



Genetics of vesicoureteral reflux and congenital anomalies of the kidney and urinary tract

Keum Hwa Lee^{1,2,3}, Heon Yung Gee⁴, Jae Il Shin^{1,2,3}

¹Department of Pediatrics, Yonsei University College of Medicine, Seoul, ²Department of Pediatric Nephrology, Severance Children's Hospital, Seoul, ³Institute of Kidney Disease Research, Yonsei University College of Medicine, Seoul, ⁴Department of Pharmacology, Brain Korea 21 PLUS Project for Medical Sciences, Severance Biomedical Science Institute, Yonsei University College of Medicine, Seoul, Korea

The definition of congenital anomalies of the kidney and urinary tract (CAKUT) is the disease of structural malformations in the kidney and/or urinary tract containing vesicoureteral reflux (VUR). These anomalies can cause pediatric chronic kidney disease. However, the pathogenesis of CAKUT is not well understood, because identifying the genetic architecture of CAKUT is difficult due to the phenotypic heterogeneity and multifactorial genetic penetrance. We describe the current genetic basis and mechanisms of CAKUT including VUR via approaching the steps and signaling pathways of kidney developmental processes. We also focus on the newly developed strategies and challenges to fully address the role of the associated genes in the pathogenesis of the disease.

Keywords: Congenital anomalies of the kidney and urinary tract; Genetic association studies; Organogenesis; Vesicoureteral reflux

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INTRODUCTION

The definition of congenital anomalies of the kidney and urinary tract (CAKUT) refers to the disease of structural malformations in the kidney and/or urinary tract [1]. CAKUT covers renal agenesis, renal hypodysplasia, multicystic dysplastic kidney, hydronephrosis, ureteropelvic junction obstruction, megaureter, ureter duplex, posterior urethral valves and vesicoureteral reflux (VUR) [1]. Its frequency and morbidity is relatively high because it can occur in about 1:500 live born children and account about 40%–50% of children with chronic kidney disease [2,3]. Among the whole feature of CAKUT, VUR has been traditionally considered the most important phenotype for urinary tract infection (UTI) and kidney damage, with possibly serious long-term consequences, such as hypertension

and decreased renal function causing reflux nephropathy [4]. It is controversial, however, that the pathogenesis of reflux nephropathy is “congenital” or “acquired.” Some studies hypothesized that patients with VUR showed congenital renal scar before birth [5,6]. It could be caused by the progression to “congenital” reflux nephropathy and renal failure, although UTI and associated kidney inflammation is absent [5,6]. Others believe that the pathogenesis of “acquired” reflux nephropathy is the high pressure caused by the refluxing urinary stream and recurrent UTI which injure the kidney parenchyma [7,8]. Like VUR or reflux nephropathy, the pathogenesis of CAKUT remains unclear, and it is not well understood why only some patients progress to develop chronic kidney disease. The cause of most CAKUT cases is unknown, therefore, many studies searching for the genes are attempted. In this article, we will

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Corresponding Author: Jae Il Shin

Department of Pediatrics, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul 03722, Korea
TEL: +82-2-2228-2050, FAX: +82-2-393-9118, E-mail: shinji@yuhs.ac

review on genetic causes of VUR and CAKUT.

CURRENT KNOWN CAKUT-CAUSING GENES RELATED TO KIDNEY DEVELOPMENT

Understanding the genetic architecture of CAKUT is difficult due to the phenotypic heterogeneity and multifactorial genetic penetrance [9]. Until recently, more than 20 human single-gene causes for CAKUT have been identified [10-13]. Understanding the genetic basis of CAKUT can be helpful to know about the kidney development. Kidney development consists of the following developmental stages: ureteric budding, metanephric kidney, mesenchymal-to-epithelial transition (MET), and nephron patterning and elongation (which include proximal and distal tubule morphogenesis and glomerulogenesis) [1,14-18]. These developmental stages are controlled by a large number of genes and signaling pathways and defects in these steps can lead to the clinical phenotypes of CAKUT (Table 1).

“BUD THEORY” RELATED GENES OF VUR AND CAKUT

VUR can be induced by multiple birth defects affecting the urinary tract development: ureteric budding, ureter differentiation and elongation, peristalsis, UVJ formation, and bladder and urethra development [19]. The ectopic ureteric orifice is related to “Bud theory” stage, which is abnormal development of the embryonic ureteric bud [20]. This theory hypothesizes that the site at which the ureteric bud grows out from the mesonephric duct is essential for development of normal kidney and urinary tract. Actually, many early development genes involved in the ureteric budding stage lead to VUR and CAKUT. This hypothesis is closely correlated to analysis of duplex kidneys which showed that a more severe hypoplasia and dysplasia were closely associated with mal-displacement of the ureteral orifice.

In fact, ureteric budding is related to signaling mechanisms including transcription factors such as *RET*, *PAX2*, *EYA1*, *SALL1*, *SIX1*, *SIX2*, *BMP4*, and *GATA3* [21-37]. They have been recognized to be mutated with CAKUT and present several extrarenal manifestations [21-37].

The *RET* proto-oncogene provides instructions for producing a protein that is involved in signaling within cells and mutations in the *RET* gene cause several diseases [38]. For example, translocations in the gene were correlated with papillary thyroid cancer [21] and multiple endocrine

neoplasia (MEN) 2A and 2B syndromes [22]. *RET* mutation is also frequently showed in humans with renal diseases, because ureteric budding is promoted through receptor *RET* by Glial cell-derived neurotrophic factor (GDNF) signaling [23]. Therefore, *RET* mutations were subsequently reported to develop CAKUT in fetuses with bilateral renal hypodysplasia/agenesis [24].

Paired box gene 2 (*PAX2*) plays a key role in the development and proliferation of multiple cell lines and development of organs [39]. Therefore, *PAX2* mutations in kidney can develop some disease spectrum of CAKUT, including VUR, renal hypodysplasia, renal cysts, and multicystic dysplastic kidneys [25].

Eyes absent homolog 1 (*EYA1*) encodes the eyes absent (*EYA*) family of proteins. The protein may play important roles in the development of eye, ear, branchial arches and kidney. *EYA1* mutations have been related with branchio-otic (BO) syndrome and congenital cataracts [26,27].

Sal-like 1 (*SALL1*) is one of the human orthologues of the spalt (*sal*) gene known in *Drosophila* [40]. *SALL1* mutations are a cause of Townes-Brocks syndrome (TBS) and branchio-oto-renal (BOR) syndrome [28,29]. TBS is an autosomal dominant syndrome containing following features: renal abnormalities (multicystic kidneys, dysplastic kidney, hypoplastic kidneys), abnormalities of the external ears (dysplastic ears, sensorineural hearing loss or deafness), anorectal malformations (rectovaginal fistula, anal stenosis, imperforate anus/absence of an anal opening), heart abnormalities (ventricular septum defects and Tetralogy of Fallot) and hand and foot abnormalities (hypoplastic or fingerlike thumbs, syndactyly, fusion of the wrist bones, overlapping foot and/or toe bones) [28,29].

SIX1 and *SIX2* genes are similar genes each other known as the *SIX* gene family. Genes in this family encode proteins that bind to DNA and control the activity of other genes [30,31]. When the *SIX1* is mutated, it impairs the normal development of tissues at fetal stage and develops the major signs and symptoms of BOR/BO syndrome similar to phenotypes resulting from *EYA1* or *SALL1* mutations [30,31].

Bone morphogenetic protein 4 (*BMP4*) produces a member of the transforming growth factor- β superfamily protein [32]. Some studies hypothesize about the role of *BMP4* that it implicates in several aspects of embryonic development by regulating cell proliferation, differentiation, and apoptosis [32-34]. *BMP4* is further continuously expressed beyond the stage of ureteric budding throughout the embryonic development of the kidney and urinary system [32-34].

Trans-acting T-cell-specific transcription factor GATA-3 (*GATA3*) gene encodes a transcription factor of the

Table 1. Human genes implicated in CAKUT

Gene	Kidney developmental stage	Location	Signals	Gene product	Disease	Inheritance type	#Gene OMIM	Reference number
Ureteric budding								
<i>RET</i>	Ureteric budding	10p13	GDNF signaling	RTK	Bilateral renal hypodysplasia/agenesis VUR, ureteral obstruction, megaureter, duplex kidney, renal abnormalities, as well as Hirschsprung's disease	AR	191830	23,82
<i>PAX2</i>	Ureteric budding	10q24.31	(-)	PAX (paired box domain) main	Papillorenal syndrome that comprises renal defects (oligomeganephronia, hypodysplasia, VUR, and cysts) and ocular defects affecting the optic nerve and/or the retina	AD	120330	39,83
<i>EYA1</i>	Ureteric budding	8q13.3	RTK signaling pathway	Eyes absent homolog 1	BO syndrome	AD	602588	26,27
<i>SALL1</i>	Ureteric budding	16q12.1	(-)	Sal-like protein 1 (also known as spalt-like transcription factor 1)	Townes-Brocks syndrome, as well as BOR syndrome	AD	107480	28,29
<i>SIX1</i>	Ureteric budding	14q23.1	(-)	SIX homeobox 1, 2 and 5	BOR syndrome, BO syndrome	AD	608389	30
<i>SIX2</i>	Ureteric budding	2p21	WNT-pathway	SIX homeobox 2	Renal hypodysplasia	NA	604994	31
<i>BMP4</i>	Ureteric budding	14q22.2	BMP-BMPR signaling system	Bone morphogenetic protein 4	Syndromic microphthalmia, orofacial cleft	AD	112262	31
<i>GATA3</i>	Ureteric budding	10p14	(-)	(-)	Hypoparathyroidism, sensorineural deafness, renal dysplasia	AD	146255	84
Development of the metanephric kidney								
<i>ROBO2</i>	Development of the metanephric kidney	3p12.3	SLIT2-ROBO2 signaling pathway	Receptor for the Slit2 ligand	VUR	NA	610878	41
<i>SLIT2</i>	Development of the metanephric kidney	4p15.31	SLIT2-ROBO2 signaling pathway	Slit protein	Cystic dysplastic kidneys, unilateral renal agenesis, and duplicated collecting system	NA	603746	41,44
<i>SRGAP1</i>	Development of the metanephric kidney	12q14.2	SLIT2-ROBO2 signaling pathway	SRGAP1 (small GTPase activating protein)	Cystic dysplastic kidneys, unilateral renal agenesis, duplicated collecting system	NA	606523	44
MET								
<i>WNT4</i>	MET	1p36.12	WNT-pathway	Protein Wnt-4	Müllerian aplasia, hyperandrogenism	AD	158330	47
<i>FGF20</i>	MET	8p22	(-)	Fibroblast growth factor 20	Renal hypodysplasia/aplasia	AR	605558	50
RAS								
<i>AGT</i>	RAS	1q42.2	RAS pathway	Angiotensinogen	Renal tubular dysgenesis	AR	106150	53
<i>REN</i>	RAS	1q32.1	RAS pathway	Renin	Renal tubular dysgenesis	AR	179820	53
<i>ACE</i>	RAS	17q23.3	RAS pathway	Angiotensin-converting enzyme	Renal tubular dysgenesis	AR	106180	53
<i>AGTR1</i>	RAS	3q24	RAS pathway	Angiotensin II receptor type 1	Renal tubular dysgenesis	AR	106165	52

Table 1. Continued

Gene	Kidney developmental stage	Location	Signals	Gene product	Disease	Inheritance type	#Gene OMIM	Reference number
Nephron patterning and elongation								
<i>Uromodulin (UMOD)</i>	Nephron patterning and elongation	16p12.3	(-)	Tamm-Horsfall protein (uromodulin)	Medullary cystic kidney disease type 2, familial juvenile hyperuricemic nephropathy, and glomerulocystic kidney disease	NA	191845	54,55
Unknown								
<i>TRAP1</i>	Unknown (possibly antiapoptotic and endoplasmic reticulum-stress signaling in proximal tubules and in thick medullary ascending limbs of Henle's loop.)	16p13.3	(-)	TRAP1 heat shock protein 75 (also known as TNF receptor associated protein 1-mitochondrial HSP90 protein)	CAKUT or CAKUT in VACTERL	NA	606219	61
<i>HNF1B</i>								
<i>HNF1B</i>	Unknown (possibly wolffian duct from a very early developmental stage of the kidney)	17q12	(-)	HNF1B protein	Renal cysts and diabetes syndrome also referred to as MODY5, renal hypodysplasia, multicystic dysplastic kidney, cystic kidney disease, single kidney, oligomeganephronia	AD	189907	83
<i>PKHD1</i>								
<i>PKHD1</i>	(-)	6p12.3-p12.2	(-)	Protein fibrocystin	Autosomal recessive polycystic kidney disease, polycystic kidney and hepatic disease	AR	606702	59
<i>KAL1</i>	(-)	Xp22.31	FGF signaling, that there is an interaction between anosmin-1 and FGFR1	Anosmin 1	VUR, hypogonadotropic hypogonadism 1 with or without anosmia (Kallmann syndrome)	XL	300836	64,65
<i>HOXA13</i>								
<i>HOXA13</i>	(-)	7p15.2	(-)	Homeobox protein	VUR, hand-foot-genital syndrome, Guttacher syndrome (preaxial deficiency, postaxial polydactyly, and hypospadias)	AD	142959	67
<i>NIPBL</i>								
<i>NIPBL</i>	(-)	5p13.2	(-)	Delangin	VUR, Cornelia de Lange syndrome	AD	608667	68

CAKUT, congenital anomalies of the kidney and urinary tract; OMIM, Online-Mendelian inheritance in man; GDNF, Glial cell line-derived neurotrophic factor; RTK, receptor tyrosine kinase; BO, branchio-otic; BOR, branchio-oto-renal; WNT, wingless and integration site growth factor; VUR, vesicoureteral reflux; AR, autosomal recessive; AD, autosomal dominant; NA, not applicable; MET, mesenchymal-to-epithelial transition; RAS, renin-angiotensin system; VACTERL, combination of at least three of the following congenital anomalies: vertebral defects (V), anorectal malformations (A), cardiac defects (C), tracheoesophageal fistula with or without esophageal atresia (TE), renal malformations (R), and limb defects (L); TNF, tumor necrosis factor; HSP, heat shock protein; FGF, fibroblast growth factor; FGFR1, FGF receptor 1; MODY, maturity onset diabetes of the young; (-): unknown.

Gata protein family that is expressed in the ureteric buds. It is regulated by the *PAX2* gene and involved in the Wolffian duct morphogenesis [35]. *GATA3* mutation can cause hydronephrosis, ectopic ureteric budding, vas deferens hyperplasia, duplex kidney, uterine agenesis as well as Hypoparathyroidism, sensorineural Deafness, and Renal disease syndrome (including renal dysplasia, unilateral kidney agenesis, and VUR) [36,37].

THE METANEPHRIC KIDNEY RELATED GENES

The metanephric kidney is formed after interaction between the ureteric bud and the metanephric mesenchyme. It starts at 4 weeks of gestation in humans, and it is regulated by Slit homolog 2 (*SLIT2*) and Roundabout homolog 2 (*ROBO2*) when the genes are expressed as a ligand and transmembrane receptor [41]. *SLIT2*-*ROBO2* signaling has been shown to play a role after initiating ureteric budding [41].

ROBO2 encodes a protein as an “immunoglobulin receptor” for the Slit protein and functions in axon guidance and cell migration. *ROBO2* mutations are related with VUR [41].

SLIT2 encodes Slit protein which is a member of secreted glycoproteins. This protein acts as “ligands” for the Robo family. *SLIT2* may associated with guiding commissural axons in the forebrain by acting as a repulsive signal preventing inappropriate midline crossing of axons projecting from the olfactory bulb [42]. Therefore, *SLIT2* can affect the formation and maintenance in the nervous system [43]. In the uretero-renal system, *SLIT2* mutations also can cause duplicated collecting system, unilateral renal agenesis, and cystic dysplastic kidneys [41,44].

SLIT2-*ROBO2* Rho GTPase-activating protein 1 (*SRGAP1*), as the gene name shows, is a small GTPase activating protein associated with the pathway mediating the repulsive signaling of Robo and Slit proteins in the cell migration process [45]. In 2015, Hwang et al. [44] identified 2 heterozygous mutations in *SRGAP1* in 2 unrelated families and described it as a novel monogenic candidate gene as causes of CAKUT in humans.

GENES INVOLVED IN OTHER DEVELOPMENTAL STAGES

After metanephric kidney stage, there are 3 steps left to complete the whole kidney development. Several genes involved in CAKUT have been described for the following process: (1) *WNT4* and *FGF20* for MET, (2) *AGT*, *REN*, *ACE*,

and *AGTR1* for renin-angiotensin system, and (3) *Uromodulin* (*UMOD*) for nephron patterning and elongation.

1. *WNT4* and *FGF20* for MET

WNT oncogene analog 4 (*WNT4*) is on chromosome 1 and structurally related to secreted signaling proteins. *WNT4* regulates kidney tubule induction and the MET within the WNT signaling pathway [14,15,46]. Because it influences both the cortical and medullary stroma during development, mutations of *WNT4* can cause kidney malformation related to Müllerian aplasia and hyperandrogenism [47]. *WNT4* is activated by *BMP4*, a known smooth muscle differentiation factor, therefore, the absence of *WNT4* can result in reduced smooth muscle [48].

Fibroblast growth factor (*FGF*) family possesses broad mitogenic and cell survival activities, and is associated with embryonic development, cell growth, morphogenesis, and tissue repair [49]. Especially *FGF20* is an important key player in MET and can cause renal hypodysplasia or aplasia [50].

2. *AGT*, *REN*, *ACE*, and *AGTR1* for renin-angiotensin system

Renal tubular dysgenesis, one of important phenotypes of CAKUT, is manifested by anuria, hypotension, and oligohydramnios, which eventually lead to Potter syndrome [51]. Several gene mutations of the renin-angiotensin system have been related to the distinct severe phenotype of CAKUT of renal tubular dysgenesis: Angiotensinogen (*AGT*), renin (*REN*), angiotensin-converting enzyme (*ACE*), and angiotensin II receptor type 1 (*AGTR1*) [52]. Gribouval et al. [53] analyzed 48 cases of renal tubular dysgenesis, and mutations in *ACE* accounted for 64.4%. Mutations in *REN*, *AGT*, and *AGTR1* were seen in 20.8%, 8.3%, and 6.3% of cases, respectively.

3. *Uromodulin* (*UMOD*) for nephron patterning and elongation

The *Uromodulin* (*UMOD*) gene encodes the Tamm-Horsfall protein, which is the most abundant urinary protein in humans, and is related to the nephron patterning and elongation. Its mutation causes glomerulocystic kidney disease, familial juvenile hyperuricemic nephropathy, medullary cystic kidney disease type 2 [54,55].

Hepatocyte nuclear factor 1B (*HNF1B*) is a homeodomain-containing transcription factor for embryogenesis of the liver, pancreas, and very early developmental stage of kidney which is expressed in the Wolffian duct [56]. *HNF1B* mutations have been reported as the cause of the Renal

Cysts and Diabetes Syndrome and reported as CAKUT spectrum malformations, such as single kidney, multicystic dysplastic kidney, renal hypodysplasia, cystic kidney disease, and autosomal recessive polycystic kidney disease (ARPKD) [57-59].

OTHER CAKUT ASSOCIATED GENES WITH UNKNOWN RELATIONSHIP OF KIDNEY DEVELOPMENT

Not only genes described above, following genes are also known as a leading cause of CAKUT in several studies: *TRAP1*, *PKHD1*, *KALI*, *HOXA13*, and *NIPBL*. Their pathogenesis of CAKUT especially associated with kidney development process, however, are not clearly revealed.

Tumor Necrosis Factor Receptor-associated Protein 1 (*TRAP1*) encodes a mitochondrial chaperone protein that is member of the heat shock protein 90 family. The protein has ATPase activity and may function in regulating cellular stress responses [60]. Mutations of *TRAP1* can develop many kinds of CAKUT phenotypes or CAKUT in VACTERL syndrome (combination of at least three of the following congenital anomalies: vertebral defects (V), anorectal malformations (A), cardiac defects (C), tracheoesophageal fistula with or without esophageal atresia (TE), renal malformations (R), and limb defects (L) [61].

Polycystic kidney and hepatic disease 1 (*PKHD1*) provides instructions for making a protein called fibrocystin. Fibrocystin exists on the cell membrane of kidney cells as a receptor and interacts with molecules and signals outside of the cell [62]. More than 270 mutations in the *PKHD1* gene have been identified in human with autosomal recessive polycystic kidney disease (ARPKD) [59].

Kallman syndrome 1 (*KALI*) gene encodes a protein called anosmin-1. Anosmin-1 is expressed in the fetal certain regions of the brain, respiratory tract, digestive system and kidneys [63]. But the precise function of anosmin-1 in kidney is not well known. It is also unclear how *KALI* gene mutations lead to signs of Kallmann syndrome, including unilateral renal agenesis [64,65].

Homeobox protein Hox-A13 (*HOXA13*) is a part of homeobox gene family, which appears to play a key role for the formation and development of the limbs (particularly the hands and feet), urinary tract, and reproductive system. At least 14 *HOXA13* gene mutations have been found in hand-foot-genital syndrome, which can have features of VUR [66,67].

Nipped-B-like protein (*NIPBL*) makes a protein named delangin, which helps the activity of chromosomes during

cell division and controls human development [68]. *NIPBL* gene mutations have been identified in people with Cornelia de Lange syndrome which is a developmental disorder affecting many parts of the body. In this syndrome, sometimes VUR is also found [69,70].

EFFORTS TO FIND OUT ADDITIONAL CAKUT RELATED GENES

Nowadays, there are many attempts and technological developments to find out additional CAKUT related genes: linkage studies, targeted next-generation sequencing (NGS), copy number variation (CNV) analysis, whole genome sequencing (WGS) and genome-wide association studies (GWAS).

Linkage analyses have been successful for mapping genetic markers by analyzing a disease in a family-based approach in insulin-dependent diabetes mellitus, Alzheimer disease, breast cancer [71-73]. The majority of published linkage studies on CAKUT have been about familial VUR, which occurs in ~1% of children [74]. However, linkage analysis is not sufficient tool to detect small effect sized loci [9].

Analysis of multiple genes using NGS in patients with CAKUT has demonstrated that <10% patients with CAKUT carry variants in previously implicated genes, such as *HNF1B*, *PAX2*, *EYA1*, *SIX5*, and *RET*. Therefore, the causative genes are not yet identified in most of CAKUT cases even though NGS technique was applied [75-77].

Another aspect of CAKUT is CNVs [78]. It can be identified by high-resolution microarrays, such as the array comparative genomic hybridization (array-CGH) and single nucleotide polymorphism (SNP) microarrays [79].

Currently, genetic studies in unexplained diseases are shifting from whole exome sequencing or CNV analysis to WGS [9]. Also, GWAS, which is known as a high-throughput genotyping technologies, makes the rapid genotyping of >1 million SNPs throughout the whole genome. GWAS can also identify associated loci that confer risk of CAKUT with large sample size [80,81].

CONCLUSIONS

Because morbidity of CAKUT may not manifest until later in life, early detection of these patients is important. CAKUT is a genetically heterogeneous group of disorders that are caused by mutations in genes related to the kidney development process. The malformation phenotypes due to gene mutations diverse from normally structured kidneys

with intact kidney function to severe hypodysplasia and end stage renal failure. Although primary VUR is one of the most common phenotype of CAKUT, its prevalence can be underestimated until it shows symptoms such as fever.

However, some studies have pointed that CAKUT always has to be considered when the patient develops small phenotypes of anomalies related to urinary tract and kidney [9-13]. The advancement in sequencing and bioinformatics technologies will show us the additional CAKUT-related genes and more relevant etiologies of disease entities than can be identified by imaging study tools or histopathology alone. Newly advancing bioinformatics technologies will show us the additional CAKUT-related genes and more relevant etiologies of disease entities. Moreover, these genetic findings will provide an opportunity to develop an ideal diagnostic CAKUT genetic test in clinical practice to facilitate early diagnosis, better management of the disease, and familial genetic counselling [9]. When the price of GWAS gets lower than that of these days and it becomes more popular, we will be able to apply genetic tests to suspected patients. Using these new tools, we could identify the patients with high risks of having the deformities in kidneys before it is too late. Understanding of CAKUT and VUR genetic bases will help the management of this condition in children.

CONFLICTS OF INTEREST

The authors have nothing to disclose.

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