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Diagnostic Yield and Benefits of Whole Exome Sequencing in CAKUT Patients Diagnosed in the First Thousand Days of Life

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Introduction: Congenital anomalies of the kidney and urinary tract (CAKUT) are the predominant cause of chronic kidney disease (CKD) and the need for kidney replacement therapy (KRT) in children. Although more than 60 genes are known to cause CAKUT if mutated, genetic etiology is detected, on average, in only 16% of unselected CAKUT cases, making genetic testing unproductive.

Methods: Whole exome sequencing (WES) was performed in 100 patients with CAKUT diagnosed in the first 1000 days of life with CKD stages 1 to 5D/T. Variants in 58 established CAKUT-associated genes were extracted, classified according to the American College of Medical Genetics and Genomics guidelines, and their translational value was assessed.

Results: In 25% of these mostly sporadic patients with CAKUT, a rare likely pathogenic or pathogenic variant was identified in 1 or 2 of 15 CAKUT-associated genes, including *GATA3*, *HNF1B*, *LIFR*, *PAX2*, *SALL1*, and *TBC1D1*. Of the 27 variants detected, 52% were loss-of-function and 18.5% *de novo* variants. The diagnostic yield was significantly higher in patients requiring KRT before 3 years of age (43%, odds ratio 2.95) and in patients with extrarenal features (41%, odds ratio 3.5) compared with patients lacking these criteria. Considering that all affected genes were previously associated with extrarenal complications, including treatable conditions, such as diabetes, hyperuricemia, hypomagnesemia, and hypoparathyroidism, the genetic diagnosis allowed preventive measures and/or early treatment in 25% of patients.

Conclusion: WES offers significant advantages for the diagnosis and management of patients with CAKUT diagnosed before 3 years of age, especially in patients who require KRT or have extrarenal anomalies.

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CAKUT comprise all structural malformations that arise from defects in the morphogenesis of the kidney and/or urinary tract, including kidney agenesis and/or duplication, kidney hypoplasia and/or dysplasia, and/or anomalies of the ureter.[1,2] Taken together, CAKUT phenotypes account for approximately 15% to 30% of all prenatally detected congenital malformations, represent the predominant

cause of CKD in infants and children, and are causative in 30% to 50% of patients requiring KRT before adult age.[3,4] The availability of KRT through dialysis or (preemptive) kidney transplantation, even in infants, has significantly improved the life expectancy and quality of life of children suffering from end-stage kidney disease. Nevertheless, children requiring KRT have a 30-fold increased risk of mortality compared with healthy children, mainly due to cardiovascular complications or infections.[5,6] A recent analysis of the United States Renal Data Systems for infants on KRT reported a patient survival of approximately 88% and 77% at 1 and 5 years, respectively.[7] Comorbidities of organs in the musculoskeletal, digestive, cardiovascular, and the central nervous systems occur in about one-third of patients with CAKUT.[8] Long-term complications of CKD and involvement of other organs negatively impact disease prognosis and play an increasingly important role in the care of patients with CAKUT.

In the last decades, the genetic basis underlying CAKUT has been increasingly elucidated. Next-generation sequencing has led to a rise of recognized monogenic causes of isolated and syndromic CAKUT.[9,10] Although over 60 genes are known to cause CAKUT if mutated, a genetic etiology has been detected, on average, in only 16% of unselected patients with CAKUT.[11] The genetically characterized CAKUT cohorts have been rather heterogeneous, often lacking detailed data on the severity of the kidney phenotypes and stages of CKD.[11] We hypothesized that the diagnostic yield for CAKUT-associated pathogenic variants may be much higher in infants diagnosed with CAKUT, especially in those requiring KRT before the age of 3 years. To this end, we applied WES in a cohort of mostly sporadic European patients with CAKUT diagnosed in the first 1000 days of life with CKD stages 1 to 5D/T, and explored whether a genetic diagnosis may impact patient care and lead to a more targeted management regimen.

METHODS

Patients

This study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Boards of Hannover Medical School, Hannover, Germany and Oslo University Hospital, Oslo, Norway. Each family provided informed consent for participation in the study. A total of 100 index cases (54% male, 46% female) with CAKUT including kidney involvement who were certain to have been diagnosed in the first 1000 days of life were enrolled in Hannover, Germany and Oslo, Norway. Patients had unilateral (31%) or bilateral (69%) CAKUT (Figure 1a). Kidney involvement included kidney agenesis, duplex kidney, crossed fused renal ectopia, multicystic dysplastic kidney (MCDK), (cystic) kidney dysplasia, kidney hypoplasia, and horseshoe kidney (Supplementary Table S1). Patients presenting with isolated vesicoureteral reflux or posterior urethral valves were excluded. Because only 6% of cases had a positive family history for CAKUT and 94% of patients had sporadic CAKUT, the analyzed cohort was designated as mostly sporadic.

Kidney function maturates quickly after birth reaching adult values at about the age of 2 years. Therefore, the estimated glomerular filtration rate during the third year of life was calculated in each patient using the revised Schwartz formula to determine the CKD stage (Figure 1b).[12] Twenty-one patients had CKD stage 5D/T and required KRT before the age of 3 years (Figure 1c).

WES and Variant Interpretation

For WES, whole blood was subjected to DNA isolation using the QIAamp DNA Blood Maxi Kit (Qiagen, Hilden, Germany). Subsequently, WES was performed using the SureSelectXT Human All Exon V4 target enrichment kit (Agilent, Santa Clara, CA) on a HiSeq 2000 sequencer (Illumina, San Diego, CA) or the SureSelectXT Human All Exon V5+UTRs target enrichment kit (Agilent) on a HiSeq 2500 sequencer (Illumina). All

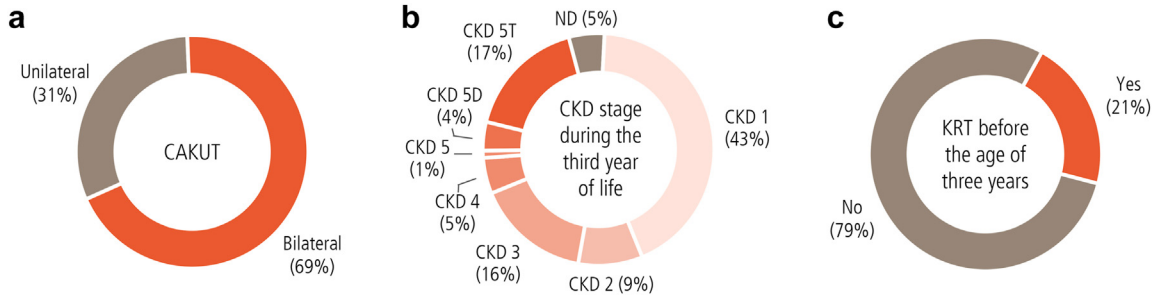

Figure 1. Characteristics of our cohort of 100 unrelated patients with CAKUT including kidney involvement diagnosed in the first 1000 days of life: (a) percentage of patients with unilateral and bilateral CAKUT, (b) distribution of CKD stages, (c) percentage of patients requiring KRT. CKD, chronic kidney disease; KRT, kidney replacement therapy; ND, not determined.

samples were sequenced to a mean target coverage of 50×. Sequencing data were aligned to the human reference genome build hg19/GRCh37, and variations were called using CLC Genomic Workbench (version 21.0.4; Qiagen). Variations were annotated and prioritized using Clinical Insight Interpret Translational (Qiagen) and our in-house data analysis workflow. Quality filters were applied (coverage ≥20, call quality ≥50, allele fraction ≥30), and nonsilent variants, that is, splice site (up to 20 bases into intron), frameshift, in-frame insertions/deletions, stop gain/loss, and nonsynonymous missense variants, which were not present in identically generated exome data of in-house controls ($n = 137$) were retained. Subsequently, only variations in established CAKUT-associated genes, that is, genes reported to be mutated in at least 3 families with CAKUT according to our in-house gene list ($n = 58$, Supplementary Table S2), were extracted, to apply stringent criteria that may be used in the clinic. Features of CAKUT genetics, such as reduced penetrance and variable expressivity, were considered when applying the American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines[13] and the American College of Medical Genetics and Genomics/Clinical Genome Resource guidelines[14] to classify the extracted variants. The minor allele frequency of the genetic variants was retrieved from the Genome Aggregation Database (gnomAD v2.1.1, https://gnomad.broadinstitute.org/). Variant pathogenicity was predicted using SIFT/PROVEAN (http://provean.jcvi.org/index.php), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), MutationTaster (http://www.mutationtaster.org/), and CADD (https://cadd.gs.washington.edu/snv). Using conventional chain termination protocols and a 3130XL Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA), (i) variants considered to be "Likely Pathogenic" (LP) or "Pathogenic" (P) were verified, (ii) familial segregation was determined, and (iii) paternity and maternity was confirmed for *de novo* variants. Copy number variations (CNVs) were analyzed for 8 genes for which CNVs have previously been associated with CAKUT (i.e., *BMP4*, *CHD1L*, *EYA1*, *GATA3*, *HNF1B*, *PAX2*, *PBX1*, and *TBX18*). The coverage of WES data at these loci in each patient with CAKUT was compared to the average coverage at the respective loci in controls ($n = 25$).

Construction of a Protein-Protein Interaction Network

A protein-protein interaction network of proteins encoded by the genes affected by LP/P variants was constructed and visualized using KidneyNetwork (https://kidney.genenetwork.nl/).[15]

Statistical Analysis

Statistical analyses were conducted using MATLAB and Statistics Toolbox Release 2018b (The MathWorks, Natick, MA) and Fisher exact test (2-tailed), whereby P values <0.05 were considered significant.

RESULTS

WES Identified LP/P Variants in CAKUT-Associated Genes in 25% of Mostly Sporadic Patients With CAKUT Diagnosed in the First 1000 Days of Life

Following WES on leukocyte DNA of all 100 patients with CAKUT, prioritization of high-quality variants by seriousness, exclusiveness, and localization in established CAKUT-associated genes ($n = 58$, Supplementary Table S2) yielded 173 nonsilent variants, that is, splice site (up to 20 bases into intron), frameshift, stop gain/loss, nonsynonymous missense variants, and small insertions/deletions, which were not present in identically generated exome data of in-house controls. CNV analysis of 8 of the CAKUT-associated genes based on WES data resulted in 1 whole-gene deletion (Supplementary Figure S1). To apply stringent criteria, only variants classified as LP or P in our cohort were considered further. According to the American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines,[13,14,16,17] 27 of the 174 variants detected in total were classified as LP or P (Table 1, Supplementary Table S3, Supplementary Figure S2). Considering that the 27 LP/P variants were identified in 25 of 100 patients, WES established a genetic diagnosis in 25% of cases diagnosed with CAKUT in the first 1000 days of life in this mostly sporadic CAKUT cohort (Figure 2a). In the few familial cases, the diagnostic yield was significantly higher than in nonfamiliar cases (5/6, 83% vs. 20/94, 21%; $P = 0.0035$; 2-tailed Fisher exact test). The diagnostic yield did not differ significantly in unilaterally or bilaterally affected cases.

In 15 of 58 CAKUT-Associated Genes, 27 Heterozygous LP/P Variants Were Detected, 18.5% of Which Were *de novo*, Digenic Inheritance was Assumed in 2 Patients

LP/P variants affected *HNF1B* and *SIX2* in 4 cases each; *GDF6*, *LIFR*, *PAX2, SALL1*, *TBC1D1*, and *UMOD* in 2 cases each; and *BMP4*, *COL4A1*, *DACT1*, *EYA1*, *GATA3*, *GREB1L*, and *ROBO1* in 1 case each (Figure 2a). Of the 27 LP/P variants, 13 were nonsynonymous missense and 14 were loss-of-function variants, that is, 5 stop gain, 7 frameshift, 1 splicing variant, and 1 whole-gene deletion (Figure 2b and c); all were heterozygous. Familial segregation analysis revealed 11 maternally inherited, 6 paternally inherited, and 5 *de novo* LP/P

Table 1. Rare (MAF ≤ 1%) variants classified as "Likely Pathogenic" (LP) or "Pathogenic" (P) according to the ACMG/AMP guidelines[13,14] in established CAKUT-associated genes ($n = 58$) detected in 100 patients with CAKUT including kidney involvement diagnosed in the first 1000 days of life; all variants are heterozygous

Patient ID, country of origin	Gene	Transcript ID	Nucleotide change	Deduced protein change	Inheritance	MAF (study cohort)	MAF (control cohort[a])	MAF comparison of study vs. control cohort (p-value)	Pathogenicity SIFT / PolyPhen-2 / MutationTaster / PROVEAN / CADD[b]	ACMG classification	Variant previously reported in
A014-01, Germany	*BMP4*	NM_001202.6	c.1085A>G	p.(Asn362Ser)	Mat	0.005	0.00002071	0.0082	Dg / PoD / DC / D / 26.2	LP	-
A001-01, Germany	*COL4A1*	NM_001845.6	c.3997G>A	p.(Asp1333Asn)	ND	0.005	0.0001169	0.0276	Dg / PrD / DC / N / 23.6	P	18
	GREB1L	NM_001142966.2	c.4939T>C	p.(Ser1647Pro)	ND	0.005	0.0001555	0.0404	T / PrD / DC / D / 25.5	LP	-
B036-01, Germany	*DACT1*	NM_016651.6	c.2005C>G	p.(Pro669Ala)	Mat	0.005	0.0	0.0047	Dg / PoD / DC / D / 24.8	LP	19
B027-01[c], Germany	*EYA1*	NM_000503.6	c.889C>T	p.(Arg297*)	ND	0.005	0.0	0.0047	- / - / DC / - / 45.0	P	20,21
A017-01, Turkey	*GATA3*	NM_001002295.2	c.1099C>T	p.(Arg367*)	Mat	0.005	0.0	0.0047	- / - / DC / - / 41.0	P	22,23
F006-01, Germany	*GDF6*	NM_001001557.4	c.746C>A	p.(Ala249Glu)	Mat	0.01	0.003166	0.1366	T / B / DC / N / 12.9	P	24–26
N079-01, Norway	*GDF6*	NM_001001557.4	c.746C>A	p.(Ala249Glu)	Pat	0.01	0.003166	0.1366	T / B / DC / N / 12.9	P	24–26
C007-01, Germany	*HNF1B*	NM_000458.4	c.949G>A	p.(Ala317Thr)	Pat	0.005	0.0	0.0047	T / B / DC / N / 22.3	LP	27
A039-01, Germany	*HNF1B*	NM_000458.4	c.1006C>G	p.(His336Asp)	Pat	0.005	0.0003747	0.0759	T / B / DC / N / 22.7	LP	28–30
F005-01[c], Germany	*HNF1B*	NM_000458.4	c.1_1674del	-	ND[d]	0.005	0.0	0.0047	-	P	31
N006-01, Norway	*HNF1B*	NM_000458.4	c.244G>A	p.(Asp82Asn)	Mat	0.005	0.001057	0.1936	Dg / PoD / DC / D / 31.0	LP	32,33
	SIX2	NM_016932.5	c.722C>T	p.(Pro241Leu)	Pat	0.02	0.003887	0.0084	T / B / DC / N / 20.6	LP	30,34
A002-01, Germany	*SIX2*	NM_016932.5	c.722C>T	p.(Pro241Leu)	Pat	0.02	0.003887	0.0084	T / B / DC / N / 20.6	LP	30,34
A010-01, Germany	*SIX2*	NM_016932.5	c.722C>T	p.(Pro241Leu)	Pat	0.02	0.003887	0.0084	T / B / DC / N / 20.6	LP	30,34
CEL004-01[c], Germany	*SIX2*	NM_016932.5	c.722C>T	p.(Pro241Leu)	Mat[e]	0.02	0.003887	0.0084	T / B / DC / N / 20.6	LP	30,34
C017-01, Germany	*LIFR*	NM_001127671.2	c.478_479 delAG	p.(Arg160fs*15)	ND	0.005	0.0	0.0047	- / - / DC / - / 24.7	P	-
A004-01, Germany	*LIFR*	NM_001127671.2	c.1273_1276 delGTTA	p.(Val425fs*2)	DNV[f]	0.005	0.0	0.0047	- / - / DC / - / 32.0	P	35
A011-01, Germany	*PAX2*	NM_003990.5	c.76delG	p.(Val26fs*3)	DNV[f]	0.005	0.0	0.0047	- / - / DC / - / 33.0	P	2,36,37
B061-01, Germany	*PAX2*	NM_003990.5	c.76dupG	p.(Val26fs*28)	Mat	0.005	0.0	0.0047	- / - / DC / - / 33.0	P	38–40
F002-01[c], Germany	*ROBO1*	NM_002941.4	c.856C>T	p.(Arg286*)	Mat[d,e]	0.005	0.0	0.0047	- / - / DC / - / 36.0	P	-
A009-01, Germany	*SALL1*	NM_002968.2	c.2759C>G	p.(Ser920*)	DNV[f]	0.005	0.0	0.0047	- / - / DC / - / 39.0	P	2
C027-01, Germany	*SALL1*	NM_002968.2	c.2801delG	p.(Ser934fs*32)	DNV[f]	0.005	0.0	0.0047	- / - / DC / - / 33.0	P	-
A007-01, Germany	*TBC1D1*	NM_015173.4	c.644_653 delACCCGCCCCA	p.(Asn215fs*93)	DNV[f]	0.005	0.0	0.0047	- / - / DC / - / 21.5	P	41
B047-01, Germany	*TBC1D1*	NM_015173.4	c.1910+2T>G	-	Mat	0.005	0.0	0.0047	- / - / DC / - / 33.0	P	-
F004-01[c], Germany	*UMOD*	NM_003361.4	c.1042C>T	p.(Gln348*)[g]	Mat[e]	0.005	0.00004676	0.0139	- / - / DC / - / 17.2	P	-
A022-01, Germany	*UMOD*	NM_003361.4	c.1538delA	p.(Asn513fs*6)[g]	Mat	0.005	0.0	0.0047	- / - / DC / - / 31.0	P	-

ACMG, American College of Medical Genetics and Genomics; B, benign; CADD, combined annotation dependent depletion; D, deleterious; DC, disease causing; Dg, damaging; DNV, *de novo* variation; LP, "Likely Pathogenic"; MAF, minor allele frequency; Mat, maternal; N, neutral; ND, not determined; P, "Pathogenic"; Pat, paternal; PoD, possibly damaging; PrD, probably damaging; T, tolerated

[a]Genome Aggregation Database European non-Finnish control cohort (gnomAD v2.1.1 controls, https://gnomad.broadinstitute.org/).

[b]A CADD Phred score ≥15 was considered pathogenic.

[c]Familial CAKUT case.

[d]Co-segregation was shown in a sibling with CAKUT.

[e]Maternal kidney ultrasound was normal.

[f]Paternity and maternity confirmed.

[g]*UMOD* loss-of-function variants were classified as pathogenic in patients with CAKUT here, whereas most *UMOD* variants causing autosomal dominant tubulointerstitial kidney disease are missense variants mainly exerting a gain-of-function effect.[42]

Reference genome build used: hg19/GRCh37.

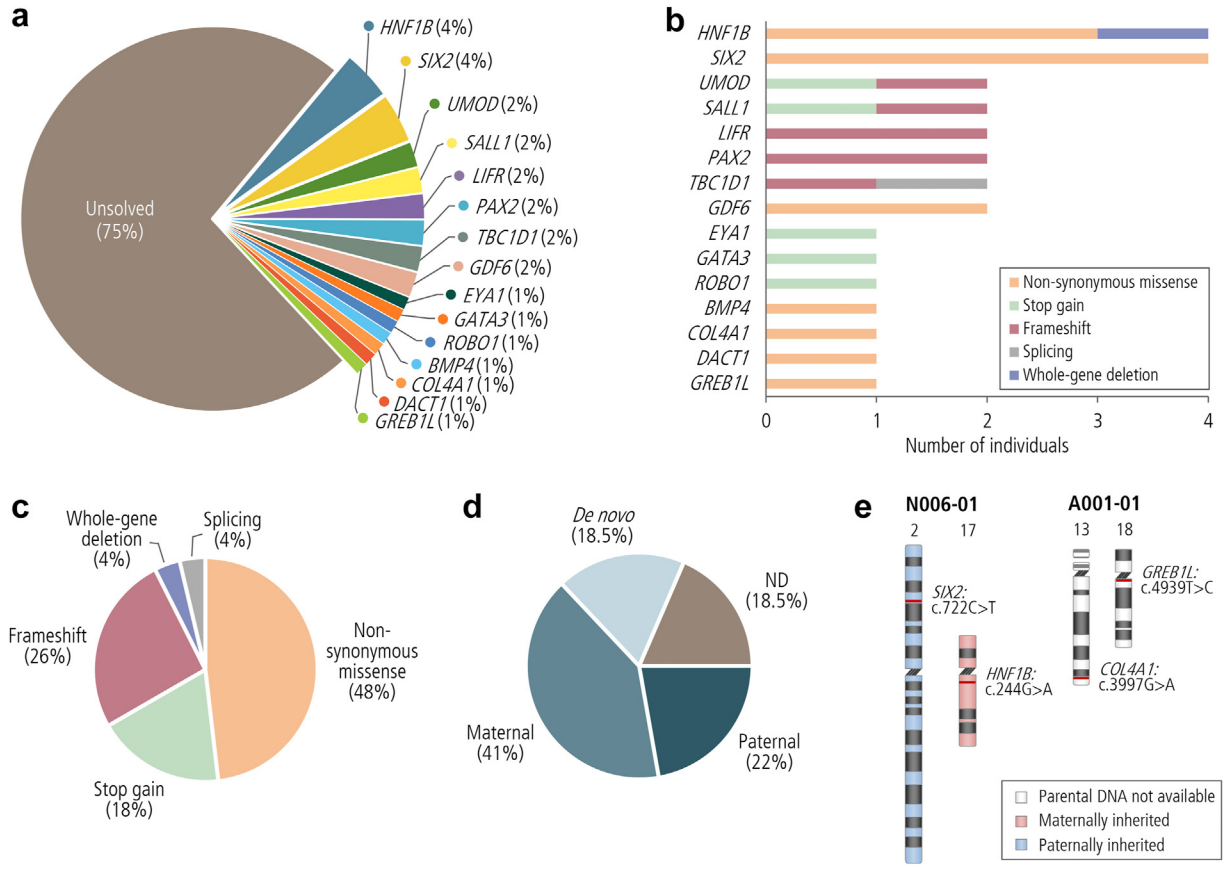

Figure 2. Genetic results in 100 patients with CAKUT including kidney involvement diagnosed in the first 1000 days of life analyzed by WES. (a) The diagnostic yield of LP/P variants in CAKUT-associated genes in the entire patient cohort was 25%. LP/P variations were identified in 15 of 58 CAKUT-associated genes in the given percentage of patients. All detected variations were heterozygous. (b, c) LP/P variants were either nonsynonymous missense variants or predicted to be loss-of-function variations, including stop gain, frameshift, splicing variants, or whole-gene deletions. (d) Inheritance of the LP/P variants. A *de novo* origin was identified in 18.5% of the variants. (e) In 2 patients, co-occurrence of LP/P variants in 2 genes suggested digenic inheritance. ND, not determined.

variants, whereas the origin of 5 variants could not be determined (Figure 2d). The fact that maternal inheritance was observed about twice as often as paternal inheritance suggests that the fertility of males carrying an LP/P variant in a CAKUT-associated gene may be compromised. Both loss-of-function variants in *SALL1*; 1 of 2 loss-of-function variants in *LIFR*, *PAX2*, and *TBC1D1*; and none of 1 or 2 loss-of-function variants in *GATA3*, *ROBO1*, and *UMOD* were found to be *de novo* (Supplementary Figure S3). In 2 patients, 2 LP/P variants each were detected; that is, in *SIX2* (paternally inherited) and *HNF1B* (maternally inherited), as well as in *COL4A1* and *GREB1L* (inheritance could not be determined), suggesting a digenic disease mechanism (Figure 2e).

The 15 affected genes primarily encode transcription factors/coactivators, ligands/secreted proteins, transmembrane receptors, or signal transduction proteins (Figure 3a) that are primarily involved in 9 different signaling pathways; that is, the particularly important GDNF/RET (https://www.wikipathways.org/index.php/Pathway:WP4830)[69] and WNT pathways, along with BMP, FAK-Src, SLIT2/ROBO1, LIF, insulin, retinoic acid or uromodulin signaling (Figure 3b). Based on human kidney-derived gene expression data (kidney.genenetwork.nl),[15] all proteins encoded by the 15 affected genes are predicted to be involved in "ureteric bud development" (GO:0001657, $P = 1.1 \times 10^{-10}$), and "ureteric bud branching" (GO:0001658, $P = 1.3 \times 10^{-10}$, Supplementary Figure S4), processes essential for normal kidney development. Knockout/mutant mouse models were available for each of the 15 affected genes, many of which had a highly similar CAKUT phenotype as patients carrying a variant in the respective gene (Supplementary Table S4).

Loss-of-Function Variants in *LIFR*, *TBC1D1*, and *UMOD* in at Least 3 Patients With CAKUT Each Provide Further Evidence for a Role of These Genes in CAKUT Pathogenesis

Two *LIFR* loss-of-function variants were detected in German patients of our cohort (Table 1), and 1 loss-of-function variant, NM_001127671.2(*LIFR*):c.2472_2476delTATGT p.(Ser824fs*41), in an Israeli patient from an

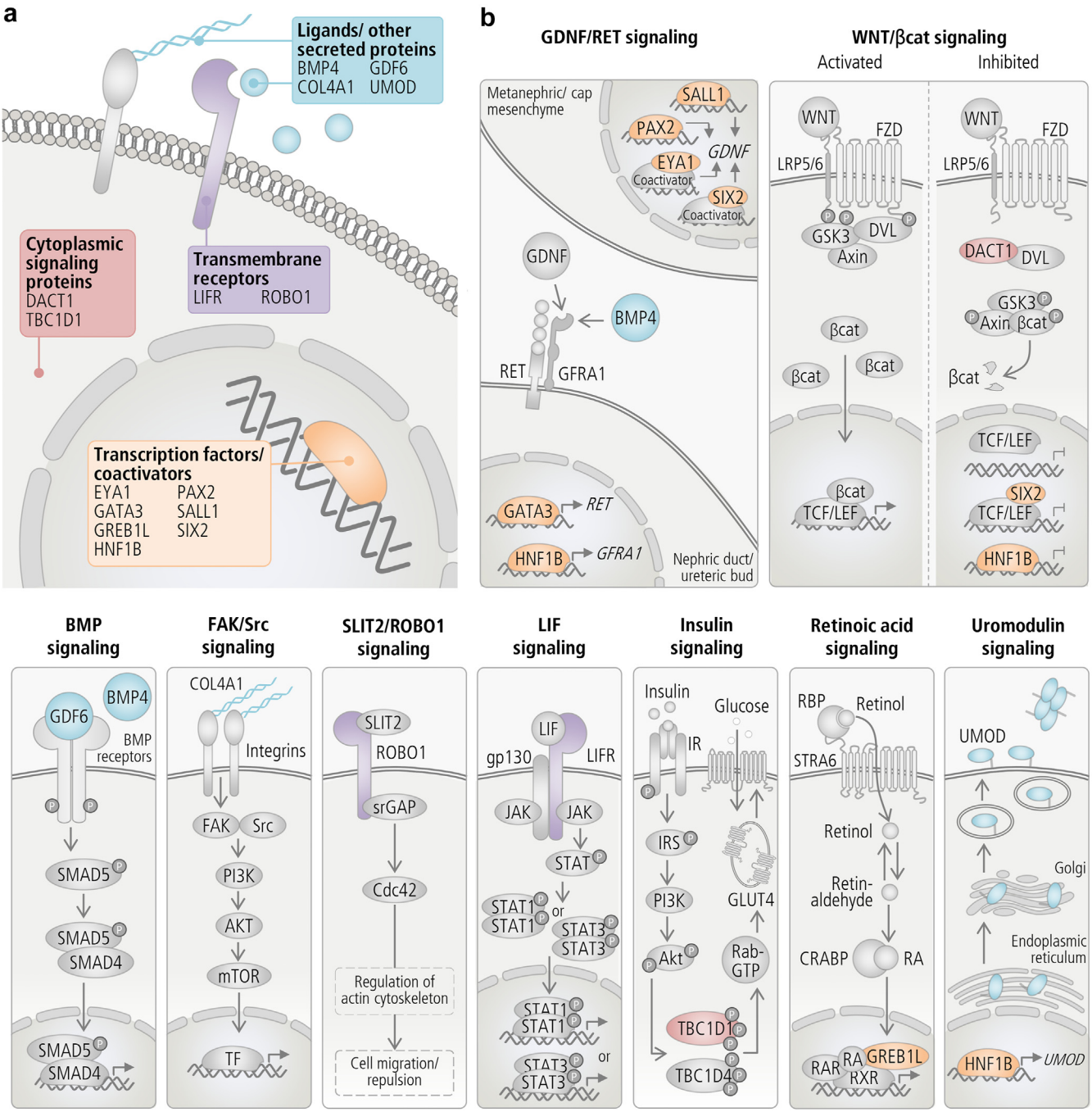

Figure 3. Proteins encoded by genes affected by LP/P variants: their cellular localization and role in cellular signaling pathways. (a) The affected genes encode transcription factors or coactivators, signal transduction proteins, transmembrane receptors, ligands or other secreted proteins. (b) GDNF/RET signaling plays key roles during kidney development, e.g., for ureteric bud emergence and kidney organogenesis. It involves transcription factors/coactivators expressed in the metanephric/cap mesenchyme, e.g., SALL1, PAX2, EYA1, SIX2, modulating *GDNF* expression, and transcription factors expressed in the nephric duct/ureteric bud, e.g., GATA3 and HNF1B, as well as ligands, e.g., BMP4, expressed in the enveloping mesenchyme regulating transmembrane receptor RET or coreceptor GFRA1 activated by GDNF.[43–50] WNT/β-catenin signaling is essential in nephrogenesis.[1] DACT1 antagonizes WNT signaling by binding Disheveled (DVL) downstream of cell surface receptor Frizzled (FZD) and inducing DVL degradation.[51,52] The nuclear repressor complex of SIX2 interacting with TCF/LEF prevents expression of WNT/β-catenin target genes.[48] HNF1B inhibits β-catenin-dependent transcription and constrains canonical WNT signaling.[53] BMP signaling plays an important role in many steps of kidney development. In canonical BMP signaling, binding of ligands, such as BMP4 or GDF6, to transmembrane serine/threonine kinases can activate SMAD signaling cascades.[54,55] FAK-Src signaling, important for kidney collecting duct morphogenesis, can be initiated by COL4A1, an extracellular matrix protein, interacting with integrins to activate the FAK-Src complex, signaling via PI3K, AKT and mTOR to regulate target gene expression.[56,57] SLIT2-ROBO signaling plays a role in ureteric bud emergence and promotes nephron development.[50,58] SLIT2 binding of the ROBO1 transmembrane receptor regulates the actin cytoskeleton, and thus cell migration and repulsion, via srGAP1 and Cdc42.[59] The LIFR transmembrane receptor mediates LIF signals, implicated in mesenchymal to epithelial (continued)

independent cohort (Supplementary Table S5). All carriers of *LIFR* frameshift variants were affected by MCDK, kidney dysplasia with hydronephrosis, or kidney agenesis (Table 2, Supplementary Table S5). Similarly, we identified 2 potential *TBC1D1* loss-of-function variants in German patients of our cohort (Table 1), and 1 loss-of-function variant, NM_015173.4(*TBC1D1*):c.643_644delAA p.(Asn215fs*32), in a Spanish patient from an independent cohort (Supplementary Table S6). One *TBC1D1* loss-of-function variant, c.2553delC p.(Arg854fs*24), was recently reported in a Chinese patient with CAKUT.[103] All carriers of *TBC1D1* loss-of-function variants were affected by kidney dysplasia, kidney agenesis or pelvic kidney (Table 2, Supplementary Table S6). Similarly, 2 *UMOD* loss-of-function variants were identified in German patients of our cohort (Table 1), and 1 loss-of-function variant, NM_003361.4(*UMOD*):c.1639C>T p.(Arg547*), in an Indian patient from an independent cohort (Supplementary Table S7). All carriers of *UMOD* loss-of-function variants were affected by MCDK, kidney hypodysplasia, or kidney agenesis; and 2 additionally by hyperuricemia (Table 2, Supplementary Table S7).

Patients With CAKUT Requiring KRT Before 3 Years of Age Were Significantly More Likely to Carry LP/P Variants in CAKUT-Associated Genes

In our cohort, 68% of patients with CAKUT presented with mild to moderate CKD (stages 1–3), 6% with advanced CKD (stages 4–5), and 21% required KRT before 3 years of age (Figure 1b and c). LP/P variants in CAKUT-associated genes were identified in 9 of 21 (43%) patients requiring KRT before 3 years of age (Figure 4a). In these patients, LP/P variants were identified in 9 genes, that is, *BMP4*, *COL4A1*, *GREB1L*, *HNF1B*, *LIFR*, *SALL1*, *TBC1D1*, *UMOD*, and recurrently in *SIX2* (Figure 4b, Table 2). Conversely, in only 16 of 79 (20%) patients not needing KRT before 3 years of age, an LP/P variant was detected (Figure 4a). Therefore, finding a genetic diagnosis by WES was significantly more likely in patients with CAKUT requiring KRT versus those not needing KRT before 3 years of age (odds ratio 2.95, $P = 0.047$, 2-tailed Fisher exact test). The CAKUT phenotypes observed more than once in patients with KRT before 3 years of age were (cystic) dysplasia, kidney duplication, or agenesis (Figure 4c). Therefore, genetic testing should be considered especially in patients with CAKUT requiring KRT at an early age.

Patients With CAKUT and Extrarenal Features Were Significantly More Likely to Carry LP/P Variants in CAKUT-Associated Genes

In our cohort, 34% of patients with CAKUT additionally presented with extrarenal features. Of these 34 patients, 14 (41%) carried LP/P variants (Figure 5a) in 11 different CAKUT-associated genes, that is, *EYA1*, *GATA3*, *GDF6*, *HNF1B*, *LIFR*, *PAX2*, *ROBO1*, *SALL1*, *SIX2*, *TBC1D1*, *UMOD* (Table 2). In contrast, in only 11 of 66 (17%) patients without extrarenal features, an LP/P variant was detected (Figure 5a). Patients with CAKUT and extrarenal anomalies were significantly more likely to carry LP/P variants than patients without extrarenal features (odds ratio 3.5, $P = 0.0136$, 2-tailed Fisher exact test). Therefore, genetic testing should be considered especially in patients with CAKUT presenting with extrarenal phenotypes.

Clinical Implications of Genetic Diagnoses and Benefits for Earlier Treatment of (Subclinical) Comorbidities

Ten of the genes affected by LP/P variants in our cohort had previously been associated with the extrarenal anomaly observed in our patients, including anomalies of the eyes, ears, brain, heart, and skeleton, or metabolic disturbances, such as hypomagnesemia, hypoparathyroidism, insulin resistance, and hyperuricemia (Figure 5b and Figure 6). The other 5 genes affected by LP/P variants in our cohort have also been associated with extrarenal phenotypes, not (yet) diagnosed in our patients (Table 2, Figure 6). Therefore, all 25 patients with CAKUT carrying an LP/P variant in 1 or 2 of these 15 genes underwent reverse phenotyping and will undergo monitoring for early detection and management of extrarenal complications known to be associated with the mutated gene (Table 2, Figure 6).

DISCUSSION

In the current study analyzing WES data of 100 mostly sporadic patients with CAKUT diagnosed in the first 1000 days of life, the diagnostic yield of LP/P variants in 1 or 2 of 58 CAKUT-associated genes was 25%. This diagnostic yield is quite a bit higher than that reported previously in unselected heterogeneous CAKUT cohorts. For example, "causal mutations" or "candidates of pathogenicity" were described in 6% of European patients with CAKUT analyzed by panel sequencing of

Figure 3. (continued) conversion and nephrogenesis induction, through JAK/STAT-regulated gene expression.[60–62] TBC1D1 regulates the insulin-induced translocation of the GLUT4 transmembrane receptor to the cell membrane.[63] GREB1L was implicated as a coactivator of retinoic acid receptor RAR and RXR-mediated gene expression.[64] Both nuclear receptors are regulated by retinoic acid (RA) generated from retinol in retinoic acid signaling.[65] Uromodulin (UMOD)/Tamm-Horsfall protein, exclusively produced by renal epithelial cells, is excreted into the urine where it forms an extracellular matrix linked to water/electrolyte balance.[42,66,67] *UMOD* expression is regulated by transcription factor HNF1B.[68]

Table 2. Clinical data of patients with CAKUT including kidney involvement diagnosed in the first 1000 days of life carrying an LP/P variant in an established CAKUT-associated gene ($n = 58$), and translational impact of the genetic findings

Patient ID	Sex	Current age	CAKUT phenotype	Age at KRT	CKD stage during third year of life[a]	Extrarenal anomalies	Aberrant gene	Relevant congenital or early-onset extrarenal anomalies reported in at least 2 patients with aberrations in the respective gene	Translational impact of the genetic findings	
									Diagnostic tests to be considered	Targeted surveillance and treatment
A014-01	F	11 y	Kidney dysplasia (r + l)	2 y	5T	None	*BMP4*	Brain including callosal agenesis, cerebellar and pituitary gland anomalies, developmental delay, eye anomalies including cataract, hearing impairment, cleft lip and palate, digital anomalies, cryptorchidism[70–72]	Neurodevelopmental follow-up, eye and ear examination, brain imaging, pituitary function tests	Neurocognitive support measures, treatment of pituitary insufficiency, vision-improvement treatment, e.g., by cataract surgery, hearing-improvement treatment, orchiopexy
A001-01	F	13 y	Dysplastic duplex kidney (r + l), ureteropelvic and ureterovesical junction obstruction (l), VUR (r + l)	3 m	5D	None	*COL4A1*	Brain anomalies including lissencephaly, hydrocephalus, seizures, cerebral aneurysm, eye anomalies including cataract, glaucoma[73,74]	Brain imaging, eye examination	Prevention of intracerebral hemorrhage, e.g., blood pressure control, restrictive use of anticoagulants, vision improvement e.g., by cataract surgery, treatment of increased intraocular pressure
							GREB1L	Hearing impairment,[75] digital anomalies, genital anomalies[76,77]	Ear examination	Hearing-improvement treatment
B036-01[b]	M	6 y	MCDK (l), dilated ureter ending in ureterocele (l)	–	1	None	*DACT1*	Developmental delay, autism, ear anomalies, genital anomalies, anal atresia[19,78]	Neurodevelopmental follow-up, ear examination	Neurocognitive support measures, hearing-improvement treatment, surgical correction of anal atresia
B027-01	F	5 y	Kidney agenesis (r), hypodysplasia, hydronephrosis (l)	–	3	Lateral branchial fistula	*EYA1*	Ear anomalies, hearing impairment, branchial defects[79]	Ear examination	Hearing-improvement treatment
A017-01	M	6 y	Kidney dysplasia (r + l), VUR (r + l)	6 y	4	Intellectual disability, ear malformation, sensorineural deafness, planovalgus deformity, muscular hypotonia, elevated liver enzymes, hypoparathyroidism	*GATA3*	Sensorineural hearing impairment, hypoparathyroidism[80]	Ear examination, endocrinological examination	Hearing-improvement treatment, management of the consequences of hypoparathyroidism by calcium and vitamin D therapy
F006-01[c]	F	7 y	Crossed fused renal ectopia (l), megaureter (r + l), hydronephrosis (r + l), VUR (r + l)	–	1	Suspected microphthalmia, auricle dysplasia and atresia of the external auditory canal (l), vertebral segmentation defects e.g., fusions, scoliosis, ventricular septal defects, patent foramen ovale, anal atresia, rectovestibular fistula	*GDF6*	Eye anomalies including microphthalmia, glaucoma, ear anomalies including hearing impairment, skeletal anomalies including vertebral, carpal, tarsal fusions, congenital heart defects[24,25,81–85]	Eye examination, ear examination, imaging of the spine, echocardiography	Vision-improvement treatment, e.g., by treatment of increased intraocular pressure, hearing-improvement treatment, treatment/ surgical correction of congenital heart defect and anal atresia
N079-01	F	5 y	Cystic kidney dysplasia (l)	–	1	Paravertebral and retrovesical cysts	*GDF6*			

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Table 2. (Continued) Clinical data of patients with CAKUT including kidney involvement diagnosed in the first 1000 days of life carrying an LP/P variant in an established CAKUT-associated gene ($n = 58$), and translational impact of the genetic findings

Patient ID	Sex	Current age	CAKUT phenotype	Age at KRT	CKD stage during third year of life[a]	Extrarenal anomalies	Aberrant gene	Relevant congenital or early-onset extrarenal anomalies reported in at least 2 patients with aberrations in the respective gene	Translational impact of the genetic findings	
									Diagnostic tests to be considered	Targeted surveillance and treatment
C007-01	F	14 y	MCDK (r)	–	1	Hyperuricemia	*HNF1B*	Pancreatic hypoplasia, early-onset diabetes mellitus, abnormal liver function, hyperuricemia and early-onset gout, hypomagnesemia, genital tract anomalies[86,87]	Screening test for diabetes mellitus, serological examination of liver enzymes, uric acid and magnesium	Prevention of diabetes by dietary intervention and lifestyle changes, insulin treatment, prevention of gout by low purine diet, sufficient amount of drinking water, urate-lowering therapy, magnesium supplementation
A039-01	F	3 y	Kidney hypoplasia (r + l)	18 m	5T	Suspected congenital lacrimal duct stenosis, atypical abdominal hernia (r+l)	*HNF1B*			
F005-01	F	22 y	Cystic kidney dysplasia (l), duplex kidney (r)	–	1	Loss of interlobular bile ducts, elevated liver enzymes, fructose/ sorbitol intolerance, hypomagnesemia, hyperhidrosis	*HNF1B*			
N006-01	F	16 y	Duplex kidney (r + l), ureterocele (r + l), VUR (r + l)	–	1	None	*HNF1B*			
							SIX2	Craniofacial anomalies, ptosis, ear anomalies and conductive hearing impairment[88–90]	Ear examination	Hearing-improvement treatment
A002-01	M	24 y	Kidney dysplasia (r + l)	1 y	5T	Mitral valve insufficiency, café-au-lait spots, hypogamma-globulinemia	*SIX2*			
A010-01	F	15 y	Cystic kidney dysplasia (r + l)	1 y	5T	None	*SIX2*			
CEL004-01	F	4 y	Duplex kidney, megaureter and ureterocele (l)	–	1	None	*SIX2*			
C017-01	M	6 y	MCDK (l)	–	1	None	*LIFR*	Attention deficit disorder[35]	Neurodevelopmental follow-up	Neurocognitive support measures
A004-01[d]	M	17 y	Kidney agenesis (r), kidney dysplasia, hydronephrosis, obstructive megaureter (l)	1 y	5T	Attention deficit disorder, prefascial testis ectopia	*LIFR*			
A011-01[e]	M	23 y	Kidney dysplasia (r + l)	5 y (KTx)	3	Myopia, hydrocele testis	*PAX2*	Developmental delay, eye anomalies including myopia, high frequency hearing impairment, joint laxity[36]	Neurodevelopmental follow-up, eye examination, ear examination	Neurocognitive support measures, vision-improvement treatment, e.g., protective eyewear to prevent retinal detachment, hearing-improvement treatment
B061-01	M	2 y	MCDK (r), renal dysplasia (l), VUR (l)	–	3	None	*PAX2*			

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Table 2. (Continued) Clinical data of patients with CAKUT including kidney involvement diagnosed in the first 1000 days of life carrying an LP/P variant in an established CAKUT-associated gene ($n = 58$), and translational impact of the genetic findings

Patient ID	Sex	Current age	CAKUT phenotype	Age at KRT	CKD stage during third year of life[a]	Extrarenal anomalies	Aberrant gene	Relevant congenital or early-onset extrarenal anomalies reported in at least 2 patients with aberrations in the respective gene	Translational impact of the genetic findings	
									Diagnostic tests to be considered	Targeted surveillance and treatment
F002-01	M	19 y	Kidney dysplasia (l)	–	1	Mild pulmonary stenosis, patent foramen ovale	*ROBO1*	Developmental delay, pituitary gland anomaly, eye anomalies including strabismus, congenital heart defect[91–94]	Neurodevelopmental follow-up, pituitary function tests, eye examination, echocardiography	Neurocognitive support measures, treatment of pituitary insufficiency, vision-improvement treatment, e.g., occlusion therapy, treatment/ surgical correction of congenital heart defect
A009-01[e]	M	26 y	Kidney dysplasia (r + l)	2 y	5T	None	*SALL1*	Developmental delay, cranial nerve palsy, eye anomalies including cataract, ear anomalies and congenital hearing impairment, rib anomalies and vertebral anomalies, congenital heart defect, gastroesophageal reflux, genital anomalies, anal atresia or stenosis, digital anomalies[95–99]	Neurodevelopmental follow-up, eye examination, ear examination, imaging of the spine, echocardiography	Neurocognitive support measures, vision-improvement treatment, e.g., by cataract surgery, hearing-improvement treatment, treatment/ surgical correction of congenital heart defect and anal atresia
C027-01	M	2 y	Kidney dysplasia (r + l)	–	3	Ear tags	*SALL1*			
A007-01[f]	M	24 y	Kidney agenesis (r), hypodysplasia (l)	1 y	5T	Insulin resistance/ prediabetes	*TBC1D1*	Obesity,[100] postprandial hyperinsulinemia[101]	–	Prevention of obesity and diabetes by dietary measures and lifestyle changes, e.g., exercise
B047-01	M	3 y	Kidney dysplasia (r + l)	–	3	None	*TBC1D1*			
F004-01	M	11 y	MCDK (r)	–	1	Cryptorchidism, phimosis, hyperuricemia	*UMOD*	Hyperuricemia and early-onset gout[102]	Serological examination of uric acid	Prevention of gout, e.g., by low purine diet, sufficient amount of drinking water, urate-lowering therapy
A022-01	F	23 y	MCDK (l), kidney hypoplasia, VUR (r)	2 y	5T	None	*UMOD*			

CKD, chronic kidney disease; D, dialysis; F, female; KTx, kidney transplantation; KRT, kidney replacement therapy; l, left; M, male; m, months; MCDK, multicystic dysplastic kidney; r, right; VUR, vesicoureteral reflux; y, years.
[a]CKD stage during third year of life determined by estimated glomerular filtration rate using the Schwartz formula.[12]
[b]Patients previously published in Christians *et al.*, 2023.[19]
[c]Patient previously published in Martens et al., 2020.[26]
[d]Patient previously published in Kosfeld et al., 2017.[35]
[e]Patients previously published in Kosfeld et al., 2018.[2]
[f]Patient previously published in Kosfeld et al., 2016.[41]

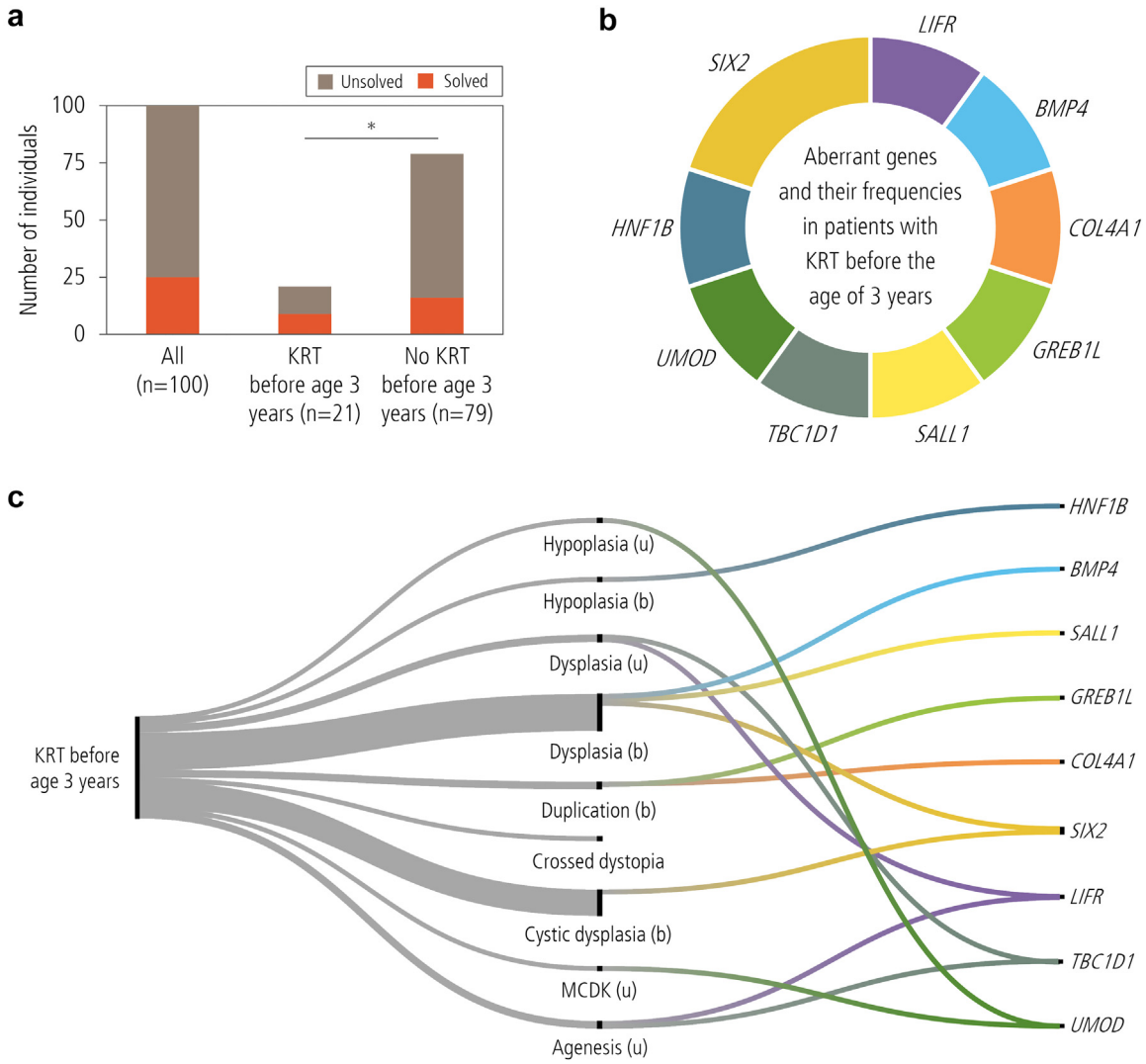

Figure 4. Results in 21 patients with CAKUT requiring kidney replacement therapy (KRT) before the age of 3 years. (a) The diagnostic yield of LP/P variants in CAKUT-associated genes by WES was 43% versus 20% in patients with CAKUT requiring versus not-requiring KRT before age 3 years. A genetic diagnosis by WES was thus more likely in patients requiring KRT ($P = 0.047$, 2-tailed Fisher exact test). (b) Genes affected by LP/P variants and their frequencies (*SIX2* was recurrently affected) in patients with CAKUT requiring KRT before age 3 years. (c) Kidney phenotype-genotype correlations in patients with CAKUT requiring KRT before age 3 years. The most frequent kidney phenotypes were (cystic) dysplasia (associated with variants in *BMP4*, *SALL1* and *SIX2*), duplication (associated with variants in *GREB1L* and *COL4A1*), and agenesis (associated with variants in *TBC1D1* and *LIFR*). MCDK, multicystic dysplastic kidney.

208 genes,[104] a "genetic diagnosis" in 10% of Chinese children with CAKUT,[105] and "causative mutations in a known/syndromic CAKUT gene" in 13% of patients with CAKUT subjected to WES,[106] or "pathogenic/likely pathogenic variants" in 14% of Korean children with CAKUT examined by targeted exome sequencing.[38] The yield of panel sequencing increases if more genes are included in the panel and CAKUT cohorts are selected. For example, "pathogenic mutations" were detected in approximately 18% of CAKUT cases, nearly half of which were severe fetal cases, analyzed by panel sequencing of 330 genes.[107] The yield of WES was similar to ours, that is, 27% in a selected CAKUT cohort with 30% familial and 7% consanguineous cases.[108] Considering that only about 15% of CAKUT cases are familial, the latter cohort is not entirely representative of the patients seen in clinics in most countries. When comparing our data with that of 12 different studies encompassing a total of 2250 patients with CAKUT yielding a genetic diagnosis on average in 16% of cases,[11] selecting patients with CAKUT including kidney involvement diagnosed in the first 1000 days of life increased the diagnostic yield to 25%. The diagnostic yield was further improved to 41% or 43%, when analyzing patients with CAKUT diagnosed in infancy with extrarenal features or patients with CAKUT requiring KRT before the age of 3 years. Of note, only 1 CNV was detected in 100 patients with CAKUT using the read depth approach that is available for CNV analysis of WES data. The variable

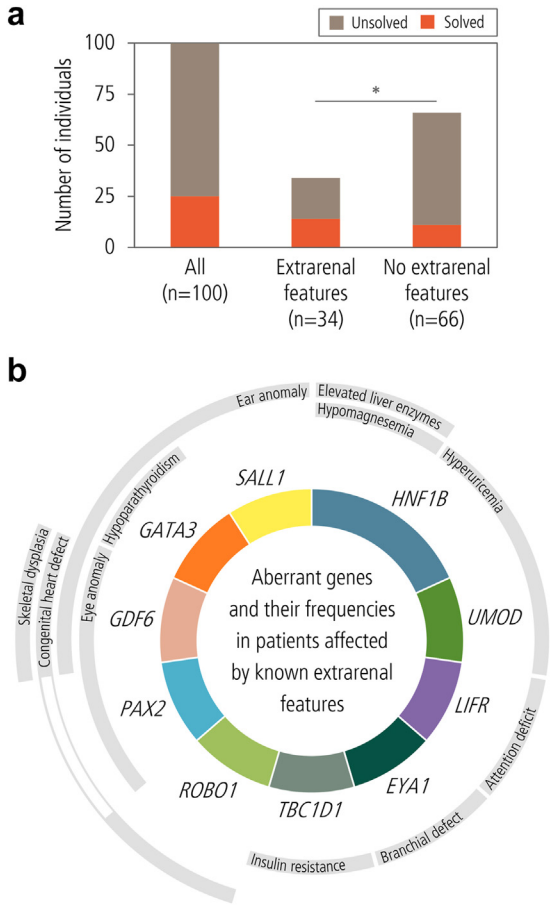

Figure 5. Results in 34 patients with CAKUT diagnosed in the first 1000 days of life with extrarenal features. (a) The diagnostic yield of LP/P variants in CAKUT-associated genes by WES was 41% versus 17% in patients with CAKUT presenting with versus without extrarenal features. A genetic diagnosis by WES was thus more likely in patients presenting with versus without extrarenal features ($P = 0.0136$, 2-tailed Fisher exact test). (b) Genes affected by LP/P variants and their frequencies (*HNF1B* was recurrently affected) in patients with CAKUT and extrarenal features that had anomalies previously described to be associated with this gene. These features are given here and in more detail in Table 2. Ear, eye, heart anomalies, and hyperuricemia are associated with more than 1 gene. The 2 patients carrying *HNF1B* variations had different known extrarenal features.

distribution of reads in WES data complicates detecting CNVs. We expect that using new technologies to detect CNVs, such as optical genome mapping or whole genome sequencing, will enhance the genetic yield in patients with CAKUT even further. Whole genome sequencing will also allow the detection of variations in the noncoding regions of the genome as novel potential causes of CAKUT.

In sporadic CAKUT cases, representing 94% of our cohort, *de novo* variants may be causative. Accordingly, a substantial proportion of *de novo* variations, that is, 5 of 27 (18.5%), was detected. *De novo* occurrence of genetic variants was higher here than in other studies of patients with CAKUT reporting 2 of 32 (6.3%)[106] or 5 of 36 (13.9%)[107] *de novo* variants, although the difference was not statistically significant. Compared to inherited variations for which the selection pressure in previous generations is high, *de novo* variations have not undergone this selection process.[109] Therefore, the damaging capacity of *de novo* variations is potentially high.[109] Accordingly, all *de novo* variants identified here were loss-of-function variants causing bilateral kidney dysplasia or unilateral agenesis and contralateral dysplasia, and CKD stages 3 to 5 during the third year of life, requiring dialysis and kidney transplantation before 6 years of age in 4 of 5 cases. *De novo* loss-of-function variants with a potentially high damaging capacity that may be associated with high penetrance affected *LIFR*, *PAX2*, *SALL1*, and *TBC1D1*, whereas inherited loss-of-function variants with a lower damaging capacity that may cause reduced penetrance affected *GATA3*, *LIFR*, *PAX2*, *ROBO1*, *TBC1D1*, and *UMOD* (Supplementary Figure S3). In line with our findings, kidney anomalies or functional impairment are identified in most patients with renal coloboma syndrome (Figure 6) caused by heterozygous pathogenic *PAX2* variants.[36] High penetrance was previously proposed for heterozygous *SALL1* loss-of-function variants with respect to features of Townes-Brocks syndrome (Figure 6), whereas functional kidney impairment with or without structural abnormalities is reported in 42% of patients.[95,96,110] Renal anomalies are detected in 60% to 72% of patients with hypoparathyroidism, deafness, and renal dysplasia syndrome caused by heterozygous deleterious *GATA3* variants.[80,111] Interestingly, *GATA3*, *PAX2*, and *SALL1* along with 2 other genes affected by loss-of-function variants in this study, that is, *EYA1* and *HNF1B*, are involved in GDNF/RET signaling governing key steps of kidney development.[43–50]

In the current report, we further establish *LIFR* and *TBC1D1* as CAKUT-associated genes in humans, and suggest that *UMOD* may be linked to human CAKUT by reporting 3 or 4 patients with CAKUT and loss-of-function variants in each of these genes (Supplementary Figure S3). Heterozygous pathogenic *UMOD* variants are known to cause medullary cystic kidney disease 2 and familial juvenile hyperuricemic nephropathy,[112–114] entities that are now grouped under the term autosomal dominant tubulointerstitial kidney disease.[42] Patients affected by autosomal dominant tubulointerstitial kidney disease, characterized by tubular damage and interstitial fibrosis in the absence of glomerular lesions, typically develop end-stage kidney disease in adulthood.[42] To date, *UMOD* variants, that is, 2 missense and 1 frameshift variant, have been linked to CAKUT, that is, MCDK, kidney hypoplasia, and vesicoureteral reflux, in 4 cases diagnosed below the age of 10 years.[104,114,115] Here, we

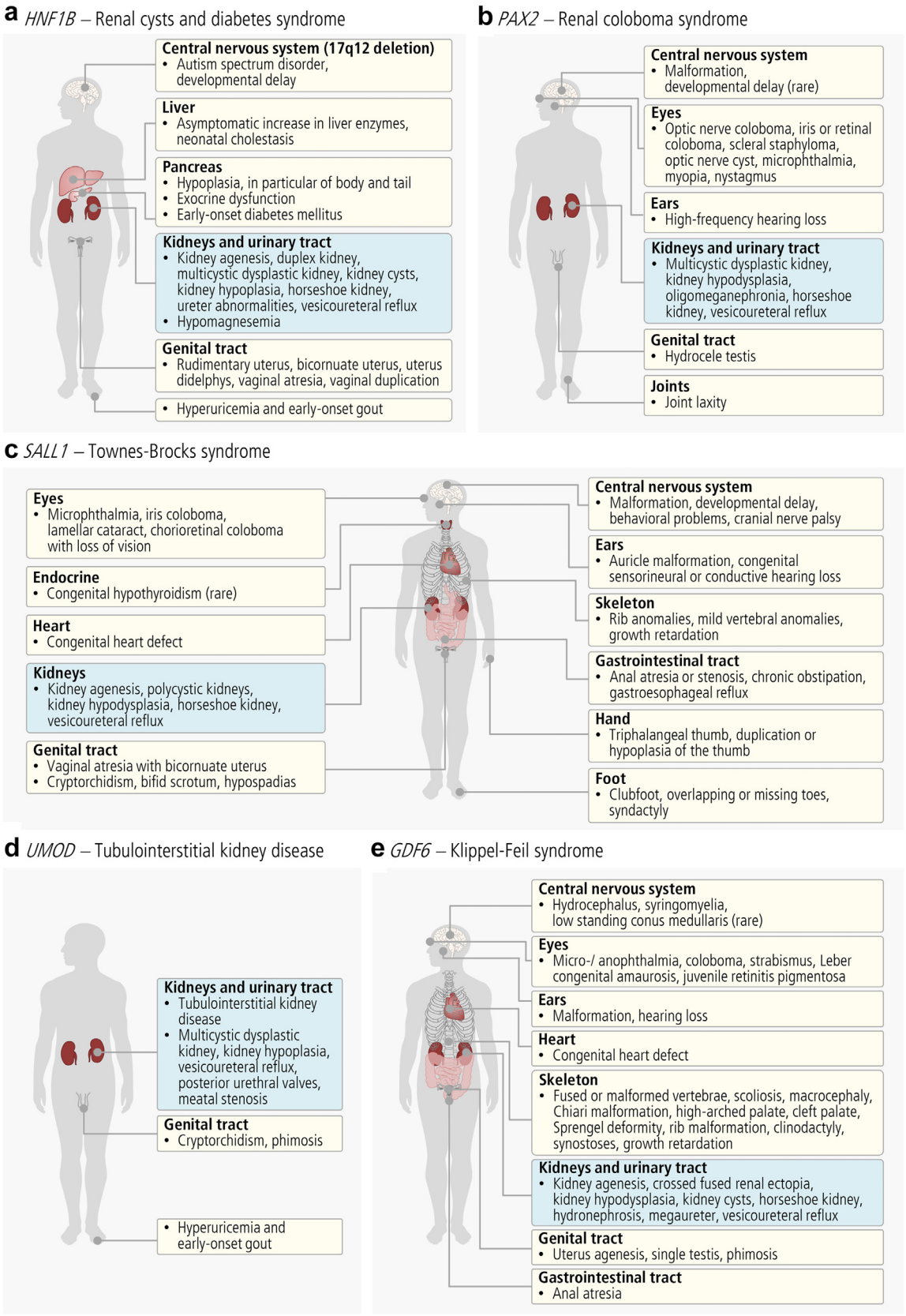

Figure 6. Schematic representation of the kidney and extrarenal anomaly spectrum commonly caused by pathogenic variants in CAKUT-associated genes selected from Figure 5b: (a) *HNF1B*-associated renal cysts and diabetes syndrome,[86] (b) *PAX2*-associated renal coloboma syndrome,[36] (c) *SALL1*-associated Townes-Brocks syndrome,[96] (d) *UMOD*-associated tubulointerstitial kidney disease,[42,66] (e) *GDF6*-associated Klippel-Feil syndrome type 1.[24,26]

report 3 more patients with *UMOD* loss-of-function variants that were affected by (i) unilateral MCDK and genital anomalies diagnosed before the age of 3 years, (ii) unilateral MCDK and contralateral kidney hypoplasia with vesicoureteral reflux and CKD stage 5T at 2 years of age, or (iii) unilateral kidney agenesis, contralateral kidney hypodysplasia diagnosed prior to 4 years of age and genital anomalies[116] allowing the unequivocal diagnosis of CAKUT (plus genital anomalies in 2 cases), not autosomal dominant tubulointerstitial kidney disease. We identified the first *LIFR* and *TBC1D1* variants in patients with CAKUT using an unbiased gene discovery approach, that is, WES and trio-based *de novo* analysis.[35,41] In these studies, only 1 CAKUT case each with a *LIFR* or *TBC1D1* loss-of-function variant was reported in patients with unilateral kidney agenesis and contralateral kidney (hypo) dysplasia. Here, we report 2 further patients with CAKUT carrying *LIFR* loss-of-function variants affected by MCDK, and 3 further patients with CAKUT carrying *TBC1D1* loss-of-function variants affected by kidney dysplasia, more strongly implicating *LIFR* and *TBC1D1* variants and LIF, insulin as well as uromodulin signaling in CAKUT pathogenesis.

The following examples highlight the potential of genetic findings and subsequent reverse phenotyping efforts to alter patient management. First, variants in the *UMOD* gene may be associated with hyperuricemia and increased risk of developing gout in the first 2 decades of life, due to lower fractional excretion of urate.[42] Consequently, we determined the serum uric acid concentrations in the 2 patients with CAKUT from our cohort carrying *UMOD* loss-of-function variants and diagnosed hyperuricemia in an 11-year-old patient. Dietary measures, that is, a low purine diet and an adequate amount of drinking water, were initiated in this patient to prevent gout, which resulted in a reduction of uric acid serum levels to the normal range. Second, up to 48% of patients carrying an aberration in the *HNF1B* gene develop maturity-onset diabetes of the young.[86,117] Other extrarenal features reported in patients with *HNF1B* variants include neurological anomalies (in patients with 17q12 deletion including *HNF1B*), abnormal liver function, pancreatic hypoplasia, genital tract malformation, hypomagnesemia, and hyperuricemia causing early-onset gout (Figure 6).[86] Reverse phenotyping in the 4 patients with CAKUT carrying *HNF1B* variations in our cohort led to the diagnosis of elevated liver enzymes and hypomagnesemia in a 22-year-old patient and hyperuricemia in a 14-year-old patient, resulting in preventive dietary measures, magnesium supplementation and treatment with allopurinol. All *HNF1B* variant carriers will be monitored with respect to early-onset diabetes mellitus, hypomagnesemia, and hyperuricemia. Third, variants in *TBC1D1* and in a gene with a related function in insulin signaling, *TBC1D4*, have been linked to obesity and postprandial hyperinsulinemia.[100,101] In reverse phenotyping, 1 of the patients with CAKUT carrying a *TBC1D1* loss-of-function variant was diagnosed with insulin resistance/prediabetes at the age of 17 years.[41] Dietary measures and lifestyle changes, e.g., exercise, were initiated in this patient, resulting in normalization of carbohydrate and insulin metabolism. Fourth, *GATA3* variants may be associated with hypoparathyroidism.[80,111] Reverse phenotyping in a 6-year-old patient with CAKUT carrying a *GATA3* variant in our cohort led to the diagnosis of hypoparathyroidism, resulting in treatment with calcium and vitamin D.

In summary, we show here that genetic testing is increasingly warranted in patients with CAKUT, because the diagnostic yield is significant in selected cohorts of patients: 25% in patients with CAKUT diagnosed in the first 1000 days of life, and as high as 41% and 43%, respectively, in patients with CAKUT presenting with extrarenal anomalies or requiring KRT before the age of 3 years. In addition, we provide evidence that, in each patient, identification of the aberrant gene underlying CAKUT may facilitate early detection and management of comorbidities, which will certainly improve clinical outcomes in patients with CAKUT.

DISCLOSURE

DH received institutional research grants from Chiesi, Kyowa Kirin, and Amgen; personal payments for lectures from Chiesi and Kyowa Kirin; and personal payments for data safety board, advisory board, and registry board from Kyowa Kirin. He serves as president of the European Society of Paediatric Nephrology, as council member of the International Pediatric Nephrology Association, and as member of the European Rare Kidney Disease Reference Network. ACG received travel expenses to the annual meeting in Heidelberg, Germany from the European Rare Kidney Disease Reference Network. All the other authors declared no competing interests.

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AUTHOR CONTRIBUTIONS

LW, HM, DH, and RGW designed the study. LW, HM, and AC carried out the study and analyzed the data. RG generated the exome sequencing raw data. LW, IH, ACG, KF, GA, SB, IVP, MK, AB, and DH contributed patient material, genetic and clinical information. LW and HM generated the figures. LW, HM, DH, and RGW wrote the manuscript with contributions from all other authors. All authors approved the submitted and published version of the manuscript.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1. Coverage analysis of whole exome sequencing reads showing the whole-gene deletion of *HNF1B* in patient F005-01.

Figure S2. Electropherograms of variants classified as "Likely Pathogenic" or "Pathogenic" and their segregation, as verified by targeted sequencing.

Figure S3. Scheme of all loss-of-function variants identified in CAKUT patients here, and their presumed damaging capacity based on the fact whether they are inherited or *de novo* variants and have thus undergone selection pressure in previous generations or not.

Figure S4. Visualization of an interaction network of the proteins encoded by the 15 genes found to be mutated in CAKUT patients diagnosed in the first 1000 days of life.

Table S1. Kidney and ureter phenotypes of 100 CAKUT patients diagnosed in the first 1000 days of life and diagnostic yield in patients with a defined CAKUT phenotype.

Table S2. Human genes ($n = 58$) mutated in ≥3 CAKUT families according to [1] and updated.

Table S3. Details of 27 heterozygous variants classified as "Likely Pathogenic" or "Pathogenic" detected in 25 CAKUT patients with kidney involvement diagnosed in the first 1000 days of life.

Table S4. Genes affected by "Likely Pathogenic" or "Pathogenic" variants in CAKUT patients diagnosed in the first 1,000 days of life, main signaling pathways the encoded proteins play a role in, variants and phenotypes in patients compared to phenotypes of mutant/knockout mouse models, and gene expression in the murine developing kidney.

Table S5. Rare heterozygous loss-of-function variants in the *LIFR* (NM_001127671.2) gene in CAKUT patients.

Table S6. Rare heterozygous loss-of-function variants in the *TBC1D1* (NM_015173.4) gene in CAKUT patients.

Table S7. Rare heterozygous loss-of-function variants in the *UMOD* (NM_003361.4) gene in CAKUT patients.

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