Spectrum of congenital anomalies of the kidney and urinary tract (CAKUT) including renal parenchymal malformations during fetal life and the implementation of prenatal exome sequencing (WES)

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Research Article

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Abstract

Objectives and Background: Congenital malformations of the kidney and urinary tract (CAKUT) have a prevalence of 4-60 in 10,000livebirths and constitute for 40-50% of all end stage pediatric kidney disease. CAKUT can have a genetic background due to monogenetic inherited disease such as PKD or ciliopathies. They can also be found in combination with extra-renal findings as part of a syndrome. Upon detection of genitourinary malformations during the fetal anomaly scan the question arises if further genetic testing is required. The purpose of this study was to determine the phenotypic presentation of CAKUT cases and the results of exome analysis (WES).

Methods: This is a retrospective analysis of 63 fetal cases with a diagnosis of CAKUT or DSD at a single center between August 2018 and December 2022.

Results: A total of 63 cases (5.6%) out of 1123 matched CAKUT phenotypes including renal parenchyma malformations. In 15out of 63WES analysis a pathogenic variant was detected (23.8%). In fetuses with isolated CAKUT the rate of detecting a pathogenic variant on exome sequencing was five out of 44 (11.4%). Ten out of 19 fetuses (52.6%) that displayed extra-renal findings in combination with CAKUT were diagnosed with a pathogenic variant.

Conclusion: WES provides an increase in diagnosing pathogenic variants in cases of prenatally detected CAKUT. Especially in fetuses with extra-renal malformations, WES facilitates a gain in information on the fetal genotype to enhance prenatal counselling and management.

What's already known about this topic?

Literature has shown that congenital malformations of the kidney and urinary tract (CAKUT) can be associated with genetic disorders. So far it has not been clear how high the yield of exome sequencing (WES) is in fetal genitourinary malformations.

What does this study add?

This study analyses cases of CAKUT and the rate of pathogenic variant in exome sequencing (WES). The results indicate that especially among fetuses with polycystic kidney disease or associated extra-renal malformations the rate of pathogenic finding is highest (29% and 52.6% respectively).

Introduction

Fetal abnormalities can be detected prenatally in around 2–3% of pregnancies [1, 2]. Upon detection, parental genetic counselling is offered together with invasive diagnostic procedures such as amniocentesis and chorionic villous sampling performing a karyotype and/or chromosomal microarray analysis (CMA). Overall, 8–10% of fetuses with an anomaly display an abnormal karyotype with an additional 6% of microdeletions and microduplications, which leaves most fetuses without a specific

genetic diagnosis [3]. Depending on the publication, exome sequencing can detect a pathogenic variant in 20–80% of cases, if the karyotype or CMA is negative [4–7]. Prenatal WES can expand the yield of diagnosing an underlying disease in prenatally detected fetal abnormalities and it has the potential to increase the knowledge on prenatal disease [8].

Congenital anomalies of the kidney and urinary tract (CAKUT) including renal parenchymal malformations are detected in 20–30% of all fetal anomalies, identified during the prenatal scan [9]. The second trimester anomaly scan has become a standard of care provided for almost all risk pregnancies in Germany [10]. Some urinary tract malformations are detected during the third trimester scan, as a form of late onset CAKUT.

CAKUT is a heterogeneous group of fetal malformations that affect the development of the kidneys and their outflow tracts (Fig. 1) [11, 12]. The prevalence is estimated to be around 4–60 in 10,000 births [13]. Extra-renal anomalies are detected in 30% of infants with CAKUT [9].

The development of the urinary tract is a multistage process, that is initiated by the ureteric bud and the metanephron at five gestational weeks. Any disturbance at any stage of the renal development can lead to different types of CAKUT with more severe defects occurring based on disruptions during early embryonic development [14–16]. The abnormal embryonic kidney development can be caused by renal parenchymal malformations (e.g. congenital cystic kidney disease), abnormal renal migration (e.g. altered position of the renal tissue) and disturbance in the developing renal collecting system (e.g. urinary tract obstruction) [17, 18]. CAKUT can be divided into non-genetic and genetic origins [19]. Certain teratogens, including drugs are known to cause an impairment of kidney development (e.g. ACE inhibitors and warfarin) [20]. The spectrum of CAKUT can range from almost no impairment to end stage renal disease requiring kidney transplantation or to a lethal condition due to pulmonary hypoplasia [15, 21]. Therefore, early detection during the prenatal ultrasound is essential to facilitate parental counselling and a close fetal and neonatal follow-up. Today, children affected by CAKUT have better chances of survival due to an improvement in early diagnosis, interventions and dialysis as well as kidney transplantation. Depending on the type of CAKUT severe comorbidities can be associated, impacting on overall survival and quality of life [19].

This study focuses on the additional information in diagnosing pathogenic variants, when performing WES after a negative karyotype and/ or microarray upon detection of CAKUT prenatally.

Material and methodology

We searched the whole exome sequencing data bank at the Center of Human Genetics in Tübingen, Germany for cases of prenatally detected CAKUT including renal parenchymal malformations. A total number of 1123 WES were conducted from August 2018 until December 2022. Patient information was systematically reviewed including demographic factors, prior analysis and outcome. The genetic analysis was performed due to either CAKUT alone or combined with extra-renal findings concerning the fetal phenotype. As keywords we searched: CAKUT, polycystic kidney disease, hydronephrosis, megaureter,

megacystis, LUTO, ureterocele, horseshoe kidney, ectopic kidney, fused kidney, kidney duplication, enlarged kidney, small kidney, hyperechogenic kidney. 63 cases out of 1123 (5.6%) matched the keywords. Descriptive analysis was performed applying SPSS version 22, IBM.

Genetic analysis

Trio exome sequencing of our own cohort DNA quantity and quality were determined using Qubit Fluorometer and NanoDrop ND-8000 (Thermo Fisher Scientific, Dreieich, Germany). Enrichment of the coding DNA sections of the test persons was carried out with one of the following enrichment methods: SureSelect Human All Exon 50Mb V6 Kit, SureSelect Human All Exon 50Mb V7 Kit (Agilent, Santa Clara, CA, USA), the Twist Human Core + Refseq exome (Twist Bioscience, San Fransisco, CA, USA) or the CeGaT Exome Xtra V1 (CeGaT GmbH, Tübingen, Germany). For Agilent enrichment kits, sequencing libraries were prepared using the SureSelectXT workflow. Library preparation and capture for all samples was performed according to the respective manufacturer's instructions. Paired-end sequencing was performed using the Novaseq6000 system (Illumina, San Diego, CA) with 2x100 base pairs (bp) read length. Sequence data were processed with Illumina bcl2fastq2. Adapter sequences were removed with Skewer and the sequences obtained were aligned to the human reference genome (hg19) with the Burrows Wheeler aligner (BWA mem). Sequences that could not be clearly assigned to a genomic position were removed, as were sequence duplicates that were probably due to amplification (internal software). The average coverage was 170x. Sequence variants (single nucleotide exchanges and short insertions/deletions) were determined from the remaining high-quality sequences (CeGaT StrataCall). Copy number variations (CNV) were computed on uniquely mapping, non-duplicate, high quality reads using an internally developed method based on sequencing coverage depth. Briefly, we used reference samples to create a model of the expected coverage that represents wet-lab biases as well as intersample variation.

Results

From August 2018 until December 2022 a total of 63 cases (5.6%) out of 1123 were analysed. The mean maternal age was 33.2 years (range 19-41 years, SD ± 5.14). The mean gestational week was 23.2 (range 14-35, SD ± 5.32). The initial genetic test that was requested upon detection of a fetal malformation was a karyotype in 69.8% and a chromosomal microarray analysis in 28.6% (in one case the initial genetic test was not specified) (table 1). Incidental findings were reported in five out of the 63 cases (7.9%). A variant of unknown / uncertain significance (VUS) was detected in two fetuses (3.2%). A trio exome sequencing (trio WES) was conducted in 61 cases (96.8%). In two fetuses a single WES was performed. The mean turnaround time was 13.3 days (median 12 days, range 6-42 days, SD ± 6.02). Sanger validation of variants was performed in 98.4%. The source of fetal DNA was through amniocentesis in 58 cases (92.1%), chorionic villous sampling in three cases (4.8%) and postmortem DNA sampling in two cases (3.1%).

Detection rate

In 15 out of 63 WES analysis a pathogenic variant was detected (23.8%) (figure 1, table 2). In fetuses with isolated CAKUT the rate of detecting a pathogenic variant by exome sequencing was five out of 44 (11.4%). Ten out of 19 fetuses (52.6%) that displayed extra-renal findings in combination with CAKUT were diagnosed with a pathogenic variant. The associated findings were of the fetal face (cleft palate), the central nervous system (vermian hypoplasia, dilated cisterna magna), of the neck (lateral cervical cysts), thorax (diaphragmatic hernia, pulmonary hypoplasia), heart (not specified), abdomen (omphalocele, gastroschisis, microcolon), extremities (club feet, arthrogryposis), fetal hydrops and genital malformation (hydrometrocolpos, cloacal malformation). Additionally, there were associated abnormal findings of the fetal growth (IUGR, macrosomia) and the amniotic fluid (polyhydramnios, oligohydramnios, anhydramnios). Three pregnancies underwent termination, among these was a case of bilateral renal agenesis, and two fetuses with LUTO.

In fetuses with isolated renal anomalies, the highest detection rate of pathogenic variants was among fetuses with isolated polycystic kidney disease (29% *PKHD1, PKHD1, PKD1, ETFA*). One fetus out of four with bilateral renal agenesis was diagnosed with *HNF1B* (25%). All other isolated CAKUT cases did not reveal a pathogenic variant in WES (table 2).

A higher detected rate was found in fetuses with CAKUT and extra-renal findings: Ten out of 19 fetuses (52.6%) were diagnosed with a pathogenic variant. Three of six fetuses with polycystic kidney disease and extra-renal abnormalities displayed a pathogenic variant (*INVS, TP63, CEP290*). Some of the CAKUT cases with associated malformations did reveal a pathogenic variant in WES (unilateral renal agenesis *Hypomethylation C2;* horseshoe kidney *KMT2A;* hydronephrosis *FREM2, KANSL1;* LUTO *CHD7;* renal hypoplasia *GREBIL, POGZ*).

Discussion

This study focuses on the detection rate of pathogenic variants in WES in fetuses with CAKUT including renal parenchymal malformations. The rate of prenatal CAKUT was relatively low with 5.6% among all performed WES at the data bank at the Center of Human Genetics in Tübingen, Germany. The overall detection rate of a pathogenic variant in our cohort was 23.8%. The highest yield of detection was among fetuses with extra-renal findings with 52.6% vs. 11.4% in fetuses with isolated CAKUT. A variety of pathogenic variants were detected with differing neonatal prognosis in our cohort (table 2).

In literature, WES can detect a pathogenic variant in 20–80% of cases when the karyotype or CMA is negative [4–7]. The diagnostic yield of WES in prenatal CAKUT has been described between 0 and 16% [22, 23]. Another publication supports our finding, that fetuses with extra renal anomalies display a higher diagnostic rate of pathogenic variants than fetuses with isolated renal findings (25% vs. 9.1% respectively) [7]. We suggest the following diagnostic chronology (Fig. 2): Initially the sonographer should distinguish between structural/ dysplastic kidneys (as in PKD, hyperechogenic kidneys) and outflow obstruction/ renal agenesis and ectopic kidneys. Upon detection one should always search for extra-renal abnormalities. Genetic counselling should be offered. In case of simple outflow obstruction/ renal

agenesis and ectopic kidneys invasive/ non-invasive genetic testing can be offered. In the case of extra-renal anomalies invasive genetic testing is advisable. If the karyotype/ CMA is negative further testing as in WES should be discussed and offered. If polycystic/ hyperechogenic kidneys with or without extra-renal anomalies are detected prenatally invasive genetic testing is advisable. Family history should always be considered. If the karyotype/ CMA is negative further testing like WES should be offered.

Sequential sonographic follow-up and individualized care is required upon detection of CAKUT. Termination of pregnancy can be discussed and offered based on national legislation in cases with poor prognosis (e.g., lung hypoplasia due to renal mal-/ dysfunction or specific pathogenic variants associated with unfavorable outcome).

Conclusion

The wide availability of exome sequencing is revolutionizing our understanding of genetic causes of prenatal abnormalities including CAKUT. This analysis is so far the largest study on the implementation of WES in CAKUT. Upon prenatal detection of fetal CAKUT we suggest a thorough ultrasonographic scan in order to exclude associated extra-renal anomalies (Fig. 2). An individualized approach is necessary based on the sonographic findings (renal findings and extra-renal findings) considering the parental preference on finding out the underlying genotype. All patients should receive genetic counselling with the offer of genetic testing (non-invasive vs. invasive). If the karyotype or CMA is negative further genetic analysis can be offered (including WES). Based on our research, the highest detection yield of relevant pathogenic variants is among fetuses with renal and extra-renal findings (43.5% vs. 12.5% in fetuses with isolated CAKUT)

Declarations

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Exome sequencing was performed as a clinical service under clinical consent forms. Retrospective collection of data from patient records has been granted a waiver of informed consent, as all clinical data contained in this report have been anonymized.

Data availability statement

The data supporting the findings of this study are available upon request.

Abbreviations

CAKUT = Congenital malformations of the kidney and urinary tract

PKD = polycystic kidney disease

DSD = Disorders of sex development

NIPT = non-invasive prenatal testing

CAH = congenital adrenal hyperplasia

WES = (whole) exome sequencing

LUTO = lower urinary tract obstruction

VUS = variant of unknown/ uncertain significance

DNA = Deoxyribonucleic acid

CMA = chromosomal microarray analysis

MCKD = Multicystic kidney disease

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Authors' contribution to the Manuscript

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Conflict of interest statement

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References

1. Centers for Disease, C. and Prevention, *Update on overall prevalence of major birth defects--Atlanta, Georgia, 1978-2005.* MMWR Morb Mortal Wkly Rep, 2008. **57**(1): p. 1-5.
2. Feldkamp, M.L., et al., *Etiology and clinical presentation of birth defects: population based study.* BMJ, 2017. **357**: p. j2249.
3. Wapner, R.J., et al., *Chromosomal microarray versus karyotyping for prenatal diagnosis.* N Engl J Med, 2012. **367**(23): p. 2175-84.
4. Majewski, J., et al., *What can exome sequencing do for you?* J Med Genet, 2011. **48**(9): p. 580-9.
5. Drury, S., et al., *Exome sequencing for prenatal diagnosis of fetuses with sonographic abnormalities.* Prenat Diagn, 2015. **35**(10): p. 1010-7.
6. Aoi, H., et al., *Whole exome sequencing of fetal structural anomalies detected by ultrasonography.* J Hum Genet, 2021. **66**(5): p. 499-507.
7. Lei, L., L. Zhou, and J.J. Xiong, *Whole-exome sequencing increases the diagnostic rate for prenatal fetal structural anomalies.* Eur J Med Genet, 2021. **64**(9): p. 104288.
8. Gabriel, H., et al., *Trio exome sequencing is highly relevant in prenatal diagnostics.* Prenat Diagn, 2022. **42**(7): p. 845-851.
9. Wiesel, A., et al., *Prenatal detection of congenital renal malformations by fetal ultrasonographic examination: an analysis of 709,030 births in 12 European countries.* Eur J Med Genet, 2005. **48**(2): p. 131-44.
10. Carmen Prodan, N., et al., *How to do a second trimester anomaly scan.* Arch Gynecol Obstet, 2022.
11. Caiulo, V.A., et al., *Ultrasound mass screening for congenital anomalies of the kidney and urinary tract.* Pediatr Nephrol, 2012. **27**(6): p. 949-53.
12. Stein, D. and E. McNamara, *Congenital Anomalies of the Kidneys and Urinary Tract.* Clin Perinatol, 2022. **49**(3): p. 791-798.
13. Tain, Y.L., et al., *Incidence and Risks of Congenital Anomalies of Kidney and Urinary Tract in Newborns: A Population-Based Case-Control Study in Taiwan.* Medicine (Baltimore), 2016. **95**(5): p. e2659.

14. Dunn, P.M., *Dr Edith Potter (1901 1993) of Chicago: pioneer in perinatal pathology.* Arch Dis Child Fetal Neonatal Ed, 2007. **92**(5): p. F419-20.
15. dos Santos Junior, A.C., D.M. de Miranda, and A.C. Simoes e Silva, *Congenital anomalies of the kidney and urinary tract: an embryogenetic review.* Birth Defects Res C Embryo Today, 2014. **102**(4): p. 374-81.
16. Capone, V.P., et al., *Genetics of Congenital Anomalies of the Kidney and Urinary Tract: The Current State of Play.* Int J Mol Sci, 2017. **18**(4).
17. Uy, N. and K. Reidy, *Developmental Genetics and Congenital Anomalies of the Kidney and Urinary Tract.* J Pediatr Genet, 2016. **5**(1): p. 51-60.
18. Rosenblum, S., A. Pal, and K. Reidy, *Renal development in the fetus and premature infant.* Semin Fetal Neonatal Med, 2017. **22**(2): p. 58-66.
19. Talati, A.N., C.M. Webster, and N.L. Vora, *Prenatal genetic considerations of congenital anomalies of the kidney and urinary tract (CAKUT).* Prenat Diagn, 2019. **39**(9): p. 679-692.
20. Morgan, T.M., D.P. Jones, and W.O. Cooper, *Renal teratogens.* Clin Perinatol, 2014. **41**(3): p. 619-32.
21. Nicolaou, N., et al., *Genetic, environmental, and epigenetic factors involved in CAKUT.* Nat Rev Nephrol, 2015. **11**(12): p. 720-31.
22. Lord, J., et al., *Prenatal exome sequencing analysis in fetal structural anomalies detected by ultrasonography (PAGE): a cohort study.* Lancet, 2019. **393**(10173): p. 747-757.
23. Petrovski, S., et al., *Whole-exome sequencing in the evaluation of fetal structural anomalies: a prospective cohort study.* Lancet, 2019. **393**(10173): p. 758-767.

Tables

Tables 1 and 2 are available in the Supplementary Files section.

Figures

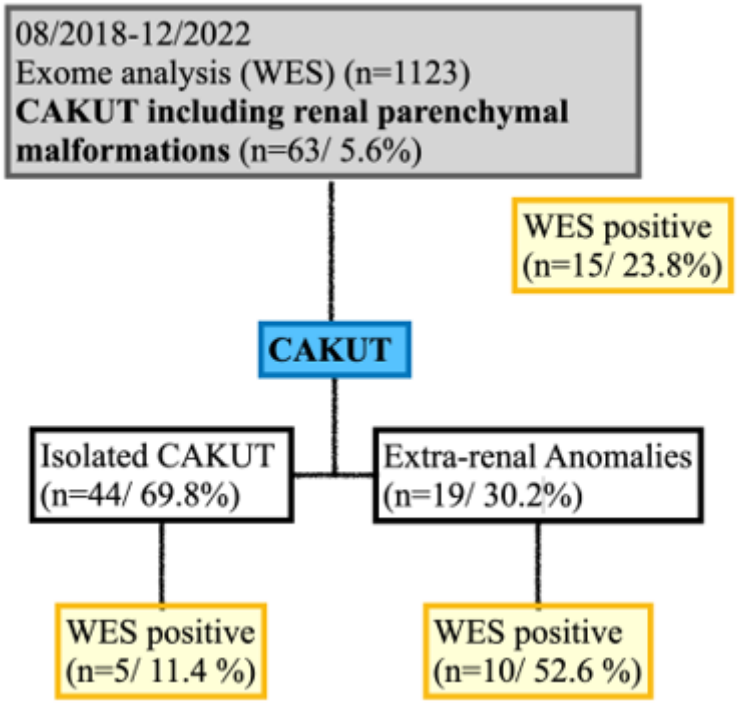

Figure 1

Description of the study population grouped according to the fetal phenotype. A total of 63 cases were analyzed during the investigation period (08/2018 – 12/2022).

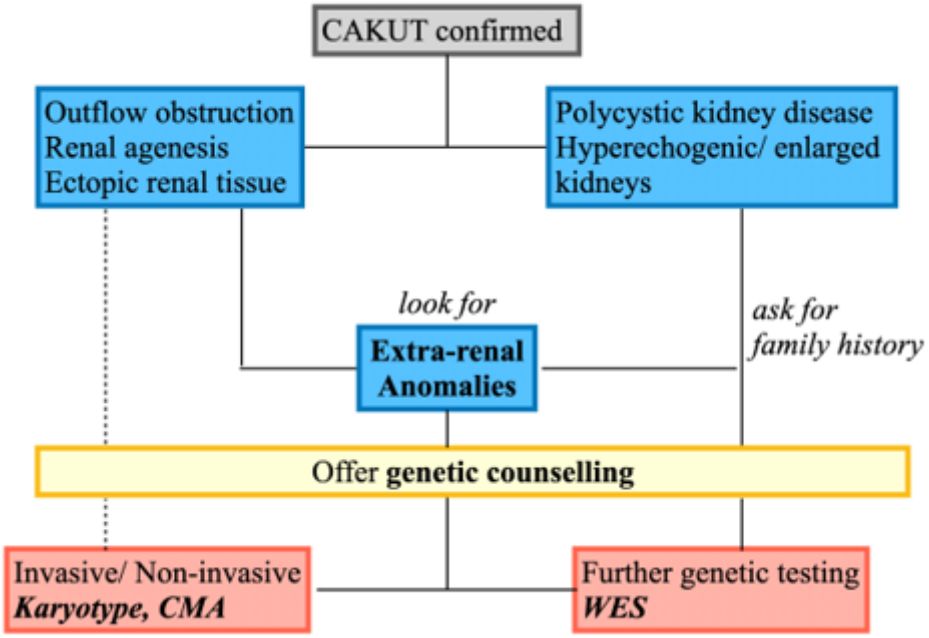

Figure 2

Counselling on further genetic testing upon prenatal detection of CAKUT. Initially one should distinguish between structural/ dysplastic kidneys (as in PKD, hyperechogenic kidneys) and outflow obstruction/

renal agenesis and ectopic kidneys. The sonographer should always search for extra-renal abnormalities. Genetic counselling should be offered. In case of simple outflow obstruction/ renal agenesis and ectopic kidneys invasive/ non-invasive genetic testing can be offered. If extra-renal anomalies are apparent invasive genetic testing is advisable. If the karyotype/ CMA is negative further texting as in WES should be discussed. If polycystic/ hyperechogenic kidneys with or without extra-renal anomalies are detected prenatally invasive genetic testing is advisable. Family history should always be considered. If the karyotype/ CMA is negative further texting as in WES should be offered if available.

Supplementary Files

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