESRD of unknown etiology. CAKUT was defined as the demonstration of any abnormality of number, size, shape, or anatomic position of the kidneys or urinary tract, and included at least one of the following: renal agenesis, renal hypoplasia/dysplasia, multicystic dysplastic kidneys, obstructive uropathy, or reflux nephropathy. SRNS was defined by the lack of response to steroid treatment in a patient with nephrotic syndrome, and included biopsy findings of FSGS and diffuse mesangial sclerosis. Chronic GN encompassed Alport syndrome, membranoproliferative GN, crescentic GN, IgA nephropathy, and hemolytic uremic syndrome. Renal cystic ciliopathies included patients diagnosed with nephronophthisis, medullary cystic disease, or other renal cystic diseases. Nephrolithiasis/nephrocalcinosis included patients with urinary stone disease.

All patients completed clinical questionnaires, which included information regarding age of disease onset, family history of renal disease, and presence of consanguinity. Information regarding extrarenal manifestations was obtained from each patient's clinical chart.

DNA Isolation and WES

Research-based WES was performed as previously described.[28] In brief, genomic DNA was isolated from blood lymphocyte or saliva samples and subjected to exome capture using Agilent SureSelect human exome capture arrays (Life Technologies), followed by next-generation sequencing on the Illumina HighSeq sequencing platform. Sequence reads were mapped to the human reference genome assembly (National Center for Biotechnology Information build 37/hg19) using the CLC Genomics Workbench software (version 6.5.2; CLC bio, Aarhus, Denmark).

Mutation Calling in Approximately 400 Genes Known to Cause CKD

After alignment to the human reference genome, variants were filtered as previously described and as summarized in Supplemental Figure 2.[6,29] In brief, variant filtering on the basis of population frequency was performed using population databases (Exome Sequencing Project [http://evs.gs.washington.edu/EVS], Exome Aggregation Consortium [http://exac.broadinstitute.org], Genome Aggregation Database [http://gnomad.broadinstitute.org], and 1000 Genomes Project [http://www.internationalgenome.org/1000-genomes-browsers]) to include only rare alleles (*i.e.*, minor allele frequency <1%). The exception to this was the *NPHS2* R229Q allele, which, despite having a minor allele frequency >1%, has been reported to be pathogenic when in *trans* with other specific *NPHS2* alleles.[30] Synonymous and intronic variants that were not located within splice site regions were excluded.

We then evaluated WES data for causative mutations in 396 genes associated with renal disease (Supplemental Table 1, A–G). This included 41 genes that cause isolated CAKUT, 50 genes for SRNS, 17 genes for chronic GN, 95 genes for renal cystic ciliopathies, and 37 genes for urinary stone disease. We also evaluated WES data for variants in an additional 145 genes that have been reported to cause syndromic CAKUT and 11 genes that have been reported to cause unspecified CKD.

A mean depth coverage of 58× was achieved in our cohort. Six of the 396 known CKD genes did not achieve a mean coverage of at least 30× (Supplemental Table 2).

Surviving variants were then ranked based on their likelihood of being disease-causing, taking into consideration evolutionary conservation among orthologs using the ENSEMBL Genome Browser (http://www.ensembl.org) and assembled using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/), as well as prediction scores from the web-based prediction programs PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2), SIFT (http://sift.bii.a-star.edu.sg/), and MutationTaster (http://www.mutationtaster.org). Remaining variants were further evaluated by review of the existing literature and determination of phenotypic match. Clinician-scientists and geneticists who had knowledge of the clinical phenotypes and pedigree structure as well as experience with exome evaluation performed mutation calling. In addition, the American College of Medical Genetics and Genomics guidelines for variant classification were applied and variants were considered to be disease-causing if they were classified as pathogenic or likely pathogenic (Supplemental Table 3).[31–33] All variants were confirmed in original patient DNA by Sanger sequencing.

CNV Analysis

For probands in whom clinical SNP arrays revealed a pathogenic copy number variant (CNV) and WES evaluation for single nucleotide variants and small insertions/deletions was negative, we performed CNV analysis on WES data using CoNIFER software in order to verify the clinical findings.[34] WES was not utilized to identify novel CNVs because of the relatively low sensitivity of this technique.

RESULTS

Clinical Characteristics

Of 272 probands who received a renal transplant at Boston Children's Hospital between 2007 and 2017, we recruited 104 individuals for WES (Supplemental Figure 1). Fifty five (52.9%) patients had a clinical diagnosis of CAKUT, which was the most common cause of ESRD in our cohort. Twenty one (20.2%) patients were clinically diagnosed with SRNS, seven (6.8%) with chronic GN, nine (8.6%) with a renal cystic ciliopathy, and three (2.9%) with nephrolithiasis. In nine out of 104 (8.6%) patients, the cause of ESRD was unknown. Sixty two (59.6%) patients were male, nine (8.7%) were from consanguineous families, and 23 (22.1%) had a family history of renal disease. Clinical characteristics of the 104 probands enrolled for WES are compared with those of the 272 total probands who were eligible for the study and 2,196 patients included in the NAPRTCS registry between 2007 and 2014 (Supplemental Table 4). The 104 enrolled probands are fairly representative of the general pediatric renal transplant cohort.

A Monogenic Cause of CKD Is Identified in 32.7% of Renal Transplant Recipients

We performed WES in 104 renal transplant recipients and identified a likely causative mutation in one of approximately 400 CKD genes in 34 (32.7%) individuals (Figure 1, Table 1). Six patients had previously undergone clinical genetic testing and already carried a molecular genetic diagnosis. In five families, targeted gene sequencing performed in our laboratory had identified a pathogenic mutation previously.[8] In both of these instances, previously identified mutations were confirmed by WES. For 23 individuals, WES provided a molecular genetic diagnosis for the first time.

In the 34 individuals in whom a molecular genetic diagnosis was established, 29 patients had monogenic mutations in 19 different genes, three patients had homozygous deletions of *NPHP1*, and two patients harbored pathogenic CNVs (Table 1). There were three patients with compound heterozygous mutations in whom segregation was not definitively established. In these situations, patients were considered to

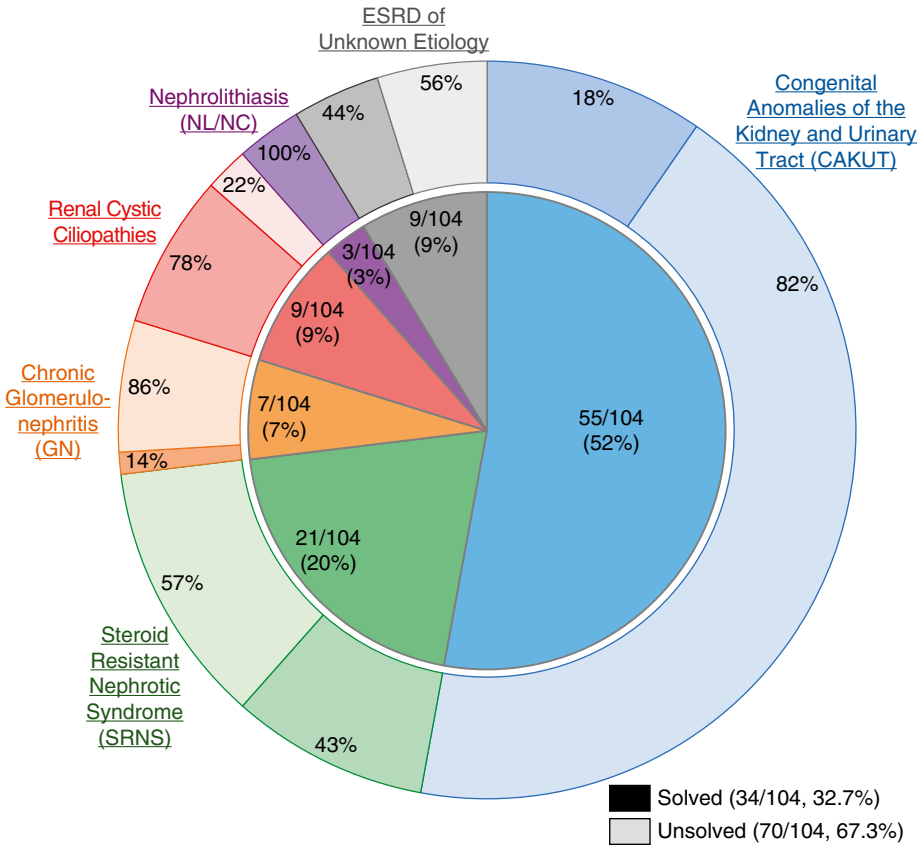

Figure 1. A monogenic cause of ESRD is identified in 32.7% of renal transplant recipients with onset of CKD before 25 years of age. Probands are categorized by clinical diagnostic group. Inner segments represent the numbers and fractions (in %) of transplant recipients that fall into one of six clinical diagnostic groups, as follows: CAKUT, 55 out of 104 (52%); SRNS, 21 out of 104 (20%); chronic GN, seven out of 104 (7%); renal cystic ciliopathies, nine out of 104 (9%); nephrolithiasis or nephrocalcinosis (NL/NC), three out of 104 (3%); and ESRD of unknown etiology, nine out of 104 (9%). Outer segments represent for each diagnostic group the relative fraction of patients in whom a molecular genetic diagnosis was established (darker colors). A disease-causing mutation was identified in 34 out of 104 families (32.7%). The distribution by clinical diagnostic group is as follows: a molecular diagnosis was established in ten out of 55 (18%) patients with CAKUT, nine out of 21 (43%) patients with SRNS, one out of seven (14%) patients with GN, seven out of nine (78%) patients with a renal cystic ciliopathy, three out of three (100%) patients with nephrolithiasis, and four out of nine (44%) patients with ESRD of unknown etiology.

be molecularly solved only if there was a clear genotype-phenotype match.

Clinical Determinants of Establishing a Molecular Diagnosis

The percentage of patients in whom we established a molecular genetic diagnosis differed across clinical diagnostic groups (Supplemental Figure 3). Three patients with urinary stone disease were clinically diagnosed with PH1, and all three were found to have mutations in *AGXT*. A molecular genetic diagnosis was identified in seven out of nine (78%) patients with a renal cystic ciliopathy, four out of nine (44%) patients with ESRD of unknown etiology, nine out of 21 (43%) patients with SRNS, ten out of 55 (18%) patients with CAKUT, and one out of seven (14%) patients with chronic GN. The broadest genetic heterogeneity was seen among patients with CAKUT, in whom mutations in six different genes and two CNVs were found in ten families.

The likelihood of establishing a molecular genetic diagnosis was also higher for patients who reported a history of consanguinity (67% compared with 30%), patients who had extra-renal manifestations (45% compared with 18%), and patients with a positive family history of renal disease (48% compared with 28%) (Supplemental Figure 4).

WES Can Provide a Precise Etiologic Diagnosis for Renal Transplant Recipients

Nine patients in our cohort were given an *a priori* clinical diagnosis of ESRD of unknown etiology, and in four of these patients, we made a molecular diagnosis through WES (Figure 1, Table 1). All four patients presented in childhood or early adolescence with advanced renal disease, and biopsies were either

Table 1. Disease-causing mutations identified in 34 out of 104 (32.7%) renal transplant recipients with onset of CKD at < 25 years of age

Family[a]	*A Priori* Clinical Diagnosis	Post-WES Diagnosis	Extrarenal Manifestations	Family History	Homozygosity (>75 MB)	Gene	Zygosity	c.Change p.Change Segregation (m, p)[b]	Conservation	PP2[c] SIFT[d]/ Mutation Taster[e]	Allele Frequency in gnomAD[f]	HGMD[g] (ACMG[h])	PMID (if previously reported)
CAKUT													
B910[i]	Wolf–Hirschhorn syndrome	Wolf–Hirschhorn syndrome	Growth failure, seizures, developmental delay	No	No	4p16.3 deletion		Heterozygous deletion (NA)				NA	#10995514
B643[i]	L UPJO R MCDK	RCAD	Autism	No	No	17q12 deletion		Heterozygous deletion (NA)				NA	#25536396
B849[i]	BOR	BOR	Cleft palate, brachial pit, hearing loss	No	No	*EYA1*	Het	c.966+1G>C Splice (NA)	N/A	N/A	None	Gene (P)	Novel
B1162	BOR	BOR	Malformed ears, hearing loss, polydactyly	Yes (mother)	No	*EYA1*	Het	c.1319G>A p.R440Q (NA)	*C.i.*	0.786 Tol/DC	None	Variant (P)	#10464653
B664	B/L MCDK	RCAD	None	No	No	*HNF1B*	Het	c.494G>A p.R165H (NA)	*C.e.*	0.999 Del/—	None	Variant (LP)	#24254850
B1137	VUR	Alagille syndrome	ADHD, scoliosis, heart murmur	Yes (mother)	No	*JAG1*	Het	c.2638T>C p.C880R (NA)	*D.m.*	0.99 Del/DC	0/1/251,430	Gene (LP)	Novel
B848	Alagille syndrome	Alagille syndrome	Tetralogy of Fallot, liver failure	No	No	*JAG1*	Het	c.2957_2958insTT p.L986Ffs*2 (NA)	N/A	N/A	None	Gene (P)	Novel
B1142	B/L renal agenesis	Feingold syndrome	Duodenal web	Yes (father)	No	*MYCN*	Het	c.1178G>A p.R393H (NA)	*D.m.*	1.00 Del/DC	None	Variant (LP)	#15821734
B934	BOR	Townes Brocks syndrome	Malformed ears, hearing loss, Duane syndrome, VSD, polydactyly	No	No	*SALL1*	Het	c.826C>T p.R276* (NA, WT)	N/A	N/A	None	Variant (P)	#9973281
B625	B/L renal dysplasia	EEC syndrome	Hypergonadotropic hypogonadism, sandal gap deformity	Yes (pat gpa)	No	*TP63*	Het	c.1012C>T p.R338C (NA; het)	*C.i.*	0.99 Del/DC	0/1/251,202	Gene (LP)	Novel

Table 1. Continued

Family[a]	*A Priori* Clinical Diagnosis	Post-WES Diagnosis	Extrarenal Manifestations	Family History	Homozygosity (>75 MB)	Gene	Zygosity	c.Change p.Change Segregation (m, p)[b]	Conservation	PP2[c] SIFT[d]/ Mutation Taster[e]	Allele Frequency in gnomAD[f]	HGMD[g] (ACMG[h])	PMID (if previously reported)
SRNS													
B284[j]	FSGS	FSGS	Charcot-Marie-Tooth	Yes (mother)	No	*INF2*	Het	c.542T>G p.V181G (het, NA)	*H.s.*	0.98 Del/DC	None	Variant (P)	#23014460
B1273[i]	CNS	CNS	Autism, seizures	No	No	*NPHS1*	Cpd het	c.139delG p.A47Pfs*81 (NA)[k]	N/A	N/A	0/3/277,638	Variant (P)	#18503012
								c.1701C>A p.C567* (NA)[k]	N/A	N/A	0/10/282,168	Variant (P)	#11317351
B144[j]	CNS	CNS	Aortic and pulmonary valve stenosis, microcephaly	Yes (sister)	Yes	*NPHS1*	Hom	c.728C>T p.P243L (het; het)	*D.r.*	1.00 Del/DC	None	Gene (LP)	Novel
B801[i]	CNS	CNS	Hypotonia, developmental delay	No	Yes	*NPHS1*	Hom	c.1379G>A p.R460Q (NA)	*M.m.*	0.48 Tol/Poly	0/1/250,476	Variant (LP)	#11317351
B350[j]	CNS	CNS	Aortic stenosis	No	No	*NPHS1*	Hom	c.1868G>T p.C623F (NA)	*D.r.*	1.00 Del/DC	0/10/282,168	Variant (P)	#9915943
B1395[i]	CNS	CNS	Polymicrogyria, arthrogryposis, developmental delay	Yes (brother)	Yes	*NPHS2*	Hom	c.503G>A p.R168H (het; het)	*D.m.*	0.99 Del/DC	0/3/221,108	Variant (P)	#15253708
B188[j]	FSGS	FSGS	None	No	No	*NPHS2*	Hom	c.855_856delAA p.R286Tfs*17 (NA)	N/A	N/A	0/20/281,474	Variant (P)	#10742096
B354	FSGS	FSGS	None	Yes (brother)	Yes	*PLCE1*	Hom	c.4978_4981delCAGA p.Q1660Lfs*9 (het; het)	N/A	N/A	None	Variant (P)	#25349199
B92[j]	Frasier syndrome	Frasier syndrome	Gonadal dysgenesis, hereditary spherocytosis	Yes (mother, sister)	No	*WT1*	Het	c.1432+4C>T Splice (het; NA)	N/A	N/A	None	Variant (P)	#9398852
Chronic GN													
B2440	Alport syndrome	Alport syndrome	Hearing loss	Yes (mother)	No	*COL4A5*	Hemi	c.4791T>G p.Y1597* (het; NA)	N/A	N/A	None	Variant (P)	#9848783

Table 1. Continued

Family[a]	*A Priori* Clinical Diagnosis	Post-WES Diagnosis	Extrarenal Manifestations	Family History	Homozygosity (>75 MB)	Gene	Zygosity	c.Change p.Change Segregation (m, p)[b]	Conservation	PP2[c] SIFT[d]/ Mutation Taster[e]	Allele Frequency in gnomAD[f]	HGMD[g] (ACMG[h])	PMID (if previously reported)
Renal cystic ciliopathies													
A4037[l]	NPHP	NPHP	Retinal degeneration, pseudotumor cerebri, macrocephaly, XXX karyotype	No	No	*CEP83*	Hom	c.2007delA p.E669Dfs*14 (het, het)	N/A	N/A	None	Variant (P)	#24882706
B659	Senior Loken syndrome	Senior Loken syndrome	Retinal degeneration	Yes (sister)	No	*CEP83*	Hom	c.2007delA p.E669Dfs*14 (NA)	N/A	N/A	None	Variant (P)	#24882706
B06	Short-rib thoracic dysplasia	Short-rib thoracic dysplasia	Restrictive lung disease, cholestatic liver disease	No	No	*DYNC2H1*	Cpd het	c.9638A>G p.Y3213C (WT; het)	*D.m.*	1.00 Del/DC	0/1/31,366	Gene (LP)	Novel
								c.12431C>G p.P4144R (het; WT)	*G.g.*	0.97 Del/DC	0/3/279,946	Gene (LP)	Novel
B1233	NPHP	RCAD	None	No	No	*HNF1B*	Het	c.857T>C p.L286P (NA)	*C.i.*	1.00 Del/—	None	Gene (LP)	Novel
B367	NPHP	NPHP	None	No	No	*NPHP1*	Hom		Homozygous deletion (NA)			Variant (P)	#9326933
B950	NPHP	NPHP	Oculomotor apraxia	No	No	*NPHP1*	Hom		Homozygous deletion (NA)			Variant (P)	#9326933
B375	ARPKD	ARPKD	Increased heterogeneity of liver, splenomegaly	No	No	*PKHD1*	Cpd het	c.10452dupT p.L3485Sfs*18 (NA)[k]	N/A	N/A	0/1/250,588	Variant (P)	#15108281
								c.11452G>T p.V3818F (NA)[k]	*X.t.*	0.6 Del/DC	0/1/251,398	Gene (LP)	Novel
Nephrolithiasis/ nephrocalcinosis													
B949	PH1	PH1	None	No	No	*AGXT*	Cpd het	c.33dupC p.K12Qfs*156 (NA)[k]	N/A	N/A	0/43/270,822	Variant (P)	#10394939
								c.508G>A p.G170R (NA)[k]	*D.m.*	1.00 Del/DC	1/136/ 252,084	Variant (P)	#23229545
B2404	PH1	PH1	Hypothyroidism	No	No	*AGXT*	Hom	c.245G>A p.G82E (het, het)	*S.c.*	1.00 Del/DC	0/5/251,218	Variant(P)	#1349575
B942	PH1	PH1	None	No	No	*AGXT*	Hom	c.473C>T p.S158L (NA)	*D.r.*	0.99 Del/DC	0/1/226,530	Variant (P)	#15849466

Table 1. Continued

Family[a]	*A Priori* Clinical Diagnosis	Post-WES Diagnosis	Extrarenal Manifestations	Family History	Homozygosity (>75 MB)	Gene	Zygosity	c.Change p.Change Segregation (m, p)[b]	Conservation	PP2[c] SIFT[d]/ Mutation Taster[e]	Allele Frequency in gnomAD[f]	HGMD[g] (ACMG[h])	PMID (if previously reported)
ESRD of unknown etiology													
B797	ESRD	NPHP	Learning disability, cerebellar hypoplasia	No	Yes	*NPHP1*	Hom	Homozygous deletion (NA)				Variant (P)	#9326933
B238	ESRD	SRNS	None	No	Yes	*NUP93*	Hom	c.575A>G p.Y192C (NA)	*X.t.*	0.03 Del/DC	0/7/282,600	Gene (LP)	Novel
B2559	ESRD	SRNS	None	No	No	*TRPC6*	Het	c.2684G>T p.R895L (WT, WT)	*D.r.*	0.90 Del/DC	None	Variant (P)	#21734084
B1873	ESRD	NPHP	Neonatal stroke, mild autism spectrum disorder	No	No	*TTC21B*	Cpd het	c.986A>T p.E329V (het, WT)[m]	*C.i.*	0.99 Del/DC	0/1/251,074	Gene (LP)	Novel
								c.1038G>A p.W346* (WT, WT)[m]	N/A	N/A	None	Gene (P)	Novel

Patients are grouped according to their *a priori* clinical diagnosis. The patients in whom WES clarified the clinical diagnosis or lead to a diagnosis for the first time are underlined. m, p, maternal allele, paternal allele; ACMG, American College of Medical Genetics and Genomics; PMID, PubMed ID; NA, not available; L, left; UPJO, ureteropelvic junction obstruction; R, right; MCDK, multicystic dysplastic kidney; RCAD, renal cysts and diabetes; BOR, branchio-oto-renal syndrome; Het, heterozygous; P, pathogenic; *C.i.*, *Ciona intestinalis*; Tol, tolerated (SIFT); DC, disease causing (MutationTaster); B/L, bilateral; *C.e.*, *Caenorhabditis elegans*; Del, deleterious (SIFT); LP, likely pathogenic; VUR, vesicoureteral reflux; ADHD, attention deficient hyperactivity disorder; *D.m.*, *Drosophila melanogaster*; VSD, ventricular septal defect; EEC, ectrodactyly, ectodermal dysplasia, and cleft lip/palate syndrome; pat gpa, paternal grandfather; *H.s.*, *Homo sapiens*; CNS, congenital nephrotic syndrome; Cpd het, compound heterozygous; Hemi, hemizygous; Hom, homozygous; *D.r.*, *Danio rerio*; *M.m.*, *Mus musculus*; Poly, polymorphism (MutationTaster); NPHP, nephronophthisis; WT, wild type; *G.g.*, *Gallus gallus*; ARPKD, autosomal recessive polycystic kidney disease; *X.t.*, *Xenopus tropicalis*; *S.c.*, *Saccharomyces cerevisiae*.

[a]Family number is underlined: WES altered or further clarified the *a priori* clinical diagnosis.

[b]Segregation listed as (maternal allele, paternal allele). If maternal and paternal DNA are unavailable, segregation is listed as NA.

[c]PolyPhen-2 score, which predicts potential effect of an amino acid change on the structure and function of a protein (http://genetics.bwh.harvard.edu/pph2). More deleterious mutations are closer to 1.000, whereas tolerant changes are closer to 0.000.

[d]SIFT, which predicts whether an amino acid change will affect protein function (http://sift.bii.a-star.edu.sg/).

[e]MutationTaster, prediction tool to determine deleteriousness of an amino acid substitution (http://www.mutationtaster.org).

[f]Genome Aggregation Database (gnomAD; http://www.gnomad.broadinstitute.org).

[g]Human Gene Mutation Database (HGMD; https://portal.biobase-international.com), listed as "Variant" if mutation is reported in HGMD or "Gene" if gene, but not specific allele is reported.

[h]ACMG classifications as described previously.[31]

[i]Proband underwent clinical genetic testing before enrollment in this study. Mutation was confirmed *via* WES.

[j]Proband underwent panel sequencing previously in our laboratory before enrollment in this study. Mutation was confirmed *via* WES.

[k]Probands with compound heterozygous mutations in whom parental DNA was unavailable for segregation. These were only considered to be molecularly solved if there was a clear genotype-phenotype correlation.

[l]Index case published previously (PMID 24882706).

[m]Inspection of WES reads demonstrate that the two alleles are in *trans* (Supplemental Figure 5).

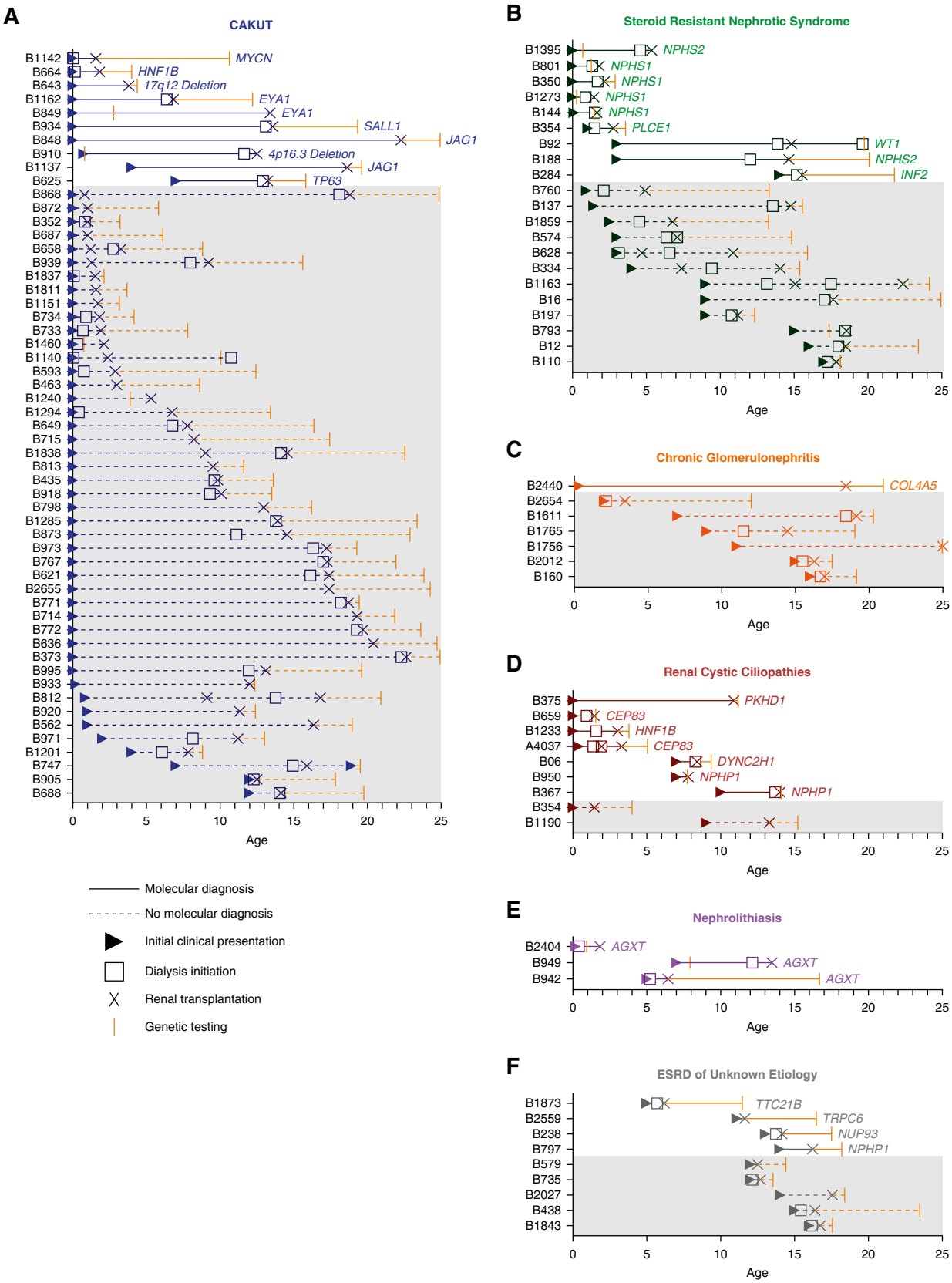

Figure 2. Relationship between the time point at which WES was performed and relevant diagnostic and treatment events in 104 renal transplant recipients. Each patient is denoted as a separate entry on the *y*-axis. Age is represented on the *x*-axis. For each patient, age

deferred or nondiagnostic. WES revealed mutations in the following genes: (*1*) *NPHP1* (homozygous deletion), which causes juvenile NPHP; (*2*) *TRPC6* (c.2684G>T, p.Arg895Leu; *de novo*), which is a cause of autosomal dominant FSGS;[35,36] (*3*) *TTC21B* (c.1038G>A, p.Trp346*; c.986A>T, p.Glu328Val), which has been reported to cause both glomerular and cystic renal diseases;[37,38] and (*4*) *NUP93* (c.575A>G, p.Tyr192Cys), which is a cause of SRNS.[28] Examination of WES reads confirmed that the compound heterozygous *TTC21B* alleles were inherited in *trans* (Supplemental Figure 5). Thus, for all four individuals, WES provided a precise diagnosis for the first time.

In addition to these four patients with ESRD of unknown etiology, WES provided a more specific molecular etiologic diagnosis for seven additional patients (underlined in Table 1). This included one patient who was initially diagnosed with a renal cystic ciliopathy, but ultimately was found to have a mutation in *HNF1B*. This patient did not have any known family history of renal disease and received a deceased donor renal transplant. There were also six patients initially thought to have isolated CAKUT, but were found to have mutations in genes that cause syndromic disease. Upon further chart review, it was noted that five of these six patients had subtle extrarenal manifestations. In total, identification of a molecular diagnosis provided clarification of a patient's clinical diagnosis for 11 out of 34 probands (32%).

Implications of Establishing a Molecular Genetic Diagnosis for Clinical Management

Because of the retrospective nature of this study, virtually all recruited patients underwent genetic testing after kidney transplantation (Figure 2). Several patients did carry an accurate clinical diagnosis, which retrospectively was confirmed by WES. However, in many cases, the clinical diagnosis was made several years after a patient's initial presentation, and early initiation of genetic testing could have had important implications for clinical care. We identified five probands, four with correct clinical diagnoses, in whom identification of a molecular genetic etiology of CKD could have had clinical consequences (Table 2). Here, we highlight several examples.

We established a molecular genetic diagnosis in nine out of the 21 patients clinically diagnosed with SRNS. Three out of these nine patients received treatment with steroids or other immunosuppressive therapy, and one patient also received plasmapheresis before transplantation, as some studies suggest that prophylactic plasmapheresis can reduce the likelihood of FSGS recurrence for high-risk patients.[39–41] It was not until after kidney transplantation that WES was performed for all three individuals. Identification of a molecular genetic diagnosis earlier in the patients' disease courses could minimize the exposure to intensive immunosuppressive medications both pre- and post-transplant.[42] In addition, it could also have obviated the need for invasive procedures, such as renal biopsy or catheter placement for plasmapheresis.

Three patients were clinically diagnosed with PH1. Although all three patients were clinically diagnosed before WES, the diagnosis was delayed by almost 1 year for patient B2404, who was initially thought to have renal dysplasia. This patient developed anuric renal failure as an infant, and required RRT shortly after birth. She did undergo biochemical evaluation for PH1 at the time of her initial presentation, but her serum oxalate levels returned normal, potentially because she was receiving intermittent hemodialysis at the time. The patient was ultimately found to have a markedly elevated serum oxalate level several months later while on peritoneal dialysis and undergoing evaluation for kidney transplantation. After her clinical diagnosis of PH1, she was initiated on a more frequent dialysis regimen to decrease her risk for systemic oxalosis and was listed for a combined liver-kidney transplant, which is the treatment of choice.[43] Performing WES at the time of her initial presentation may have led to a more rapid diagnosis and earlier initiation of these treatment measures. In particular, WES has an advantage over traditional screening methods, such as urinary oxalate levels, which can vary depending on age and diet, and can also be inaccurate in advanced CKD.[43] Furthermore, WES would reduce the need for more invasive procedures, such as renal or liver biopsy.

Finally, one patient with ESRD of unknown etiology was found to have a *de novo* mutation in *TRPC6*. This patient initially presented in adolescence with hypertensive crisis, severely depressed renal function, and laboratory studies suggestive of a thrombotic microangiopathy. There was concern for atypical hemolytic uremic syndrome, and she received treatment with eculizumab for several months. After thoughtful consideration, eculizumab was discontinued before renal transplantation, and there was no recrudescence of the prior microangiopathic process. However, WES at the time of the patient's initial presentation may have prevented the need for prolonged treatment with eculizumab, which increases the risk for severe infection. Additionally, identification of a genetic cause for this patient's renal disease provides closure for the family and will also be important for future genetic counseling.

A summary of the potential implications for each of the genes in which a mutation was found in our cohort is provided in Supplemental Table 5.

at clinical presentation (triangles), dialysis initiation (open squares), renal transplantation (X), and genetic testing (orange vertical hatch) are depicted. Patients in whom a molecular genetic diagnosis was made are on the top, and depicted as solid lines on a white background. Patients in whom no genetic mutations were identified are shown as dashed lines on a gray background. Some patients received a renal transplant preemptively and never required dialysis. For most patients, genetic testing on a research basis was completed after kidney transplantation because of the retrospective nature of this study. Patient data are grouped by clinical diagnosis within the same categories depicted in Figure 1, *i.e.*, (A) CAKUT, (B) SRNS, (C) chronic GN, (D) renal cystic ciliopathies, (E) nephrolithiasis/nephrocalcinosis, and (F) ESRD of unknown etiology.

Table 2. Five probands in whom early genetic diagnosis could have affected clinical care

Family	Gene	Gender, Ethnicity	Age at Presentation/ Age at ESRD	Clinical Details	Biopsy (if done)	Retrospective or Prospective Implications after WES
B2404	*AGXT*	Female, Indian	2 mo/2.5 mo	• Presented with advanced CKD in infancy • Initial work-up included normal serum oxalate level • Presumed diagnosis of renal dysplasia • PH1 diagnosis made at 1 yr of age during evaluation for LRD transplant	None	• Early initiation of daily hemodialysis to decrease risk for systemic oxalosis • Earlier listing for combined liver-kidney transplantation
B188	*NPHS2*	Female, white	3 yr/12 yr	• Presented with edema, proteinuria, and hypoalbuminemia • Treatment with steroids, cyclophosphamide, cyclosporine, and ACE inhibitors • Received plasmapheresis before transplantation to reduce risk of FSGS recurrence[8,39-41]	FSGS	• Avoidance of pretransplant IS and pheresis catheter placement • Using a lower-risk (*e.g.*, steroid minimization) protocol for IS given low risk for recurrence
B354	*PLCE1*	Male, Pakistani	12 mo/15 mo	• Presented with edema and proteinuria • No response to steroids or calcineurin inhibitors	Biopsy 1: mesangial proliferative GN Biopsy 2: DMS	• Avoidance of pretransplant IS (steroids and cyclosporine)
B2559	*TRPC6*	Female, white	11 yr/11 yr	• Presented with malignant hypertension, anemia, thrombocytopenia, and elevated LDH • Received treatment with eculizumab because of concern for atypical HUS and initiated on hemodialysis	Global and segmental sclerosis, tubular atrophy, interstitial fibrosis	• No need for treatment with eculizumab • Genetic counseling given autosomal dominant inheritance and 50% risk of passing along mutant allele
B92	*WT1*	Female, white	4 yr/13 yr	• Treatment with steroids, cyclophosphamide, and ACE inhibitors • Gonadal dysgenesis diagnosed at 18 yr of age during work-up for primary amenorrhea	FSGS	• Earlier initiation of hormone therapy and prophylactic gonadectomy

LRD, living related donor; ACE, angiotensin-converting enzyme; IS, immunosuppression; DMS, diffuse mesangial sclerosis; LDH, lactate dehydrogenase; HUS, hemolytic uremic syndrome.

DISCUSSION

In this study, we performed WES in 104 probands who developed CKD before 25 years of age and who received a renal transplant at Boston Children's Hospital between 2007 and 2017. We show that a molecular genetic diagnosis can be established in 32% of pediatric renal transplant recipients.

It has been demonstrated previously that a causal mutation can be detected in approximately 20% of all children and young adults who present with CKD before 25 years of age.[2] To our knowledge, this is the first study to systematically assess the diagnostic yield of WES in pediatric renal transplant recipients. We determined that the percentage of patients with a molecular diagnosis is slightly higher than that for patients with milder degrees of CKD. This suggests that patients with genetic forms of CKD may have a more severe disease course, although future studies with more patients will be needed for definitive conclusions to be made. In addition, consistent with prior literature, the likelihood of identifying a genetic mutation depended upon a patient's clinical disease group, as well as the presence of consanguinity or extrarenal manifestations.[3,13] With the exception of the cohort of patients with urinary stone disease, in which all three patients had mutations in *AGXT*, the molecular diagnostic rate for each clinical group in our study was slightly higher than, but overall similar to, those that have been previously reported.[3–15,19]

Although research-based genetic testing for most of our patients was performed after kidney transplantation, we identified both retrospective and prospective clinical implications for five patients in whom a molecular diagnosis was established. In 11 cases, identification of a genetic mutation provided a more precise etiological cause for the patient's CKD. This is perhaps most salient for the four patients with ESRD of unknown etiology in whom a genetic mutation was identified. Additionally, genetic testing may also lead to more rapid diagnoses for certain patients, such as the patient with PH1 in whom an accurate diagnosis was delayed for 1 year. In fact, it is not uncommon for diagnoses of PH1 to be delayed or even missed entirely, as reports suggest that up to 10% of patients with PH1 are diagnosed only after allograft failure after isolated kidney transplantation.[44] A timely diagnosis of PH1 is paramount for patient care, as increased frequency of dialysis, combined liver-kidney transplantation, and, for some patients, a trial of pyridoxine, can all help to reduce the systemic oxalate burden.[45–47]

Establishing a precise molecular genetic diagnosis can also allow for preemptive screening for extrarenal manifestations. Recent studies suggest that patients clinically diagnosed with isolated CAKUT can have mutations in genes that cause syndromic disease.[48] In some cases, this can be attributed to the differential effects of hypomorphic mutations, as compared with null mutations, or to a gene's variable expressivity.[49] Family members with the same *JAG1* mutation, for instance, can have varying clinical manifestations, ranging from isolated renal disease to severe cholestatic liver disease.[33,50] In other situations, extrarenal manifestations may manifest later in life or, alternatively, subtle phenotypes may be initially overlooked and only identified retrospectively once a genetic diagnosis is made.[48] In these cases of reverse phenotyping, identification of a genetic mutation can lead to preemptive screening for extrarenal manifestations and earlier treatment when available.

Establishing a molecular genetic diagnosis also allows for tailoring of pre- and post-transplant treatment. As another example to the specific therapy for PH1 discussed above, immunosuppression regimens can also be adjusted for patients found to have genetic causes of SRNS. For example, reports suggest that up to 20%–30% of patients with SRNS experience disease-recurrence after kidney transplantation; however, this risk is only 4%–8% in patients who have genetic forms of SRNS.[10,42,51] Certain patients with hereditary SRNS, therefore, could be candidates for a steroid minimization protocol at the time of transplantation given their low risk for immunologic disease.[52] This is particularly pertinent in the pediatric population, in whom corticosteroids can have significant adverse effects on linear growth, body habitus, and self-image, and in whom compliance is a large issue. One exception is in patients who have the Fin(major) *NPHS1* allele (c.121delCT), who have been reported to develop anti-nephrin antibodies post-transplantation, and are at slightly increased risk for SRNS recurrence.[53] It is likely that future studies will elucidate further genotype-phenotype correlations, which would allow us to provide more personalized care for each patient.

Finally, there are examples in which establishing a causal molecular genetic diagnosis helps to guide living donor ascertainment. It has been established that women with heterozygous mutations in *COL4A5*, which causes X-linked Alport syndrome, are at a higher risk for developing CKD later in life. Thus, appropriate genetic counseling should be provided for potential donors who are found to harbor deleterious heterozygous *COL4A5* mutations.[54,55] Similarly, autosomal dominant causes of *FSGS* often manifest later in life and can be associated with variable expressivity. Family members of an individual found to carry a mutation in one of these genes should be offered genetic testing, and those in whom a mutation is identified should potentially be precluded from living kidney donation, given their risk for developing disease. Finally, although allograft survival is improved after living renal donation when compared with deceased donation, there often may be hesitancy in pursuing living donor transplantation in patients with FSGS because of the risk of recurrent disease. If a monogenic cause of FSGS is discovered in a patient, concerns regarding living donation may be alleviated because of the reduced risk of recurrence.[56]

Limitations of our study include a relatively small sample size and single center design. Because our study was conducted at a large, tertiary referral center, our results may not be generalizable to all medical centers worldwide. In addition, although we took a nonbiased approach in recruiting patients with a primary renal disease who received a transplant at our center, there may be potential ascertainment bias because of clinical differences between the patients who declined to participate and those who were enrolled. Finally, WES may miss mutations in introns and promotor regions, certain CNVs, and mutations in exons with low coverage. We additionally predict

that the percentage of individuals in whom a causal diagnosis can be made would be higher if formal CNV analysis is added. However, given the progressively declining costs of WES and utility demonstrated in many clinical scenarios,[57,58] it is becoming an efficient and cost-effective diagnostic study. Thus, given the effect that identification of a genetic mutation can have on pre- and post-transplant care for renal transplant recipients, we propose that WES be considered for patients who develop CKD at 25 years of age or younger.

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DISCLOSURES

Dr. Hildebrandt is a cofounder, SAC member, and holds stock in Goldfinch-Bio.

SUPPLEMENTAL MATERIAL

This article contains the following supplemental material online at http://jasn.asnjournals.org/lookup/suppl/doi:10.1681/ASN.2018060575/-/DCSupplemental.

Supplemental Table 1A. Forty one genes that represent monogenic causes of human isolated CAKUT, if mutated.

Supplemental Table 1B. Fifty genes that represent monogenic causes of human nephrotic syndrome, if mutated.

Supplemental Table 1C. Seventeen genes that represent monogenic causes of human nephritis, if mutated.

Supplemental Table 1D. Ninety five genes that represent monogenic causes of human nephronophthisis or cystic kidney disease, if mutated.

Supplemental Table 1E. Thirty seven genes that represent monogenic causes of human nephrolithiasis or nephrocalcinosis, if mutated.

Supplemental Table 1F. One hundred forty five genes that represent monogenic causes of human syndromic CAKUT, if mutated.

Supplemental Table 1G. Eleven genes that represent rare monogenic causes of human kidney disease (miscellaneous category), if mutated.

Supplemental Table 2. Information on six out of 396 known CKD genes that did not achieve a mean coverage of at least 30×.

Supplemental Table 3. ACMG guidelines for variant calling.

Supplemental Table 4. Clinical characteristics of probands at Boston Children's Hospital who underwent renal transplantation between 2007 and 2017 compared with patients in the NAPRTCS registry.

Supplemental Table 5. Clinical consequences after establishment of a molecular genetic diagnosis for 21 genes identified in 104 families.

Supplemental Figure 1. Recruitment strategy and likelihood of detecting a monogenic cause of CKD in 104 renal transplant recipients.

Supplemental Figure 2. Variant filtering process for the identification of causative mutations in genes known to cause CKD.

Supplemental Figure 3. Percentage of patients with a molecular genetic diagnosis varies by clinical diagnostic group.

Supplemental Figure 4. The likelihood of identifying a molecular diagnosis is higher when there is consanguinity, extrarenal manifestations, or a positive family history.

Supplemental Figure 5. *TTC21B* compound heterozygous alleles are inherited in *trans* for patient B1873.

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