의학박사 학위논문

표적유전자 염기서열분석을 이용한 선천성 신 요로 기형의 원인 유전자 탐색

Targeted exome sequencing provided comprehensive genetic diagnosis of congenital anomalies of the kidney and urinary tract

2020년 8월

서울대학교 대학원

의학과 중개의학 전공

안 요 한

A thesis of the Degree of Doctor of Philosophy

Targeted exome sequencing provided comprehensive genetic diagnosis of congenital anomalies of the kidney and urinary tract

표적유전자 염기서열분석을 이용한 선천성 신 요로 기형의 원인 유전자 탐색

August 2020

Translation Medicine, College of Medicine

The Graduate School

Seoul National University

Yo Han Ahn

Abstract

Targeted exome sequencing provided comprehensive genetic diagnosis of congenital anomalies of the kidney and urinary tract

Yo Han Ahn

Translation Medicine, College of Medicine

The Graduate School

Seoul National University

Congenital anomalies of the kidney and urinary tract (CAKUT) are the most common cause of chronic kidney disease in children. The search for genetic causes of CAKUT has led to genetic diagnosis in approximately 5–20% of CAKUT patients from Western countries. In this study, genetic causes of CAKUT in Korean children were sought using targeted exome sequencing (TES) of CAKUT-related genes. A total of 94 patients with CAKUT were recruited. We performed TES of 60 genes that had been reported to cause CAKUT in human or murine models. Copy number variations (CNVs) of targeted genes were assessed using in-house relative comparison method. Pathogenic single nucleotide variants (SNVs) and CNVs were re-confirmed by Sanger sequencing and array comparative genomic hybridization, respectively. We identified genetic causes in 13.8% of the 94 recruited patients. Pathogenic SNVs of five known disease-causing genes, *HNF1B*, *PAX2*, *EYA1*,

UPK3A, and *FRAS1* were found in 7 cases. Pathogenic CNVs of 6 patients were found in *HNF1B*, *EYA1*, and *CHD1L*. *HNF1B* mutation was the most common genetic cause and was associated with various renal phenotypes. Genetic abnormality types did not significantly differ according to CAKUT phenotypes. Patients with pathogenic variants of targeted genes had syndromic features more frequently than those without (P <0.001). Kaplan-Meier survival curves showed that the presence of genetic mutations did not affect the renal survival (Log-rank test, P =0.280). This is the first genetic analysis study of Korean patients with CAKUT. We showed that TES could provide a cost-effective means for detecting both SNV and CNV for the genetic diagnosis of CAKUT. However, only one-seventh of patients were found to have pathogenic mutations in known CAKUT-related genes, indicating that there are more CAKUT-causing genes or environmental factors to discover.

Keywords: Congenital anomalies of the kidney and urinary tract, Genetic analysis, Single nucleotide variant, Copy number variant

Student number: 2015 - 30009

Contents

List of Tables and Figures

Introduction

Chronic kidney disease (CKD) is a worldwide public health problem with an increase in its prevalence (1). Although CKD in children is not common, it can be a devastating illness with long-term consequences. While CKD in adults commonly comes from diabetes and hypertension along with primary glomerulopathy, the major causes of CKD in children are different; Congenital anomalies of the kidney and urinary tract (CAKUT) are the most common cause of CKD in children, accounting for two-thirds of pediatric CKD (2, 3). In representative pediatric CKD cohorts, CAKUT accounts for 48% in the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS), 41% in the Chronic Kidney Disease in Children (CKiD), KoreaN cohort study for Outcomes in patients With Pediatric Chronic Kidney Disease (KNOW-PedCKD) also reported that the most common CKD diagnoses were related to CAKUT (43%) (2-4). CAKUT represent any abnormalities in number, size, shape, or anatomic position of the kidneys or other parts of the urinary tract, such as renal agenesis, renal hypodysplasia, multicystic dysplastic kidney, vesicoureteral reflux, ureteropelvic junction obstruction, ureterovesical junction obstruction, and posterior urethral valves. While some CAKUT, such as isolated vesicoureteral reflux or other primary urinary tract anomalies, do not impair renal function, others with a primary defect in kidney parenchymal development often progress to CKD. In addition, patients with CAKUT often have extrarenal manifestations as well, such as hearing loss, neurocognitive disorders, and cardiac anomalies, which affect growth and development. Therefore, many children with CKD resulting from CAKUT experience extrarenal manifestations. This complicates the long-standing CKD of

children; pediatric CKD are known to be complicated by physical and psychosocial development. Nearly 50% of pediatric patients with CKD suffer from chronic complications such as cardiovacular disease, metatolic bone disease, and anemia (5). Baek *et al*. (6) reported that patients with a higher CKD stage had worse quality of life in physical, emotional, and school functioning categories, and Kang *et al*. (7) showed that about 20% of chilren with CKD had significant mental health problems and psychosocial adjustment problems. Furthermore, CKD patients have high morbidity and mortality as adult patients (1), and the mortality rate of Korean pediatric CKD was previously reported as 19.9 per 1,000 patient–years (8). Extrarenal manifestations of CAKUT would exacerbate the morbidity and mortality of pediatric CKD.

Disruption of renal development, caused by environmental factors or the dysfunction of genes involved in this process, can lead to CAKUT (9). Epidemiology studies revealed that maternal diet, maternal conditions, and maternal substance use during pregnancy are associated with CAKUT (10). On the other hand, genetic causes of CAKUT have been revealed since 1995, when a mutation in *PAX2* was first discovered to cause optic nerve coloboma, renal hypodysplasia, and vesicoureteral reflux (11). Previous studies have identified over 40 genomic disorders, and more than 50 genes have been reported to be associated with CAKUT (12, 13). Currently, up to 18% of patients with CAKUT can be explained by monogenic causes, most of which have a dominant pattern of inheritance (13, 14). Moreover, three large cohort studies using chromosomal microarrays identified that 4.5–16.6% of patients with CAKUT harbor genomic imbalances, especially in patients with renal hypodysplasia (15-17). Most copy number variations (CNVs) causing CAKUT have been previously reported to be

associated with other developmental disorders, such as developmental delay, neurocognitive disorders, and cardiac malformations (12). Since many patients with CAKUT have extrarenal anomalies, CAKUT can be classified as syndromic form and isolated form, as suggested by Vivante *et al*. and Sanna-Cherchi *et al* (Table 1)(12, 14). In addition, 38 genomic disorders associated with CAKUT are known to be accompanied by neurodevelopmental disorders and/or congenital heart defects; therefore CAKUT with neurodevelopmental disorders and/or congenital heart defects also can be classified as syndromic forms of CAKUT. Among the 38 genomic disorders previously mentioned, the most frequently identified genomic disorder was chromosome 17q12 deletion causing renal cysts and diabetes syndrome (RCAD), followed by chromosome 22q11.2 deletion, causing velocardiofacial syndrome, and chromosome 1q21 deletion (12).

Genetic diagnosis of CAKUT can help physicians correctly diagnose the extent of the problem and evaluate patients for extrarenal manifestations, in addition to enabling family evaluation and genetic counseling. However, it remains challenging because of genetic and phenotypic heterogeneity and incomplete penetrance of CAKUT. In the literature, diagnostic yield of genetic testing of patients with CAKUT is approximately 5–20%, representing either single nucleotide variants (SNVs) or CNVs of the relevant genes (15-23). Most of the large studies on CAKUT are from Western countries, therefore genetic background of CAKUT in Asian populations is yet to be elaborated (24, 25). Moreover, optimal and cost-effective method of genetic diagnosis in CAKUT that can detect both SNV and CNV still needs to be found.

In this study, we aimed to elucidate the genetic causes of CAKUT in Korean children using targeted exome sequencing (TES) of CAKUT-related genes.

Table 1. Syndromic forms of congenital anomalies of the kidney and urinary tract.

Gene	OMIM gene ID	Inheritance	OMIM syndrome name	CAKUT phenotype	Extra-renal phenotype
BMP4	112262	AD	Microphthalmia, syndromic 6	RHD	Cleft lip, micro-ophthalmia
EYA1, SIX1, SIX5	601653, 601205, 600963	AD	Branchio-oto-renal syndrome	RHD	Sensorineural hearing loss, preauricular pits, branchial cysts, microtia
GATA3	131320	AD	Hypoparathyroidism, sensorineural deafness, and renal dysplasia	RHD	Hypoparathyroidism, sensorineural deafness, genital malformations
HNF1B	189907	AD	Renal cysts and diabetes syndrome	RHD	Maturity onset diabetes in the young type 5, hyperuricemia, hypomagnesemia
PAX2	167409	AD	Renal coloboma syndrome	RHD	Retinal and optic nerve coloboma
RET	164761	AD	Multiple endocrine neoplasia IIA/IIB	Renal agenesis	Medullary thyroid carcinoma, pheochromocytoma, central hypoventilation syndrome
SALL1	602218	AD	Townes-Brocks syndrome	RHD, VUR, renal ectopia, PUV	Thumb abnormalities, facial dysmorphisms, gastrointestinal malformations, genital malformations
TNXB	600985	AD	Ehlers-Danlos syndrome	VUR	Joint hypermobility
FRAS1	607830	AR	Fraser syndrome (homozygous loss-of function variants)	RHD, VUR, PUV	Facial dysmorphisms, developmental delay, genital malformations
FREM1	608944	AR	Bifid nose with or without anorectal and renal anomalies (Fraser-associated)	RHD, VUR, PUV	Bifid nose, developmental delay, gastrointestinal malformations, genital malformations

FREM2	608945	AR	Fraser syndrome (homozygous loss-of function variants)	RHD, VUR, PUV	Facial dysmorphisms, developmental delay, genital malformations
GDNF	605710	AD	Hirschsprung disease	RHD	Absent enteric ganglia, pheochromocytoma, central hypoventilation syndrome
KAL1	300836	XLR	Hypogonadotropic hypogonadism with or without anosmia (Kallmann syndrome)	RHD, obstructive uropathy	Hyposmia/anosmia, genital malformations, skeletal malformations, endocrine abnormalities
NOTCH2	600275	AD	Alagille syndrome	RHD, cysts	Facial dysmorphisms, cardiac malformations, cholestatic liver disease, skeletal abnormalities
UMOD	191845	AD	Hyperuricemic nephropathy, familial juvenile 1	Medullary cystic kidney disease	Hyperuricemia
WNT4	603490	AD	Mullerian aplasia and hyperandrogenism	RHD, renal ectopia	Genital malformations, endocrine abnormalities
ACE	106180	AR	Renal tubular dysgenesis	Renal tubular dysgenesis	Pulmonary hypoplasia, facial dysmorphisms
AGT	106150	AR	Renal tubular dysgenesis	Renal tubular dysgenesis	Pulmonary hypoplasia, facial dysmorphisms
AGTR1	106165	AR	Renal tubular dysgenesis	Renal tubular dysgenesis	Pulmonary hypoplasia, facial dysmorphisms

OMIM, online mendelian inheritance in man; AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive; RHD, renal hypodysplasia; VUR, vesicoureteral reflux; PUV, posterior urethral valves.

Methods

1. Study Participants

Patients with CAKUT were recruited from the pediatric nephrology clinic at Seoul National University Children's Hospital. All patients were diagnosed with CAKUT on the basis of renal imaging studies. Inclusion criteria were renal hypodysplasia, renal agenesis, multicystic dysplastic kidney, bilateral vesicoureteral reflux, and bilateral obstructive uropathy (ureteropelvic junction obstruction, ureterovesical junction obstruction, or posterior urethral valves). Syndromic CAKUT was defined as conditions that are associated with other congenital anomalies, such as RCAD syndrome, branchio-oto-renal (BOR) syndrome, renal coloboma syndrome, and Fraser syndrome, as previously described (12) (Table 1). In addition, CAKUT with neurodevelopmental disorders and/or congenital heart defects were also classified as syndromic forms of CAKUT.

This study was approved by the Institutional Review Board of Seoul National University Hospital. Informed consent was obtained from all individual participants and/or their parents.

2. Targeted Exome Sequencing and Bioinformatics Analysis

Through a literature review, 60 genes that had been reported to cause CAKUT in humans or murine models were selected (Table 2). We designed baits covering all exon regions of the targeted 60 genes (Twist Custom Panels; Twist Bioscience, San Francisco, CA). Genomic DNA was extracted from whole blood and sequencing libraries were prepared using Twist modular library preparation kits. Targeted sequencing was performed with 2x 101 bp paired-end reads on an Illumina MiSeq platform (Illumina, San Diego, CA). The average depths of coverage in sequencing

were 106x (range 106-144x). Bioinformatics analyses using sequenced reads were performed as previously described (26-29). Strict depth filtering was initially applied to select candidate variants, and if there were samples with no selected variants, the depth criteria was lowered to identify candidate variants again. Variants were annotated with various information using ANNOVAR (Annotate Variation) (30) utilizing (i) population databases such as 1000 genome phase III, ExAC (Exome Aggregation Consortium), and KRGDB (Korean Reference Genome Database)(http://coda.nih.go.kr/coda/KRGDB/), (ii) disease databases such as OMIM (Online Mendelian Inheritance in Man) and sequencing databases such as RefSeqGene, and (iii) in silico predictive algorithms such as FATHMM (Functional Analysis through Hidden Markov Models), MutationAssessor, MutationTaster, SIFT, Polyphen, GERP, and Phylop for interpretation and classification of variants according to the American College of Medical Genetics and Genomics (ACMG) guidelines (31). Workflow of prioritization and filtering the annotated variants is shown in Table 3. Variants classified as pathogenic or likely pathogenic based on ACMG guidelines were confirmed by Sanger sequencing (Figure 1). CNVs were calculated using aligned read counts in target regions by an in-house relative comparison method. Although exon-level CNV detection was performed, we reported only gene-level CNVs because of following reasons; (i) the read depth in TES is lower in exon-level than in gene-level, which can have a negative effect on the overall accuracy of CNV detection, (ii) read breakpoints are often located in introns, which are not sequenced by TES. Interpretation of detected CNVs was based on size, gene content, and overlap with known disease-associated regions according to the ACMG guidelines for postnatal CNV calling [28]. Classified pathogenic CNVs were re-confirmed by array

comparative genomic hybridization (array CGH; Cytoscan 750k array; Affymetrix, Santa Clara, CA). Whenever possible, genotypes of relevant genes were obtained from the parents.

3. Statistical Analysis

To determine statistical differences between groups with or without pathogenic variants, categorical variables were analyzed using the chi-square test or Fisher's exact test and continuous variables were compared using the t-test or Mann-Whitney U test. All values were reported as a median (interquartile range; IQR). Kaplan-Meier analysis was used to assess renal survival. *P* values <0.05 were considered statistically significant. Statistical analysis was performed using SPSS version 23.0 (IBM, Armonk, NY, USA).

Table 2. The 60 genes included in the gene panel design.

Gene	OMIM gene ID	Inheritance	Renal phenotype	Ref
ACE	106180	AR	Renal tubular dysgenesis	(32)
ACVR2B	602730	UK	Renal agenesis and renal hypoplasia in mice	(33)
AGT	106150	AR	Renal tubular dysgenesis	(32)
AGTR1	106165	AR	Renal tubular dysgenesis	(23, 32)
AGTR2	300034	AR	UPJO	(34)
BICC1	614295	AD	Cystic renal dysplasia	(35)
BMP4	112262	AD	Renal hypodysplasia	(36)
BMP7	112267	AD	Renal dysplasia	(21, 37)
CDC5L	602868	AD	Multicystic renal dysplasia	(21, 38)
CHD1L	613039	AD	Renal hypodysplasia, VUR, and UPJO	(21, 39)
DLX5	600028	AR	Urethral malformation in mice	(40)
DLX6	600030	UK	Urethral malformation in mice	(40)
DSTYK	612666	AD	Renal hypodysplasia and UPJO	(41)
EMX2	600035	UK	Urinary tract anomalies in mice compound heterozygous for Pax2 and Emx2	(42)
EYA1	601653	AD	Branchio-oto-renal syndrome, MCDK, and renal hypoplasia	(43, 44)

FGF20	605558	AR	Renal agenesis	(45)
FOXC1	601090	AD	Renal hypodysplasia	(46)
FRAS1	607830	AR	Fraser syndrome	(47, 48)
FREM2	608945	AR	Fraser syndrome	(48, 49)
GATA3	131320	AD	Hypoparathyroidism, sensorineural deafness, and renal disease (HDR) syndrome	(50)
GDF11	603936	UK	Candidate gene	(49)
GDNF	600837	AD	Renal agenesis	(51, 52)
GFRA1	601496	AD	Renal agenesis	(52)
GREM1	603054	AR	Renal agenesis	(48)
HNF1B	189907	AD	Renal hypodysplasia, MCDK, and renal cyst	(53)
HOXC11	605559	UK	Renal agenesis and renal hypodysplasia in mice	(54)
HOXA11	142958	UK	Renal agenesis and renal hypodysplasia in mice	(54)
HOXD11	142986	UK	Renal agenesis and renal hypodysplasia in mice	(54)
KAL1	300836	XLR	Renal agenesis and Kallman syndrome	(55)
MMP-1	120353	AR	Obstructive uropathy	(56)
MMP-3	185250	UK	Obstructive uropathy	(56)
MMP-8	120355	UK	Obstructive uropathy	(56)
MUC1	158340	AD	Medullary cystic kidney disease type 1	(57)

NOTCH2	600275	AD	Alagille syndrome and Hajdu-Cheney syndrome with renal anomalies	(58, 59)
PAX2	167409	AD	Renal coloboma syndrome and renal hypodysplasia	(60)
PKD1	601313	AD	Autosomal dominant polycystic kidney disease	(61)
PKD2	173910	AD	Autosomal dominant polycystic kidney disease	(61)
PKHD1	606702	AR	Autosomal recessive polycystic kidney disease	(62)
REN	179820	AR	Renal tubular dysgenesis	(32)
RET	164761	AD	Renal agenesis	(51)
ROBO2	602431	AD	VUR	(63, 64)
SALL1	602218	AD	Renal hypodysplasia	(44)
SIX1	601205	AD	Branchio-oto-renal syndrome	(65)
SIX2	604994	AD	Renal hypodysplasia	(36)
SIX4	606342	UK	Candidate gene	(49)
SIX5	600963	AD	Branchio-oto-renal syndrome	(66)
SLIT2	603746	AD	Renal agenesis and MCDK	(67)
SOX17	610928	AD	VUR and UPJO	(68)
SPRY1	602465	UK	Candidate gene	(49)
TBX18	604613	AD	Urinary tract anomalies	(69)
TFAP2A	107580	AD	Branchio-oto-renal syndrome	(70)
TNXB	600985	AD	VUR	(71)
TRAP1	606219	AR	VUR, renal agenesis	(72)

TSC2	191092	AD	Cystic kidney disease in mice	(73)
UMOD	191845	AD	Medullary cystic kidney disease type 2 and hyperuricemic nephropathy	(74)
UPK3A	611559	AD	Renal adysplasia	(75)
USF2	600390	AD	Renal hypodysplasia	(76)
WNT4	603490	AD	Renal hypodysplasia	(77)
WT1	607102	AD	Diffuse mesangial sclerosis	(78)
XPNPEP3	613553	AR	Renal cysts and dysplasia	(79)

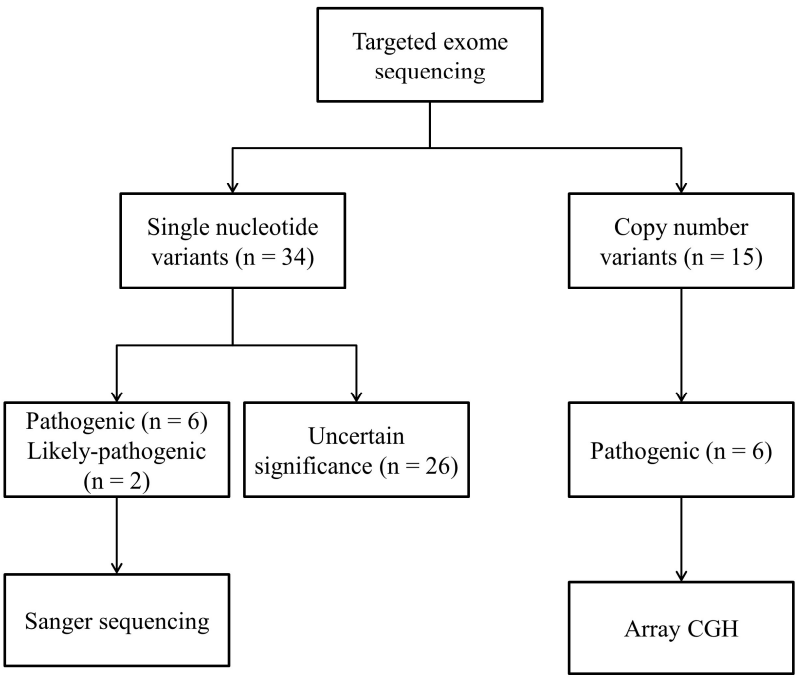
OMIM, online mendelian inheritance in man; Ref, reference; AR, autosomal recessive; UK, unknown; UPJO, ureteropelvic junction obstruction; AD, autosomal dominant; VUR, vesicoureteral reflux; MCDK, multicystic dysplastic kidney; XLR, X-linked recessive; VACTERL, vertebral defects, anal atresia, cardiac defects, tracheo-esophageal fistula, renal anomalies, and limb abnormalities.

Table 3. Single nucleotide variant prioritization and filtering workflow.

Step		Process	Variants (average)
1		Joint call variants	828,905
	1-1	Variants in coding region	18,398
	1-2	Not synonymous variants	12,078
	1-3	Rare variants (minor allele frequency < 1%) in 1000G, ExAC, and KRGDB	3,698
2		Sum of variants in each sample	5,285 (56.2)
	2-1	Variants in targeted 60 genes	620 (6.6)
	2-2	Qualified variants (Depth ≥ 5, VAF > 0.3, GQ ≥ 20)	358 (3.8)
3		Variant classification	
		Pathogenic	6
		Likely pathogenic	2
		Variants of uncertain significance	26
		Benign	324

1000G, 1000 Genomes Project; ExAC, Exome Aggregation Consortium; KRGDB, Korean Reference Genome Database; VAF, variant allele frequency; GQ, genotype quality.

Figure 1. Schematic workflow for the identification of single nucleotide variants and copy number variants.



Results

A total of 94 unrelated Korean patients (M:F 78:16) with CAKUT were recruited (Table 4). The most common phenotype of CAKUT was bilateral renal hypodysplasia (n = 44), followed by unilateral renal hypodysplasia/renal agenesis/multicystic dysplastic kidney with contralateral other anomalies (n = 27), bilateral vesicoureteral reflux (n = 14), unilateral renal hypodysplasia/renal agenesis/multicystic dysplastic kidney (n = 5), and bilateral obstructive uropathy (n = 4). A renal anomaly of each kidney was counted independently as a single unit. Among the 188 renal units of 94 patients, the most common anomaly was renal hypodysplasia (n = 111), followed by vesicoureteral reflux (n = 69), obstructive uropathy (n = 10), multicystic dysplastic kidney (n = 10), renal agenesis (n = 9), and hydronephrosis (n = 4) (Table 4).

Five patients had a family history of CAKUT, of which two had siblings with CAKUT and four had family members with CAKUT in previous generations (mother and grandparents). Sixty-two patients had extrarenal manifestations, such as perinatal problems including prematurity, oligohydramnios, and intrauterine growth retardation (n = 31), neurodevelopmental disorders (n = 16), cardiac disease (n = 10), genital anomalies (n = 9), eye diseases (n = 8), hearing loss (n = 8), diabetes (n = 6), and hypothyroidism (n = 5) (Table 4). Eighty-eight patients (93.6%) exhibited impaired renal function (defined as estimated glomerular filtration rate < 90 mL/min/1.73 m^2) at their last visit and 40 patients (42.6%) developed end stage renal disease (ESRD) at the median age of 13.8 (IQR, 8.9–19.8) years.

Table 4. Clinical presentation of study participants.

ID	Sex	Enrolled age (yr)	Right kidney	Left kidney	Extra-renal phenotype	Renal outcome	Parental sampling
1	F	21.1	Renal agenesis	Renal cortical cysts	No	ESRD at 20.8 yr	Father and mother
2	M	18.5	RHD	RHD	No	CKD stage 3 at 21.0 yr	ND
3	M	20.6	RHD	RHD and VUR	Oligohydramnios	ESRD at 10.4 yr	NA
4	M	21.8	RHD	RHD	Preterm, oligohydramnios, developmental delay, microcephaly, spastic quadriplegia, inguinal hernia, and post-transplant diabetes mellitus	ESRD at 6.8 yr	ND
5	F	19.4	RHD and UPJO	RHD and UPJO	No	ESRD at 18.7 yr	ND
6	F	19.3	No	Renal agenesis	No	Normal at 21.7 yr	ND
7	M	18.4	RHD	RHD	Morning glory discs, dilated cardiomyopathy, and hypothyroidism	ESRD at 10.4 yr	NA
8	M	17.0	Renal cortical cysts and VUR	Renal agenesis	Testicular cystic mass and epididymal cysts	CKD stage 3 at 19.5 yr	NA
9	M	17.0	RHD	RHD and renal cortical cysts	No	ESRD at 16.9 yr	ND

10	M	17.3	No	RHD and VUR	Thyroid colloid cysts	CKD stage 2 at 20.1 yr	ND
11	M	20.0	RHD and renal cortical cysts	RHD and renal cortical cysts	Oligohydramnios	ESRD at 16.9 yr	ND
12	M	15.3	RHD	RHD	No	CKD stage 3 at 18.0 yr	ND
13	M	8.5	MCDK	VUR	Imperforate anus, duodenal obstruction, and choledochal cyst	Normal at 9.8 yr	NA
14	M	19.1	RHD	RHD	Epilepsy, attention deficit hyperactivity disorder, and post-transplant diabetes mellitus	ESRD at 17.2 yr	NA
15	F	14.5	RHD	RHD	Pre-auricular pit and hearing loss	ESRD at 5.4 yr	ND
16	M	16.3	RHD	RHD	Birth asphyxia, nephrocalcinosis, hearing loss, periventricular leukomalacia, and cardiomyopathy	CKD stage 5 at 17.3 yr	NA
17	F	17.6	RHD	No	Hearing loss	CKD stage 2 at 19.8 yr	Father and mother
18	M	0.8	RHD and VUR	RHD	Oligohydramnios	CKD stage 3 at 3.5 yr	NA
19	M	20.1	RHD	RHD	No	CKD stage 3 at 22.8 yr	NA
20	M	16.0	No	Renal agenesis	Micropenis	CKD stage 5 at 16.0 yr	NA

21	M	9.5	RHD	RHD	No	CKD stage 2 at 12.3 yr	NA
22	M	8.3	Renal cortical cysts and VUR	RHD and VUR	Oligohydramnios, post-transplant diabetes mellitus	ESRD at 6.6 yr	NA
23	M	9.1	RHD	RHD	Preterm, oligohydramnios, and atrial septal defect	ESRD at 6.0 yr	NA
24	M	7.8	Renal agenesis	Duplicated ureter and hydronephrosis	Preterm, oligohydramnios, tetralogy of Fallot, imperforate anus, anorectal malformation, patent urachus, strabismus, and inguinal hernia	CKD stage 2 at 10.2 yr	NA
25	F	13.7	RHD and renal cortical cysts	RHD and renal cortical cysts	Preterm and oligohydramnios	ESRD at 7.4 yr	NA
26	M	6.1	RHD	RHD	Preterm and oligohydramnios	CKD stage 3 at 8.9 yr	NA
28	M	5.9	RHD and VUR	RHD and VUR	Preterm and oligohydramnios	CKD stage 3 at 8.7 yr	NA
29	M	5.3	RHD and renal cortical cysts	RHD and renal cortical cysts	Preterm, oligohydramnios, inguinal hernia, and developmental delay	ESRD at 1 mo	Father and mother
30	F	4.8	RHD and renal cortical cysts	RHD and renal cortical cysts	Atrial septal defect, short stature, hearing loss, and hypothyroidism	ESRD at 2.9 yr	NA
31	M	5.5	Renal agenesis	Hydronephrosis	Developmental delay, retroperitoneal pseudocyst, and hearing loss	CKD stage 3 at 8.3 yr	NA
32	M	8.3	RHD and VUR	VUR	No	CKD stage 2 at 11.2 yr	ND

33	M	15.9	RHD	Renal agenesis and VUR	No	CKD stage 5 at 18.7 yr	ND
34	M	12.2	RHD and renal cortical cysts	RHD and renal cortical cysts	Preterm, imperforate anus, hypospadias, and choledocal cyst	CKD stage 3 at 13.6 yr	ND
35	M	10.9	RHD	RHD	Preterm, oligohydramnios, branchial cleft cyst, lymphoma, developmental delay, autism, and inguinal hernia	ESRD at 12.1 yr	NA
36	M	4.6	RHD and renal cortical cysts	RHD and renal cortical cysts	Oligohydramnios, hereditary multiple exostoses and congenital ptosis	CKD stage 3 at 7.5 yr	ND
37	M	12.8	RHD and renal cortical cysts	RHD and renal cortical cysts	Preterm, oligohydramnios, attention deficit hyperactivity disorder	ESRD at 12.2 yr	ND
38	M	13.8	RHD and renal cortical cysts	RHD, renal cortical cysts, and VUR	Preterm, mental retardation, attention deficit hyperactivity disorder, hearing loss, and intermittent exotropia	CKD stage 3 at 16.6 yr	ND
39	F	22.1	RHD and renal cortical cysts	RHD and renal cortical cysts	No	ESRD at 13.4 yr	ND
40	M	11.2	RHD and renal cortical cysts	RHD and renal cortical cysts	Nail dysplasia	ESRD at 5.2 yr	ND
41	M	18.1	RHD and renal cortical cysts	RHD and renal cortical cysts	Patent ductus arteriosus, ureter stone, pancreatic hypoplasia, common bile-duct dilatation, and hepatic cyst	CKD stage 5 at 20.3 yr	NA
42	M	9.8	RHD and renal cortical cysts	RHD and renal cortical cysts	Preterm, jejunal atresia, cerebral palsy (periventricular leukomalacia)	CKD stage 3 at 12.8 yr	ND

43	M	15.4	RHD and renal cortical cysts	RHD and renal cortical cysts	Hyperuricemia	CKD stage 3 at 15.8 yr	ND
44	M	24.5	RHD	Renal cortical cysts	Hyperuricemia	CKD stage 2 at 27 yr	ND
45	F	10.2	RHD and renal cortical cysts	RHD and renal cortical cysts	Preterm, oligohydramnios, hypothyroidism and Fanconi syndrome	CKD stage 5 at 13.2 yr	ND
46	M	23.2	RHD and renal cortical cysts	RHD	Thrombocytopenia	ESRD at 18.5 yr	ND
48	F	28.0	RHD	RHD	Hypothyroidism	ESRD at 27.9 yr	ND
49	M	1.6	VUR	RHD and VUR	Preterm and hypospadias	Normal at 4.4 yr	ND
50	M	26.1	Mid-ureteral obstruction	MCDK	Post-transplant diabetes mellitus	ESRD at 17.6 yr	ND
51	M	11.9	No	MCDK and ureterocele	Preterm, atrial septal defect, bilateral undescended testis, and omphalocele	Normal at 11.9 yr	ND
52	F	12.2	RHD and renal cortical cysts	MCDK	Preterm and oligohydramnios	ESRD at 11 mo	ND
53	M	21.3	RHD	RHD and VUR	No	ESRD at 7.2 yr	ND
54	M	20.0	VUR	VUR	No	ESRD at 20.0 yr	ND

55	M	19.9	VUR	VUR	No	CKD stage 3 at 22.4 yr	NA
56	M	18.2	VUR	VUR	Intermittent exotropia	CKD stage 3 at 21.1 yr	ND
57	M	17.6	VUR	VUR	No	ESRD at 16.7 yr	ND
58	M	22.8	MCDK and VUR	VUR	Parietal lobe epilepsy, multiple neuropathic pain, hypomagnesemia, and hyperuricemia	ESRD at 21.1 yr	NA
59	M	16.8	VUR	VUR	No	CKD stage 5 at 19.8 yr	ND
60	M	17.3	RHD and VUR	RHD and VUR	No	ESRD at 17.0 yr	ND
61	M	19.3	VUR	VUR	Optic disc coloboma and strabismus	CKD stage 5 at 22.4 yr	ND
62	M	22.3	VUR	VUR	No	CKD stage 3 at 23.6 yr	ND
63	M	23.2	RHD	RHD and VUR	No	ESRD at 20.1 yr	NA
64	M	14.2	VUR	RHD and VUR	No	ESRD at 5.6 yr	ND
65	M	25.2	VUR	VUR	No	ESRD at 16.9 yr	ND

66	M	18.9	VUR	VUR	No	CKD stage 3 at 21.3 yr	NA
67	M	12.0	RHD and VUR	VUR	Atrial septal defect and epiblepharon	CKD stage 4 at 14.9 yr	ND
68	M	12.1	RHD and VUR	RHD and VUR	Oligohydramnios	ESRD at 8.9 yr	ND
69	M	21.6	RHD	RHD	No	ESRD at 20.0 yr	ND
70	M	21.2	RHD and VUR	VUR	Intellectual and developmental delay, nocturnal enuresis, and insomnia	CKD stage 4 at 21.2 yr	ND
71	M	9.0	VUR	VUR	Preterm	CKD stage 2 at 11.8 yr	ND
72	F	22.9	VUR	VUR	bilateral superior vena cava	ESRD at 21.6 yr	ND
73	M	12.9	RHD and VUR	RHD and VUR	Post-transplant diabetes mellitus	ESRD at 13.6 yr	NA
74	M	8.6	RHD	VUR	Preterm, umbilical hernia, inguinal hernia, and cryptorchidism	CKD stage 2 at 10.6 yr	ND
75	M	7.9	VUR	VUR	Preterm	CKD stage 4 at 10.8 yr	ND
76	M	19.7	RHD and VUR	VUR	No	CKD stage 2 at 19.7 yr	NA

77	M	11.9	RHD	RHD and VUR	No	CKD stage 4 at 11.9 yr	ND
78	M	4.9	RHD and VUR	VUR	No	ESRD at 4 mo	ND
79	M	15.5	VUR	VUR	Complex febrile seizure and inguinal hernia	CKD stage 5 at 18.2 yr	NA
80	M	9.3	RHD, renal cortical cysts, and VUR	RHD, renal cortical cysts, and VUR	Oligohydramnios, developmental delay and sensory neural hearing loss	ESRD at 2 mo	ND
81	M	15.8	VUR	VUR	No	ESRD at 15.5 yr	NA
82	M	15.5	RHD and renal cortical cysts	RHD and renal cortical cysts	Diabetes mellitus	Normal at 18.1 yr	ND
83	M	16.9	PUV	PUV	Hearing loss	CKD stage 3 at 19.8 yr	ND
84	M	5.3	PUV and RHD	PUV and RHD	Preterm and oligohydramnios	CKD stage 4 at 8.2 yr	ND
85	M	4.3	PUV and RHD	PUV and RHD	Preterm, oligohydramnios, hypothyroidism, inguinal hernia, hypertrophic cardiomyopathy, auricular benign cyst, and developmental delay	ESRD at 5 mo	ND
86	F	20.6	Renal agenesis	UVJO	Hematometra with upper vaginal obstruction	CKD stage 2 at 23.6 yr	NA

87	M	13.8	UPJO	UPJO	No	ESRD at 13.4 yr	ND
88	F	3.1	VUR	Renal agenesis	No	CKD stage 2 at 5.8 yr	ND
89	M	14.7	Hydronephrosis	MCDK	No	CKD stage 2 at 5.1 yr	ND
90	F	14.4	UPJO	MCDK	Partial bicornuate uterus or partial septated uterus	CKD stage 2 at 15.4 yr	ND
91	F	10.7	MCDK	Hydronephrosis	Preterm, oligohydramnios, imperforated anus, vaginal anomaly, and ambiguous genitalia	CKD stage 2 at 13.1 yr	ND
92	M	10.4	RHD and renal cortical cysts	MCDK	Oligohydramnios, choledochal cyst, and hypomagnesemia	CKD stage 2 at 13.0 yr	Father and mother
93	M	3.5	Renal cortical cysts	MCDK	Preterm and portal vein thrombosis	Normal at 3.5 yr	ND
94	M	15.6	RHD and renal cortical cysts	RHD and renal cortical cysts	Developmental delay, Atrial septal defect, cleft palate, cerebral palsy, congenital patellar dislocation, congenital blepharoptosis, and skeletal dysplasia	CKD sate 2 at 18.1 yr	ND
95	M	14.5	RHD and renal cortical cysts	RHD and renal cortical cysts	Optic disc anomaly, hemolytic anemia, and attention deficit hyperactivity disorder	ESRD at 3.2 yr	Father and mother
96	M	10.9	VUR and ureterocele	RHD	No	CKD stage 3 at 13.9 yr	ND

Yr, years; F, female; M, male; ESRD, end stage renal disease; RHD, renal hypodysplasia; CKD, chronic kidney disease; ND, not done; NA, not available; VUR, vesicoureteral reflux; UPJO, ureteropelvic junction obstruction; MCDK, multicystic dysplastic kidney; mo, months; UVJO, ureterovesical junction obstruction; PUV, posterior urethral valves.

1. Single Nucleotide Variants

We identified pathogenic or likely pathogenic SNVs of five known disease-causing genes in seven cases of *HNF1B* (n = 2), *PAX2* (n = 2), *EYA1* (n = 1), *UPK3A* (n = 1), and *FRAS1* (n = 1; compound heterozygous) (Table 5). These genes are known to have autosomal dominant inheritance, except for *FRAS1*, which exhibits autosomal recessive inheritance (13). The inheritance pattern was confirmed in one patient (ID 29), who inherited his *HNF1B* mutation from his father; however, the father has no kidney problems or extrarenal manifestations, while the patient's grandfather had juvenile-onset diabetes mellitus (genetic test not available). In two patients (ID 92 and 95), variants were confirmed as de novo after confirming the absence of the variants in their parents. Five SNVs in genes with autosomal dominant inheritance were frameshift or stop-gain mutations, suggesting loss of function. These truncating mutations were classified as pathogenic SNVs based on the absence in the population databases, in silico prediction of deleterious effect, and patients' clinical phenotypes (Table 5). Pathogenic or likely-pathogenic variants identified in the patients and their parents (when available) were confirmed by Sanger sequencing. The rest of the twenty-six missense mutations of 21 patients in known CAKUT-causing genes were classified as variants of uncertain significance (Table 6).

Renal phenotypes of mutations in *HNF1B*, *PAX2*, and *EYA1* were bilateral renal hypodysplasia or unilateral renal hypodysplasia with contralateral multicystic dysplastic kidney. Two patients with bilateral renal hypodysplasia and optic nerve anomalies carried variants in *PAX2* (p.Arg115Pro and p.Val26Glyfs*28, respectively), causing renal coloboma syndrome. A missense mutation at Arg-115

in the *PAX2* gene was determined as a likely pathogenic variant based on its absence in the population data, a deleterious effect predicted by in silico analysis, and the compatible phenotype of the patient. A patient carrying a novel truncating variant (p.Gly563*) in *EYA1*, which is known to cause BOR, had bilateral renal hypodysplasia and underwent excision of branchial cleft cyst at the age of 11.8 years. This patient was born prematurely with small sized kidneys and oligohydramnios noted before birth, and developed lymphoma at the age of 2.7 years.

A truncating mutation of *UPK3A* (p.Leu156Valfs*85) was expressed as bilateral vesicoureteral reflux with stage 2 CKD at the age of 4.0 years. A patient with right renal agenesis and left renal cysts carried compound heterozygous mutations in *FRAS1* (p.Tyr2273* from the father and p.Gly3456Asp from the mother). Her older brother who had left MCDK, right ureteropelvic junction obstruction, and posterior urethral valves developed stage 3 CKD at the age of 18.8 years (genetic test not available).

All patients with pathogenic or likely pathogenic SNVs developed CKD during childhood.

Table 5. Pathogenic or likely pathogenic single nucleotide variants.

ID	Sex/ Age (yr)	Gene	Nucleotide	Amino Acid	Segregation	Frequency		Renal Phenotype	Extrarenal Phenotype	Renal Function	Ref	Classification
						KRGDB	ExAC					
29	M/ 5.3	*HNF1B*	c.541C>T	p.Arg181*	Father	0	0	Bilateral RHD and renal cysts	Preterm, oligohydramnios, inguinal hernia, and developmental delay	ESRD at 1 mo	(80)	Pathogenic
92	M/ 10.4	*HNF1B*	c.1103_1116del	p.His368Argfs*27	De novo	0	0	Left MCDK and right RHD with renal cysts	Oligohydramnios, choledochal cyst, and hypomagnesemia	CKD	No	Pathogenic
07	M/ 18.4	*PAX2*	c.344G>C	p.Arg115Pro	NA	0	0	Bilateral RHD	Morning glory optic discs, dilated cardiomyopathy, and hypothyroidism	ESRD at 10.4 yr	No	Likely pathogenic
95	M/ 14.5	*PAX2*	c.76dupG	p.Val26Glyfs*28	De novo	0	0	Bilateral RHD and renal cysts	Optic nerve anomaly, hemolytic anemia, and ADHD	ESRD at 3.2 yr	No	Pathogenic

35	M/ 10.9	*EYA1*	c.1582G>T	p.Gly563*	NA	0	0	Bilateral RHD	Preterm, oligohydramnios, branchial cleft cyst, lymphoma, developmental delay, autism, and inguinal hernia	ESRD at 12.1 yr	No	Pathogenic
55	M/ 19.9	*UPK3A*	c.466_467del	p.Leu156Valfs*85	NA	0	0.0004	Bilateral VUR	No	CKD	No	Pathogenic
1	F/ 21.1	*FRAS1*	c.6819T>A c.10367G>A	p.Tyr2273* p.Gly3456Asp	Father Mother	0.00050	0.0020	Right renal agenesis and left renal cysts	No	ESRD at 20.8 yr	No No	Pathogenic Likely pathogenic

Transcript number; *HNF1B*, NM_000458.4; *PAX2*, NM_000278.5; *EYA1*, NM_000503.6; *UPK3A*, NM_006953.4; *FRAS1*, NM_025074.7. Yr, years; KRGDB, Korean Reference Genome Database; ExAC, Exome Aggregation Consortium; Ref, reference; RHD, renal hypodysplasia; mo, months; ESRD, end stage renal disease; MCDK, multicystic dysplastic kidney; CKD, chronic kidney disease; NA, not available; ADHD, attention deficit hyperactivity disorder; VUR, vesicoureteral reflux.

Table 6. Variants of uncertain significance.

ID	Gene Transcript number	Nucleotide	Amino acid	Segregation	Frequency		Mutation Taster	Renal phenotype	Extra-renal phenotype	Renal function	Sanger sequencing
					KRGDB	ExAC					
3	*RET* NM_020975.6	c.1921G>A	p.Ala641Thr	NA	0.0014	0.00004	DC	Bilateral RHD, horseshoe kidney, and left VUR	Oligohydramnios	ESRD at 10.4 yr	Yes
8	*TNXB* NM_019105.8	c.9749C>T	p.Thr3250Met	NA	0	0.00006	DC	Left renal agenesis, right renal cysts, and right VUR	Testicular cystic mass and epididymal cysts	CKD	No
8	*ROBO2* NM_002942.5	c.3553T>C	p.Trp1185Arg	NA	0	0	NA	Left renal agenesis, right renal cysts, and right VUR	Testicular cystic mass and epididymal cysts	CKD	No
13	*TNXB* NM_019105.8	c.2090T>C	p.Leu697Pro	NA	0.0018	0.00006	DC	Right MCDK and left VUR	Imperforate anus, duodenal obstruction, and choledochal cyst	Normal at 9.8 yr	No
14	*RET* NM_020975.6	c.1618A>G	p.Arg540Gly	NA	0.0009	0.00004	DC	Bilateral RHD	Epilepsy, ADHD, and post-transplant diabetes	ESRD at 17.2 yr	No

16	*TNXB* NM_019105.8	c.1364G>A	p.Gly455Asp	NA	0.0009	0.00001	DC	Bilateral RHD	Birth asphyxia, hearing loss, periventricular leukomalacia, and cardiomyopathy	CKD	No
17	*RET* NM_020975.6	c.874G>A	p.Val292Met	Mother	0.0018	0.0006	DC	Right RHD	Hearing loss	CKD	Yes
20	*NOTCH2* NM_024408.4	c.5684G>A	p.Arg1895His	NA	0.0009	0.0001	DC	Left renal agenesis	Micropenis	CKD	Yes
22	*ROBO2* NM_002942.5	c.1435C>T	p.Arg479Trp	NA	0	0.00003	DC	Left RHD, right renal cysts, and bilateral VUR	Oligohydramnios, post-transplant diabetes mellitus	ESRD at 6.6 yr	No
28	*TNXB* NM_019105.8	c.2030A>G	p.Asp677Gly	NA	0.0045	0.0025	DC	Bilateral RHD and bilateral VUR	Preterm and oligohydramnios	CKD	No
28	*NOTCH2* NM_024408.4	c.5557G>C	p.Asp1853His	NA	0	0.00002	DC	Bilateral RHD and bilateral VUR	Preterm and oligohydramnios	CKD	No
30	*CHD1L* NM_004284.6	c.1841G>A	p.Arg614Gln	NA	0.0077	0.0001	DC	Bilateral RHD and renal cysts	Atrial septal defect, short stature, hearing loss, and hypothyroidism	ESRD at 2.9 yr	No

31	*CHD1L* NM_004284.6	c.2345T>C	p.Leu782Ser	NA	0.0036	0.00005	DC	Right renal agenesis and left hydronephrosis	Developmental delay, retroperitoneal pseudocyst, and hearing loss	CKD	No
41	*HNF1B* NM_000458.4	c.439C>G	p.Gln147Glu	NA	0	0	DC	Bilateral RHD with renal cysts and renal stone	Patent ductus arteriosus, pancreatic hypoplasia, common bile-duct dilatation, and hepatic cyst	CKD	Yes
55	*ROBO2* NM_002942.5	c.3585G>T	p.Gln1195His	NA	0	0.00001	NA	Bilateral VUR	No	CKD	Yes
58	*SLIT2* NM_004787.4	c.674G>A	p.Arg225His	NA	0.0005	0.0001	DC	Right MCDK and bilateral VUR	Parietal lobe epilepsy, multiple neuropathic pain, hypomagnesemia, and hyperuricemia	ESRD at 21.1 yr	No
63	*HNF1B* NM_000458.4	c.313G>A	p.Glu105Lys	NA	0.0014	0.0001	DC	Bilateral RHD and left VUR	No	ESRD at 20.1 yr	Yes
66	*SLIT2* NM_004787.4	c.1046C>T	p.Ser349Phe	NA	0.0005	0	DC	Bilateral VUR	No	CKD	No

73	*SIX2* NM_016932.5	c.707C>T	p.Pro236Leu	NA	0.0005	0.0002	DC	Bilateral RHD and bilateral VUR	Post-transplant diabetes mellitus	ESRD at 13.6 yr	No
73	*SLIT2* NM_004787.4	c.4488G>T	p.Arg1496Ser	NA	0.0014	0.00002	DC	Bilateral RHD and bilateral VUR	Post-transplant diabetes mellitus	ESRD at 13.6 yr	No
76	*TNXB* NM_019105.8	c.2030A>G	p.Asp677Gly	NA	0.0045	0.0025	DC	Right RHD and bilateral VUR	No	CKD	No
76	*NOTCH2* NM_024408.4	c.5557G>C	p.Asp1853His	NA	0	0.0002	DC	Right RHD and bilateral VUR	No	CKD	No
79	*DSTYK* NM_015375.3	c.1718T>C	p.Ile573Thr	NA	0	0	DC	Bilateral VUR	Complex febrile seizure and inguinal hernia	CKD	No
81	*ACE* NM_000789.4	c.2186G>A c.2803C>T	p.Arg729Gln p.Pro935Ser	NA	0.0009 0.0005	0.0003 0.00002	DC DC	Bilateral VUR	No	ESRD at 15.5 yr	Yes
86	*CHD1L* NM_004284.6	c.968A>T	p.Asp323Val	NA	0.0027	0.0001	DC	Right renal agenesis and left UVJO	Hematometra with upper vaginal obstruction	CKD	No

KRGDB, Korean Reference Genome Database; ExAC, Exome Aggregation Consortium; NA, not available; DC, disease causing; RHD, renal hypodysplasia; ESRD, end stage renal disease; CKD, chronic kidney disease; MCDK, multicystic dysplastic kidney; ADHD, attention deficit hyperactivity disorder; VUR, vesicoureteral reflux; UVJO, ureterovesical junction obstruction.

2. Copy Number Variants

Six patients showed pathogenic CNVs (deletions in 4 and duplications in 2) in the following targeted genes, *HNF1B* (n = 4), *EYA1* (n = 1), and *CHD1L* (n = 1) (Table 7). The size of the rearrangements ranged from 1.48 to 2.20 Mb.

Four patients had a deletion or duplication of chromosome 17q12 containing *HNF1B* with variable renal manifestations including renal hypodysplasia, multicystic dysplastic kidney, vesicoureteral reflux, ureteral obstruction, and renal cortical cysts. All patients had variable extrarenal manifestations including diabetes mellitus, choledochal cyst, hypomagnesemia, hyperuricemia, and epilepsy. Among the four patients with a CNV of chromosome 17q12, one patient with a duplication (ID 82) had normal renal function at last follow up at 18.1 years, while the others with deletions developed CKD during childhood. Two patients with the 17q12 deletion (ID 50 and 82) were diagnosed with diabetes at the age of 23.7 and 18.1 years, respectively, while another patient with the same deletion (ID 34) had not developed diabetes at last follow up at 13.6 years. Regarding extrarenal symptoms other than diabetes, a patient with a 17q12 duplication (ID 58) complained of multiple neuropathic pain, weakness, and tremors after renal transplantation at the age of 21.1 years and was suspected to have parietal lobe epilepsy or psychiatric disorders. Patient ID 34 with a 17q12 deletion had multiple congenital anomalies including imperforate anus, hypospadias, and choledochal cysts. He had undergone several surgeries for these anomalies and experienced operation-related complications.

A patient with a deletion of 8q13.3 containing *EYA1* (ID 15) had bilateral renal hypodysplasia with pre-auricular pits and severe hearing loss requiring hearing aids

from the age of 6.0 years. One patient with bilateral renal cortical cysts and renal stones (ID 41) had a duplication of 1q21.1 encompassing *CHD1L*. His extrarenal manifestation was patent ductus arteriosus, which required device closure at the age of 7.6 years.

Table 7. Pathogenic copy number variants.

ID	Sex/ Age(Yr)	Chromosome	CNV Type	Start (Mb)	End (Mb)	Size (Mb)	Involved OMIM Genes	Renal Phenotype	Extrarenal Phenotype	Renal Function	Ref
34	M/ 12.7	17q12	Del	34.82	36.38	1.56	*ZNHIT3*, *MYO19*, *PIGW*, *GGNBP2*, *DHRS11*, *MRM1*, *LHX1*, *AATF*, *ACACA*, *TADA2A*, *DUSP14*, *SYNRG*, *DDX52*, *HNF1B*, and *TBC1D3*	Bilateral RHD with renal cysts	Preterm, imperforate anus, hypospadias, and choledochal cyst	CKD	(16)
50	M/ 26.1	17q12	Del	34.47	36.24	1.76	*TBC1D3B*, *CCL3L3*, *CCL4L2*, *TBC1D3C*, *CCL3L1*, *TBC1D3H*, *TBC1D3G*, *ZNHIT3*, *MYO19*, *PIGW*, *GGNBP2*, *DHRS11*, *MRM1*, *LHX1*, *AATF*, *ACACA*, *TADA2A*, *DUSP14*, *SYNRG*, *DDX52*, and *HNF1B*	Left MCDK and right mid-ureteral obstruction	Post-transplant diabetes mellitus	ESRD at 17.6 yr	(16)
58	M/ 22.8	17q12	Dup	34.82	36.37	1.55	*ZNHIT3*, *MYO19*, *PIGW*, *GGNBP2*, *DHRS11*, *MRM1*, *LHX1*, *AATF*, *ACACA*, *TADA2A*, *DUSP14*, *SYNRG*, *DDX52*, *HNF1B*, and *TBC1D3*	Bilateral VUR and right MCDK	Parietal lobe epilepsy, multiple neuropathic pain, hypomagnesemia, and hyperuricemia	ESRD at 21.1 yr	(16)

82	M/ 15.5	17q12	Del	34.82	36.30	1.48	*ZNHIT3*, *MYO19*, *PIGW*, *GGNBP2*, *DHRS11*, *MRM1*, *LHX1*, *AATF*, *ACACA*, *TADA2A*, *DUSP14*, *SYNRG*, *DDX52*, and *HNF1B*	Bilateral RHD with renal cysts	Diabetes mellitus	Normal	(16)
15	F/ 14.5	8q13.3	Del	71.94	74.15	2.20	*EYA1*, *MSC*, *TRPA1*, *KCNB2*, and *TERF1*	Bilateral RHD	Pre-auricular pit and hearing loss	ESRD at 5.4 yr	(81)
41	M/ 18.1	1q21.1	Dup	146.0	147.99	1.99	*NBPF12*, *PRKAB2*, *FMO5*, *CHD1L*, *BCL9*, *ACP6*, *GJA5*, *GJA8*, *GPR89B*, and *NBPF11*	Bilateral RHD with renal cysts and renal stone	Patent ductus arte riosus, pancreatic hypoplasia, comm on bile-duct dilata tion, and hepatic cyst	CKD	(23)

Yr, years; CNV, copy number variant; OMIM, Online Mendelian Inheritance in Man; Ref, reference; Del, deletion; RHD, renal hypodysplasia; CKD, chronic kidney disease; MCDK, multicystic dysplastic kidney; ESRD, end stage renal disease; Dup, duplication; VUR, vesicoureteral reflux.

3. Genotype and Phenotype Correlations

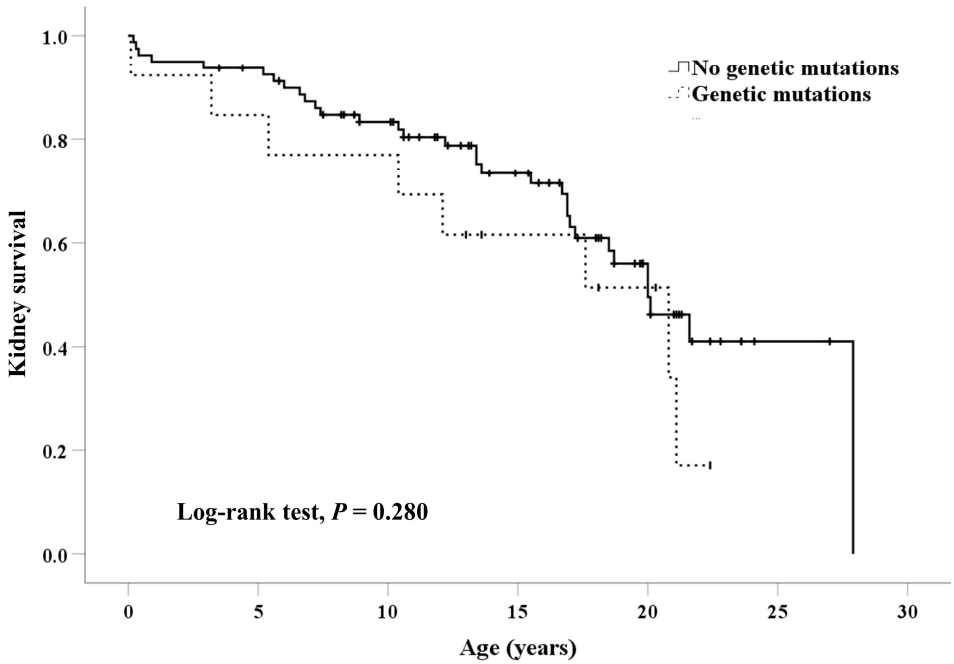
The types of genetic abnormalities did not significantly differ according to CAKUT phenotypes. Pathogenic variants were identified in three of nine patients with unilateral multicystic dysplastic kidney and other contralateral anomalies, with a relatively high detection rate. Bilateral anomalies of kidneys were more frequent in patients with pathogenic variants (76.9%) than those without pathogenic variants (51.9%), but the difference was not statistically significant (P =0.091). Patients with pathogenic variants had syndromic features more frequently (84.6%) than those without variants (25.9%; P <0.001; Table 8). Family history of CAKUT was rare in both groups with or without mutations. Perinatal problems also did not differ between the two groups. Kaplan–Meier survival curves showed that the presence of genetic mutations did not affect renal survival (Log-rank test, P =0.280; Figure 2).

Table 8. Comparison between patients with and without pathogenic/likely pathogenic variants.

Characteristic	Pathogenic or Likely Pathogenic Variants		*P* Value
	Positive (n = 13)	Negative (n = 81)	
Male sex	11 (84.6)	67 (82.7)	1.000
Age at enrollment, years	15.5 (12.2–19.9)	15.3 (9.3–19.3)	0.360
Age at last follow up, years	18.1 (13.8–22.4)	17.2 (11.9–21.3)	0.324
Renal phenotype			0.293
Bilateral lesions			
bRHD with/without Others[a]	8 (61.5)	36 (44.4)	
uAgenesis + cOthers[a]	1 (7.7)	6 (7.4)	
uMCDK + cOthers[a]	3 (23.1)	6 (7.4)	
uRHD + cOthers[a]	0	11 (13.6)	
bVUR	1 (7.7)	13 (16.0)	
bObstructive uropathy[b]	0	4 (4.9)	
Unilateral lesions	0	5 (6.2)	
Bilateral renal anomalies	10 (76.9)	42 (51.9)	0.091
Kidney function			0.282
Normal	1 (7.7)	5 (6.2)	
CKD	4 (30.8)	44 (54.3)	
ESRD	8 (61.5)	32 (39.5)	
Age at diagnosis of ESRD, years	13.6 (10.4–20.3)	13.9 (8.9–19.7)	0.914
Age at 50% kidney survival[c], years	20.8 (10.2–31.4)	20.0 (17.2–22.8)	0.280
Family history of CAKUT[d]	1 (7.7)	4 (4.9)	0.533
Syndromic CAKUT	11 (84.6)	21 (25.9)	<0.001
Premature birth	3 (23.1)	20 (24.7)	1.000
Small for gestational age	3 (23.1)	14 (17.3)	0.698
Oligohydramnios	3 (23.1)	19 (23.5)	1.000

Values are expressed as numbers (%) and median (interquartile range). [a]Including renal hypodysplasia, multicystic dysplastic kidney, and renal agenesis. [b]Including posterior urethral valve (n = 3) and bilateral ureteropelvic junction obstruction (n = 1). [c]The median (95% confidence interval) estimated by Kaplan–Meier survival analysis. [d]Reporting by the patients and parents. b, bilateral; u, unilateral; c, contralateral; RHD, renal hypodysplasia; VUR, vesicoureteral reflux; MCDK, multicystic dysplastic kidney; CKD, chronic kidney disease; ESRD, end stage renal disease; CAKUT, congenital anomalies of the kidney and urinary tract.

Figure 2. Kidney survival according to the presence of pathogenic variants.



Discussion

This is the first report on the genetic diagnosis in a Korean patient cohort of CAKUT, which has long-term follow up data on renal outcome. Using TES, our study identified pathogenic SNVs and CNVs in 7.4% and 6.4% of patients with CAKUT, respectively. The detection rate of genetic causes in CAKUT is lower than that of other causes of early-onset CKD, partially because genetic analysis of CAKUT is difficult due to genetic heterogeneity, incomplete penetrance, and variable expressivity (13, 82). The genetic diagnosis rate of 13.8% is similar to previous studies employing various detection methods. TES achieved a 1–6% positive result in patients with several types of CAKUT (20, 21), while whole exome sequencing (WES) studies found causative mutations of known CAKUT genes in 11–14% of patients (18, 19, 25) and mutations of novel genes in 8% of families (18). Previous CNV studies reported that 4.5–16.6% of patients with variable CAKUT were carrying pathogenic CNVs (15-17, 22, 23). While most previous studies tried to identify only SNVs or CNVs, we hypothesized that simultaneous evaluation of pathogenic SNVs and CNVs using TES would increase the overall diagnostic yield. Although we achieved a better diagnostic yield than did other studies using TES, the yield was not higher than that of other studies using WES. Another study that had used such an integrated approach employed WES and evaluated not only SNVs and but also CNVs using two CNV detection tools (19). Therefore, we showed that incorporation of CNV evaluation is possible as well as helpful for the genetic diagnosis of CAKUT via TES. In addition, the number of targeted genes in a gene panel is also important for the detection rate; while previous reports tested 208 and 17 genes, our gene panel included 60 genes,

though the detection rate of SNVs is similar compared to previous studies that employed TES (20, 21). Differences in inclusion/exclusion criteria of subjects might also have affected the detection rate of causative genetic variants in a disease population. Selectively testing severe and syndromic CAKUT cases or patients from consanguineous families would result in a higher detection rate compared to testing a general cohort of CAKUT encompassing the whole spectrum of all CAKUT phenotypes. To increase diagnostic yield, our study included only patients with severe CAKUT phenotypes, such as renal agenesis, renal hypodysplasia, and cystic dysplasia, which are expected to profoundly impair long-term renal survival (16, 51, 83). In this study, 93.6% of patients developed CKD during childhood. One pediatric CKD cohort study revealed that a subset of patients diagnosed with renal hypodysplasia was particularly enriched for known genomic disorders (10.5%) (16). Another study demonstrated that stillborn fetuses with renal agenesis or severe dysplasia had a high positive rate (30%) of disease-causing mutations, although only *RET*, *GDNF*, and *GFRA1* genes were evaluated (51). We theorized that genetic abnormalities would be detected more frequently in patients with severe forms of CAKUT than those with mild forms or unilateral anomalies. However, baseline clinical characteristics and renal survival did not differ regardless of the presence of genetic mutations, which is similar to what was previously reported (16, 24).

In our study, mutations of the *HNF1B* gene were the most commonly identified genetic cause, accounting for 46.2% of patients with pathogenic variants. This result is in line with previous studies reporting that *HNF1B* mutations were the most prevalent in patients with CAKUT (15, 16, 21, 84). Moreover, the most

common pathogenic CNVs of CAKUT are 17q12 deletions; the chromosome 17q12 region containing *HNF1B* is highly susceptible to genomic rearrangement by non-allelic homologous recombination between flanking segmental duplications (85, 86). *HNF1B* encodes hepatocyte nuclear factor 1-β, which is a member of the homeodomain-containing superfamily of transcription factors. Early expression of *HNF1B* is seen in the kidney, liver, pancreas, bile ducts, genital tract, lung, thymus, and gut, where it plays an important role in organogenesis and tissue-specific regulation gene expression in these organs (87). *HNF1B* plays a critical role in ureteric bud branching and renal tubular development in early nephrogenesis (88, 89). Renal malformations associated with *HNF1B* mutations vary and include renal cysts, renal hypodysplasia, renal agenesis, cystic renal dysplasia, and ureteral defects. Extrarenal features of *HNF1B* mutations include early-onset diabetes mellitus, pancreatic hypoplasia, developmental delay, genital tract malformations, abnormal liver function, hypomagnesemia, hyperuricemia, and early-onset gout. Therefore, *HNF1B*-associated disease is considered a multi-system disorder (53). In this study, patients with *HNF1B* mutations also presented with variable, multi-systemic phenotypes. Interestingly, one patient with a 17q12 duplication manifested atypical symptoms of suspected neuropsychiatric disorders; indeed, patients with 17q12 deletions or duplications have been reported to have a broad range of psychiatric and neurologic features (90). This may be a contiguous-gene syndrome, as the chromosome 17q12 region includes *LHX1* (15, 16), which encodes LIM homeobox 1, a transcriptional factor that functions in the development of neural cells (91, 92).

Pathogenic variants in *PAX2* and *EYA1* were common in our study, which is

similar to previous findings (19, 20). Mutations in *PAX2* and *EYA1* cause the autosomal dominant disorders renal coloboma syndrome and BOR syndrome, respectively. *PAX2*, paired box gene 2, is a transcription factor playing key roles on the development of the kidneys, eyes, ears, and urogenital tract. During renal development, *PAX2* suppresses apoptosis in the developing ureteric bud; therefore *PAX2* mutations would increase apoptosis during development of the kidney and urinary tract, which may underlie decreased nephron number, hypertrophy of remaining nephrons, and renal hypodysplasia (93-95). Transcription factor *PAX2* also plays a critical role in the differentiation of the optic nerve during eye development (96). Bower *et al*. (60) reported that 77% of 173 patients with *PAX2* mutations have ophthalmological abnormalities of the optic nerve, retina, macula, and lens. Meanwhile, *EYA1* has protein phosphatase function for regulating genes encoding growth control and signaling molecules in the development of kidney, muscle, and inner ear (97). *EYA1* mutations account for 30–35% of BOR syndrome, which is characterized by branchial defects, malformations of the outer, middle, and inner ear associated with deafness, and renal anomalies (43, 65). In our study, patients with these genetic variants also presented with these characteristic clinical manifestations.

In this study, pathogenic variants in *UPK3A*, *FRAS1*, and *CHD1L* gene, which are known CAKUT-causing genes, were found in one case each. Uroplakins (UPK) including UPK3A are integral membrane proteins that assemble into 2-dimensional crystalline arrays and cover the apical urothelial surface (98). Jenkin *et al.* reported that four patients with isolated CAKUT had de novo heterozygous mutations in the *UPK3A* gene (75). Our patient with a *UPK3A* mutation also had severe bilateral

vesicoureteral reflux without extra-renal manifestations (isolated CAKUT). *FRAS1* encodes an extracellular matrix protein that appears to function in the regulation of epidermal basement membrane adhesion and organogenesis during development (99). Fraser syndrome caused by mutations in the *FRAS1* gene is characterized by cryptophthalmos, cutaneous syndactyly, kidney anomalies, and genital malformations. Kohl *et al*. identified biallelic missense mutations in the *FRAS1* gene in patients with isolated CAKUT (48). Our patient carrying compound heterozygous mutations in *FRAS1* had unilateral renal genesis with no syndromic manifestations. *CHD1L* encodes chromodomain helicase DNA-binding protein 1-like protein, a DNA helicase, which plays a role in chromatin-remodeling following DNA damage. *CHD1L* expression was high in fetal kidneys and was four times higher in fetal compared to adult kidneys, suggesting that it is of particular importance in the developing kidney (39). Weber *et al*. reported a duplication in 1q21.1 including the *CHD1L* gene was detected in a patient presenting with renal hypoplasia combined with mental retardation, macrocephaly, and ear anomalies (23). In this study, one patient with syndromic CAKUT had a duplication of 1q21.1 encompassing *CHD1L*; he had patent ductus arteriosus, pancreatic hypoplasia, common bile-duct dilatation, and hepatic cyst.

Compared with previous studies employing Caucasian patients with CAKUT, our study did not find pathogenic variants in *RORO2*, *SALL1*, *FREM2*, or *RET*, which were also often identified in previous studies (18, 21, 48, 51). Our findings are similar to a Japanese study, where *HNF1B* and *PAX2* were reported as the most common causative genes and none of the above four genes were found (24). On the other hand, a Chinese study identified pathogenic variants in *HNF1B*, *UMOD*,

NEK8, and *BBS2* genes (25). Therefore, these difference may be associated with ethnic differences.

Our study revealed syndromic CAKUT had a higher rate of pathogenic variants than did isolated CAKUT (34.4% vs. 3.2%). In this regard, extrarenal manifestations may provide an important clue for uncovering the genetic cause of CAKUT. Some syndromic CAKUT encompass characteristic extrarenal manifestations, such as hearing loss and neck anomalies in BOR syndrome (*EYA1*), optic nerve coloboma in renal coloboma syndrome (*PAX2*), and hyperuricemia and diabetes in RCAD syndrome (*HNF1B*). Evaluation of patients with CAKUT should include screening for associated extrarenal manifestations and detailed history taking of the patient and family to help identify the underlying genetic cause. On the other hand, molecular diagnosis can help physicians screen and identify hidden or subtle clinical manifestations of other organs, which significantly affect the management and prognosis of patients. Some CAKUT-causing gene mutations are associated with the risk of developmental delay, learning disabilities, and other neuropsychiatric disorders that benefit from early detection and intervention. In this study, 4 of 13 (30.7%) patients with pathogenic variants had neurodevelopmental disorders. Some extrarenal manifestations, including hearing loss, diabetes, hyperuricemia, and infected branchial cleft cyst, may present later. If physicians are aware of the possible clinical features associated with genetic defects, targeted workup or surveillance based on current recommendations can be established (16). Therefore, genetic analysis for CAKUT genes is highly recommended for CAKUT patients, especially with syndromic CAKUT. In addition, clinicians should closely monitor for the development of neurocognitive

disorders in CAKUT patients because developmental delay and intellectual disability are difficult to diagnose in younger children.

In this study, only one-seventh of the patients obtained genetic diagnosis using TES. It suggests that other genes not included in our gene panel or non-genetic factors have contributed to the occurrence of CAKUT. Clearly, epigenetic and environmental factors would affect the development of CAKUT (9); large studies revealed that CAKUT were associated with prenatal risk factors, such as gestational diabetes, maternal obesity, and low birth weight (100, 101). In this study, perinatal problems, including preterm birth, small for gestational age, and oligohydramnios were not different between the two groups with or without pathogenic variants. Reporting by parents, maternal hypertension and diabetes during pregnancy did not differ between the two groups (data not shown). However, we could not investigate other maternal factors such as diet, obesity, and substance use, which are known to be associated with CAKUT. Marked variation of the clinical phenotype and severity of CAKUT among individuals carrying the same mutation also indicates that complex genetic or non-genetic mechanisms are involved in the pathogenesis of CAKUT. In addition, as suggested for other complex developmental traits such as cleft palate and congenital heart disease (102, 103), the polygenic model might also be able to explain the vast heterogeneity of CAKUT.

Our genetic testing approach is cost-effective for several reasons. Although WES and whole-genome sequencing (WGS) provides good coverage of known disease-associated human genes, their high costs and long turnaround times limit their application in a clinical setting. TES can overcome these two major problems and

serve as a routine clinical genetic test for a wide range of CAKUT. The overall cost of our TES is about 30% to 50% cheaper than those of WES and WGS. Short turnaround time is achieved by processing a large number of patients with various types of CAKUT at the same time. In addition, sequencing depth can become even greater for a lower cost by using a targeted gene panel that has a selected number of disease-causing genes. This greater depth provides more sufficient information to detect low-frequency mutations that could not be well identified by WES and WGS. In patients with CAKUT, TES can be used in clinical care to provide greater confidence while keeping the cost down.

There are several limitations associated with this study. First, while we assessed CNVs, only those involving captured areas were searched, as we employed TES instead of WES. Therefore, we may have missed variations in noncoding regions of tested genes, as well as those involving other regions that are not covered by TES. Due to the lack of resources, only patients with CNVs were assessed by array CGH (n = 6). Therefore, other subjects may also possess CNVs that were not detected because we did not perform array CGH for all subjects. Second, the discovery of novel genes is not possible when employing TES instead of WES or WGS. With advances in technology, especially for the genetic diagnosis of such disease groups of CAKUT with genetic heterogeneity and multiple causative CNVs, whole-genome sequencing may be the better technique. Third, trio samples were not available for all patients; therefore assessment of penetrance or segregation was not sufficient. Lastly, functional studies of novel mutations were not performed. Nonetheless, this is the first report on the genetic diagnosis of CAKUT in Korean patients and one of the few studies reporting data on Asia patients. In addition, our

longitudinal outcome data on this unique Korean CAKUT patient cohort could bring new opportunities for future studies such as the development of predictive models and further investigations of yet-to known genetic abnormalities, including variants of uncertain significance.

This is the first genetic analysis study conducted on Korean patients with CAKUT using TES. We showed that TES could provide a cost-effective means for detecting both SNV and CNV for the genetic diagnosis of CAKUT. However, only one-seventh of the patients were found to have pathogenic mutations of known CAKUT-related genes, indicating that there are still more CAKUT-inducing genes or environmental factors to discover. Nonetheless, the identified mutations are important, enabling us to predict outcomes and provide proactive care and adequate genetic counseling for patients and families with CAKUT.

References

1. Collaboration GBDCKD. Global, regional, and national burden of chronic kidney disease, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet. 2020;395(10225):709-33.

2. Harambat J, van Stralen KJ, Kim JJ, Tizard EJ. Epidemiology of chronic kidney disease in children. Pediatric nephrology. 2012;27(3):363-73.

3. Kang HG, Choi HJ, Han KH, Kim SH, Cho HY, Cho MH, et al. KNOW-Ped CKD (KoreaN cohort study for outcomes in patients with pediatric CKD): Design and methods. BMC Nephrol. 2016;17:35.

4. Fathallah-Shaykh SA, Flynn JT, Pierce CB, Abraham AG, Blydt-Hansen TD, Massengill SF, et al. Progression of pediatric CKD of nonglomerular origin in the CKiD cohort. Clin J Am Soc Nephrol. 2015;10(4):571-7.

5. Groothoff JW. Long-term outcomes of children with end-stage renal disease. Pediatric nephrology. 2005;20(7):849-53.

6. Baek HS, Kang HG, Choi HJ, Cheong HI, Ha IS, Han KH, et al. Health-related quality of life of children with pre-dialysis chronic kidney disease. Pediatric nephrology. 2017;32(11):2097-105.

7. Kang NR, Ahn YH, Park E, Choi HJ, Kim SH, Cho H, et al. Mental health and psychosocial adjustment in pediatric chronic kidney disease derived from the KNOW-Ped CKD study. Pediatric nephrology. 2019.

8. Chang HJ, Han KH, Cho MH, Park YS, Kang HG, Cheong HI, et al. Outcomes of chronic dialysis in Korean children with respect to survival rates and causes of death. Korean J Pediatr. 2014;57(3):135-9.

9. Nicolaou N, Renkema KY, Bongers EM, Giles RH, Knoers NV. Genetic, environmental, and epigenetic factors involved in CAKUT. Nat Rev Nephrol. 2015;11(12):720-31.

10. Murugapoopathy V, Gupta IR. A Primer on Congenital Anomalies of the Kidneys and Urinary Tracts (CAKUT). Clin J Am Soc Nephrol. 2020;15(5):723-31.

11. Sanyanusin P, Schimmenti LA, McNoe LA, Ward TA, Pierpont ME, Sullivan MJ, et al. Mutation of the PAX2 gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux. Nature genetics. 1995;9(4):358-64.

12. Sanna-Cherchi S, Westland R, Ghiggeri GM, Gharavi AG. Genetic basis of human congenital anomalies of the kidney and urinary tract. The Journal of clinical investigation. 2018;128(1):4-15.

13. van der Ven AT, Vivante A, Hildebrandt F. Novel Insights into the Pathogenesis of Monogenic Congenital Anomalies of the Kidney and Urinary Tract. Journal of the American Society of Nephrology : JASN. 2018;29(1):36-50.

14. Vivante A, Kohl S, Hwang DY, Dworschak GC, Hildebrandt F. Single-gene causes of congenital anomalies of the kidney and urinary tract (CAKUT) in humans. Pediatric nephrology. 2014;29(4):695-704.

15. Sanna-Cherchi S, Kiryluk K, Burgess KE, Bodria M, Sampson MG, Hadley D, et al. Copy-number disorders are a common cause of congenital kidney malformations. American journal of human genetics. 2012;91(6):987-97.

16. Verbitsky M, Sanna-Cherchi S, Fasel DA, Levy B, Kiryluk K, Wuttke M, et al. Genomic imbalances in pediatric patients with chronic kidney disease. The Journal of clinical investigation. 2015;125(5):2171-8.

17. Westland R, Verbitsky M, Vukojevic K, Perry BJ, Fasel DA, Zwijnenburg PJ, et al. Copy number variation analysis identifies novel CAKUT candidate genes in children with a solitary functioning kidney. Kidney international. 2015.

18. van der Ven AT, Connaughton DM, Ityel H, Mann N, Nakayama M, Chen J, et al. Whole-Exome Sequencing Identifies Causative Mutations in Families with Congenital Anomalies of the Kidney and Urinary Tract. Journal of the American Society of Nephrology : JASN. 2018;29(9):2348-61.

19. Bekheirnia MR, Bekheirnia N, Bainbridge MN, Gu S, Coban Akdemir ZH, Gambin T, et al. Whole-exome sequencing in the molecular diagnosis of individuals with congenital anomalies of the kidney and urinary tract and identification of a new causative gene. Genetics in medicine : official journal of the American College of Medical Genetics. 2017;19(4):412-20.

20. Nicolaou N, Pulit SL, Nijman IJ, Monroe GR, Feitz WF, Schreuder MF, et al. Prioritization and burden analysis of rare variants in 208 candidate genes suggest they do not play a major role in CAKUT. Kidney international. 2016;89(2):476-86.

21. Hwang DY, Dworschak GC, Kohl S, Saisawat P, Vivante A, Hilger AC, et al. Mutations in 12 known dominant disease-causing genes clarify many congenital anomalies of the kidney and urinary tract. Kidney international. 2014;85(6):1429-33.

22. Caruana G, Wong MN, Walker A, Heloury Y, Webb N, Johnstone L, et al. Copy-number variation associated with congenital anomalies of the kidney and urinary tract. Pediatric nephrology. 2015;30(3):487-95.

23. Weber S, Landwehr C, Renkert M, Hoischen A, Wuhl E, Denecke J, et al. Mapping candidate regions and genes for congenital anomalies of the kidneys and urinary tract (CAKUT) by array-based comparative genomic hybridization. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2011;26(1):136-43.

24. Ishiwa S, Sato M, Morisada N, Nishi K, Kanamori T, Okutsu M, et al. Association between the clinical presentation of congenital anomalies of the kidney and urinary tract (CAKUT) and gene mutations: an analysis of 66 patients at a single institution. Pediatric nephrology. 2019;34(8):1457-64.

25. Lei TY, Fu F, Li R, Wang D, Wang RY, Jing XY, et al. Whole-exome sequencing for prenatal diagnosis of fetuses with congenital anomalies of the kidney and urinary tract. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2017;32(10):1665-75.

26. Youn J, Lee C, Oh E, Park J, Kim JS, Kim HT, et al. Genetic variants of PARK genes in Korean patients with early-onset Parkinson's disease. Neurobiology of aging. 2019;75:224 e9- e15.

27. McKenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome research. 2010;20(9):1297-303.

28. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics. 2009;25(16):2078-9.

29. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics. 2009;25(14):1754-60.

30. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38(16):e164.

31. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in medicine : official journal of the American College of Medical Genetics. 2015;17(5):405-24.

32. Gribouval O, Moriniere V, Pawtowski A, Arrondel C, Sallinen SL, Saloranta C, et al. Spectrum of mutations in the renin-angiotensin system genes in autosomal recessive renal tubular dysgenesis. Human mutation. 2012;33(2):316-26.

33. Oh SP, Li E. The signaling pathway mediated by the type IIB activin receptor controls axial patterning and lateral asymmetry in the mouse. Genes Dev. 1997;11(14):1812-26.

34. Nishimura H, Yerkes E, Hohenfellner K, Miyazaki Y, Ma J, Hunley TE, et al. Role of the angiotensin type 2 receptor gene in congenital anomalies of the kidney and urinary tract, CAKUT, of mice and men. Molecular cell. 1999;3(1):1-10.

35. Kraus MR, Clauin S, Pfister Y, Di Maio M, Ulinski T, Constam D, et al. Two mutations in human BICC1 resulting in Wnt pathway hyperactivity associated with cystic renal dysplasia. Human mutation. 2012;33(1):86-90.

36. Weber S, Taylor JC, Winyard P, Baker KF, Sullivan-Brown J, Schild R, et al. SIX2 and BMP4 mutations associate with anomalous kidney development. Journal of the American Society of Nephrology : JASN. 2008;19(5):891-903.

37. Dudley AT, Lyons KM, Robertson EJ. A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. Genes Dev. 1995;9(22):2795-807.

38. Groenen PM, Vanderlinden G, Devriendt K, Fryns JP, Van de Ven WJ. Rearrangement of the human CDC5L gene by a t(6;19)(p21;q13.1) in a patient with multicystic renal dysplasia. Genomics. 1998;49(2):218-29.

39. Brockschmidt A, Chung B, Weber S, Fischer DC, Kolatsi-Joannou M, Christ L, et al. CHD1L: a new candidate gene for congenital anomalies of the kidneys and urinary tract (CAKUT). Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2012;27(6):2355-64.

40. Suzuki K, Haraguchi R, Ogata T, Barbieri O, Alegria O, Vieux-Rochas M, et al. Abnormal urethra formation in mouse models of split-hand/split-foot malformation type 1 and type 4. European journal of human genetics : EJHG. 2008;16(1):36-44.

41. Sanna-Cherchi S, Sampogna RV, Papeta N, Burgess KE, Nees SN, Perry BJ, et al. Mutations in DSTYK and dominant urinary tract malformations. The New England journal of medicine. 2013;369(7):621-9.

42. Boualia SK, Gaitan Y, Murawski I, Nadon R, Gupta IR, Bouchard M. Vesicoureteral reflux and other urinary tract malformations in mice compound heterozygous for Pax2 and Emx2. PloS one. 2011;6(6):e21529.

43. Orten DJ, Fischer SM, Sorensen JL, Radhakrishna U, Cremers CW, Marres HA, et al. Branchio-oto-renal syndrome (BOR): novel mutations in the EYA1 gene, and a review of the mutational genetics of BOR. Human mutation. 2008;29(4):537-44.

44. Weber S, Moriniere V, Knuppel T, Charbit M, Dusek J, Ghiggeri GM, et al. Prevalence of mutations in renal developmental genes in children with renal hypodysplasia: results of the ESCAPE study. Journal of the American Society of Nephrology : JASN. 2006;17(10):2864-70.

45. Barak H, Huh SH, Chen S, Jeanpierre C, Martinovic J, Parisot M, et al. FGF9 and FGF20 maintain the stemness of nephron progenitors in mice and man. Dev Cell. 2012;22(6):1191-207.

46. Nakano T, Niimura F, Hohenfellner K, Miyakita E, Ichikawa I. Screening for mutations in BMP4 and FOXC1 genes in congenital anomalies of the kidney and urinary tract in humans. The Tokai journal of experimental and clinical medicine. 2003;28(3):121-6.

47. van Haelst MM, Maiburg M, Baujat G, Jadeja S, Monti E, Bland E, et al. Molecular study of 33 families with Fraser syndrome new data and mutation review. American journal of medical genetics Part A. 2008;146A(17):2252-7.

48. Kohl S, Hwang DY, Dworschak GC, Hilger AC, Saisawat P, Vivante A, et al. Mild recessive mutations in six Fraser syndrome-related genes cause isolated congenital anomalies of the kidney and urinary tract. Journal of the American Society of Nephrology : JASN. 2014;25(9):1917-22.

49. Saisawat P, Tasic V, Vega-Warner V, Kehinde EO, Gunther B, Airik R, et al. Identification of two novel CAKUT-causing genes by massively parallel exon

resequencing of candidate genes in patients with unilateral renal agenesis. Kidney international. 2012;81(2):196-200.

50. Ali A, Christie PT, Grigorieva IV, Harding B, Van Esch H, Ahmed SF, et al. Functional characterization of GATA3 mutations causing the hypoparathyroidism-deafness-renal (HDR) dysplasia syndrome: insight into mechanisms of DNA binding by the GATA3 transcription factor. Human molecular genetics. 2007;16(3):265-75.

51. Skinner MA, Safford SD, Reeves JG, Jackson ME, Freemerman AJ. Renal aplasia in humans is associated with RET mutations. American journal of human genetics. 2008;82(2):344-51.

52. Chatterjee R, Ramos E, Hoffman M, VanWinkle J, Martin DR, Davis TK, et al. Traditional and targeted exome sequencing reveals common, rare and novel functional deleterious variants in RET-signaling complex in a cohort of living US patients with urinary tract malformations. Human genetics. 2012;131(11):1725-38.

53. Clissold RL, Hamilton AJ, Hattersley AT, Ellard S, Bingham C. HNF1B-associated renal and extra-renal disease-an expanding clinical spectrum. Nat Rev Nephrol. 2015;11(2):102-12.

54. Wellik DM, Hawkes PJ, Capecchi MR. Hox11 paralogous genes are essential for metanephric kidney induction. Genes Dev. 2002;16(11):1423-32.

55. Albuisson J, Pecheux C, Carel JC, Lacombe D, Leheup B, Lapuzina P, et al. Kallmann syndrome: 14 novel mutations in KAL1 and FGFR1 (KAL2). Human mutation. 2005;25(1):98-9.

56. Djuric T, Zivkovic M, Milosevic B, Andjelevski M, Cvetkovic M, Kostic M, et al. MMP-1 and -3 haplotype is associated with congenital anomalies of the kidney and urinary tract. Pediatric nephrology. 2014;29(5):879-84.

57. Kirby A, Gnirke A, Jaffe DB, Baresova V, Pochet N, Blumenstiel B, et al. Mutations causing medullary cystic kidney disease type 1 lie in a large VNTR in MUC1 missed by massively parallel sequencing. Nature genetics. 2013;45(3):299-303.

58. Kamath BM, Bauer RC, Loomes KM, Chao G, Gerfen J, Hutchinson A, et al. NOTCH2 mutations in Alagille syndrome. Journal of medical genetics. 2012;49(2):138-44.

59. Narumi Y, Min BJ, Shimizu K, Kazukawa I, Sameshima K, Nakamura K, et al. Clinical consequences in truncating mutations in exon 34 of NOTCH2: report of six patients with Hajdu-Cheney syndrome and a patient with serpentine fibula polycystic kidney syndrome. American journal of medical genetics Part A. 2013;161A(3):518-26.

60. Bower M, Salomon R, Allanson J, Antignac C, Benedicenti F, Benetti E, et al. Update of PAX2 mutations in renal coloboma syndrome and establishment of a locus-specific database. Human mutation. 2012;33(3):457-66.

61. Rossetti S, Consugar MB, Chapman AB, Torres VE, Guay-Woodford LM, Grantham JJ, et al. Comprehensive molecular diagnostics in autosomal dominant polycystic kidney disease. Journal of the American Society of Nephrology : JASN. 2007;18(7):2143-60.

62. Ward CJ, Hogan MC, Rossetti S, Walker D, Sneddon T, Wang X, et al. The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor-like protein. Nature genetics. 2002;30(3):259-69.

63. Bertoli-Avella AM, Conte ML, Punzo F, de Graaf BM, Lama G, La Manna A, et al. ROBO2 gene variants are associated with familial vesicoureteral reflux. Journal of the American Society of Nephrology : JASN. 2008;19(4):825-31.

64. Lu W, van Eerde AM, Fan X, Quintero-Rivera F, Kulkarni S, Ferguson H, et al. Disruption of ROBO2 is associated with urinary tract anomalies and confers risk of vesicoureteral reflux. American journal of human genetics. 2007;80(4):616-32.

65. Krug P, Moriniere V, Marlin S, Koubi V, Gabriel HD, Colin E, et al. Mutation screening of the EYA1, SIX1, and SIX5 genes in a large cohort of patients harboring branchio-oto-renal syndrome calls into question the pathogenic role of SIX5 mutations. Human mutation. 2011;32(2):183-90.

66. Hoskins BE, Cramer CH, Silvius D, Zou D, Raymond RM, Orten DJ, et al. Transcription factor SIX5 is mutated in patients with branchio-oto-renal syndrome. American journal of human genetics. 2007;80(4):800-4.

67. Hwang DY, Kohl S, Fan X, Vivante A, Chan S, Dworschak GC, et al. Mutations of the SLIT2-ROBO2 pathway genes SLIT2 and SRGAP1 confer risk for congenital anomalies of the kidney and urinary tract. Human genetics. 2015;134(8):905-16.

68. Gimelli S, Caridi G, Beri S, McCracken K, Bocciardi R, Zordan P, et al. Mutations in SOX17 are associated with congenital anomalies of the kidney and the urinary tract. Human mutation. 2010;31(12):1352-9.

69. Vivante A, Kleppa MJ, Schulz J, Kohl S, Sharma A, Chen J, et al. Mutations in TBX18 Cause Dominant Urinary Tract Malformations via Transcriptional Dysregulation of Ureter Development. American journal of human genetics. 2015;97(2):291-301.

70. Li H, Sheridan R, Williams T. Analysis of TFAP2A mutations in Branchio-Oculo-Facial Syndrome indicates functional complexity within the AP-2alpha DNA-binding domain. Human molecular genetics. 2013;22(16):3195-206.

71. Gbadegesin RA, Brophy PD, Adeyemo A, Hall G, Gupta IR, Hains D, et al. TNXB mutations can cause vesicoureteral reflux. Journal of the American Society of Nephrology : JASN. 2013;24(8):1313-22.

72. Saisawat P, Kohl S, Hilger AC, Hwang DY, Yung Gee H, Dworschak GC, et al. Whole-exome resequencing reveals recessive mutations in TRAP1 in individuals with CAKUT and VACTERL association. Kidney international. 2014;85(6):1310-7.

73. Hernandez O, Way S, McKenna J, 3rd, Gambello MJ. Generation of a conditional disruption of the Tsc2 gene. Genesis. 2007;45(2):101-6.

74. Hart TC, Gorry MC, Hart PS, Woodard AS, Shihabi Z, Sandhu J, et al. Mutations of the UMOD gene are responsible for medullary cystic kidney disease 2 and familial juvenile hyperuricaemic nephropathy. Journal of medical genetics. 2002;39(12):882-92.

75. Jenkins D, Bitner-Glindzicz M, Malcolm S, Hu CC, Allison J, Winyard PJ, et al. De novo Uroplakin IIIa heterozygous mutations cause human renal adysplasia leading to severe kidney failure. Journal of the American Society of Nephrology : JASN. 2005;16(7):2141-9.

76. Groenen PM, Garcia E, Debeer P, Devriendt K, Fryns JP, Van de Ven WJ. Structure, sequence, and chromosome 19 localization of human USF2 and its rearrangement in a patient with multicystic renal dysplasia. Genomics. 1996;38(2):141-8.

77. Vivante A, Mark-Danieli M, Davidovits M, Harari-Steinberg O, Omer D, Gnatek Y, et al. Renal hypodysplasia associates with a WNT4 variant that causes aberrant canonical WNT signaling. Journal of the American Society of Nephrology : JASN. 2013;24(4):550-8.

78. Jeanpierre C, Denamur E, Henry I, Cabanis MO, Luce S, Cecille A, et al. Identification of constitutional WT1 mutations, in patients with isolated diffuse mesangial sclerosis, and analysis of genotype/phenotype correlations by use of a computerized mutation database. American journal of human genetics. 1998;62(4):824-33.

79. O'Toole JF, Liu Y, Davis EE, Westlake CJ, Attanasio M, Otto EA, et al. Individuals with mutations in XPNPEP3, which encodes a mitochondrial protein, develop a nephronophthisis-like nephropathy. The Journal of clinical investigation. 2010;120(3):791-802.

80. Bellanne-Chantelot C, Chauveau D, Gautier JF, Dubois-Laforgue D, Clauin S, Beaufils S, et al. Clinical spectrum associated with hepatocyte nuclear factor-1beta mutations. Ann Intern Med. 2004;140(7):510-7.

81. Brophy PD, Alasti F, Darbro BW, Clarke J, Nishimura C, Cobb B, et al. Genome-wide copy number variation analysis of a Branchio-oto-renal syndrome cohort identifies a recombination hotspot and implicates new candidate genes. Human genetics. 2013;132(12):1339-50.

82. Vivante A, Hildebrandt F. Exploring the genetic basis of early-onset chronic kidney disease. Nat Rev Nephrol. 2016;12(3):133-46.

83. Sanna-Cherchi S, Ravani P, Corbani V, Parodi S, Haupt R, Piaggio G, et al. Renal outcome in patients with congenital anomalies of the kidney and urinary tract. Kidney international. 2009;76(5):528-33.

84. Thomas R, Sanna-Cherchi S, Warady BA, Furth SL, Kaskel FJ, Gharavi AG. HNF1B and PAX2 mutations are a common cause of renal hypodysplasia in the CKiD cohort. Pediatric nephrology. 2011;26(6):897-903.

85. Mefford HC, Clauin S, Sharp AJ, Moller RS, Ullmann R, Kapur R, et al. Recurrent reciprocal genomic rearrangements of 17q12 are associated with renal disease, diabetes, and epilepsy. American journal of human genetics. 2007;81(5):1057-69.

86. Sharp AJ, Hansen S, Selzer RR, Cheng Z, Regan R, Hurst JA, et al. Discovery of previously unidentified genomic disorders from the duplication architecture of the human genome. Nature genetics. 2006;38(9):1038-42.

87. El-Khairi R, Vallier L. The role of hepatocyte nuclear factor 1beta in disease and development. Diabetes Obes Metab. 2016;18 Suppl 1:23-32.

88. Massa F, Garbay S, Bouvier R, Sugitani Y, Noda T, Gubler MC, et al. Hepatocyte nuclear factor 1beta controls nephron tubular development. Development. 2013;140(4):886-96.

89. Lokmane L, Heliot C, Garcia-Villalba P, Fabre M, Cereghini S. vHNF1 functions in distinct regulatory circuits to control ureteric bud branching and early nephrogenesis. Development. 2010;137(2):347-57.

90. Rasmussen M, Vestergaard EM, Graakjaer J, Petkov Y, Bache I, Fagerberg C, et al. 17q12 deletion and duplication syndrome in Denmark-A clinical cohort of 38 patients and review of the literature. American journal of medical genetics Part A. 2016;170(11):2934-42.

91. Nagamani SC, Erez A, Shen J, Li C, Roeder E, Cox S, et al. Clinical spectrum associated with recurrent genomic rearrangements in chromosome 17q12. European journal of human genetics : EJHG. 2010;18(3):278-84.

92. Moreno-De-Luca D, Consortium S, Mulle JG, Simons Simplex Collection Genetics C, Kaminsky EB, Sanders SJ, et al. Deletion 17q12 is a recurrent copy number variant that confers high risk of autism and schizophrenia. American journal of human genetics. 2010;87(5):618-30.

93. Porteous S, Torban E, Cho NP, Cunliffe H, Chua L, McNoe L, et al. Primary renal hypoplasia in humans and mice with PAX2 mutations: evidence of increased apoptosis in fetal kidneys of Pax2(1Neu) +/- mutant mice. Human molecular genetics. 2000;9(1):1-11.

94. Dziarmaga A, Clark P, Stayner C, Julien JP, Torban E, Goodyer P, et al. Ureteric bud apoptosis and renal hypoplasia in transgenic PAX2-Bax fetal mice mimics the renal-coloboma syndrome. Journal of the American Society of Nephrology : JASN. 2003;14(11):2767-74.

95. Dziarmaga A, Eccles M, Goodyer P. Suppression of ureteric bud apoptosis rescues nephron endowment and adult renal function in Pax2 mutant mice. Journal of the American Society of Nephrology : JASN. 2006;17(6):1568-75.

96. Nornes HO, Dressler GR, Knapik EW, Deutsch U, Gruss P. Spatially and temporally restricted expression of Pax2 during murine neurogenesis. Development. 1990;109(4):797-809.

97. Li X, Oghi KA, Zhang J, Krones A, Bush KT, Glass CK, et al. Eya protein phosphatase activity regulates Six1-Dach-Eya transcriptional effects in mammalian organogenesis. Nature. 2003;426(6964):247-54.

98. Kong XT, Deng FM, Hu P, Liang FX, Zhou G, Auerbach AB, et al. Roles of uroplakins in plaque formation, umbrella cell enlargement, and urinary tract diseases. J Cell Biol. 2004;167(6):1195-204.

99. McGregor L, Makela V, Darling SM, Vrontou S, Chalepakis G, Roberts C, et al. Fraser syndrome and mouse blebbed phenotype caused by mutations in FRAS1/Fras1 encoding a putative extracellular matrix protein. Nature genetics. 2003;34(2):203-8.

100. Hsu CW, Yamamoto KT, Henry RK, De Roos AJ, Flynn JT. Prenatal risk factors for childhood CKD. Journal of the American Society of Nephrology : JASN. 2014;25(9):2105-11.

101. Parikh CR, McCall D, Engelman C, Schrier RW. Congenital renal agenesis: case-control analysis of birth characteristics. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2002;39(4):689-94.

102. Yu Y, Zuo X, He M, Gao J, Fu Y, Qin C, et al. Genome-wide analyses of non-syndromic cleft lip with palate identify 14 novel loci and genetic heterogeneity. Nat Commun. 2017;8:14364.

103. Cordell HJ, Bentham J, Topf A, Zelenika D, Heath S, Mamasoula C, et al. Genome-wide association study of multiple congenital heart disease phenotypes identifies a susceptibility locus for atrial septal defect at chromosome 4p16. Nature genetics. 2013;45(7):822-4.

국문초록

선천성 신 요로 기형은 소아 만성신질환의 가장 흔한 원인으로 서구 국가에서 약 5-20%에서 유전적 원인이 밝혀졌으나 한국인에서 이에 관한 연구가 없었다. 본 연구는 표적유전자 염기서열분석을 통해 한국 소아 선천성 신 요로 기형 환자에서 유전적 원인을 찾고자 하였다. 문헌 검색을 통해 선천성 신 요로 기형의 원인 유전자로 알려진 60 개의 표적유전자를 선정하였다. 영상검사를 통해 선천성 신 요로 기형으로 진단된 환자 94명을 대상으로 표적유전자 염기서열분석을 시행하여 단일염기변이와 복제수 변이를 확인하였다. 표적유전자 염기서열분석을 통해 밝혀진 단일염기변이와 복제수 변이는 각각 생거 염기서열분석과 배열 비교 유전체 혼성화법으로 확인하였다. 94명의 환자 중 13명 (13.8%)에서 유전적 원인을 확인하였다. 7명의 환자에서는 *HNF1B*, *PAX2*, *EYA1*, *UPK3A*, *FRAS1* 유전자의 단일염기변이가 확인되었고 6명의 환자에서는 *HNF1B*, *EYA1*, *CHD1L* 유전자를 포함한 복제수 변이가 확인되었다. *HNF1B* 유전자 변이가 가장 흔했고 해당 유전자 변이를 가지고 있는 환자는 다양한 형태의 선천성 신 요로 기형과 신장 외 질환을 동반하였다. 유전자 이상의 종류와 선천성 신 요로 기형의 표현형은 연관성이 없었다. 증후군성 선천성 신 요로 기형 환자에서는 유전적 이상이 빈번하였다 (P <0.001). 유전자 이상 여부에 따른 신기능 생존에 차이는 없었다 (Log-rank test, P =0.280). 본 연구는 선천성 신 요로

기형 한국인 환자에서 유전자 이상을 확인한 최초의 연구이다. 선천성 신 요로 기형의 유전자 진단에서 표적유전자 염기서열분석이 단일염기변이와 복제수 변이를 모두 검출하는데 효과적인 방법일 수 있다. 선천성 신 요로 기형 관련 유전자 검사에서 환자의 7분의 1만이 병원성 변이를 갖고 있는 것으로 확인되었기 때문에 향후 밝혀지지 않은 다른 유전자 이상 또는 환경적 요인의 영향을 밝히기 위한 추가적인 연구가 향후 필요하다.

주요어: 선천성 신 요로 기형, 단일염기변이, 복제수 변이

학 번: 2015 – 30009

감사의 글

박사학위 과정을 통해 존경하는 정해일 선생님, 하일수 선생님, 강희경 선생님께 배울 수 있어서 영광이었습니다. 항상 올바른 가르침으로 저에게 큰 깨달음을 주셨고 지치지 않도록 격려해주셔서 학위논문을 완성할 수 있었습니다. 저의 스승이신 선생님들께 감사 드립니다.

박사학위 논문심사를 맡아주신 주권욱 선생님, 고정민 선생님, 임형은 선생님께도 감사 드립니다. 논문심사 기간 동안 많은 조언을 통해 학위논문의 의미를 깊이 깨닫게 해주셨고 좀더 의미 있는 논문으로 완성할 수 있도록 해주셨습니다.

학위를 지속할 수 있도록 격려해주고 응원해준 아내와 아이들에게 고마운 마음을 전합니다. 가족 모두를 마음 깊이 사랑합니다.

This research was supported by a grant of the Korea Health Technology Research & Development Project through the Korea Health Industry Development Institute, funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI18C0013).