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Genetics of Congenital Abnormalities of Kidney and Urinary Tract (CAKUT)

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Abstract

Congenital Abnormalities of Kidney and Urinary Tract (CAKUT) are amongst the most common malformations in humans. Most CAKUT are sporadic in origin though single gene mutations have been identified in syndromic and some non-syndromic CAKUT. This article briefly reviews the recent advances in the genetics of CAKUT.

Introduction

Congenital Abnormalities of Kidney and Urinary Tract (CAKUT) encompass a wide range of structural malformations, which occur due to a defect in the morphogenesis of the kidney and urinary tract. CAKUT are among the most common birth defects, accounting for 20-30% of all birth defects (Loane et al., 2011). The estimated incidence of CAKUT is 3 to 6 per 1000 live births (Nicolaou et al., 2015). CAKUT account for 40-50% of children with chronic kidney disease (Vivante et al., 2014). CAKUT are the most frequent malformations detected by antenatal ultrasound scan (Wiesel et al., 2005)

The phenotypes included under CAKUT are structural abnormalities like renal agenesis, renal hypoplasia (renal length less than 2SD below the mean for age with normal architecture), renal dysplasia (malformation of tissue elements), multicystic dysplastic kidneys, horse shoe kidney, vesicoureteric reflux (VUR), megaureter, duplex collecting system, ectopic ureter, ureterocele and posterior urethral valve. These anomalies can exist as single entities or can occur concurrently as in VUR and duplex collecting system. These abnormalities may be seen in an orthotopic kidney or ectopic kidney. Around 50% of CAKUT have associated lower urinary tract abnormalities and uretero-pelvic junction obstruction is the most common phenotype seen in 20% (Capone et al., 2017). Most of these abnormalities arise due to a disruption of normal nephrogenesis either due to environmental factors or due to dysfunction of the genes involved in that process.

Understanding the genetics of CAKUT is essential for early diagnosis and early initiation of treatment and for prevention of end stage renal disease, especially in children. This brief review summarizes the classification of CAKUT, genetic approaches used to identify genes implicated in CAKUT and the genes causing syndromic and non-syndromic CAKUT.

Classification

CAKUT can be classified into syndromic and non-syndromic types, based on whether systems other than the renal system are involved. CAKUT can occur as part of a known genetic syndrome with congenital abnormalities outside the urinary tract. For example, renal agenesis can occur as part of the Townes-Brocks syndrome (OMIM #107480), Kallmann syndrome (OMIM #308700) or Branchiotorenal syndrome (OMIM # 113650). Some of the common syndromic causes for CAKUT along with the implicated genes are listed in Table 1. In non-syndromic CAKUT structural abnormalities are limited to the kidney and urinary tract.

Evidence for a genetic etiology in CAKUT

Genetic basis for CAKUT was suspected because of familial segregation of renal anomalies like renal agenesis, renal hypodysplasia and multicystic dysplastic kidneys. Many such families have been described in literature, suggesting autosomal dominant inheritance with reduced penetrance (Monn & Nordshus, 1984; McPherson et al.,1987; Kalpan et al., 1989). Known syndromes with CAKUT and other extra-renal manifestations with a single gene

Table 1 Some syndromic causes for CAKUT and their renal phenotypes.

Syndrome	Genes	Renal phenotype
Alagille syndrome	*JAG1, NOTCH2*	Cystic kidneys
Branchiootorenal syndrome	*EYA1, SIX5*	Unilateral or bilateral renal agenesis, hypoplasia, collecting system abnormalities
Campomelic dysplasia	*SOX9*	Hydronephrosis
Fraser syndrome	*FRAS1, FREM2, GRIP1*	Renal agenesis or hypoplasia
Kallmann syndrome	*KAL1, PROKR2*	Renal aplasia
Meckel-Gruber syndrome	*MKS1, TMEM216, TMEM67, CEP290, TMEM231, TMEM107*	Renal agenesis, cystic kidneys, duplicated ureter, hypoplastic bladder
Pallister-Hall syndrome	*GLI3*	Renal ectopia, renal dysplasia
Papillorenal syndrome/ Renal coloboma syndrome	*PAX2*	Renal hypoplasia, cysts, Multicystic dysplastic kidneys, VUR
Townes-Brocks syndrome	*SALL1, DACT1*	Ectopic kidney, hypoplastic, multicystic dysplastic kidneys

etiology also point to the existence of a genetic basis for CAKUT. Monogenic mouse models, which show a CAKUT phenotype, also indicate a genetic basis for these disorders.

Etiopathogenesis of CAKUT

The etiology of CAKUT is complex with environmental, genetic and epigenetic factors playing a role in disease causation.

- Environmental factors: Renal agenesis was shown to have a significant association with pregestational maternal diabetes mellitus. A fetus with an early exposure to diabetes in utero has an increased risk of CAKUT (Dart et al., 2015) and it has been recommended that maternal diabetes should be included in the evaluation of CAKUT. Maternal intake of angiotensin converting enzyme inhibitors (ACEI) during the first trimester is associated with an increased risk of renal dysplasia in the fetus (Cooper et al., 2006). In utero exposure to cocaine and alcohol have been linked to a higher occurrence of CAKUT in fetuses. (Yosipiv., 2012)

- Epigenetic factors: Whole exome sequencing, exome data-based copy number variants (CNV) analysis and bisulphite sequencing were done on a pair of monozygotic twins, who were discordant for congenital renal agenesis. The analysis showed 514 differentially methylated regions with no differential single nucleotide polymorphism or CNV, suggesting that epigenetic modification can be an explanation for environmental factors causing CAKUT (Jin et al.,2014). Data suggest that epigenetic phenomena could influence nephrogenesis, predetermine disease susceptibility and account for the variable penetrance seen in CAKUT.

- Genetic factors: Before the advent of molecular diagnostic techniques, the classical anatomy theory, which highlighted the importance of the position of ureteric bud, was used to describe the etiopathogenesis of CAKUT. But with evidence from mouse models, newer insights to the molecular mechanism of development of CAKUT have been obtained.

Nephrogenesis: Nephrogenesis can be divided into the following stages:

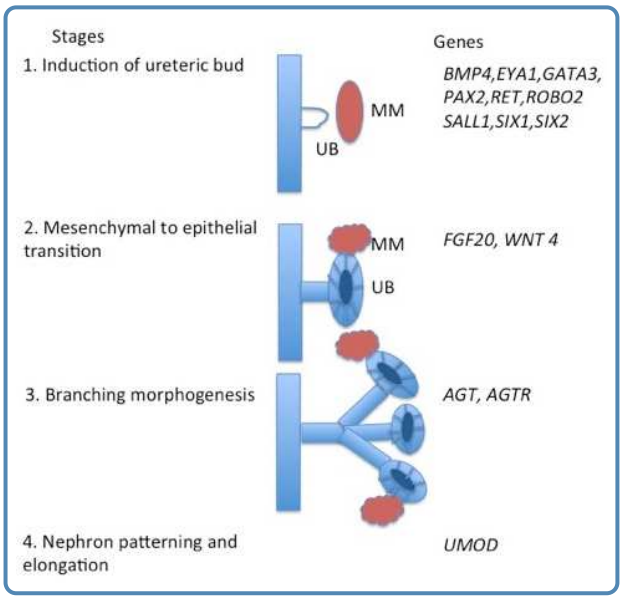
- ureteric bud induction
- mesenchymal-to-epithelial transition (MET)
- renal branching morphogenesis
- nephron patterning and elongation (which include proximal and distal tubule morphogenesis and glomerulogenesis)

This complex process is controlled by a large number of genes and signaling pathways, and alterations in these genes have been identified to cause CAKUT. The various genes implicated in the different developmental stage of nephrogenesis in mice models and human beings are shown in Figure 1 (Vivante et al., 2014).

GDNF/RET pathway: (Figure 2)

GDNF/RET is the most frequently studied pathway to understand the pathogenesis of CAKUT

(Capone et al., 2017). During embryonic development, Glial Derived Neurotropic Factor (GDNF) is expressed in metanephric mesenchyme along the length of the mesonephric duct. Tyrosine kinase receptor, RET and co receptor, GDNF alpha 1 (GDNFA1) are expressed in the mesonephric duct and when GDNF binds to RET and GDNFA1, ureteric bud is formed (Puri et al., 2011). Transcription factors like PAX2 (paired box gene 2), GATA3 (trans-acting T- cell-specific transcription factor GATA 3), EYA1 (eyes absent homolog 1), SI X1 (sine oculis-related homeoBox 1 homolog protein SI X1), SALL1 (Sal-like 1) and HOX11 (homeoBox 11) act as positive regulators of GDNF. The expression of the protein WNT11 in the epithelial tip of the ureteric bud propagates mesenchymal GDNF signaling.



UB: Ureteric bud; MM: Metanephric mesoderm

Figure 1 Nephrogenesis and human genes implicated in various stages.

GDNF activity is restricted by transcription factors like FOXC1/FOXC2 (forkhead box C1 and C2) transcription factors and the SLIT2–ROBO2 (slit homolog 2–roundabout homolog 2). The receptor tyrosine kinase antagonist Sprouty1 (SPRY1) negatively regulates GDNF/RET signaling. Negative regulation of GDNF is important to ensure a single ureteric bud and mutations involving negative regulators have shown to cause multiple ureteric buds in mouse models. Transforming growth factor β1 (TGFB1) and bone morphogenic protein 4 (BMP4) are endogenous inhibitors of the GDNF/RET signaling pathway and restrict the outgrowth of the ureteric bud to a single location. FGFR2, independent of the GDNF/RET pathway, stimulates ureteric budding and WT1 also induces ureteric bud formation in an independent manner. Angiotensin type II receptor (AGT2R) is essential for early stages of ureteric bud morphogenesis.

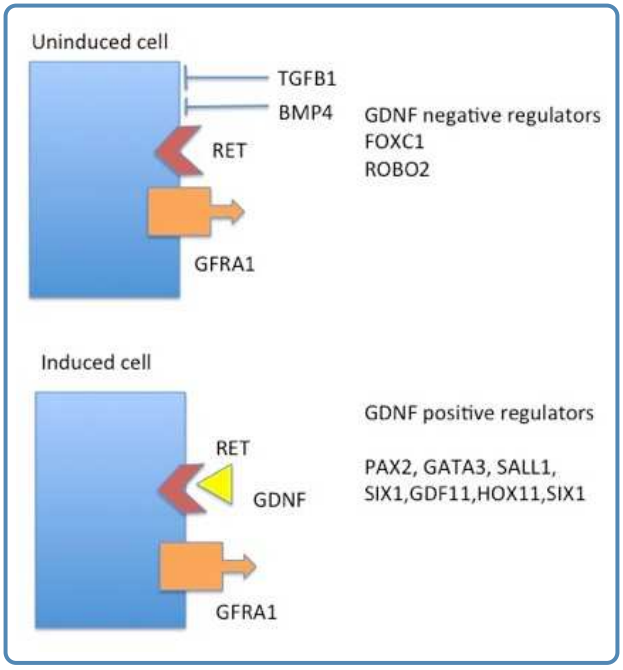


Figure 2 GDNF- RET pathway.

Strategies used to study genetic susceptibility

i. Candidate gene studies: Even though the genes identified for non-syndromic CAKUT are lesser in number, knock out mice models have enabled to identify some of the candidate genes which were validated in human beings. Candidate gene approach has enabled identification of causative heterozygous gene mutations in 6-20% of patients. (Nicolaou et al., 2015). Although different studies have identified mutations in genes like *BMP7, CHD1L, CDC5L, EYA1, GATA3, RET, ROBO2, SALL1, SIX2, SIX5, FRAS1* and *FREM2*, functional evidence is still lacking to prove causality. Even though functional characterization was done for variants in some genes like *WNT4* and *RET*, some of those variants were identified in unaffected parents of patients, indicating incomplete or lower degree of penetrance or even multifactorial etiology for CAKUT.

ii. Linkage analysis: This approach has been tried

in familial VUR and 60% of the 12 families with VUR showed linkage in chromosome 12p11- q13, even though no specific gene was identified (Weng et al., 2009).

iii. Genome wide association studies: Association studies done in sporadic cases of CAKUT like VUR have been limited by the sample size to attain statistical significance. (Capone et al., 2017)

iv. Targeted next generation sequencing: By massive parallel sequencing of multiple genes, the cause of CAKUT was elucidated in around 10%. (Capone et al., 2017).

v. Whole exome (Bekhernia et al., 2017) and whole genome sequencing: Many new genes have been implicated and the need for functional analysis of variants in various genes is on the rise.

vi. Analysis of copy number variants by chromosomal microarray: Nephrogenesis is highly sensitive to gene dosages and it has been shown that 16% of CAKUT are due to CNV's (Capone et al., 2017). De novo microdeletions of chromosome 17q12 (which contains *HNF1B*) have been implicated in patients with CAKUT with or without diabetes mellitus.

Genetics of non–syndromic CAKUT

Identification of genes in non-syndromic forms of CAKUT has been difficult. The first single gene defect identified as causing CAKUT was a deletion in *PAX2* gene in a family with optic coloboma, renal hypoplasia and VUR (Nicolaou et al., 2015). The second gene to be implicated in CAKUT was *HNF1B* in a family with two children with diabetes and renal cysts. *PAX2* and *HNF1B* were later identified as the two most common genes implicated in CAKUT, contributing to 15% of all patients with CAKUT (Nicolaou et al., 2015). The ESCAPE study, which analyzed the renal developmental genes like *PAX2, HNF1B, SALL1, EYA1 and SIX1* in a large cohort of children with renal hypodysplasia, showed a high prevalence of mutations in *PAX2* and *HNF1B* (Capone et al., 2017).

Mutations have been identified in patients with CAKUT in *BMP4, RET, DSTYK, WNT4* and *SIX2*. Though *UMOD* gene has been implicated in familial juvenile hyperuricemic nephropathy (FJHN), glomerulocystic kidney disease (GCKD) and autosomal dominant medullary cystic kidney disease 2, *UMOD* mutations were not identified in patients with isolated CAKUT, implying that it may represent a very rare etiology for this condition. In a study by Hwang et al., mutations in known CAKUT-causing genes were identified in 16% of the total 749 patients and the most commonly mutated genes were *SALL1, HNF1B* and *PAX2* (Hwang et al., 2014). Nicolaou et al, analysed the largest number of genes (a total of 208 candidate genes) by targeted NGS in phenotypically heterogeneous 453 patients with CAKUT and found only five disease causing variants (in *HNF1B, PAX2, SIX5* and *UMOD*) and concluded that many of the previously implicated genes contributed to the pathogenesis much less than what was expected (Nicolaou et al., 2016).

All these findings imply that the majority of the causes of CAKUT are still unknown, while novel variants in genes like *FRAS1, FREM2, GRIP1, ITGA8* and *TRAP1* are being identified and need functional validation. Recessive mutations in these genes have been previously characterized and could imply autosomal recessive inheritance in some cases of CAKUT.

Clinical presentation

Clinical outcomes of CAKUT are highly variable and range from asymptomatic to chronic kidney disease requiring renal replacement during a period ranging from newborn period to adulthood. With the widespread use and a sensitivity of around 80%, many malformations of kidney and urinary tract are recognized as early as 18 to 20 weeks of gestation by antenatal ultrasound scan. Oligohydramnios and altered morphology of kidney or urinary tract could indicate CAKUT. In the newborn period, CAKUT can present as part of other malformation syndromes or as a palpable abdominal mass or as respiratory distress in a newborn due to pulmonary hypoplasia. Abnormalities of the outer ear and single umbilical artery are associated with an increased risk of CAKUT.

Genetic evaluation of a patient with CAKUT

If a patient is suspected to have any syndrome, which is associated with CAKUT, specific investigation and molecular diagnosis for that particular syndrome can be attempted. In non-syndromic CAKUT, chromosomal microarray may be done to look for CNVs. Since most disease-causing variants have been identified in *PAX2* and *HNF1B*, these two genes may be screened for pathogenic variants. With the advent of NGS-based technology, targeted sequencing of previously implicated genes, whole exome sequencing or whole genome sequencing may be done to look for pathogenic variants,

but the yield of such investigations still remains low and whether such investigations need to be advised routinely for patients with non-syndromic sporadic CAKUT is debatable.

Genetic counseling of families with CAKUT

Majority of CAKUT are sporadic and cannot be explained by monogenic inheritance. The empirical recurrence risk of CAKUT has been shown to range from 4-20% in various studies (Capone et al., 2017). If a specific pathogenic variant has been identified in a proband, the family should be counseled appropriately regarding the risk of recurrence and availability of prenatal diagnosis. Antenatal ultrasound scan can be used to detect renal structural abnormalities as early as 18 to 20 weeks of gestation.

Renal abnormalities are seen in asymptomatic close relatives in 10% of patients with CAKUT. Close relatives may be screened by ultrasound scan for renal structural abnormalities.

Conclusions and future prospects

CAKUT are complex malformations, which occur due to interplay of genetic, environmental and epigenetic factors. *PAX2* and *HNF1B* are the two most commonly implicated genes in non-syndromic CAKUT. However more evidence is needed to establish the monogenic inheritance of CAKUT. Seven miRNAs with a potential role in CAKUT have been identified and await functional validation before defining the precise role of miRNAs as biomarkers for diagnosis and prognosis of CAKUT. With the decreasing cost of sequencing and increasing collaboration among researchers, in the near future, it would be possible to delineate the genetics of CAKUT and devise a method for predicting the severity and prognosis of the renal phenotype based on the genotype.

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