Supplementary methods

Study participants

This study was approved by the institutional review board (IRB) of Boston Children's Hospital as well as the institutional review boards of institutions at which families with CAKUT were recruited after obtaining informed consent from January 2010 to January 2019. 551 different families were enrolled and had WES performed on their DNA samples. All patients with CAKUT were referred to us by their pediatric nephrologist or urologist, who had 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, gonads, or other parts of the genital urinary tract that included at least one of the following: renal agenesis, renal hypo-/dysplasia, multicystic dysplastic kidney, hydronephrosis, ureteropelvic junction obstruction, hydroureter, vesicoureteral reflux (VUR), ectopic or horseshoe kidney, duplex collecting system, ureterovesical junction obstruction, epi-/hypospadias, posterior urethral valves (PUV), and cryptorchidism.

Whole exome sequencing (WES) and variant calling

WES was performed as previously described [1]. In brief, genomic DNA was isolated from blood lymphocytes 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 HiSeq sequencing platform. Sequence reads were mapped to the human reference genome assembly (NCBI build 37/hg19), and

variants were called using CLC Genomics Workbench (version 6.5.2) software (CLC Bio, Aarhus, Denmark).

Variant filtering

Genetic variants were filtered for their potential CAKUT-causing role as judging their deleteriousness, as previously described [2]. Variants with minor allele frequencies (MAF) >1% were excluded because they were unlikely to be deleterious. MAF was estimated using combined datasets incorporating all available data from the 1,000 Genomes Project, the Exome Variant Server (EVS) project, dbSNP145, the Exome Aggregation Consortium (ExAC) and gnomAD. Synonymous exonic and all intronic variants that were not located within splice site regions were excluded. Retained variants, which included nonsynonymous variants and all strong splice site variants, were then further analyzed as previously described.

Screening for genes known to cause CAKUT

We evaluated WES data for causative pathogenic variants in the 40 monogenic genes that are currently known to cause non-syndromic CAKUT, and in the currently known 179 monogenic genes for syndromic CAKUT (**Supplementary Tables S1 and S2**).

Variant evaluation to identify potential novel monogenic causes of CAKUT

Variants with a minor allele frequency (MAF) <1% were analyzed using predefined criteria as previously described [3-5]: 1) potential truncating variants, 2) obligatory

splice-site variants, 3) strong to moderate evolutionary conservation of altered amino acid residue, 4) predicted to be deleterious by two out of three *in-silico* prediction programs, 5) present in fewer than 20 heterozygous alleles on gnomAD. After above evaluations, candidate variants were ranked using the ACMG criteria [6].

References

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