

Diagnostic Utility of Exome Sequencing Among Israeli Children With Kidney Failure



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Introduction: Genetic etiologies are estimated to account for a large portion of chronic kidney diseases (CKD) in children. However, data are lacking regarding the true prevalence of monogenic etiologies stemming from an unselected population screen of children with advanced CKD.

Methods: We conducted a national multicenter prospective study of all Israeli pediatric dialysis units to provide comprehensive "real-world" evidence for the genetic basis of childhood kidney failure in Israel. We performed exome sequencing and assessed the genetic diagnostic yield.

Results: Between 2019 and 2022, we recruited approximately 88% ($n = 79$) of the children on dialysis from all 6 Israeli pediatric dialysis units. We identified genetic etiologies in 36 of 79 (45%) participants. The most common subgroup of diagnostic variants was in congenital anomalies of the kidney and urinary tract causing genes (e.g., *EYA1*, *HNF1B*, *PAX2*, *COL4A1*, and *NFIA*) which together explain 28% of all monogenic etiologies. This was followed by mutations in genes causing renal cystic ciliopathies (e.g., *NPHP1*, *NPHP4*, *PKHD1*, and *BBS9*), steroid-resistant nephrotic syndrome (e.g., *LAGE3*, *NPHS1*, *NPHS2*, *LMX1B*, and *SMARCAL1*) and tubulopathies (e.g., *CTNS* and *AQP2*). The genetic diagnostic yield was higher among Arabs compared to Jewish individuals (55% vs. 29%) and in children from consanguineous compared to nonconsanguineous families (63% vs. 29%). In 5 participants (14%) with genetic diagnoses, the molecular diagnosis did not correspond with the pre-exome diagnosis. Genetic diagnosis has a potential influence on clinical management in 27 of 36 participants (75%).

Conclusion: Exome sequencing in an unbiased Israeli nationwide dialysis-treated kidney failure pediatric cohort resulted in a genetic diagnostic yield of 45% and can often affect clinical decision making.

Kidney Int Rep (2023) 8, 2126–2135; <https://doi.org/10.1016/j.ekir.2023.07.019>

KEYWORDS: children; dialysis; ESKD; exome sequencing; kidney failure; monogenic

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Received 14 May 2023; revised 18 July 2023; accepted 24 July 2023; published online 31 July 2023

Childhood CKD covers a wide range of structural and functional disorders that often result from different genetic etiologies. Knowledge about the underlying genetic basis of childhood CKD has dramatically progressed over the last 2 decades with the identification of numerous disease-causing gene alterations and novel pathomechanisms.^{1,2} Understanding

the genetic underpinnings of childhood CKD, which often enables personalized surveillance and therapies, has increased significantly due to the growing availability of genetic testing as well as clinician's awareness. So far, hundreds of CKD-causing genes have been reported, estimated to explain up to 30% of cases among children^{1,2} and approximately 10% in adults.³ Nevertheless, the genetic diagnostic yield varies markedly across different study cohorts, depending on pre-selection criteria, clinical presentation, geographic region, and ethnicity. Furthermore, in clinical practice, genetic kidney diseases might be overlooked due to its variable expressivity, incomplete penetrance, low index of suspicion, lack of overt symptoms at early disease stages, and insufficient availability of next generation sequencing methods. Consequently, data are lacking regarding the true prevalence of monogenic CKD etiologies stemming from a non-selected large scale population screen of children with advanced CKD.

We therefore conducted a national multicenter study of all Israeli pediatric dialysis units to provide comprehensive evidence for monogenic etiologies of childhood kidney failure. We report the results from exome sequencing in 79 children with kidney failure treated by dialysis, comprising 88% of the total of 90 children treated with dialysis in Israel during the study period.

METHODS

Study Participants

We conducted a national multicenter collaboration study of all Israeli dialysis units and established the Israeli Kidney Failure Genetic Cohort. The overarching aim of this collaboration is to collect clinical data and DNA samples for genetic studies to provide comprehensive real-world evidence for kidney failure's genetic basis at a national level. By utilizing the Israeli end-stage kidney disease registry, we identified and located all pediatric units in which Israeli children under the age of 18 years are treated with maintenance dialysis. During the study period (September 2019–January 2022), clinical and pedigree data from all patients were collected by administering a standardized questionnaire, after obtaining informed consent. Blood samples of individuals on dialysis and their parents, if available, were collected. Approval for human subject research was obtained from the Institutional Review Boards of Sheba Medical Center and the Israeli Ministry of Health, as well as from ethics review boards of the participating centers.

Clinical Assessment and Diagnosis

Single or multiple primary clinical diagnoses were recorded for each participant based on their medical

records and their primary pediatric nephrologist's assessment. In addition, all participants underwent a clinical interview at the time of recruitment to the study, which included a comprehensive review of their medical files, imaging studies, and kidney biopsy reports, when available. The primary kidney clinical diagnoses were classified as either congenital anomalies of the kidneys and urinary tract (CAKUT), nephrotic syndrome, renal cystic ciliopathy, tubulointerstitial disease, nephrolithiasis-related kidney disease, and CKD of unknown etiology. Furthermore, information regarding the medical history, family history, ethnicity, age at dialysis initiation, consanguinity, and presence of extrarenal manifestations were obtained (Table 1).

Exome Sequencing

DNA was isolated from peripheral blood lymphocytes using the DNeasy Blood and Tissue Kit (Qiagen). Proband exome sequencing was performed on genomic DNA for all affected individuals using an Agilent v5 SureSelect Capture Kit and Illumina 2500 sequencing technology. For each sample, paired end-reads (2 × 150 bp) were obtained, processed, and mapped to the genome. We used the BWA-MEM algorithm (Illumina, San Diego, CA) version 0.7.1216 to align the sequence reads to the human reference genome (hg38). The HaplotypeCaller

Table 1. Clinical characteristics and pre-exome sequencing clinical diagnoses among 79 children with kidney failure

Characteristics	Value	Percentage
Gender		
Male	35	44%
Female	44	56%
Phenotype		
CAKUT	30	38%
SRNS	20	25%
Renal cystic ciliopathy	9	11%
Unknown	7	9%
Glomerulopathy	6	8%
Tubulopathy	4	5%
Nephrolithiasis-related kidney disease	2	3%
Other	1	1%
Biopsied		
Yes	22	28%
No	57	72%
Age at dialysis initiation (yrs)		
0–5	28	35%
5–10	17	22%
11–15	23	29%
15–18	11	14%
Parental consanguinity		
First cousins	38	48%
Family history of ESKD		
Yes	22	28%
No	57	72%

CAKUT, congenital anomalies of the kidney and urinary tract; ESKD, end-stage kidney disease; SRNS, steroid-resistant nephrotic syndrome.

algorithm of GATK V.3.8 was applied for variant calling, as recommended in the best practice pipeline.⁴ KGG-seq version 1.1 was used for annotating identified variants.⁵ In addition, in-house scripts were applied for filtering, based on family pedigree and local datasets of variants detected in previous sequencing projects (in-house cohort of ~5000 samples) and the Genome Aggregation Database data set (gnomAD),⁶ as well as from 30,000 exome sequencing data samples of ethnically matched controls (16,000 Jews and 14,000 Arabs) available via the Franklin Platform (Genoox, Tel-Aviv, Israel, available at <https://franklin.genoox.com/clinical-db/home>).

Diagnostic Analysis

We evaluated exome sequencing data for all rare non-synonymous and splice variants (minor allele frequencies of 1% or less) in genes present in the OMIM database. In addition, we generated a list of previously reported CKD-causing genes and performed a second targeted analysis for this predefined CKD gene panel (Supplementary Table S1). Mutation calling was performed independently by 3 teams: bioinformaticians, geneticists, and a team of clinician-scientists with expertise in nephrogenetics. Exome sequencing data was filtered using the Franklin Platform (Genoox, Tel-Aviv, Israel, available at <https://franklin.genoox.com/clinical-db/home>) Exome data were interpreted according to the American College of Genetic and Genomic Medicine guidelines.⁷ We classified variants as pathogenic, likely pathogenic, variants of unknown significance, likely benign, or benign. American College of Genetic and Genomic Medicine standards and guidelines recommend 28 criteria for variant pathogenicity classification. The Genoox Classification Engine ran 17 of them automatically. The artificial intelligence-based variant classification engine automates rules PVS1, PS1, PM1, PM2, PM4, PM5, PP2, PP3, PP5, BA1, BS1, BS2, BP1, BP3, BP4, BP6, and BP7.⁸ The remaining rules cannot be automated because they require clinical information specific to the patient genotype, e.g., familial data, *de novo* evidence (PS2, PM6), segregation data (PP1, BS4), and/or allelic data (PM3, BP2). Therefore, the remaining criteria were manually classified. We evaluated all variants reported pathogenic or likely pathogenic by Clinvar and by the Genoox Classification Engine, all rare (minor allele frequency < 1%) homozygous variants and all rare (minor allele frequency < 1%) heterozygous variants found in our in-house CKD panel. When a trio exome was available, we also performed analysis for *de novo* variants.

Sanger sequencing was performed to confirm likely pathogenic and pathogenic variants in original DNA samples and when available, to test for familial segregation of the phenotype with the genotype. In

addition, copy number variants were called using the DRAGEN software, which identifies copy number variants by comparing the average coverage and improperly paired reads per area of a sample to data from a cohort of normal samples. Areas with significant differences relative to the cohort were considered copy number variants. For further validation, we additionally compared the average coverage per exon of each sample to a cohort of similar samples using in-house scripts. Sequence variants that remained after the exome sequencing evaluation process were examined for segregation in available samples of affected and unaffected family members.

RESULTS

Patient Characteristics

During the study period, 90 children were treated with maintenance dialysis in Israel. The parents of 11 children chose not to participate in the study. The characteristics of the study population, which includes 79 children (45% male) from 78 different families, who underwent exome sequencing are shown in Table 1. Single, duo, and trio exome analysis were performed in 55%, 19%, and 26% of cases, respectively. Fifty-one (65%) children are of Arab descent and 28 (35%) are Jewish. Overall, 22 participants (28%) had a relative with kidney failure. Consanguinity was evident in 38 participants (48%) of the cohort, predominantly among patients of Arab descent. Mean age at dialysis initiation was 8.5 years (range 1 month–18 years; median = 9 years). The most prevalent mode of dialysis was hemodialysis (75% of the cohort). The most common primary clinical diagnosis was CAKUT followed by nephrotic syndrome and renal cystic ciliopathies (Table 1).

Exome Sequencing Reveals Monogenic Etiology in Approximately 45% of Children on Dialysis

We detected diagnostic single-gene etiologies in 36 of the 79 participants (45%), encompassing 21 distinct monogenic disorders (Table 2, Figure 1). Of these, 13 (36%) are diseases with autosomal dominant inheritance, 22 (61%) with autosomal recessive inheritance, and 1 is an X-linked disease. In addition, 2 participants had compound heterozygous diagnostic pathogenic variants. Among the 36 diagnostic variants that were detected, 24 variants were previously reported as disease-causing and 12 were novel. Overall, 16 of 27 (59%) disease-causing variants had allele frequencies of zero in the Exome Sequencing Project and the Genome Aggregation Database. The mean age of dialysis initiation was comparable between patients with monogenic kidney failure and those with negative exome

Table 2. Thirty-six participants with kidney failure for whom exome sequencing yielded a molecular genetic diagnosis

Patient	Clinical data	Post-ES diagnosis	Gene/region	Annotation	Zygoty	CADD score	Allele frequency ^a	ACMG class	ACMG criteria
21–112	Muslim Arab female with cystic kidney disease; kidney failure at 14 y; no consanguinity	Nephronophthisis 4	<i>NPHP4</i>	c.3325C>T p.Arg1109Ter NM_015102.5	Homo	38	0.002%	P	PVS1, PM2, PP5
21–114	Muslim Arab female with cystic kidney disease and chronic lung disease; kidney failure at 1.8 y; consanguinity	Autosomal recessive polycystic kidney disease	<i>PKHD1</i>	c.2279G>A p.Arg760His NM_138694.4	Homo	33	0.0013%	P	PM2, PP3, PP5
21–115	Muslim Arab male with dysplastic kidneys. Kidney failure at 5.5 y; no consanguinity	CAKUT	<i>COL4A1</i>	c.1807C>T p.Pro603Ser NM_001845.6	Het	25.2	0.0019%	LP	PM2, PP3, PM1, PP5 PP2
21–117	Muslim Arab female with nephrotic syndrome, dysmorphism, Intellectual disability, short stature, and skeletal deformity; kidney failure at 7 y; consanguinity	Schimke immuno- osseous dysplasia	<i>SMARCAL1</i>	c.1682G>A p.Arg561His NM_014140.4	Homo	31	0%	LP	PP3, PM2, PP4, PP5
21–139	Arab Druze female with hypoplastic kidney, polydactyly, obesity, strabismus; kidney failure at 18 y; consanguinity	Bardet-Biedl syndrome type 9	<i>BBS9</i>	c.1063C>T p.Gln355Ter NM_198428.3	Homo	40	0%	P	PVS1, PM2, PP5
21–146	Arab Muslim male with nephronophthisis. Kidney failure at 8 y; consanguinity	Nephronophthisis type 1	<i>NPHP1</i> deletion	chr2 deletion: 110,123, 780-110,204,978	Homo	NA		P	
21–147	Arab Muslim male with tubulopathy and primary hypothyroidism. Kidney failure at 14 y; consanguinity	Cystinosis	<i>CTNS</i>	c.681G>A p.Glu227Glu NM_004937.3	Homo	23	0.0007%	LP	PM2, PP3, PP5, PP4
21–154	Arab Muslim male with nephrotic syndrome, Intellectual disability and microcephaly. Kidney failure at 2 y; consanguinity	Galloway-Mowat syndrome	<i>LAGE3</i>	c.317+4A>G NM_006014.5	Homo	22	0%	LP	PM2, PP3, PP5, PM6, PP4
21–174	Jewish male with CAKUT; kidney failure at 2 y; no consanguinity	Branchiootic syndrome 1	<i>EYA1</i>	c.1597+2T>C NM_000503.6	Het	33	0%	LP	PVS1, PM2
21–182	Arab Muslim male with nephrogenic DI, vesicoureteral reflux, uropathy, recurrent UTIs; kidney failure at 16 y; consanguinity	Nephrogenic DI	<i>AQP2</i>	c.83T>C p.Leu28Pro NM_000486.6	Homo	27	0%	LP	PM2, PM1 PP3, PP2, PP5
21–183	Arab Muslim male with nephrotic syndrome; kidney failure at 6 y; no consanguinity	Nephrotic syndrome type 2	<i>NPHS2</i>	c.714G>T p.Arg238Ser NM_014625.4 c.412C>T p.Arg138Ter NM_014625.4	Comp het	25.2 44	0.0032% 0.0006%	P P	PS1, PM2, PP3, PP2, PP5 PSV1, PM2, PP5
21–213	Arab Muslim female with kidney and liver cystic disease; kidney failure at 3 y; consanguinity	Autosomal recessive polycystic kidney disease	<i>PKHD1</i>	c.2279G>A p.Arg760His NM_138694.4	Homo	33	0.0013%	P	PM2, PP3, PP5
21–218	Arab Muslim male with CKDu, Intellectual disability, short stature, skeletal deformity, convulsions; kidney failure at 15 y; consanguinity	CAKUT	<i>TBX6</i> + 29 genes deletion	chr16 deletion: 29,802, 071-30,199,161 (16p11.2)	Het	NA		P	
21–219	Arab Muslim male with polycystic kidney, developmental delay, elevated liver enzymes; kidney failure at 1.5 y; no consanguinity	HNF1B- related disease	<i>HNF1B</i> + 28 genes deletion	chr17 deletion: 36,459,737-38,222,841 (17q12-q21.2)	Het	NA		P	

(Continued on following page)

Table 2. (Continued) Thirty-six participants with kidney failure for whom exome sequencing yielded a molecular genetic diagnosis

Patient	Clinical data	Post-ES diagnosis	Gene/region	Annotation	Zygoty	CADD score	Allele frequency ^a	ACMG class	ACMG criteria
21–225	Jewish female with renal aplasia and imperforated anus; kidney failure at 2 months; no consanguinity	Branchiootic syndrome 1	<i>EYA1</i>	c.1586C>A p.Ala529Glu NM_000503.6	Het	29	0%	LP	PP3, PM2
21–226	Arab Muslim female with cystinosis; kidney failure at 5.5 y; consanguinity	Cystinosis	<i>CTNS</i>	c.587dup p.Asn196fs NM_004937.3	Homo	NA	0%	P	PVS1, PM2, PP5, PP4
21–250	Arab Muslim female with cystinosis; kidney failure at 8 y; consanguinity	Cystinosis	<i>CTNS</i>	c.587dup p.Asn196fs NM_004937.3	Homo	NA	0%	P	PVS1, PM2, PP5, PP4
21–253	Arab Muslim female with suspected nephronophthisis; kidney failure at 3 y; no consanguinity	CAKUT	<i>NFIA</i>	c.39C>G p.Tyr13Ter NM_001145512.2	Het	31	0%	LP	PVS1, PM2
21–262	Arab Muslim male with hyperoxaluria, skeletal deformity, developmental delay; kidney failure at 3.5 months; consanguinity	Primary hyperoxaluria type 1	<i>AGXT</i>	c.997A>T p.Arg333Ter NM_000030.3	Homo	43	0%	P	PVS1, PM2, PP5, PP4
21–263	Arab Muslim male with glomerulopathy and deafness; kidney failure at 14 y; no consanguinity	Alport syndrome	<i>COL4A5</i>	c.3319_3335del p.Gly1107fs NM_033380.3	Hemi	NA	0%	LP	PVS1, PM2
21–264	Arab Muslim male with hyperoxaluria, skeletal deformity, severe growth delay, splenomegaly; kidney failure at 2.5 mo; consanguinity	Primary hyperoxaluria type 1	<i>AGXT</i>	c.358+1G>A NM_000030.3	Homo	33	0%	P	PVS1, PM2, PP4
21–314	Arab Muslim female with cystic kidney disease; kidney failure at 17 y; consanguinity	Nephronophthisis 1	<i>NPHP1</i> deletion	chr2 deletion: 110,881,357-110,962,555	Homo	NA		P	
21–315	Jewish male with CAKUT and Intellectual disability; kidney failure at 14 y; no consanguinity	CAKUT	<i>COL4A1</i> deletion	chr13 deletion: 110,152,323-110,214,025 ring chromosome 13	Het	NA		LP	
21–354	Jewish male with CAKUT; kidney failure at 2.5 y; no consanguinity	CAKUT	AIFM3 + 76 genes duplication	chr22 duplication: 18516490-21046135 (22q11.21 dup)	Het	NA		P	
21–481	Arab Muslim male nephrotic syndrome; kidney failure at 18 months; consanguinity	Nephrotic syndrome, type 1	<i>NPHS1</i>	c.2104G>A p.Gly702Arg NM_004646.4	Homo	23.6	0%	LP	PM2, PP1 PS1
21–482	Arab Muslim male with cystic kidney disease, liver cysts and bronchiectasis; kidney failure at 14.5 y; consanguinity	Autosomal recessive polycystic kidney disease	<i>PKHD1</i>	c.2279G>A p.Arg760His NM_138694.4 c.4870C>T p.Arg1624Trp NM_138694.4	Comp het	33 26	0.0013% 0.0059%	P LP	PM2, PP3, PP5 PM2, PP5
21–483	Jewish male with nephrotic syndrome; kidney failure at 11.5 y; no consanguinity	Nail-patella syndrome	<i>LMX1B</i>	c.737G>A p.Arg246Gln NM_001174147.2	Het	31	0%	P	PM2, PM1, PP2, PM5, PP3, PP5, PM6
21–484			<i>SMARCAL1</i>		Homo	25.2	0.0006%	P	

(Continued on following page)

Table 2. (Continued) Thirty-six participants with kidney failure for whom exome sequencing yielded a molecular genetic diagnosis

Patient	Clinical data	Post-ES diagnosis	Gene/region	Annotation	Zygoty	CADD score	Allele frequency ^a	ACMG class	ACMG criteria
	Arab Muslim female with nephrotic syndrome, failure to thrive, osseous dysplasia; kidney failure at 11 y; consanguinity	Schimke immuno-osseous dysplasia		c.2290C>T p.Arg764Trp NM_014140.4					PM2, PM5, PP3, PP5, PP4
21–487	Arab Muslim female with glomerulopathy, coloboma, cardiomyopathy; kidney failure at 6 y; consanguinity	Papillorenal syndrome	<i>PAX2</i>	c.76dup p.Val26fs NM_000278.5	Het	NA	0%	P	PVS1, PM2, PP5
21–509	Jewish female with cystinosis; kidney failure at 13.5 y; no consanguinity	Cystinosis	<i>CTNS</i>	c.1015G>A p.Gly339Arg NM_004937.3	Homo	26.6	0.0032%	P	PP3, PM2, PP5, PP4
21–680	Arab Muslim male with hydronephrosis and dysplastic kidneys, mild intellectual disability; kidney failure at 17.5 y; consanguinity	Bardet-Biedl syndrome 9	<i>BBS9</i>	c.385C>T p.His129Tyr NM_198428.3	Homo	23	0%	VUS ^b	PM2, PP3
21–763	Jewish female (per phenotype) with XY DSD, proteinuria, CNS cystic lesions; kidney failure at birth; consanguinity	Denys-Drash syndrome	<i>WT1</i>	c.1316G>T p.Arg439Leu NM_024426.6	Het	32	0%	LP	PM2, PM5, PM1, PP2, PP5
21–848	Arab Muslim female with nephrotic syndrome; kidney failure at 15 y; consanguinity	Nephrotic syndrome, type 2	<i>NPHS2</i>	c.851C>T p.Ala284Val NM_014625.4	Homo	26	0.0039%	P	PM2, PM1, PP2, PP3, PP5
21–878	Jewish female with large hyperechoic kidneys and severe oligohydramnios; kidney failure at birth; no consanguinity	Autosomal recessive polycystic kidney disease	<i>PKHD1</i>	c.3766del p.Gln1256fs NM_138694.4	Homo	NA	0.02%	P	PVS1, PM2, PP5,
21–511	Arab Muslim male with renal dysplasia, elevated CK, hyperlipidemia, fatty liver, short stature, GH deficiency, normal eye examination. Kidney failure at 13.5 y; consanguinity	Papillorenal syndrome	<i>PAX2</i>	c.76dup p.Val26fs NM_000278.5	Het	NA	0%	P	PVS1, PM2, PP5,
22–511	Arab Muslim male with renal hypoplasia, elevated CK, normal eye examination; kidney failure at 2.5 y; consanguinity	Papillorenal syndrome	<i>PAX2</i>	c.76dup p.Val26fs NM_000278.5	Het	NA	0%	P	PVS1, PM2, PP5,

ACMG, American College of Medical Genetics and Genomics; CADD, combined annotation dependent depletion; CAKUT, congenital anomalies of the kidney and urinary tract; CK, creatine kinase; CKDu, chronic kidney disease of unknown origin; Comp, compound; DI, diabetes insipidus; DSD, disorder of sex development; ES, exome sequencing; GH, growth hormone; Hemi, hemizygous; Het, heterozygous; Homo, homozygous; LP, likely pathogenic; P, pathogenic; UTI, urinary tract infection; y, years.

^aAllele frequency was determined using the genome AD website (<https://gnomad.broadinstitute.org>).

^bAlthough this variant is designated as VUS per ACMG criteria, in the context of the specific clinical findings and absence of controls, it was considered as disease-causing.

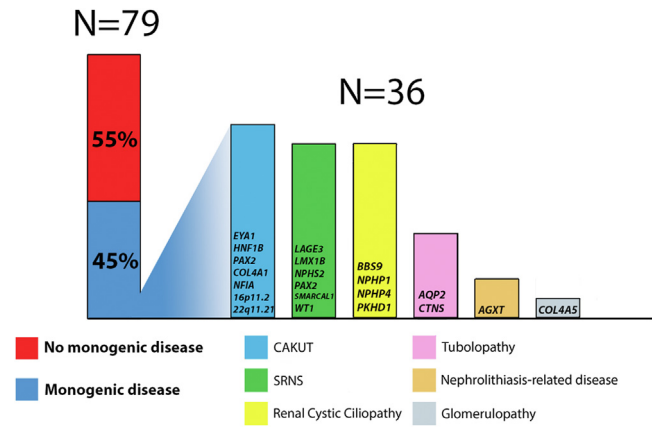


Figure 1. Diagnostic yield of exome sequencing in Israeli children on dialysis. In 36 (45%) participants (blue), we identified monogenic CKD-related diagnostic variants. For the 36 solved cases, the different genes of the diagnostic variants are grouped into sub-categories and displayed on the right side. CAKUT, congenital anomalies of the kidney and the urinary tract; CKD, chronic kidney disease; SRNS, steroid-resistant nephrotic syndrome.

findings (7.9 vs. 9.05 years, respectively; $P = 0.21$). The yield of positive molecular diagnosis resulting from exome sequencing for each CKD category is presented in Figure 2a. Whereas the overall genetic diagnostic yield was approximately 45%, clinical diagnoses of ciliopathies, tubulopathies, and nephrolithiasis-related kidney disease exhibited the highest probability for an underlying genetic condition (89%–100% yield). Furthermore, a genetic diagnosis was made in 2 of 7 participants (29%) with kidney failure of unknown cause (Figure 2a). Higher yields of genetic diagnosis were achieved in individuals with syndromic features compared to those without syndromic features (52% vs. 32%, respectively), in individuals with Arabic ethnicity compared to Jewish ethnicity (55%

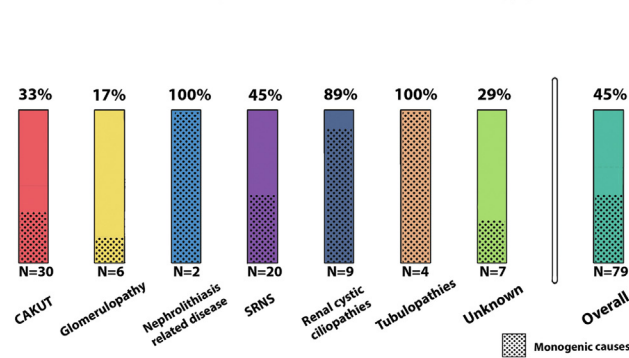
vs. 29%, respectively), and among consanguineous compared to nonconsanguineous families (63% vs. 29%, respectively) (Figure 2b). Nevertheless, patients with none of the above-mentioned risk factors for monogenic kidney disease had an approximately 20% likelihood for an identification of an underlying genetic diagnosis. The distribution of the various monogenic etiologies in relation to age at dialysis initiation is shown in Supplementary Figure S1.

Our results demonstrate significant phenotypic and genetic heterogeneity in childhood-onset CKD. For example, in 3 unrelated Arab Muslim participants, we detected the *PKHD1* p.Arg760His alteration, potentially suggesting a founder mutation effect. This mutation exhibits phenotypic heterogeneity in terms of kidney failure timing and the resulting dialysis initiation age, which was different for each individual (1.5 and 3 years). Similarly, 3 participants from 2 different Arab Muslim families, were found to harbor the heterozygous *PAX2* p.Val26fs alteration, known to reside in a genetic hotspot.^{9,10} Whereas 2 participants presented clinically with CAKUT, the third participant presented with steroid-resistant nephrotic syndrome and focal segmental glomerulosclerosis.¹¹

Clinical Implications of Molecular Diagnosis

Overall, the molecular genetic diagnosis corresponded with the primary pediatric nephrologists’ pre-exome clinical diagnoses in 31 of 36 (~86%) cases, often confirming the diagnosis of a specific CKD etiology. Furthermore, for all genetically solved cases, the molecular diagnosis enabled validation of a final diagnosis and the performance of targeted formal genetic counseling to the participants and their family members. This also allowed for the provision of specific therapies,

a Proportion of monogenic CKD across different etiology groups



b Proportion of monogenic CKD across different patient characteristics

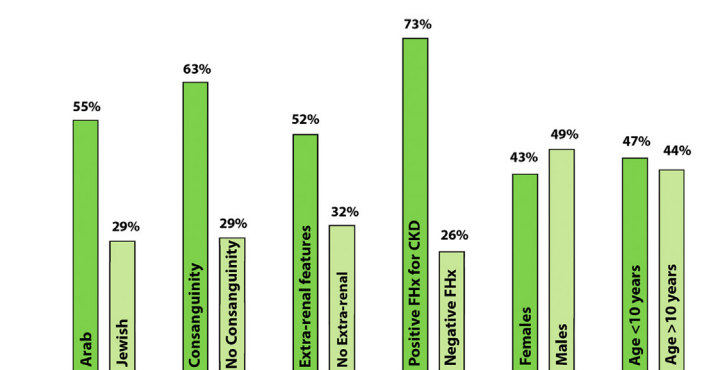


Figure 2. Proportion of monogenic CKD etiologies across different clinical categories. (a) Proportion of monogenic CKD across different etiology groups. Among patients with tubulopathies, nephrolithiasis-related kidney disease and renal cystic ciliopathies, the yield was very high ~90% to 100% compared with lower yields of ~17% to 30% among patients with glomerulopathy, CAKUT, and CKDu. One patient with kidney failure secondary to acute kidney injury (AKI) is not displayed in the left panel. (b) Proportion of monogenic CKD across different patient’s characteristics. CAKUT, congenital anomalies of the kidney and the urinary tract; CKD, chronic kidney disease; CKDu, CKD of unknown etiology; FHx, family history; SRNS, steroid-resistant nephrotic syndrome.

if available, which require confirmed molecular diagnoses. For example, establishing a genetic diagnosis of hyperoxaluria type 1 enables the initiation of the RNA interference–based therapy that is approved for this subtype of hyperoxaluria.¹² Although the diagnosis can also be suggested upon biochemical parameters, genetic diagnosis should always be established for hyperoxaluria.^{13,14}

Altogether, in 27 of 36 (75%) genetically confirmed cases, the molecular diagnosis has a potential influence on the clinical management (Table 3). The molecular genetic diagnosis guided clinical decision making in terms of disease surveillance, kidney transplant related regimen, introduction of disease-specific treatments, and performance of preimplantation genetic diagnosis for disease prevention in the context of family planning (Table 3).

In 5 cases, the genetic diagnosis did not correspond with the pre-exome clinical diagnosis of the primary nephrologist (participants no. 21–218, 21–182, 21–680, 21–253, and 21–112; Table 2). These included cases with an unknown diagnosis and cases where the distinction between CAKUT and renal cystic cilopathies was challenging solely based on the phenotype and imaging. Consequently, cilopathies were incorrectly classified as CAKUT and vice-versa.

DISCUSSION

In the present nationwide exome sequencing study involving nearly all dialysis-treated Israeli children with kidney failure, we detected diagnostic variants in 36 of 79 participants (45%). The study cohort included children from different ethnicities who were all exclusively treated in 1 of 6 Israeli pediatric dialysis units across the country. These findings show a high prevalence of monogenic etiologies in childhood-onset kidney failure and emphasize the extent to which genetic testing can help resolve underlying diagnostic etiologies. This allows for gene-specific management and monitoring of the affected children with advanced CKD and their families.

Our study has several limitations that should be considered. First, we did not perform a chromosomal microarray-based copy-number variation analysis. This, to some extent, may affect our ability to detect hemizygous conditions or microdeletion syndromes and potentially, might have resulted in an underestimation of the overall genetic diagnostic yield. Nonetheless, using the exome data, we were still able to detect medium and large size gene deletions in 6 patients. Second, trio exome analysis was not available for all probands. This can affect, to some degree, the detection of novel *de novo* mutations. Specifically, it can

result in a more conservative exome sequencing molecular diagnostic yield, because identifying *de novo* variants can increase the strength of evidence to classify variants as pathogenic. Ideally, a trio genome sequencing study would be most effective because it could uncover additional variants such as mitochondrial genome variants, copy number variants, structural variants, and single nucleotide variants in noncoding regions. Finally, our study was limited to Israeli children with different ethnic backgrounds and with relatively high rates of consanguinity; therefore, its generalizability may be limited.

The strength of our study includes its nationwide and comprehensive nature, which included a non-selected cohort reflecting “real world” clinical utility of exome sequencing of all dialysis-treated Israeli children.

The observed genetic detection of 45% is a relative high yield compared with that previously reported for single-center or selected pediatric CKD cohorts.^{1,15,16} However, this relatively high genetic diagnostic yield is comparable to other Israeli nationwide exome sequencing studies performed in children with neurodevelopmental disorders.¹⁷ Moreover, we found high percentages of monogenic cases in children even when there was no positive family history, consanguinity, or syndromic features (26%, 29%, and 32% respectively). This implies that a significant proportion of all childhood-onset CKD can be attributed to genetic causes, which are often overlooked given the lack of overt risk factors for an underlying genetic condition. Similarly, in a single-center experience of exome sequencing in 104 pediatric recipients of kidney transplantation, Mann *et al.* reported a genetic diagnostic yield of 32.7%.¹⁶

International comparisons demonstrate that Israel is one of the leading countries in childhood kidney failure prevalence mainly among non-Jewish minorities.^{18,19} We have previously hypothesized that this, in part, is secondary to widespread and underappreciated genetic CKD etiologies in Israeli minorities.¹⁸ The current study results support this hypothesis and highlight the need for targeted screening and prevention strategies for high-risk populations.

Genetic diagnoses enabled identification of potential prospective consequences for many patients and could have enabled earlier diagnosis in the course of their disease, which might have mitigated negative outcomes. Detection of monogenic causes of CKD enables personalized surveillance and treatments, spares unnecessary diagnostic procedures such as kidney biopsies, prevents inappropriate therapy use, and might prevent incompatible transplantation from affected relatives. In addition, it allows family genetic consulting and early diagnosis of asymptomatic family

Table 3. Twenty-seven patients (75%) for whom the molecular genetic diagnosis influenced subsequent clinical management

Patient	Gene	Clinical management	
21-112	<i>NPHP4</i>	Changed the initial pre-ES clinical diagnosis of CAKUT. Subsequently, the patient was referred for ophthalmologic evaluation to monitor for the potential development of retinitis pigmentosa.	Surveillance
21-139	<i>BBS9</i>	The genetic diagnosis prompted specialized ophthalmologic surveillance at a tertiary hospital due to a lack of local expertise.	Surveillance
21-174	<i>EYA1</i>	Surveillance and ENT evaluation to identify and address any potential hearing problems.	Surveillance
21-253	<i>NFIA</i>	Prompted the referral for brain MRI.	Surveillance
21-263	<i>COL4A5</i>	Surveillance and ENT evaluation to identify and address any potential hearing problems. Necessitates regular ophthalmologic evaluations to monitor for the potential development of ophthalmic diseases such as anterior lenticonus.	Surveillance
21-354	<i>22q11.21 dup</i>	Led to completion of a comprehensive multidisciplinary evaluation, comprising of immunology and cardiology investigations, which ultimately led to a better comprehension of the entire clinical profile of chronic lung disease and bronchiectasis, which the patient exhibited.	Surveillance
21-763	<i>WT1</i>	The genetic diagnosis, in this orthodox Jewish family, revealed the actual gender of the offspring, impacting matters related to Jewish legal practices and customs related to gender within their community and led to comprehensive multidisciplinary targeted evaluation and monitoring.	Surveillance
21-117	<i>SMARCA1</i>	The diagnosis led to a change in the patient's pretransplant regimen. Instead of using Tacrolimus, Cyclosporine was used, as patients with this condition are at an elevated risk for developing infections due to their weakened immunity. Additionally, there is ongoing research and discussion about the potential benefits of combining a bone marrow transplant with a kidney transplant in this population to further improve results.	Pharmacological treatment Transplant
21-262	<i>AGXT</i>	Led to the implementation of a specific pharmacological treatment (Lumasiran), and prompted the need for ongoing surveillance by a multidisciplinary team.	Pharmacological treatment Surveillance
21-264	<i>AGXT</i>	Led to the implementation of specific pharmacological treatment (Lumasiran), and prompted the need for ongoing surveillance by a multidisciplinary team.	Pharmacological treatment Surveillance
21-147	<i>CTNS</i>	Led to the implementation of a specific pharmacological treatment (Cysteamine), and prompted the need for ongoing surveillance by a multidisciplinary team.	Pharmacological treatment Surveillance
21-226	<i>CTNS</i>	Led to the implementation of a specific pharmacological treatment (Cysteamine), and prompted the need for ongoing surveillance by a multidisciplinary team.	Pharmacological treatment Surveillance
21-250	<i>CTNS</i>	Led to the implementation of a specific pharmacological treatment (Cysteamine), and prompted the need for ongoing surveillance by a multidisciplinary team.	Pharmacological treatment Surveillance
21-509	<i>CTNS</i>	Led to the implementation of a specific pharmacological treatment (Cysteamine), and prompted the need for ongoing surveillance by a multidisciplinary team.	Pharmacological treatment Surveillance
21-182	<i>AQP2</i>	Led to the implementation of a specific pharmacological treatment.	Pharmacological treatment
21-183	<i>NPHS2</i>	The diagnosis obviated the need for immunosuppressive drugs.	Pharmacological treatment
21-848	<i>NPHS2</i>	The diagnosis obviated the need for immunosuppressive drugs.	Pharmacological treatment
21-481	<i>NPHS1</i>	The diagnosis obviated the need for immunosuppressive drugs.	Pharmacological treatment
21-483	<i>LMX1B</i>	The diagnosis obviated the need for immunosuppressive drugs.	Pharmacological treatment
21-213	<i>PKHD1</i>	Impacted the evaluation process prior to transplantation by requiring a thorough examination of the liver. Led to the determination to undertake a combined transplantation of both the liver and kidney.	Transplant
21-219	<i>HNF1B</i>	Following the genetic diagnosis, genetic testing of the parents was conducted, and the father was identified as an asymptomatic carrier of the mutation. As a result, the mother was chosen as the living donor for the transplant.	Transplant
21-482	<i>PHKD1</i>	Impacted the evaluation process prior to transplantation requiring a thorough examination of the liver. Led to a combined liver and kidney transplant.	Transplant
21-680	<i>BBS9</i>	An inadequate evaluation of the bladder during the initial transplant procedure led to implant failure due to urological complications. It was not until 5 years after the initial transplant that the underlying urological issues were identified and addressed. In retrospect, the molecular diagnosis of a BBS variant known to cause urological problems could have prompted a more thorough urological evaluation and surveillance, potentially preventing the failure of the initial transplant and the need for a second transplant.	Transplant Surveillance
21-154	<i>LAGE3</i>	The genetic diagnosis facilitated the successful use of PGD in the following pregnancy.	Pre-implantation Genetic diagnosis
21-511	<i>PAX2</i>	The genetic diagnosis facilitated the successful use of PGD in the third child, preventing the transmission of the disorder. Additionally, the patient was sent for an ophthalmological evaluation to identify and address any potential visual problems.	Pre-implantation Genetic diagnosis Surveillance
22-511	<i>PAX2</i>	The genetic diagnosis facilitated the successful use of PGD in the third child, preventing the transmission of the disorder. Additionally, the patient was sent for an ophthalmological evaluation to identify and address any potential visual problems.	Pre-implantation Genetic diagnosis Surveillance
21-878	<i>PKHD1</i>	The genetic diagnosis facilitated the successful use of PGD in a following pregnancy.	Pre-implantation Genetic diagnosis

BBS, Bardet-Biedl Syndrome; CAKUT, congenital anomalies of the kidney and urinary tract; Dup, duplication; ENT, ear, nose, and throat; MRI, magnetic resonance imaging; PGD, preimplantation genetic diagnosis.

members. As a result, we and others have recently launched with the Israeli Ministry of Health, a national government supported pilot for publicly funded trio exome sequencing testing for all Israeli pediatric patients with kidney failure.

The significant prevalence of monogenic etiologies among children on dialysis seen here implies an even greater number of undiagnosed patients in the considerably more prevalent antecedent CKD stages.

Overall, our study suggests that exome sequencing should be a standard and routine diagnostic tool for all children with advanced CKD.

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

We thank all the families and referring physicians who submitted samples for testing. This work was carried out in partial fulfillment of the PhD degree requirements for OS. This research was supported by the Israel Science Foundation (grant No. 2773/19). AV is supported by an European Research Council Starting Grant (StG ERC grant #101040267-GeneCKD). The funding organizations had no role in the design and conduct of the study, collection, management, analysis, and interpretation of the data, preparation, review, or approval of the manuscript, and decision to submit the manuscript for publication.

SUPPLEMENTARY MATERIALS

Supplementary Files (PDF)

Figure S1. Age of kidney failure for each genetic etiology.

Table S1. Monogenic CKD gene panel.

STROBE Statement.

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