# Whole-Exome Sequencing Identifies Causative Mutations in Families with Congenital Anomalies of the Kidney and Urinary Tract

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## ABSTRACT

**Background** Congenital anomalies of the kidney and urinary tract (CAKUT) are the most prevalent cause of kidney disease in the first three decades of life. Previous gene panel studies showed monogenic causation in up to 12% of patients with CAKUT.

**Methods** We applied whole-exome sequencing to analyze the genotypes of individuals from 232 families with CAKUT, evaluating for mutations in single genes known to cause human CAKUT and genes known to cause CAKUT in mice. In consanguineous or multiplex families, we additionally performed a search for novel monogenic causes of CAKUT.

**Results** In 29 families (13%), we detected a causative mutation in a known gene for isolated or syndromic CAKUT that sufficiently explained the patient's CAKUT phenotype. In three families (1%), we detected a mutation in a gene reported to cause a phenocopy of CAKUT. In 15 of 155 families with isolated CAKUT, we detected deleterious mutations in syndromic CAKUT genes. Our additional search for novel monogenic causes of CAKUT in consanguineous and multiplex families revealed a potential single, novel monogenic CAKUT gene in 19 of 232 families (8%).

**Conclusions** We identified monogenic mutations in a known human CAKUT gene or CAKUT phenocopy gene as the cause of disease in 14% of the CAKUT families in this study. Whole-exome sequencing provides an etiologic diagnosis in a high fraction of patients with CAKUT and will provide a new basis for the mechanistic understanding of CAKUT.

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Congenital anomalies of the kidney and urinary tract (CAKUT) constitute the most common cause of CKD in the first three decades of life.[1,2] CAKUT can present as an isolated renal condition or as part of a clinical syndrome.[3–7] Despite large differences in clinical manifestation, these conditions likely share a pathogenic origin in dysregulation of renal morphogenesis.[8,9]

We hypothesized that human CAKUT may be caused by mutations in distinct single monogenic genes. Previous supporting evidence for this hypothesis include (*1*) familial occurrence of CAKUT; (*2*) the presence of CAKUT as part of the phenotypic manifestation of known monogenic, multiorgan syndromes; (*3*) the presence of monogenic mouse models with CAKUT; (*4*) the congenital nature of CAKUT; and (*5*) the knowledge that specific master genes govern renal morphogenesis.[4,10–12] To date, 40 monogenic causes for isolated CAKUT have been identified (Supplemental Table 1).[5,6,12–37,39–41]. We previously showed by gene panel sequencing that >10% of CAKUT were monogenic in origin,[42] whereas another 2% of patients were explained by mutations in the Fraser complex of genes.[33] With novel CAKUT gene discovery proceeding at an accelerating rate[43,44] and considering that whole-exome sequencing (WES) is not limited to detection of a prespecified list of candidate genes, we hypothesize that, in >12% of patients with CAKUT, a monogenic cause can be detected by WES.

We and others have previously shown that a significant subset of patients with a clinical diagnosis of isolated CAKUT harbor mutations in known disease genes for syndromic forms of CAKUT (Supplemental Table 2).[33,45,46] These patients did not exhibit syndromic CAKUT on clinical examination and are clinically indistinguishable from other patients with CAKUT.[33,45,46] Two reasons were identified as possible explanations for this genotype-phenotype discrepancy. There may be mild extrarenal manifestations of the respective syndrome that are only unveiled after careful clinical reevaluation after establishment of a molecular diagnosis.[45,46] Alternatively, this broad phenotypic variability in the presence of mutations in syndromic disease genes can be due to an allelism of the underlying gene (Supplemental Figure 1).[33] We and others have evaluated WES data from individuals with CAKUT; however the focus was often on specific subcategories of CAKUT.[36,43,47–49] To date, only one publication has systemically evaluated WES in 62 CAKUT families.[45]

We attempted to quantify the prevalence of mutations in known CAKUT genes in a large cohort. On the basis of previous observations, we hypothesized that a significant proportion of individuals with a clinical diagnosis of isolated CAKUT will harbor disease-causing mutations in syndromic CAKUT genes as well as murine and novel "candidate" genes. We show that, in 14% of families with CAKUT, a likely pathogenic mutation in a known CAKUT gene or CAKUT phenocopy gene can be identified. Furthermore, WES facilitates the discovery of candidate variants for CAKUT as seen in 16% of families with CAKUT (Figure 1C-E).

**Significance Statement**

Congenital anomalies of the kidney and urinary tract (CAKUT) are the most common cause of CKD in the first three decades of life. Several lines of evidence support a monogenic disease hypothesis for CAKUT. This manuscript describes the utility of whole-exome sequencing for the identification of likely disease-causing mutations in a large pediatric cohort of 232 families with CAKUT. We find that, in 14% of families, a monogenic disease-causing CAKUT gene can be identified. Furthermore, WES provides the opportunity for identifying novel candidate genes for CAKUT, which will provide insights into the underlying pathogenesis of CAKUT.

## METHODS

### Human Subjects

The study was approved by the institutional review board of the University of Michigan and Boston Children's Hospital as well as the institutional review boards of institutions where we have recruited families. From January 2010 to January 2017, patients with CAKUT were enrolled after obtaining informed consent. A total of 488 individuals (319 affected and 169 reportedly unaffected) from 232 different families were enrolled and had WES performed on DNA samples. All patients with CAKUT were referred to us by their pediatric nephrologist or urologist who made a clinical diagnosis of CAKUT on the basis of renal imaging studies. CAKUT was defined as demonstration of any abnormality of number, size, shape, or anatomic position of the kidneys or other parts of the urinary tract that included at least one of the following: renal agenesis, renal hypo-/dysplasia, multicystic dysplastic kidneys, hydronephrosis, ureteropelvic junction obstruction, hydroureter, vesicoureteral reflux, ectopic or horseshoe kidney, duplex collecting system, ureterovesical junction obstruction, epi-/hypospadias, posterior urethral valves, and cryptorchidism.

For evaluation using WES, families were divided into subgroups as follows: (*1*) reportedly consanguineous (50 of 232); (*2*) likely consanguineous (origin in a region with a high degree of remote consanguinity; 43 of 232); (*3*) syndromic manifestation of CAKUT (one or more extrarenal features; 16 of 232); (*4*) severe manifestation of CAKUT (*i.e.*, unilateral renal agenesis or renal dysplasia; six of 232); (*5*) patients with multiplex cases of CAKUT (40 of 232); (*6*) parental DNA available for analysis (60 of 232); or (*7*) other (17 of 232) (Figure 2). Before being considered for WES, a selection of individuals

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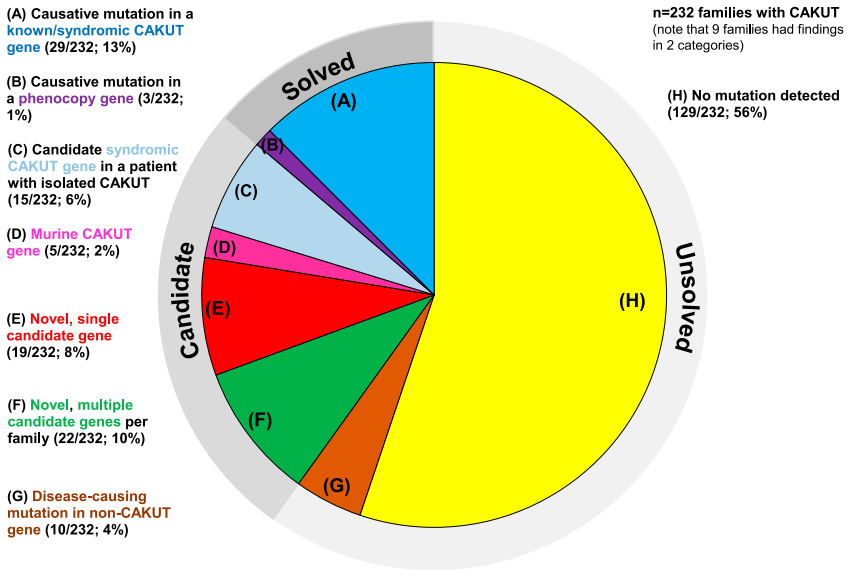


**Figure 1.** Number and percentage of 232 congenital anomalies of the kidney and urinary tract (CAKUT) families in which a causative mutation in a known monogenic CAKUT gene (14%) or a candidate gene(s) (16%) was detected by whole-exome sequencing. Blue color denotes that a mutation in a single causative gene was detected in a known isolated or syndromic CAKUT gene (dark blue), and purple color denotes that a mutation in a causative gene was known to phenocopy CAKUT (purple). Light blue denotes candidate mutations in a known syndromic CAKUT gene in families with isolated CAKUT (light blue). Pink was chosen if a candidate variant in a murine CAKUT gene was identified. Red was chosen if one potential novel CAKUT gene was detected in a family, or green was chosen if multiple novel candidate genes for CAKUT were detected in a family. (A) In 29 of 232 (13%) families with CAKUT (dark blue), a causative mutation was detected in one of 40 isolated CAKUT genes (Supplemental Table 1) or one of 179 known syndromic CAKUT genes (Supplemental Table 2). The individuals with mutations in a syndromic CAKUT gene exhibited the corresponding syndromic CAKUT phenotype. (B) In three of 232 (1%) families, a mutation was identified in a gene causing a kidney disease that may represent a phenocopy of CAKUT (purple; *i.e.*, small kidneys of non-CAKUT origin). (C) In 15 of 232 (6%) families with predominantly isolated CAKUT, candidate mutations were detected in one of 179 syndromic CAKUT genes (light blue), indicating a "hypomorphic" effect of these mutations. (D) In five of 232 (2%) families, mutations in a known gene for murine CAKUT were identified (pink). (E) In 19 of 232 (8%) families, a single potential novel candidate gene for CAKUT was identified per family (red). (F) In 22 of 232 (9%) families, multiple potential novel candidate genes remained per family (green). (G) In ten of 232 (4%) families, we identified mutations in genes known to be causative of monogenic non-CAKUT diseases (brown). (H) In 129 of 232 (56%) families, no causative or candidate mutations were detected (yellow).

with suggestive phenotypes were prescreened for mutations in the CAKUT genes *EYA1*, *PAX2*, *HNF1B*, *GATA3*, *SIX1*, and *SIX5* using targeted sequencing approaches. In total, a causative mutation was identified in 70 families (78 individuals) after screening of 958 families (7.3%; 1111 affected individuals and 269 unaffected parents), and this information is not included in this study but is published elsewhere.[21,33,42,50]

## WES AND VARIANT CALLING

WES was performed as previously described.[51] In brief, genomic DNA was isolated from blood lymphocyte or saliva samples and subjected to exome capture using Agilent SureSelect human exome capture arrays (Life Technologies) followed by next generation sequencing on the Illumina HighSeq sequencing platform. Sequence reads were mapped to the human reference genome assembly (NCBI build 37/hg19) using CLC Genomics Workbench (version 6.5.2) software (CLC Bio, Aarhus, Denmark). After alignment to the human reference genome, variants were filtered for most likely deleterious variants as previously described.[52,53] Variants with minor allele frequencies >1% in the dbSNP (version 147) or the 1000 Genomes Project (1094 subjects of various ethnicities; May 2011 data release) databases were excluded, because they were unlikely to be deleterious. Synonymous and intronic variants that were not located within splice site regions were excluded. Kept variants, which included nonsynonymous variants and splice site variants, were then analyzed (Supplemental Figure 2).

### Mutation Calling in Known Genetic Causes of Isolated Human CAKUT, Syndromic Human CAKUT, and Murine CAKUT Candidate Genes

We evaluated WES data for causative mutations in 40 monogenic genes for isolated CAKUT known at the time (Supplemental Table 1), 179 single-gene candidates for monogenic

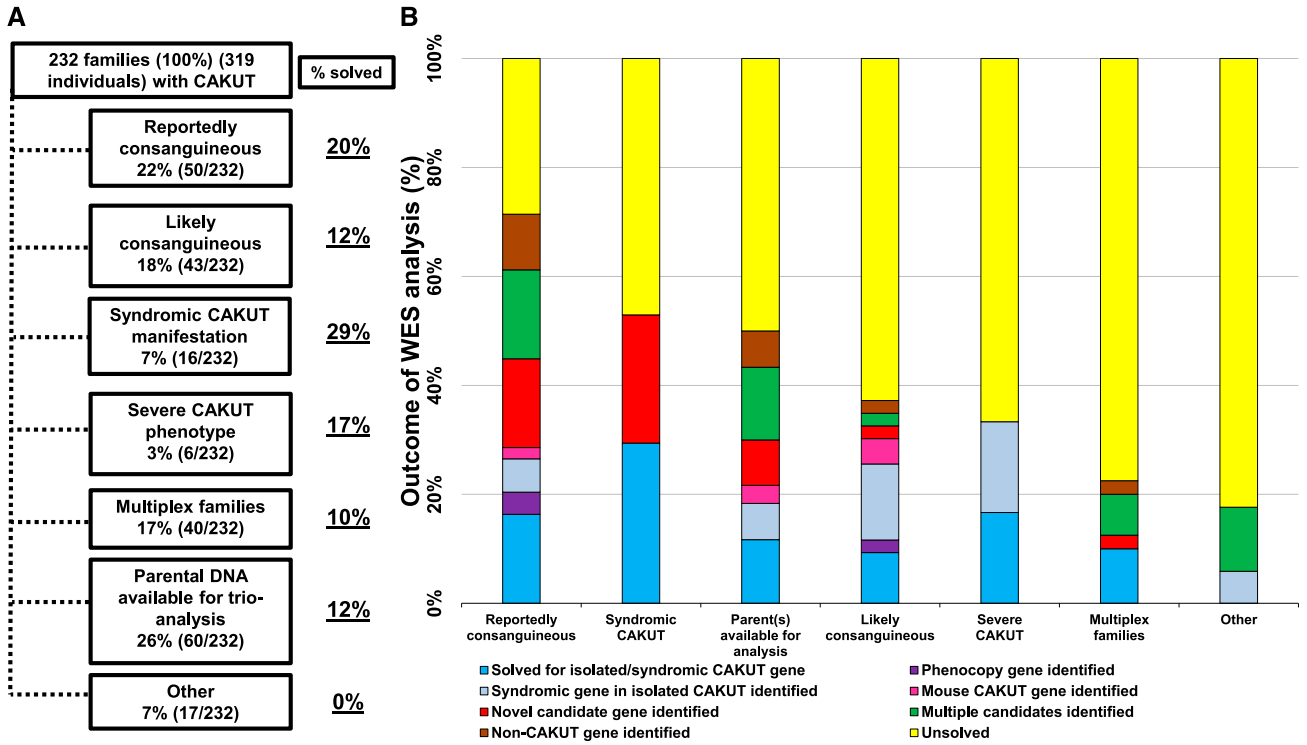


**Figure 2.** Inclusion criteria for 232 families to perform whole-exome sequencing (WES). (A) Individuals with congenital anomalies of the kidney and urinary tract (CAKUT) were prioritized for inclusion in WES on the basis of the following criteria: (*1*) reportedly consanguineous (50 of 232; 22%); (*2*) reportedly nonconsanguineous but origin in countries with increased rate of consanguinity and therefore, considered likely consanguineous (43 of 232; 18%); (*3*) syndromic CAKUT phenotype (16 of 232; 7%); (*4*) severe manifestation of CAKUT (renal agenesis or renal dysplasia; six of 232; 3%); (*5*) families with multiple affected family members (40 of 232; 17%); (*6*) DNA of additional family members available for a duo, trio, or quad analysis (60 of 232; 26%); and (*7*) other reasons to include in WES (*e.g.*, family potentially related to a family to which the other criteria applied; 17 of 232; 7%). Outcome of WES analysis by "recruitment group." (B) The seven recruitment groups for inclusion in CAKUT WES are sorted horizontally starting with the group with the lowest percentage of unsolved families and going to the group with the highest percentage unsolved. Each group is further subsorted into categories of identified genes. Categories are similar to those in Figure 1, and the colors used are the same as well: (*1*) solved for isolated or syndromic CAKUT gene (dark blue), (*2*) phenocopy gene (purple), (*3*) syndromic gene identified in patients with isolated CAKUT (light blue), (*4*) mouse CAKUT gene identified (pink), (*5*) single novel candidate gene identified (red), (*6*) multiple candidate genes identified (green), (*7*) non-CAKUT gene identified (brown), and (*8*) unsolved (yellow).

forms of known syndromic CAKUT (Supplemental Table 2), and 185 candidate genes for mutations in genes for murine CAKUT (Supplemental Table 3). Details on evaluation strategy are in Supplemental Figures 3 and 4. Remaining variants were ranked on the basis of their probable effect on the function of the encoded protein considering evolutionary conservation among orthologs across phylogeny using ENSEMBL Genome Browser and assembled using Clustal Omega as well as the web-based prediction programs PolyPhen-2, SIFT, and MutationTaster. Variant filtering on the basis of population frequency was performed using population databases (EVS server, ExAC, gnomAD, and 1000-genomes) to include only rare alleles (*i.e.*, minor allele frequency $<1\%$). Phenotype and functional aspects of each mutation/gene were discussed in a nephrogenetic panel with a minimum of five members for each of the 232 families before final candidate decisions were made (A.T.v.d.V, D.M.C, H.I., N.M., J.C., A.V., S.S., and F.H.) (Supplemental Figure 4). All variant calling was performed using our stringent *a priori* criteria (Supplemental Figure 4) along with the standards and guidelines set out by the American College of Medical Genetics.[54]

Remaining variants were confirmed in original patient DNA by Sanger sequencing. Whenever familial DNA (parents or siblings) was available, segregation analysis was performed. Although identification of copy number variants by WES is limited, WES data were analyzed using Conifer software to detect pathogenic copy number variants.

### Targeted Search for Homozygously Mutated Novel Genetic Causes of CAKUT in Families with Significant Levels of Homozygosity

If no causative mutation was found in a monogenic cause of isolated, syndromic, or murine CAKUT and a family had significant levels of detected homozygosity (megabase pairs) after homozygosity mapping ($\geq$60 megabase pairs (Mbp)), we proceeded to evaluate WES data for homozygous variants (Supplemental Figure 4). Homozygosity mapping data were generated from WES data using downstream processing of aligned BAM files using

Picard and samtools.[55] Single-nucleotide variant calling was performed using the Genome Analysis Toolkit (GATK),[56] and the generated VCF file was subsequently used in the homozygosity mapper.[57] All single heterozygous variants were excluded on the basis of an *a priori* recessive hypothesis. Remaining variants were ranked as described previously (Supplemental Figures 2 and 4).

### Identification of Novel Genetic Causes of CAKUT by Familial Analysis (Duo, Trio, or Quad Analysis)

Data processing of FASTQs was performed by the Genomics Platform at the Broad Institute of Harvard and Massachusetts Institute of Technology (Broad Institute, Cambridge, MA). Single-nucleotide polymorphisms and insertions/deletions were jointly called across all samples using the GATK HaplotypeCaller. Default filters were applied to single-nucleotide polymorphisms and insertion/deletion calls using the GATK Variant Quality Score Recalibration approach. Lastly, the variants were annotated using Variant Effect Predictor.[58] The variant call set was uploaded onto Seqr for analysis of the WES output.

## RESULTS

### WES Identifies a Likely Pathogenic Monogenic Cause of CAKUT in 14% of Families with CAKUT

We performed WES in 232 families with CAKUT (319 affected individuals). Clinical characteristics for the 319 affected individuals are outlined in Supplemental Figure 5, Supplemental Table 5, and Table 1. In 14% (32 of 232) of CAKUT families, we identified mutations in 22 different monogenic genes known to cause isolated or syndromic CAKUT or phenocopies of CAKUT (32 different mutations in 22 genes) (Figure 1A and B, Table 2). Of the 32 different mutations identified in these 22 CAKUT genes, 16 of 32 (50%) are novel mutations not previously described in the literature. Specifically, we detected likely causative mutations in the following subgroup of CAKUT families.

### Detecting Mutations in Known Genes for Isolated or Syndromic CAKUT in 13% of Families with a Corresponding Phenotype

In 13% of CAKUT families (29 of 232), we detected a mutation in a gene that is known to cause isolated or syndromic CAKUT in patients exhibiting the corresponding isolated or syndromic CAKUT phenotype (Figure 1A, dark blue segment). In patients with isolated CAKUT, we detected mutations in 13 genes (five recessive [*FRAS1*, *TRAP1*, *FREM2*, *ETV4*, and *HPSE2*] and eight dominant [*SALL1*, *SRGAP1*, *ROBO2*, *TBX18*, *HNF1B*, *NRIP1*, *GATA3*, and *GREB1L*]) from the 40 genes that are known to cause isolated CAKUT when mutated (Supplemental Table 1). In addition, we detected six monogenic causes of syndromic CAKUT in patients with the corresponding syndromic CAKUT phenotype (three recessive [*FAT4*, *CTU2*, and *TRPS1*] and three dominant [*ACTG1*, *KMT2D*, and *KAT6B*]) as well as *Trisomy 18* and *Trisomy 20*.

**Table 1.** Clinical characteristics of the 319 individuals (232 families) with congenital anomalies of the kidneys and urinary tract who were submitted for whole-exome sequencing analysis

| Patient Characteristics | Total Cohort | |
|---|---|---|
| | *n* | Percentage |
| Sex | | |
| Women | 129 | 40 |
| Men | 189 | 59 |
| Unknown | 1 | <1 |
| Total | 319 | 100 |
| Extrarenal manifestations | | |
| Yes | 79 | 25 |
| No | 240 | 75 |
| Total | 319 | 100 |
| Reported consanguinity | | |
| Yes | 59 | 18 |
| No | 260 | 82 |
| Total | 319 | 100 |
| Homozygosity on mapping ≥60 Mbp | | |
| Yes | 50 | 16 |
| No | 240 | 75 |
| Not enough SNPs to generate mapping | 29 | 9 |
| Total | 319 | 100 |
| CAKUT phenotype | | |
| Unilateral CAKUT[a] | 130 | 41 |
| Bilateral concordant CAKUT[a] | 111 | 35 |
| Bilateral discordant CAKUT[a] | 32 | 10 |
| Undefined CAKUT phenotype | 21 | 7 |
| Isolated PUV or epi-/hypospadias | 11 | 3 |
| PUV with an additional CAKUT phenotypes | 14 | 4 |
| Total | 319 | 100 |

SNP, single-nucleotide polymorphism; CAKUT, congenital anomalies of the kidney and urinary tract; PUV, posterior urethral value.
[a]Supplemental Figure 5 and Supplemental Table 5 have a breakdown of the CAKUT pathologies in individuals with unilateral or bilateral pathology.

### Detecting a Mutation in a Phenocopy Gene in 1% of Families with CAKUT

In three of the 232 families (1%), mutations in genes were identified that, if mutated, give rise to conditions that may phenocopy CAKUT (Figure 1B, purple segment). This pertained mostly to bilateral small kidneys that were thought to represent the CAKUT phenotype of renal hypo-/dysplasia but in fact, represented small cystic kidneys due to mutations in renal ciliopathy genes (*NPHP1*, *NPHP4*, and *TMEM213*). The molecular diagnosis after WES, therefore, differed from the previous clinical diagnosis in these three families.

### Identifying Hypomorphic Mutations in Known Genes for Syndromic CAKUT in 6% of Families with Isolated CAKUT

Because we previously found that null mutations in certain monogenic genes cause syndromic forms of CAKUT, whereas hypomorphic mutations in the same genes may cause isolated CAKUT (Supplemental Figure 1),[33] we evaluated WES data for mutations in one of the 179 known causes of syndromic CAKUT in families with isolated CAKUT phenotypes. We detected deleterious

**Table 2.** Information on identified mutations in congenital anomalies of the kidney and urinary tract genes known to cause isolated or syndromic congenital anomalies of the kidney and urinary tract or mutations in genes known to phenocopy congenital anomalies of the kidney and urinary tract and the corresponding clinical phenotype

| Family Identification[a] | Gene | Mode of Transmission | Nucleotide Change | Amino Acid Change | State | Evolutionary Conservation[b] | PP2 SIFT MT | CADD Score | EVS[c] | gnomAD[c] | ACMG[d] | HGMD[e] | Phenotypes | Segregation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A617[f] | *SALL1*[g] | Dominant | c.703G>A | p.Ala 235Thr | Het | *Danio rerio* | 0.782 Del. D.C. | 18 | / | 0/3/277166 | Likely pathogenic | DM | Townes Brock syndrome<br>BL VUR R duplex | Variant inherited from father (affection status unknown) |
| A1041[h] | *SRGAP1*[g] | Dominant | c.1993C>A | p.Pro 665Thr | Het | *Caenorhabditis elegans* | 0.309 Del. D.C. | 24 | / | / | Pathogenic | DM | L horseshoe kidney<br>R MCDK<br>Cleft palate<br>Intellectual disability | Variant inherited from mother (affection status unknown) |
| A1147 | *GATA3*[g] | *De novo* dominant | c.708_709 insT | p.Ser 237Glnfs*67[i] | Het | — | / | NA | / | / | Pathogenic | Gene | BL VUR<br>Septate uterus<br>Hearing loss<br>Progressive renal impairment | *De novo* variant (paternity and maternity confirmed) |
| A1160[j] | Trisomy 20p[g] | *De novo* | | | | — | | | | / | / | | L RA<br>Mitral regurgitation<br>Hypotonia<br>Intellectual disability | *De novo* variant (paternity and maternity confirmed) |
| A3346[k] | *TRPS1*[g] | Dominant | c.2795C>T | p.Ala 932Val | Het | *Saccharomyces cerevisiae* | 0.997 Del. D.C. | 21 | / | / | Likely pathogenic | DM | Trichorhinophalangeal syndrome<br>BL RHD, VUR | Variant inherited from affected father (trichorhinophalangeal syndrome) |
| A4450 | *KMT2D*[g] | Dominant | c.6638G>A | p.Gly2213Asp | Het | Xenopus tropicalis | 0.186 Tol. D.C. | 4 | 0/1/4085 | 0/6/171078 | Likely pathogenic | DM | BL VUR<br>Cleft palate<br>Facial dysmorphism<br>Protruding ears<br>Delayed development | Variant inherited from father (mild facial dysmorphism) |
| A4478[l] | *FAT4*[g]<br>*FAT4*[g] | Compound het | c.9279A>C<br>c.9313A>G | p.Gln3093His<br>p.Ser3105Gly | Het<br>Het | *D. rerio*<br>*Xenopus tropicalis* | Tol. D.C.<br>Tol .D.C. | 16<br>4 | /<br>/ | 0/10/244826<br>0/5/245198 | Likely pathogenic | DM | L RA, R UVJO<br>Facial dysmorphism<br>Psychomotor delay<br>Intellectual disability<br>Hypotonia<br>Sprengel deformity<br>Other skeletal deformities<br>BL cryptorchism | Yes (unaffected parents het carriers, variant confirmed in affected siblings) |
| A4672[f,m] | *HNF1B*[g] | Dominant | c.1024T>C | p.Ser 342Pro | Het | *D. rerio* | 0.767 Del. D.C. | 8 | / | 0/1/243686 | Likely pathogenic | DM | R RHD<br>Cystinuria | Variant inherited from mother (affection status unknown) |
| A4732[h] | *SRGAP1*[g] | Dominant | c.806G>A | p.Cys 269Tyr | Het | *D. rerio* | 0.840 Tol. D.C. | 25 | / | / | Pathogenic | DM | R MCDK<br>R ureterocele | Variant inherited from affected mother (R duplicated kidney) |
| A3403[n] | *TRAP1*[g] | Recessive | c.1406G>A | p.Arg 469His | Hom | *S. cerevisiae*[o] | 0.997 Del. D.C. | 33 | 0/66/4234 | 11/1552/269946 | Pathogenic | DM | BL VUR | Yes (unaffected parents het carriers, variant segregates in two affected siblings) |
| A3880[p] | *TBX18*[g] | Dominant | c.1010delG | p.Gly 337Val fs*19[i] | Het | — | / | NA | / | / | Likely pathogenic | DM | UPJO | Yes (segregates in multiple affected family members) |
| HAG[q] | *NRIP1*[g] | Dominant | c.279del | p.Trp 93* (stop gain)[i] | Het | — | / | NA | / | / | Likely pathogenic | DM | RHD<br>MCDK<br>Hydronephrosis | Yes (segregates in multiple affected family members) |
| A1023[r] | *FREM2*[g] | Compound het | c.4031G>A | p.Arg 1344His | Het | *D. rerio* | 0.085 Del./ | 16 | 0/23/4277 | 12/102/277164 | Uncertain significance | DM | R RA | NA |
| | *FREM2*[g] | | c.7535G>A | p.Arg 2512His | Het | *D. rerio* | 0.929 Del./ | 18 | 0/10/4290 | 0/132/276840 | Uncertain significance | DM | Bladder calculi | |
| A1220[f] | *ROBO2*[g] | Dominant | c.292G>T | p.Gly 98Trp | Het | *C. elegans* | 0.880 Del. D.C. | 19 | / | / | Uncertain significance | DM | R UPJO<br>Renal stones | NA |
| A1232[r] | *FREM2*[g] | Compound het | c.649C>T | p.Arg 217Cys | Het | *X. tropicalis* | 0.836 Del./ | 17 | / | 0/1/244270 | Uncertain significance | DM | PUV, R VUR, L UPJO | NA |
| | *FREM2*[g] | | c.4031G>A | p.Arg 1344His | Het | *D. rerio* | 0.085 Del./ | 16 | 0/23/4277 | 12/102/277164 | Uncertain significance | DM | Progressive renal impairment | |

**Table 2.** Continued

| Family Identification[a] | Gene | Mode of Transmission | Nucleotide Change | Amino Acid Change | State | Evolutionary Conservation[b] | PP2 SIFT MT | CADD Score | EVS[c] | gnomAD[c] | ACMG[d] | HGMD[e] | Phenotypes | Segregation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| B24[s] | *ETV4*[g] | Recessive | c.1244G>A | p.Arg 415His | Hom | *Drosphilia melanogaster* | 1.00 Del. D.C. | 36 | 0/1/4299 | 0/23/245730 | Likely pathogenic | Gene | R VUR | Variant het in unaffected mother, paternal DNA NA |
| B196 | *CTU2*[g] | Recessive | c.1399C>T | p.Arg 467Cys | Hom | *D. melanogaster*[t] | 0.926 Del. D.C. | 21 | / | 0/1/245354 | Likely pathogenic | Gene | L hydronephrosis<br>Facial dysmorphism<br>Microcephaly<br>Intellectual disability<br>Growth retardation<br>Pulmonary stenosis<br>Imperforate anus<br>Absent uterus | Variant het in unaffected mother, paternal DNA NA |
| B268[u] | *HPSE2*[g] | Recessive | c.457C>T | p.Arg 153*[l] | Hom | — | / | 10 | / | 0/3/245274 | Pathogenic | DM | Urofacial syndrome<br>L RHD, L UPJO | NA |
| A3837 | *TBX18*[g] | Dominant | c.1802A>G | p.Gln 601Arg | Het | *Ciona intestinalis* | 0.932 Del. D.C. | 14 | / | 0/4/217866 | Uncertain significance | Gene | PUV, BL VUR | NA |
| A3900 | *FRAS1*[g] | Compound het | c.3998T>C | p.Val 1333Ala | Het | *C. elegans*[v] | 0.086 Tol. D.C. | 15 | / | 0/12/244984 | Uncertain significance | Gene | PUV | NA |
| | *FRAS1*[g] | | c.8131T>C | p.Tyr 2711His | Het | *C. elegans* | 0.928 Del. D.C. | 13 | / | 1/29/191356 | Uncertain significance | Gene | | |
| B211 | Trisomy 18[g] | *De novo* dominant | | | | — | | | | | / | / | R MCDK, L RHD<br>Facial dysmorphism<br>Short palpebral fissures<br>Very small low-set ears<br>High arched palate<br>Congenital cardiopathy<br>Esophageal atresia and trachoesophageal fistula<br>High position of the anus<br>Generalized nail hypoplasia<br>Syndactyly of the feet | NA |
| B630 | *HNF1B*[g] | Dominant | 1.5-Mb deletion chromosome 17q12 | | | | | | | | Pathogenic | DM | BL MCDK<br>Hyperuricemia ADHD<br>Developmental delay | NA |
| B1434 | *CTU2*[g] | Recessive | c.1399C>T | p.Arg 467Cys | Hom | *D. melanogaster*[w] | 0.926 Del. D.C. | 21 | / | 0/1/245354 | Likely pathogenic | Gene | R MCDK, L hydronephrosis<br>Global developmental delay<br>Brain MRI; cave of septum pellucidium | NA |
| B1435 | *ACTG1*[g] | *De novo* | c.464C>T | p.Ser 155Phe | Het | *S. cerevisiae* | 1.00 Del. D.C. | 18 | / | / | Pathogenic | DM | L partial duplex kidney, L hydroureter, L hydronephrosis,<br>Facial dysmorphism, lissencephaly, Dany–Walker malformation, global developmental delay<br>Growth retardation | NA |
| B1439 | *SALL1*[g] | Dominant | c.1666G>A | p.Gly 556Ser | Het | *D. rerio* | 0.999 Del. D.C. | 20 | / | 0/3/245992 | Uncertain significance | Gene | BL VUR, BL hydronephrosis | NA |
| B1316 | *GREB1L*[g] | Dominant | c.4276G>A | p.Val 1426Ile | Het | *D. rerio*[y] | 0.079 Tol. D.C. | 14 | / | / | Likely pathogenic | Gene | BL RHD<br>Facial dysmorphism<br>Short neck<br>Single transverse palmar crease<br>Brachydactyly | NA |
| A1261[x] | *GREB1L*[g] | Dominant | c.5068G>A | p.Val1690Met | Het | *X. tropicalis* | 0.681 Del. D.C. | 30 | / | / | Likely pathogenic | Gene | BL VUR<br>L RA<br>Supernumerary nipple | Yes (segregates in multiple affected family members) |

**Table 2.** Continued

| Family Identification[a] | Gene | Mode of Transmission | Nucleotide Change | Amino Acid Change | State | Evolutionary Conservation[b] | PP2 SIFT MT | CADD Score | EVS[c] | gnomAD[c] | ACMG[d] | HGMD[e] | Phenotypes | Segregation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| F1436 | *KAT6B*[g] | Dominant/*de novo* | c.4285G>A | p.Glu1429Lys | Het | *D. rerio* | 0.995 Tol. D.C. | 8 | / | / | Likely pathogenic | Gene | BL VUR, BL RHD<br>Facial dysmorphism<br>Microcephaly<br>Developmental delay<br>Dysplastic ears<br>Muscle weakness (pectoralis and trapezius with limited mobility of shoulder) | NA |
| B1650 | *HPSE2*[g] | Recessive | c.1099–2A>G | 100% ESS | Hom | — | / | NA | / | / | Pathogenic | Gene | Urofacial syndrome "inverted smile"<br>BL VUR<br>Hinman syndrome | Yes (unaffected parents het carriers, variant segregates in two affected siblings) |
| A3962 | *NPHP1*[z] | Recessive | c.1804delA | p.Ser 602Val fs*4[i] | Hom | — | / | NA | / | 0/1/245974 | Pathogenic | Gene | BL RHD | NA |
| A4235 | *TMEM 231*[z] | Recessive | c.119T>G | p.Leu40Arg | Hom | *C. elegans* | 0.951 Del. D.C. | 23 | / | / | Likely pathogenic | Gene | BL RHD, Facial dysmorphism, microcephaly, intellectual disability, polysyndactyly, heart anomalies, growth retardation. | Yes (segregates in two affected family members) |
| B1306 | *NPHP4*[z] | Compound het | c.3983C>T | p.Pro1328Leu | Het | *C. elegans* | 0.999 Del. / | 26 | 0/4/2047 | 0/62/268266 | Likely pathogenic | Gene | BL RHD | NA |
| | *NPHP4*[z] | | c.2021G>A | p.Arg674His | Het | *C. elegans* | 0.999 Del. / | 29 | 0/1/2089 | 0/8/277216 | Likely pathogenic | Gene | Growth retardation<br>Patent ductus arteriosus<br>Facial dysmorphism | |

PP2, PolyPhen 2; SIFT, Sorting Intolerant from Tolerant; MT, Mutation Taster; CADD, Combined Annotation Dependent Depletion; EVS, Exome Variant Server; gnomAD, Genome Aggregation Database; ACMG, American College of Medical Genetics; Het, heterozygous; Del., deleterious; D.C., disease causing; /, data not available; DM, disease mutation; BL, bilateral; VUR, vesicoureteric reflux; R, right; L, left; MCDK, multicystic dysplastic kidney; —, not applicable; NA, not available; RA, renal agenesis; RHD, renal/hypodysplasia; Tol., tolerated; UVJO, ureterovesical junction obstruction; Hom, homozygous; UPJO, ureteropelvic junction obstruction; PUV, posterior urethral valve; ADHD, Attention Deficit Hyperactivity Disorder; Mb, megabase; MRI, magnetic resonance imaging; ESS, essential splice site.

[a]For families in which the disease-causing variant has previously been reported in the literature, the corresponding reference is provided.

[b]Evolutionary conservation was assessed across phylogeny over eight species: *Mus musculus*, *Gallus gallus*, *Xenopus tropicalis*, *Danio rerio*, *Caenorhabditis elegans*, *Ciona intestinalis*, *Drosphilia melanogaster*, and *Saccharomyces cerevisiae*. If conservation is interrupted in one species but otherwise preserved across phylogeny, additional information is provided.

[c]Variant frequencies listed for homozygous/hemizygous (if applicable)/heterozygous/total alleles detected in the population.

[d]ACMG American College of Human Genetics Standards and Guidelines Classification as pathogenic, likely pathogenic, or uncertain significance.[66]

[e]HGMD, Human Gene Mutation Database; (https://portal.biobaseinternational.com/hgmd). If the exact variant has previously been reported and classified as a pathogenic mutation that is disease causing, the variant is denoted as DM. If the gene but not the exact variant has been reported for the corresponding phenotype, gene is indicated.

[f]Ref. 42.

[g]Mutations in the isolated or syndromic gene identified in families with the corresponding phenotype.

[h]Ref. 21.

[i]Frameshift, stop loss, stop gain, or nonsense variant.

[j]Ref. 64.

[k]Ref. 68.

[l]Ref. 69.

[m]Finding in more than two categories.

[n]Ref. 36.

[o]Interruption in conservation due to leucine present in C. intestinalis.

[p]Ref. 23.

[q]Ref. 43.

[r]Ref. 33.

[s]Ref. 32.

[t]Interruption in conservation due to serine present in C. elegans.

[u]Ref. 46.

[v]Interruption in conservation due to arginine present in D. rerio.

[w]Interruption in conservation due to serine present in C. elegans.

[x]Ref. 44.

[y]Interruption in conservation due to methionine present in M. musculus.

[z]Mutations in phenocopy gene.

mutations in 6% (15 of 232) of families in one of the following 12 dominant genes: *AMER1*, *KAT6B*, *NOTCH2*, *KMT2D*, *EP300*, *NSDHL*, *TP63*, *OFD1*, *FGFR1*, *FGFR3*, *HOXA13*, and *FLNA* (Figure 1C, light blue segment, Supplemental Table 6).

### Identifying Novel Human CAKUT Genes Using Murine CAKUT Candidate Genes

Having detected likely causative mutations or candidate variants in 20% (47 of 232) of families (Figure 1, A–C), we proceeded to evaluate WES data of the remaining 185 of 232 unsolved families (80%) for mutations in potentially novel genetic causes of human CAKUT. By applying a search in the 185 known monogenic causes of murine CAKUT genes (Supplemental Table 3), we identified deleterious variants in 2% (five of 232) of families with CAKUT in three recessive genes (*LAMA5*, *MEGF8*, and *TNS1*) and one dominant gene (*FOXC1*) (Figure 1D, Supplemental Table 4, pink segment). Mutations in these genes have not yet been implicated in human CAKUT. However, given the corresponding phenotype in mouse models Supplemental Table 3, we consider them likely novel genes for human CAKUT.

### Discovering 19 Potential Novel Unique Candidate Genes for CAKUT in 8% of Families

Having detected likely causative mutations or candidate variants in 22% of CAKUT families (Figure 1, A–D), we proceeded to evaluate 102 of 232 families (44%) that either were consanguineous or had a duo/trio/quad pedigree structure. We evaluated for either (*1*) novel recessive genes by evaluation of homozygous regions in consanguineous families (37 of 232; 16%) or (*2*) recessive and/or dominant mutations by duo, trio, or quad analysis depending on pedigree structure (65 of 232; 28%) (Supplemental Figures 3 and 4). After filtering of variants on the basis of *a priori* genetic criteria (Methods), we arrived at a single novel candidate gene in 8% of families (19 of 232) with CAKUT (Figure 1E, Supplemental Table 4, red segment). Search for additional CAKUT families with variants in these 19 novel candidate genes by GeneMatcher[59] did not yield any additional families to date, a finding that is not uncommon in monogenic forms of CAKUT.[38]

### Identifying Multiple Potential Novel Candidate Genes per Family in 10% of Families with CAKUT

In 10% of the families (22 of 232), multiple candidate CAKUT genes were identified after *a priori* filtering criteria (Methods, Figure 1F, Supplemental Table 4, green segment). No single gene per family could be prioritized on the basis of genetic criteria (*e.g.*, nonsense versus missense variant) or protein information obtained from the literature.

### Detecting Monogenic Causes for Non-CAKUT Diseases in Families with CAKUT

In 8% of CAKUT families (18 of 232), we detected mutations in 21 disease-causing genes known to be causative of non-CAKUT diseases (Supplemental Table 7). Twelve of these genes were identified in ten of 232 CAKUT families (4%); in these families, no CAKUT-causing mutation could be identified (Figure 1G). However, nine were identified in eight CAKUT families; in these families, we detected either a CAKUT-causing gene (Figure 1A and B) or a CAKUT candidate gene (Figure 1, C–F). These mutations are coded in brown in Supplemental Table 7 but highlighted to indicate their additional status as families with a CAKUT-causing mutation that was detected. These causative mutations in nonrenal disease genes were reported back to the referring physician according to the American College of Medical Genetics guidelines.[60–62]

### Spectrum of Mutations in Known CAKUT Genes

It is known that, in consanguineous pedigrees, the likelihood of detecting a homozygous causative mutation in a recessive gene rather than compound heterozygous mutations rises with the degree of relatedness or homozygosity across the genome.[63] We, therefore, plotted homozygosity in descending order for families in which we identified a likely causative mutation or a candidate mutation in a CAKUT gene or a CAKUT phenocopy gene (Figure 3). In seven of 47 families that exhibited significant levels of homozygosity ($\geq$100 Mbp), we identified four homozygous recessive mutations; in the 38 families that had homozygosity $<$100 Mbp, four had a compound heterozygous recessive mutation, whereas 25 had a dominant heterozygous mutation. Of note, in three families (B1316, B1439, and B1435) with homozygosity level of $\geq$60 Mbp, a single heterozygous disease-causing mutation in established isolated or syndromic CAKUT genes was detected (*SALL1*, *ACTG1*, and *GREB1L*) (Figure 3). Overall, the solve rate varies by pedigree structure, ranging from 10% in outbred multiplex families, 12% in pedigrees with a trio structure, 12% in families from regions where consanguinity is high, 17% in patients with severe CAKUT, and 20% in families that are consanguineous to 29% in families with syndromic CAKUT (Figure 2).

### Syndromic CAKUT Genes Constitute Promising Candidate Genes for Isolated CAKUT Phenotypes

We previously described an allelic genotype-phenotype correlation, in which null mutations in known syndromic CAKUT genes (*e.g.*, protein truncating) cause syndromic CAKUT phenotypes, whereas hypomorphic mutations in the same subset of syndromic CAKUT genes (*e.g.*, missense) cause isolated CAKUT phenotypes.[33] We, therefore, evaluated WES data for mutations in 40 genes that are known to cause isolated (*i.e.*, nonsyndromic) CAKUT (Supplemental Table 1). Conversely, we also evaluated WES data for mutations in 179 genes that are known to cause syndromic CAKUT (Supplemental Table 2) in both patients with the corresponding phenotype and as a candidate gene hypothesis, additionally in families with an isolated CAKUT phenotype.

We detected likely causative mutations that were concordant (*i.e.*, mutations in an isolated CAKUT gene in families with isolated CAKUT or mutations in syndromic CAKUT genes in families with syndromic CAKUT) in 13% of families (29 of 232) (Figure 1A). Interestingly, we also detected causative mutations that were discordant (*i.e.*, mutations in syndromic CAKUT genes in families with isolated CAKUT) in 6%

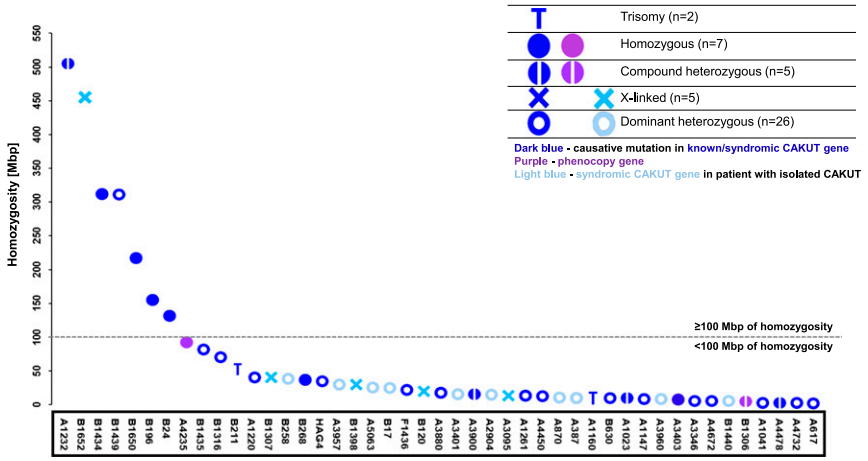


**Figure 3.** Relationship between measured homozygosity and disease-causing mutations in congenital anomalies of the kidney and urinary tract (CAKUT). Homozygosity mapping was performed on the basis of single-nucleotide polymorphisms generated from whole-exome sequencing data. Data are shown for families in which a CAKUT-causing gene or a gene known to phenocopy CAKUT was identified (32 of 232) and families with isolated CAKUT in which a candidate gene in a known syndromic CAKUT gene was identified (15 of 232). A representative individual from each family was plotted from the highest to the lowest level of total homozygosity (megabase pairs) across the genome. In total, seven individuals had homozygosity ≥100 Mbp, whereas 38 individuals had homozygosity of <100 Mbp, which is denoted by the gray dashed line. In two families, homozygosity mapping could not be generated due to low coverage, and therefore, they are not included in this graph (A3887: *TBX18* dominant heterozygous mutation and A2962: *NPHP1* homozygous variant). Causative mutations in isolated/syndromic genes identified in CAKUT families with the corresponding phenotype are denoted by a dark blue color, phenocopy genes are denoted by a purple color, and candidate mutations in syndromic CAKUT genes identified in families with isolated CAKUT are denoted by a light blue color. Homozygous variants are denoted by filled circles, compound heterozygous variants are denoted by two half circles, dominant heterozygous variants are denoted by unfilled circles, X-linked variants are denoted by an "X," and complex chromosomal rearrangements are denoted by a "T." Note that, in the part of our cohort with homozygosity of ≥100 Mbp (the cluster left of the *x* axis), paradoxically, we identified causative mutations in heterozygous genes (*e.g.*, B1439; *SALL1*). In patients with rare cases with extreme homozygosity, heterozygous disease-causing mutations can be identified. Such patients have previously been described in the literature.[67]

of families (15 of 232) (Figure 1C). Strikingly, 13 of these 15 mutations were hypomorphic mutations (*i.e.*, 13 missense and two splice variants) (Supplemental Table 6, Table 3). The literature on 13 of these 15 mutations supports the genotype-phenotype correlation, in which hypomorphic mutations cause isolated CAKUT phenotypes (as shown here), whereas syndromic CAKUT was caused by null mutations in those same genes in 35%–97% of patients in the literature (Supplemental Table 6, column 7).

## DISCUSSION

### WES Can Identify Likely Pathogenic Mutations in 14% of Families

We applied WES to a large cohort of 232 families with CAKUT. We showed that, in this patient cohort, WES detects a specific deleterious mutation in a known CAKUT or CAKUT phenocopy gene in 32 of 232 (14%) families with CAKUT. Mutations were identified in known genes for isolated or syndromic manifestations of CAKUT in 13% (29 of 232) of families exhibiting the corresponding phenotype (Figure 1A). In addition, we identified causative mutations in genes that may cause phenocopies of CAKUT in three of the 232 families (1%) (Figure 1B). Gene panel studies have shown that monogenic causation accounts for approximately 12% of patients with CAKUT.[33,42] WES has the added advantage that detection of mutations is not limited to a prespecified list of candidate genes. We show here the utility of WES for the identification of monogenic, likely pathogenic mutations in 14% of families with CAKUT.

### Candidate Genes Can Be Derived from WES

In 6% (15 of 232) of CAKUT families with an isolated CAKUT phenotype, we were able to identify candidate mutations in a known syndromic CAKUT gene (Figure 1C). In five families (2%), we identified four novel murine candidate genes (Figure 1D). Additionally, in 19 families (8%), we identified single novel CAKUT candidates using targeted search for homozygously mutated genes in homozygous families or by trio evaluation in families in which parental DNA was available (Figure 1E). So far, mutation analyses have not yielded mutations in these genes in additional families with CAKUT. This rarity is not unexpected, because in many of recently identified CAKUT genes, very few families with mutations have been identified. Additional genetic and experimental evidence will help determine whether mutations in these newly identified

**Table 3.** Information on identified candidate mutations in congenital anomalies of the kidney and urinary tract genes known to cause isolated or syndromic congenital anomalies of the kidney and urinary tract

| Family Identification[a] | Gene | Mode of Transmission | Nucleotide Change | Amino Acid Change | State | Evolutionary Conservation[b] | PP2 SIFT MT | CADD Score | EVS[c] | gnomAD[c] | ACMG[d] | HGMD[e] | Phenotypes | Segregation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A387 | *KAT6B*[f] | Dominant | c.2152C>T | p.Arg 718Trp | Het | *Saccharomyces cerevisiae*[g] | 0.983 Del. D.C. | 16 | / | / | Uncertain significance | Gene | L RA, R hydronephrosis; progressive renal impairment | Variant inherited from father (affection status unknown) |
| A870 | *NOTCH2*[f] | Dominant | c.3556T>A | p.Tyr 1186Asn | Het | *Danio rerio* | 0.854 Del. D.C. | 18 | 0/1/4299 | 0/12/277048 | Uncertain significance | Gene | BL VUR nevus pigmentosus R supranummery nipple | Variant inherited from mother (affection status unknown) |
| A3401 | *NOTCH2*[f] | Dominant | c.6767G>A | p.Arg 2256His | Het | *Ciona intestinalis*[h] | 0.862 Del. D.C. | 15 | 0/1/4299 | 0/12/246058 | Uncertain significance | Gene | R RHD, L UVJO PUV | Variant inherited from father (affection status unknown); segregates in two affected siblings |
| B17 | *KMT2D*[f] | Dominant | c.13190G>T | p.Gly 4397Val | Het | *Drosphilia melanogaster* | 0.960 Del. D.C. | 11 | / | / | Uncertain significance | Gene | L RA | NA |
| A3095 | *NSDHL*[f] | X-linked recessive | c. 842G>A | p.Arg 281His | Hemi | *X. tropicalis* | 0.899 Del. D.C. | 12 | / | 0/6/8/199924 | Uncertain significance | Gene | Prune belly syndrome L RA | NA |
| A5063 | *EP300*[f] | Dominant | c.1781C>T | p.Thr594Met | Het | *D. melanogaster*[j] | 0.999 Del. D.C. | 32 | / | 0/17/246260 | Uncertain significance | Gene | BL MCDK | Variant inherited from father (affection status unknown); segregates in two affected siblings |
| B258 | *TP63*[f] | Dominant | c.799G>A | p.Val267Ile | Het | *C. intestinalis*[i] | 0.802 Tol. D.C. | 22 | / | 0/3/246070 | Uncertain significance | Gene | L UPJO | NA |
| A3957 | *NOTCH2*[f] | Dominant | c.6892C>T | p.Arg2298Trp | Het | *D. rerio* | 0.609 Del. D.C. | 11 | / | 0/1/245696 | Uncertain significance | Gene | BL hydronephrosis Anorectal malformation | NA |
| A3960 | *FGFR1*[f] | Dominant | c.1426C>T | p.Arg476Trp | Het | *D. rerio* | 1.000 Del. D.C. | 6 | / | 0/4/277084 | Uncertain significance | Gene | L RA, R VUR Hypospadias Bladder extrophy | NA |
| B1307 | *AMER1*[f] | Dominant | c.185G>T | p.Gly62Val | Het | *D. rerio* | 0.813 Tol. D.C. | 19 | 0/1/1/2427 | 0/4/9/200427 | Uncertain significance | Gene | R MCDK | NA |
| B1398[k] | *OFD1*[f] | X-linked | c.936–2A>G | 100% ESS | Het | — | / | NA | 0/0/1/2427 | 0/8/23/198443 | Uncertain significance | Gene | BL VUR R hydronephrosis | Variant inherited from mother (affection status unknown) |
| B1440 | *HOXA13*[f] | Dominant | c.25C>T | p.Pro9Ser | Het | *D. rerio* | 0.992 Tol. D.C. | 18 | / | 0/28/226114 | Uncertain significance | Gene | BL hydronephrosis BL RHD, PUV Facial dysmorphism | NA |
| A2904 | *FGFR3*[f] | Dominant | c.1663G>A | p.Val555Met | Het | *D. melanogaster* | 0.985 Del. D.C. | 19 | / | 0/48/274072 | Likely pathogenic | DM | R RHD, VUR | NA |
| B120 | *OFD1*[f] | Dominant | c.517+1G>A | 100% ESS | Het | | / | NA | / | / | Pathogenic | Gene | R MCDK L duplex L VUR, ureterocoele | NA |
| B1652[k] | *FLNA*[f] | X-linked recessive | c.6348C>G | p.His2116Gln | Hemi | *Caenorhabditis elegans* | 0.955 Del. D.C. | 12 | / | / | Uncertain significance | Gene | Prune belly syndrome Neurogenic bladder BL VUR | Variant inherited from mother (affection status unknown) |

PP2, PolyPhen 2; SIFT, Sorting Intolerant from Tolerant; MT, Mutation Taster; CADD, Combined Annotation Dependent Depletion; EVS, Exome Variant Server; gnomAD, Genome Aggregation Database; ACMG, American College of Medical Genetics; Het, heterozygous; Del., deleterious; D.C., disease causing; /, data not available; L, left; RA, renal agenesis; R, right; BL, bilateral; VUR, vesicoureteric reflux; RHD, renal/hypodysplasia; UVJO, ureterovesical junction obstruction; PUV, posterior urethral valve; NA, not available; Hemi, hemizygous; MCDK, multicystic dysplastic kidney; Tol., tolerated; UPJO, ureteropelvic junction obstruction; —, not applicable; ESS, essential splice site; DM, disease mutation.

[a]Unique family identification number.

[b]Evolutionary conservation was assessed across phylogeny over eight species: *Mus musculus*, *Gallus gallus*, *Xenopus tropicalis*, *Danio rerio*, *Caenorhabditis elegans*, *Ciona intestinalis*, *Drosphilia melanogaster*, and *Saccharomyces cerevisiae*. If conservation is interrupted in one species but otherwise preserved across phylogeny, additional information is outlined.

[c]Variant frequencies listed for homozygous/hemi (if applicable)/het/total alleles detected in the population.

[d]ACMG American College of Human Genetics Standards and Guidelines Classification as pathogenic, likely pathogenic, or uncertain significance.[66]

[e]HGMD Human Gene Mutation Database; (https://portal.biobaseinternational.com/hgmd). If the exact variant has previously been reported and classified as a pathogenic mutation that is disease causing, the variant is denoted as DM. If the gene but not the exact variant has been reported for the corresponding phenotype, gene is indicated.

[f]Mutations in syndromic genes identified in families with an isolated syndromic congenital anomaly of the kidney and urinary tract phenotype.

[g]Interruption in conservation due to glutamine present in *D. rerio* and *D. melanogaster*.

[h]Interruption in conservation due to leucine present in *D. rerio*.

[i]Interruption in conservation due to glutamate present in *C. elegans*.

[j]Interruption in conservation due to asparagine present in *X. tropicalis*.

[k]Finding in more than two categories.

genes are indeed disease causing in CAKUT. In 22 of the 232 families (9%), we were unable to identify a unique, potentially novel gene per family, but rather, after filtering of variants, we were left with multiple potential causative genes (Figure 1F).

### Limitations of the Study

In total, 129 of 232 families (56%) remained without any findings (Figure 1H), the reason for which is likely multifold. First, it has been shown that up to 16.6% of individuals with CAKUT have a molecular diagnosis attributable to copy number variants, which can be difficult to detect using WES.[64] Second, the coverage distribution across the exome is variable, which means that variants in some low-coverage areas may be missed.

Because our cohort was prescreened for CAKUT genes, this likely led to an overall underestimation of the true prevalence of monogenic causation within our CAKUT cohort.

In relation to causality, although we performed variant calling according to our stringent *a priori* criteria (which have been extensively published[23,44,65]) that adhere to standard classification as per the American College of Medical Genetics,[66] functionality of each detected variant was not individually tested.

We show the success of WES in terms of obtaining a molecular diagnosis in families with CAKUT. The finding that, in 14% of families, a likely pathogenic gene can be identified is significant, further supporting the hypothesis that CAKUT is caused by mutations in monogenic genes.

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## DISCLOSURES

D.M.C., A.T.v.d.V., H.I., N.M., M.N., J.C., A.V., D.y.H., J.S., D.A.B., J.M.S., D.S., R.S., J.K.W., A.D., A.J.M., W.T., T.J.S., T.H., E.W., S.A., A.A., C.A.H., H.H., T.M.K., F.K., C.M.K., R.D., and S.S., performed whole exome evaluation and mutation analysis. L.S., K.A., D.R.S., M.A.B., M.J.G.S., N.M.R., M.A.F., A.Z.T., G.H.D., R.B., N.S., N.A.S., J.A.K., S.E.D, H.M.F., D.M., M.A.S., H.S.A, L.A.E., A.K., P.S., S.S.C., R.S.L., S.B.B., W.L., H.M.R., V.T. and F.H. recruited patients and gathered detailed clinical information for the study. S.S., H.L.R., D.G.M. A., M.L., K.M.L., M.W.W., S.M.M., and R.P.L. performed whole exome sequencing and downstream data analysis. F.H. conceived of and directed the entire study and wrote the manuscript with D.M.C. and A.T.v.d.V.

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