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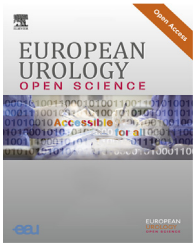

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Copy Number Variation Analysis Facilitates Identification of Genetic Causation in Patients with Congenital Anomalies of the Kidney and Urinary Tract

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Abstract

Background: Congenital anomalies of the kidneys and urinary tract (CAKUT) are the most common cause of chronic kidney disease among children and adults younger than 30 yr. In our previous study, whole-exome sequencing (WES) identified a known monogenic cause of isolated or syndromic CAKUT in 13% of families with CAKUT. However, WES has limitations and detection of copy number variations (CNV) is technically challenging, and CNVs causative of CAKUT have previously been detected in up to 16% of cases.

Objective: To detect CNVs causing CAKUT in this WES cohort and increase the diagnostic yield.

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Design, setting, and participants: We performed a genome-wide single nucleotide polymorphism (SNP)-based CNV analysis on the same CAKUT cohort for whom WES was previously conducted.
Outcome measurements and statistical analysis: We evaluated and classified the CNVs using previously published predefined criteria.
Results and limitations: In a cohort of 170 CAKUT families, we detected a pathogenic CNV known to cause CAKUT in nine families (5.29%, 9/170). There were no competing variants on genome-wide CNV analysis or WES analysis. In addition, we identified novel likely pathogenic CNVs that may cause a CAKUT phenotype in three of the 170 families (1.76%).
Conclusions: CNV analysis in this cohort of 170 CAKUT families previously examined via WES increased the rate of diagnosis of genetic causes of CAKUT from 13% on WES to 18% on WES + CNV analysis combined. We also identified three candidate loci that may potentially cause CAKUT.
Patient summary: We conducted a genetics study on families with congenital anomalies of the kidney and urinary tract (CAKUT). We identified gene mutations that can explain CAKUT symptoms in 5.29% of the families, which increased the percentage of genetic causes of CAKUT to 18% from a previous study, so roughly one in five of our patients with CAKUT had a genetic cause. These analyses can help patients with CAKUT and their families in identifying a possible genetic cause.

1. Introduction

Congenital anomalies of the kidney and urinary tract (CAKUT) are the most prevalent cause of chronic kidney disease (CKD) in the first three decades of life [1]. CAKUT can present as an isolated renal condition or as part of a clinical syndrome [2–6]. Despite large differences in clinical manifestation, these conditions probably share a pathogenic origin in dysregulation of renal morphogenesis [6,7].

We hypothesized that a large proportion of human CAKUT cases may be caused by variants in distinct single monogenic genes. Previous supporting evidence for this hypothesis includes (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 [2,8,9]. To date, 40 monogenic causes of isolated CAKUT and 232 monogenic causes of syndromic CAKUT have been identified [3,4,10–17] (Supplementary Tables 1 and 2).

In a previous study, we used whole-exome sequencing (WES) analysis to determine the proportion of individuals with CAKUT for whom a causative variant could be identified in a cohort of 232 families with CAKUT [18]. We found that in 13% of the families, CAKUT could be attributed to one of the known monogenic genes for isolated or syndromic CAKUT [18].

WES has limitations and detection of the presence of a copy number variation (CNV) is technically challenging [19,20]. Genetic causation may also be represented by pathogenic CNVs in addition to point variants or small insertions or deletions. In a previous study, known pathogenic CNVs were detected in up to 10.5% of patients with CAKUT [21].

Here we performed a genome-wide CNV analysis on the same cohort of 232 families with CAKUT in whom we previously conducted WES analysis [18]. Of the 232 families, 170 had DNA amounts and quality sufficient to perform CNV analysis, among which we detected a pathogenic CNV as the likely cause of CAKUT in nine families (5.29%). This increased the diagnostic rate for genetic causes of CAKUT from 13% on WES alone [18] to 18% on WES + CNV analysis combined.

2. Patients and methods

2.1. Human subjects

This study was approved by the institutional review board (IRB) of Boston Children's Hospital as well as the IRBs of institutions where we recruited families. All patients with CAKUT were referred to us by their pediatric nephrologist or urologist, who made the clinical diagnosis of CAKUT on the basis of renal imaging studies.

CAKUT is defined as demonstration of any abnormality of number, size, shape, or anatomical position of the kidneys or other parts of the urinary tract that included at least one of the following: renal agenesis, renal hypoplasia/dysplasia, multicystic dysplastic kidneys, hydronephrosis, ureteropelvic junction obstruction, hydroureter, vesicoureteral reflux, ectopic or horseshoe kidney, duplex collecting system, ureterovesical junction obstruction, epispadias/hypospadias, posterior urethral valves, or cryptorchidism [22]. Syndromic CAKUT is defined as a condition that affects multiple body systems with CAKUT.

2.2. Genotyping and CNV calling

Genomic DNA was isolated from peripheral blood lymphocytes. SNP genotyping was performed on all cases using the Infinium Expanded Multi-Ethnic Genotyping Array (MegaEx; Illumina, San Diego, CA, USA). CNV analysis was performed as previously described using the same set of population controls encompassing 21 498 individuals with no reported disease association to nephropathy and developmental dis-

orders [23]. In brief, raw genotyping data were preprocessed with Illumina GenomeStudio v2011 to obtain intensity data that included probe-level logR-ratio and B allele frequency (BAF) values. Cases with a mismatched self-declared gender and estimated genotyped gender were removed from further analysis. CNV calling was initially performed on hg18 assembly coordinates and subsequently converted to the hg19 assembly coordinates using UCSC liftOver tool (https://genome.ucsc.edu/cgi-bin/hgLiftOver). PennCNV (version 2011-05-03) [24] was used to identify CNVs using the *-test*, *-confidence*, and *-minconf 30* parameters in the *detect_cnv.pl* function, retaining high-quality CNVs with a minimum confidence score of 30 for downstream analysis only.

2.3. CNV analysis and classification

CNVs were classified as pathogenic (GD-CNV) or likely pathogenic (candidate GD-CNV) on the basis of previously reported criteria [23]. In brief, regions within predicted CNV boundaries were annotated with RefSeq (https://www.ncbi.nlm.nih.gov/refseq), annotated with known syndromic CNVs [23] curated from the Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources (DECIPHER) [25,26] and the International Standards for Cytogenomic Arrays (ISCA) databases [27], and annotated with genes causing kidney disease and CAKUT curated from the Online Mendelian Inheritance in Man (OMIM, https://www.omim.org/) and the Mouse Genome Informatics (http://www.informatics.jax.org/) databases [23].

As previously described, a CNV was defined as pathogenic if it overlapped at least 70% of a known syndromic CNV [23] or as likely pathogenic when a large CNV of at least 100 kb intersected an exon, occurred in less than 0.02% of population controls, and did not overlap (<70%) a clinically interpreted benign or likely benign CNV in the ISCA database. The following additional criteria were also included: (1) CNV boundaries overlapped at least 70% with a reported pathogenic or likely pathogenic CNV in the ISCA database, (2) intersected a causative autosomal-dominant gene for CAKUT in humans or mice, and/or (3) was the reciprocal of a known GD-CNV (coordinates with ≥70% overlap) [23]. A flowchart of CNV analysis and evaluation is depicted in Figure 1.

3. Results

3.1. Patient characteristics

A total of 488 individuals with CAKUT (319 affected, 169 reportedly unaffected) from 232 different families were previously enrolled in our study of WES in CAKUT [18]. Of these 232 CAKUT families, 170 had sufficient DNA samples to perform CNV analysis. We performed SNP microarray and CNV analysis in one individual (proband) for each family.

The cohort of 170 families had a diverse spectrum of CAKUT phenotypes; 116 families (68%) had isolated CAKUT and 54 families (32%) had syndromic CAKUT. The clinical characteristics of the cohort are summarized in Table 1.

3.2. Identification of known pathogenic CNVs in families with CAKUT

Genome-wide CNV analysis identified a pathogenic CNV known to cause CAKUT (GD-CNV) in nine of the 170 families (5.29%). Details of the pathogenic CNVs and clinical features are outlined in Table 2. The logR ratio and B allele frequency graph for each CNV are presented in Supplementary Figure 1. In particular, for each patient there was no competing CNV that can be attributed to a cause of the CAKUT presentation. Likewise, there was no competing variant detected via WES analysis that may otherwise explain the cause of the CAKUT.

Among these nine pathogenic CNVs, seven were large deletions and two were large duplications (Table 2). Two patients were identified as having DiGeorge syndrome (also known as 22q11 deletion syndrome), and *RCAD* deletion (renal cysts and diabetes) was detected in two patients. A 22q11 duplication was detected for one patient (Table 2).

3.3. Identification of novel likely pathogenic CNVs in families with CAKUT

Identification of likely pathogenic CNVs (“novel” CNVs) was performed using the previously described criteria [23] (Fig. 1). We identified likely pathogenic CNVs that may cause a CAKUT phenotype in three of the 170 families (1.76%; Table 3). Details of the likely pathogenic CNVs and clinical features are outlined in Table 3, while the logR ratio and B allele frequency graph for each CNV are presented in Supplementary Figure 2.

Similar to the identification of pathogenic CNVs, the likely pathogenic CNVs identified were unique to each family, with no competing genetic explanation. All of the three CNVs identified are duplications; details of these likely pathogenic CNVs are outlined in Table 3.

4. Discussion

We identified known pathogenic CNVs in 5.29% of families with CAKUT, and likely pathogenic CNVs in 1.76% (Table 1, Table 2, and Supplementary Table 3). Owing to the known nature of variable expressivity, we used broad CAKUT as the phenotype in this study, which is more heterogeneous and includes any abnormality of the number, size, shape, or anatomical position of the kidneys or other parts of the urinary tract [22].

Another paper using broad CAKUT as the phenotype [23] identified known pathogenic CNVs in 4.0% of families with CAKUT and likely pathogenic CNVs in 1.7% [23], which is similar to our study.

Sanna-Cherchi et al. [21] limited the CAKUT phenotypes to renal aplasia, agenesis, hypoplasia, and dysplasia (referred to together as renal hypodysplasia), and identified known pathogenic CNVs in 10.5% of patients, and likely pathogenic CNVs in 6.1%. Verbitsky et al. [28] limited the phenotypes to vesicoureteral reflux, and identified known pathogenic CNVs in 2% of patients, and likely pathogenic CNVs in 0.92%. The difference in CNV detection can be attributed to the difference in the inclusion criteria.

Of note, individuals B26-21 and B630-21 had the same pathogenic SNV at chr17:34815551-36249430 (hg19), known as *RCAD* deletion. This 1.4-Mb deletion is consistent with the known recurrent deletion at chromosome 17q12 [29,30]. The two individuals each carry other different nonpathogenic/non-likely pathogenic CNVs, and thus they are not likely to be from the same family or have a sample or technical error. Calls for the proximal and distal breakpoints are based on the first and last SNPs showing the CNV, respectively. The exact CNV breakpoints can sit between

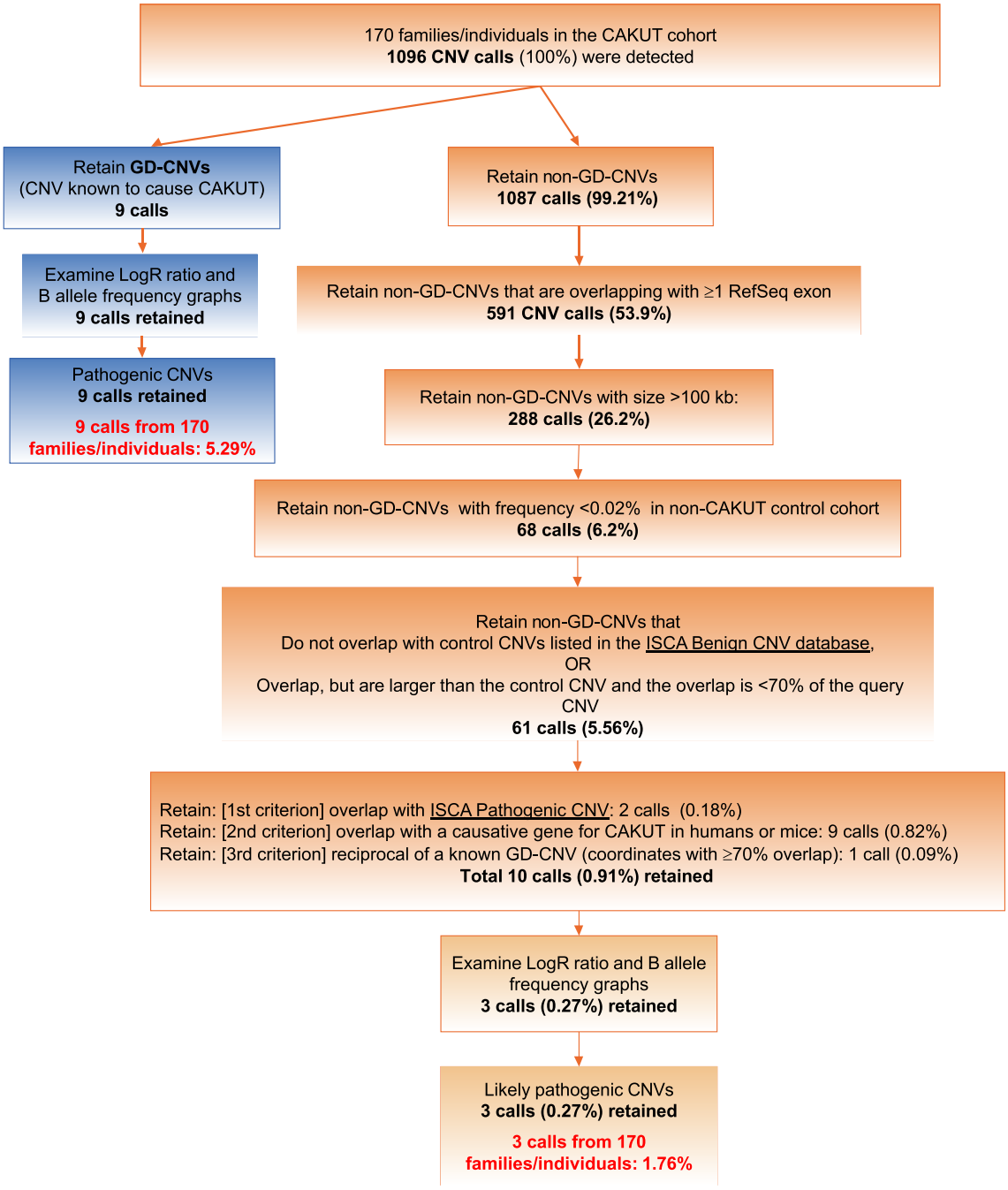

Fig. 1 – Flowchart of CNV evaluation and classification. The flowchart illustrates the CNV evaluation algorithm, which is based on Verbitsky et al [23]. LogR ratio and B allele frequency evaluations are based on Peiffer et al [31]. Blue boxes indicate pathogenic CNVs known to cause CAKUT phenotype (GD-CNVs). Yellow boxes indicate the process for filtering out CNVs not known to cause CAKUT phenotype (non-GD-CNVs) to likely pathogenic CNVs. Red boxes indicate likely pathogenic CNVs. The proportions in black bold font represent the percentages of the number of the CNV calls in that box compared to the original total CNV calls (n = 1096). The proportions in red bold font represent the percentages of the number of families/individuals with pathogenic or likely pathogenic CNVs compared to the total families/individuals (n = 170). CAKUT = congenital anomalies of the kidneys and urinary tract; CNV = copy number variation; GD-CNVs = genomic disorder-copy number variation (pathogenic CNVs known to cause CAKUT phenotype); ISCA = International Standards for Cytogenomic Arrays (http://www.iscaconsortium.org/); RefSeq = NCBI Reference Sequence Database (https://www.ncbi.nlm.nih.gov/refseq/).

the SNP called and the next SNP, which can vary from a few kb or less to more, depending on the density of the array at this area. Therefore, even if the calls for the two CNVs look the same, the exact breakpoints may not be identical.

The unique point of our study is that we used the same cohort previously analyzed via WES [18] in a new analysis via CNVs.

In our previous study using WES technology, we found that CAKUT could be attributed to one of the known monogenic genes for isolated or syndromic CAKUT in 13% of the families [18]. In this study, using CNV analysis we identified an additional 5.29% of families whose CAKUT could be attributed to a monogenic cause. Therefore, CNV analysis increased the diagnostic rate for genetic causes of CAKUT

Table 1 – Clinical characteristics of the 170 individuals (from 170 families) with CAKUT who underwent evaluation of copy number variation

Parameter	Result, *n* (%)
Gender, *n* (%)	
Female	58 (34)
Male	111 (65)
Unknown	1 (<1)
Total	170 (100)
Extrarenal manifestations	
Yes	54 (32)
No	116 (68)
Total	170 (100)
Reported consanguinity	
Yes	35 (21)
No	135 (79)
Total	170 (100)
Homozygosity on mapping ≥60 Mbp[a]	
Yes	31 (18)
No	129 (76)
Not enough single-nucleotide polymorphisms to generate a map	10 (6)
Total	170 (100)
CAKUT phenotype	
Unilateral CAKUT	71 (42)
Bilateral concordant CAKUT	59 (35)
Bilateral discordant CAKUT	22 (13)
Undefined CAKUT phenotype	7 (4)
Isolated posterior urethral valve or epispadias/ hypospadias	2 (<1)
Posterior urethral valve with additional CAKUT	9 (5)
Total	170 (100)

CAKUT = congenital anomalies of the kidneys and urinary tract
[a] In addition to self-reports of consanguinity, we used homozygosity mapping ≥60 Mbp as an objective measurement to determine consanguinity.

from 13% to 18%. WES and CNV analyses complement each other to increase the genetic diagnostic rate for patients with CAKUT. We recommend running both platforms to identify both sequencing variants and CNVs in the work-up for genetic causes of CAKUT.

5. Conclusions

In summary, we conducted genome-wide CNV analysis on a cohort of CAKUT families for whom we previously performed WES analysis [18]. We identified a pathogenic CNV as the likely cause of CAKUT in nine out of 170 families (5.29%). This increased the diagnosis rate for genetic causes of CAKUT from 13% diagnosed on WES [18] to 18% diagnosed on WES + CNV combined. WES and CNV analyses complement each other to increase the genetic diagnostic rate for patients with CAKUT. We recommend running both platforms to identify both sequencing variants and CNVs as part of the patient work-up to identify a genetic cause of CAKUT.

Author contributions: Friedhelm Hildebrandt had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Hildebrandt, Sanna-Cherchi, Wu, Lim.
Acquisition of data: Stajic, Soliman, Kari, El Desoky, Fathy, Milosevic, Turudic, Al Saffar, Awad, Eid, Ramanathan, Senguttuvan, Mane, Lee, Bauer, Lu, Hilger, Tasic.

Table 2 – Information on nine pathogenic CNVs known to cause a CAKUT phenotype (GD-CNVs) identified in the cohort

Individual ID	CAKUT phenotype	Extrarenal phenotype	CNV position (hg19)	CNV length (bp)	CN	Known pathogenic CNV	Genes involved
A1955-21	Bilateral VUR grade III	None reported	chr1:146067632-147825769	1 758 137	1	1q21.1 class I deletion	21
A2903-21	Bilateral renal dysplasia, ESRD	Hirschsprung's disease	chr7:141888080-159122659	17 234 579	1	7q36 deletion	176
A693-21	Horseshoe kidney	Anal atresia, cryptorchidism	chr15:30950529-32513897	1 563 368	3	15q13.3 duplication	11
F0126_735	VUR	None reported	chr16:15122812-16362651	1 239 839	1	16p13.11 deletion	20
B26-21	Bilateral glomerulocystic KD	None reported	chr17:34815551-36249430	1 433 879	1	RCAD deletion	20
B630-21	Bilateral multicystic dysplastic kidney	Hyperurecimia, ADHD, DD, asthma	chr17:34815551-36249430	1 433 879	1	RCAD deletion	20
B378-21	Left renal agenesis	Cerebral palsy	chr22:20740778-21461607	720 829	1	DiGeorge B-D nested deletion	22
B1004-21	Bilateral VUR, scrotal hypoplasia	Facial dysmorphy, rib hypoplasia, hypoplastic nails	chr22:20740778-36077803	15 337 025	3	22q11.2 distal duplication	242
A2037-21	Left renal agenesis, left cryptorchidism	None reported	chr22:21052014-21461607	409 593	1	DiGeorge B-D nested deletion	17

ADHD = attention-deficit/hyperactivity disorder; CAKUT = congenital anomalies of the kidneys and urinary tract; CN = copy number; CNV = copy number variation; DD = developmental delay; ESRD = end-stage renal disease; GD-CNV = genomic disorders copy number variation; hg19 = human genome assembly 19 (Genome Reference Consortium human build 37); KD = kidney disease; RCAD = renal cysts and diabetes; VUR = vesicoureteral reflux.

Table 3 – Information on three likely pathogenic CNVs identified in the cohort

Individual ID	CAKUT phenotype	Extrarenal phenotype	CNV position (hg19)	CNV length (bp)	CN	Genes involved
A976-21	Right multicystic dysplastic kidney	ASD, PFO	chr6:136639035-	927 908	3	10
PAD4	Left renal agenesis	None reported	chr18:733474-1855370	1 121 896	3	3
B26-21	Bilateral glomerulocystic KD	None reported	chr22:18892575-	1 416 225	3	45

ASD = atrial septal defect; CAKUT = congenital anomalies of the kidneys and urinary tract; CN = copy number; CNV = copy number variation; hg19 = human genome assembly 19 (Genome Reference Consortium human build 37); KD = kidney disease; PFO = patent foramen ovale.

Analysis and interpretation of data: Wu, Lim, Wang, Seltzsam, Zheng, Schierbaum, Schneider, Mann, Connaughton, Nakayama, van der Ven, Dai, Kolvenbach, Kause, Ottlewski.
Drafting of the manuscript: Wu, Lim.
Critical revision of the manuscript for important intellectual content: Hildebrandt, Sanna-Cherchi.
Statistical analysis: Wu, Lim, Shril.
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Appendix A. Supplementary data

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