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

Chen-Han Wilfred Wu, Tze Y. Lim, Chunyan Wang, Steve Seltzman, Bixia Zheng, Luca Schierbaum, Sophia Schneider, Nina Mann, Dervla M. Connaughton, Makiko Nakayama, Amelie T. van der Ven, Rufeng Dai, Caroline M. Kolvenbach, Franziska Kause, Isabel Ottlewska, Natasa Stajic, Nevena A. Soliman, Jameela A. Kari, Sherif El Desoky, Hanan M. Fathy, Danko Milosevic, Daniel Turudici, Muna Al Saffar, Hazem S. Awad, Loai A. Eid, Aravind Ramanathan, Prabha Senguttuvan, Shrikant M. Mane, Richard S. Lee, Stuart B. Bauer, Weining Lu, Alina C. Hilger, Velibor Tasic, Shirlee Shri, Simone Sanna-Cherchi, Friedhelm Hildebrandt

*Department of Pediatrics, Boston Children’s Hospital, Harvard Medical School, Boston, MA, USA; Department of Urology, Case Western Reserve University and University Hospitals, Cleveland, OH, USA; Department of Genetics and Genome Sciences, Case Western Reserve University and University Hospitals, Cleveland, OH, USA; Division of Nephrology, Columbia University Irving Medical Center, New York, NY, USA; Department of Pediatric Nephrology, Institute for Mother and Child Health Care, Belgrade, Serbia; Department of Pediatrics, Center of Pediatric Nephrology & Transplantation, Cairo University, Egyptian Group for Orphan Renal Diseases, Cairo, Egypt; Department of Pediatrics, King AbdulAziz University, Jeddah, Saudi Arabia; Pediatric Nephrology Unit, University of Alexandria, Alexandria, Egypt; Department of Pediatric Nephrology, University Hospital Center Zagreb, Zagreb, Croatia; Department of Pediatrics, Boston Children’s Hospital, Harvard Medical School, Boston, MA, USA; Department of Paediatrics, College of Medicine and Health Sciences, United Arab Emirates University, Al Ain, United Arab Emirates; Pediatric Nephrology Department, Dubai Hospital, Dubai, United Arab Emirates; Department of Pediatrics, Dubai Medical College and Kidney Centre of Excellence, Al Jallia Children’s Specialty Hospital, Dubai, United Arab Emirates; Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, USA; Department of Pediatric Nephrology, Dr. Mehta’s Multi-Speciality Hospital, Chennai, India; Department of Genetics, Yale University School of Medicine, New Haven, CT, USA; Department of Urology, Boston Children’s Hospital and Harvard Medical School, Boston, MA, USA; Renal Section, Department of Medicine, Boston University Medical Center, Boston, MA, USA; Department of Pediatric and Adolescent Medicine, Friedrich Alexander University Erlangen-Nurnberg, Erlangen, Germany; Medical Faculty Skopje, University Children’s Hospital, Skopje, Macedonia

**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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1 These authors contributed equally to this work.

* Corresponding author. Division of Nephrology, Boston Children’s Hospital, 300 Longwood Avenue, Boston, MA 02115, USA. Tel. +1 617 35556129; Fax: +1 617 8300385. E-mail address: friedhelm.hildebrandt@childrens.harvard.edu (F. Hildebrandt).
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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 Cyto genetic 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 (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
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                           |               |
| 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* |               |
| Yes                              | 31 (18)       |
| No                               | 129 (76)      |
| Total                            | 170 (100)     |

CAKUT = congenital anomalies of the kidneys and urinary tract

* In addition to self-reports of consanguinity, we used homozygosity mapping ≥60 Mbp as an objective measurement to determine consanguinity.

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   | None reported        | chr1:146067632-147825769 | 1758 137 | 1 | 1q21.1 class I deletion | 21 |
| A2903-21      | Bilateral renal dysplasia, ESRD | Hirschsprung’s disease | chr7:141888080-159122659 | 17234579 | 1 | 7q36 deletion | 176 |
| A693-21       | Horseshoe kidney | Anal atresia, cryptorchidism | chr15:30950529-32513897 | 1563368 | 3 | 15q13.3 duplication | 11 |
| F0126, 735    | VUR             | None reported        | chr16:15122812-16362651 | 1239 839 | 1 | 16p13.11 deletion | 20 |
| B26-21        | Bilateral glomerulocystic KD | None reported | chr17:34815551-36249430 | 1433 879 | 1 | RCAD deletion | 20 |
| B630-21       | Bilateral multicystic dysplastic kidney | Hyperurecimia, ADHD, DD, asthma | chr17:34815551-36249430 | 1433 879 | 1 | RCAD deletion | 20 |
| B378-21       | Left renal agenesis | Cerebral palsy | chr22:20740778-21461607 | 720829 | 1 | DiGeorge B-D nested deletion | 22 |
| B1004-21      | Bilateral VUR, scrotal hypoplasia | Facial dysmorphism, rib hypoplasia, hypoplastic nails | chr22:20740778-36077803 | 15337025 | 3 | 22q11.2 distal duplication | 242 |
| A2037-21      | Left renal agenesis, left cryptorchidism | None reported | chr22:21052014-21461607 | 409593 | 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 | chr6:136639035-147825769 | 927908 | 3 | 10 |
| PAD4          | Left renal agenesis | None reported | chr18:733474-1855370 | 1121896 | 3 | 3 |
| B26-21        | Bilateral glomerulocystic KD | None reported | chr22:18892575-1416225 | 416225 | 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.

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.
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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.euros.2022.08.004.

References

[1] Chesnaye N, Bonthuis M, Schafer F, et al. Demographics of paediatric renal replacement therapy in Europe: a report of the ESPN/ERA-EDTA registry. Pediatr Nephrol 2014;29:2403–10. https://doi.org/10.1007/s00467-014-2884-6.

[2] Vivante A, Kohl S, Hwang D-Y, Dworschak GC, Hildebrandt F. Single-gene causes of congenital anomalies of the kidney and urinary tract (CAKUT) in humans. Pediatr Nephrol 2014;29:695–704. https://doi.org/10.1007/s00467-013-2684-4.

[3] Sanyanupin S, Schimmelt I, Mencoe L, et al. Mutation of the Pax2 gene in a family with optic-nerve colobomas, renal anomalies and vesicoureteral reflux. Nat Genet 1995;9:358–64. https://doi.org/10.1038/ng0495-358.

[4] Lindner TH, Njolstad PR, Horikawa Y, Bostad L, Bell GI, Sovik O. A novel syndrome of diabetes mellitus, renal dysfunction and genital malformation associated with a partial deletion of the pseudo-POU domain of hepatocyte nuclear factor-1 beta. Hum Mol Genet 1999;8:2001–8. https://doi.org/10.1093/hmg/8.11.2001.

[5] Soliman NA, Ali RI, Gharib EA, Habib EI, Zada AM. Pattern of clinical presentation of congenital anomalies of the kidney and urinary tract among infants and children. Nephron 2015;20:413–8. https://doi.org/10.1111/nep.12414.

[6] Ichikawa I, Kuwayama F, Pope JC, Stephens FD, Miyazaki Y. Paradigm shift from classic anatomic theories to contemporary cell biological views of CAKUT. Kidney Int 2002;61:889–98. https://doi.org/10.1046/j.1525-1755.2002.00188.x.

[7] Costantini F. Genetic controls and cellular behaviors in branching morphogenesis of the renal collecting system. Wiley Interdiscip Rev Dev Biol 2012;1:693–713. https://doi.org/10.1002/wdev.52.

[8] Davies JA. Mesenchyme to epithelium transition during development of the mammalian kidney tubule. Acta Anat 1996;156:187–201.

[9] Weber S, Thiele H, Mir S, et al. Muscarinic acetylcholine receptor M3 mutation causes urinary bladder disease and a prune- belly-like syndrome. Am J Hum Genet 2011;88:668–74. https://doi.org/10.1016/j.ajhg.2011.10.007.

[10] Hwang D-Y, Dworschak GC, Kohl S, et al. Mutations in 12 known dominant disease-causing genes clarify many congenital anomalies of the kidney and urinary tract. Kidney Int 2014;85:1429–33. https://doi.org/10.1038/jkini.2013.508.

[11] Hoskins BE, Cramer CH, Silviu D, et al. Transcription factor SIX5 is mutated in patients with branchio-oto-renal syndrome. Am J Hum Genet 2007;80:800–4. https://doi.org/10.1086/513322.

[12] Ruf RG, Xu PX, Silviu D, et al. SIX1 mutations cause branchio-oto-renal syndrome by disruption of EYA1-SIX1-DNA complexes. Proc Natl Acad Sci U S A 2004;101:8090–5. https://doi.org/10.1073/pnas.0308475101.

[13] Kohlhase J, Wischermann A, Reichenbach H, Froster U, Engel W. Mutations in the SALL1 putative transcription factor gene cause Townes-Brocks syndrome. Nat Genet 1998;18:81–3. https://doi.org/10.1038/ng0198-81.

[14] Vivante A, Hildebrandt F. Exploring the genetic basis of early-onset chronic kidney disease. Nat Rev Nephrol 2016;12:133–46. https://doi.org/10.1038/nrendo.2015.205.

[15] Hardelin J, Levilliers J, Delcastillo I, et al. X-chromosome-linked Kallmann syndrome — stop mutations validate the candidate gene. Proc Natl Acad Sci U S A 1992;89:8190–4. https://doi.org/10.1073/pnas.89.17.8190.

[16] Saisawat P, Kohl S, Hilger AC, et al. Whole-exome sequencing reveals recessive mutations in TRAP1 in individuals with CAKUT and VACTERL association. Kidney Int 2014;85:1310–7. https://doi.org/10.1038/kid.2013.417.

[17] Humbert C, Silbermann F, Morar B, et al. Integrin alpha 8 recessive mutations are responsible for bilateral renal agenesis in humans. Am J Hum Genet 2014;94:288–94. https://doi.org/10.1016/j.ajhg.2013.12.017.

[18] van der Ven AT, Connaughton DM, Ityel H, et al. whole-exome sequencing identifies causative mutations in families with congenital anomalies of the kidney and urinary tract. J Am Soc Nephrol 2018;29:2348–61. https://doi.org/10.1681/ASN.2017121265.

[19] Vestergaard LK, Oliveira DNP, Hegdall CK, Hegdall EV. Next generation sequencing technology in the clinic and its challenges. Cancers 2021;13:1751. https://doi.org/10.3390/cancers13081751.

[20] Sathirapongsasuti JF, Lee H, Horst BAJ, et al. Exome sequencing-based copy-number variation and loss of heterozygosity detection: ExomeCNV. Bioinformatics 2011;27:2648–54. https://doi.org/10.1093/bioinformatics/btr362.

[21] Sanna-Cherchi S, Kiryluk K, Burgess KE, et al. copy-number disorders of the kidney and urinary tract. Kidney Int 2014;85:1429–33. https://doi.org/10.1038/jkini.2013.508.

[22] Vivante A, Hildebrandt F. Exploring the genetic basis of early-onset chronic kidney disease. Nat Rev Nephrol 2016;12:133–46. https://doi.org/10.1038/nrendo.2015.205.

[23] Hardelin J, Levilliers J, Delcastillo I, et al. X-chromosome-linked Kallmann syndrome — stop mutations validate the candidate gene. Proc Natl Acad Sci U S A 1992;89:8190–4. https://doi.org/10.1073/pnas.89.17.8190.

[24] Saisawat P, Kohl S, Hilger AC, et al. Whole-exome sequencing reveals recessive mutations in TRAP1 in individuals with CAKUT and VACTERL association. Kidney Int 2014;85:1310–7. https://doi.org/10.1038/kid.2013.417.

[25] Humbert C, Silbermann F, Morar B, et al. Integrin alpha 8 recessive mutations are responsible for bilateral renal agenesis in humans. Am J Hum Genet 2014;94:288–94. https://doi.org/10.1016/j.ajhg.2013.12.017.

[26] van der Ven AT, Connaughton DM, Ityel H, et al. whole-exome sequencing identifies causative mutations in families with congenital anomalies of the kidney and urinary tract. J Am Soc Nephrol 2018;29:2348–61. https://doi.org/10.1681/ASN.2017121265.

[27] Vestergaard LK, Oliveira DNP, Hegdall CK, Hegdall EV. Next generation sequencing technology in the clinic and its challenges. Cancers 2021;13:1751. https://doi.org/10.3390/cancers13081751.

[28] Sathirapongsasuti JF, Lee H, Horst BAJ, et al. Exome sequencing-based copy-number variation and loss of heterozygosity detection: ExomeCNV. Bioinformatics 2011;27:2648–54. https://doi.org/10.1093/bioinformatics/btr362.

[29] Sanna-Cherchi S, Kirbyuk K, Burgess KE, et al. copy-number disorders are a common cause of congenital kidney malformations. Am J Hum Genet 2012;91:987–97. https://doi.org/10.1016/j.ajhg.2012.10.007.

[30] Schiedl A. Renal abnormalities and their developmental origin. Nat Rev Genet 2007;8:791–802. https://doi.org/10.1038/nrg2205.

[31] Verbitsky M, Westland R, Perez A, et al. The copy number variation landscape of congenital anomalies of the kidney and urinary tract. Nat Genet 2019;51:117–27. https://doi.org/10.1038/s41588-018-0281-y.

[32] Wang K, Li M, Hadley D, et al. PennCNV: an integrated hidden Markov model designed for high-resolution copy number variation
[25] Firth HV, Richards SM, Bevan AP, et al. decipher: database of chromosomal imbalance and phenotype in humans using Ensembl resources. Am J Hum Genet 2009;84:524–33. https://doi.org/10.1016/j.ajhg.2009.03.010.

[26] Swaminathan GJ, Bragin E, Chatzimichali EA, et al. DECIPHER: web-based, community resource for clinical interpretation of rare variants in developmental disorders. Hum Mol Genet 2012;21:R37–44. https://doi.org/10.1093/hmg/ddr362.

[27] Miller DT, Adam MP, Aradhya S, et al. Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies. Am J Hum Genet 2010;86:749–64. https://doi.org/10.1016/j.ajhg.2010.04.006.

[28] Verbitsky M, Krithivasan P, Batourina E, et al. copy number variant analysis and genome-wide association study identify loci with large effect for vesicoureteral reflux. J Am Soc Nephrol 2021;32:805–20. https://doi.org/10.1681/ASN.2020050681.

[29] Mefford HC, Claunin S, Sharp AJ, et al. Recurrent reciprocal genomic rearrangements of 17q12 are associated with renal disease, diabetes, and epilepsy. Am J Hum Genet 2007;81:1057–69. https://doi.org/10.1086/522591.

[30] Nagamani SCS, Erez A, Shen J, et al. Clinical spectrum associated with recurrent genomic rearrangements in chromosome 17q12. Eur J Hum Genet 2010;18:278–84. https://doi.org/10.1038/ejhg.2009.174.

[31] Peiffer DA, Le JM, Steemers FJ, et al. High-resolution genomic profiling of chromosomal aberrations using Infinium whole-genome genotyping. Genome Res 2006;16:1136–48.