Clinical application of next-generation sequencing for the diagnosis of suspected renal disease

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Clinical application of next-generation sequencing for the diagnosis of suspected renal disease

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Abstract

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Background: Recent development of genetic diagnosis, including next-generation sequencing (NGS) evolved spectacularly, it has broadened diagnostic opportunities for unrecognized diseases as providing single-step analysis for the targeted numerous genes simultaneously. In many of renal disorders, clinical symptoms or laboratory findings are nonspecific for the diagnosis even after a renal biopsy that can hamper treatment modality. Understanding genetic causes of undefined clinical phenotypes and heterogenous symptoms can be helpful to determine therapeutic strategies and improving prognosis of the diseases. To identify the genetic background of renal disorders, NGS panel was designed and tested to patients with non-specific

nephrogenic symptoms to confirm the diagnosis and to evaluate the efficacy of NGS panel test as a diagnostic tool.

Methods: In total, 30 patients with suspected inherited renal disease were tested using a NGS panel including 167 genes those were known to be associated with kidney disease, as well as diseases originating in other organs that may present with common symptoms of kidney disease.

Results: Thirty patients underwent NGS panel test due to different reasons such as urinalysis abnormalities, cystic kidney disease detected through imaging studies, steroid-resistant nephrotic syndrome, renal failure and electrolyte imbalance with/without metabolic acidosis. We detected 16 pathogenic or likely pathogenic variants of 14 different genes in nine patients and 142 variants of unknown significance (VUS) of 70 genes in all patients. Final molecular diagnostic rate in the study group was shown to be 46.7 % (14/30) and they were diagnosed as follows; 5 patients with electrolyte imbalance were including Bartter syndrome(2), Gitelman's syndrome(2) and chloride secreting diarrhea(1) and 5 patients with isolated hematuria or hematuria and proteinuria were all diagnosed as Alport syndrome. Five patients who referred with a cystic kidney were 4 cases of the autosomal dominant polycystic kidney (ADPKD) and 1q36 deletion syndrome (1). Ten patients (33.3%) were clearly matched between the initial clinical impression and molecular diagnosis.

Conclusions: Based on the results of this study, NGS panel testing showed the

possibility as a comprehensive and fast diagnostic tool for differential genetic diagnosis of various kidney diseases. Especially, this testing method is very advantageous for noninvasive in-depth diagnosis even when compared with renal biopsy. Accurate and comprehensive interpretation followed by extensive NGS analysis, including CNV detection, will help to increase the diagnostic yield of this technique.

Keywords: Next-generation sequencing (NGS), Copy number variation (CNV), kidney disease, renal disease, gene test

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I. Introduction

Inherited renal disorder represents a heterogeneous group of diseases, including monogenic disorders such as autosomal dominant/recessive polycystic kidney disease (ADPKD/ARPKD) as well as complex genetic disorders such as steroid resistance nephrotic syndrome (SRNS), Alport syndrome, and congenital anomalies of the kidney and urinary tract (CAKUT) [1]. Ten percent of adults and almost all children with inherited kidney diseases are receiving renal replacement therapy. To improve patient outcomes, quick and accurate diagnosis is desirable, but this can be difficult due to its non-specific and overlapping symptoms of kidney diseases. Additionally, some diseases originating from other organs can present with

symptoms common in kidney disease, including electrolyte imbalance and metabolic acidosis or alkalosis. Renal cysts can also present in various multi-systemic diseases such as tuberous sclerosis complex, oral-facial-digital syndrome, and coloboma syndrome [2,3]. Therefore, it can be challenging to diagnose the precise underlying cause of nephrogenic symptoms of the disease by using conventional laboratory and imaging diagnostic tools. An invasive procedure involving renal biopsy could be performed for identifying underlying etiology of a disease, but it is limited in the range of conditions it can successfully confirm, and it carries a risk of complications [4-7]. Moreover, the histologic diagnosis could only be made in limited cases.

Genetic testing is one of the most useful diagnostic tools for identifying the cause of such diseases. However, many of genes and non-specific symptoms with genetic heterogeneity involved in heritable kidney diseases make the diagnosis difficult, and examination of selected genes using Sanger sequencing-based technology has been costly and time-consuming. Next-generation sequencing (NGS) technique has integrated for diagnostic applications as well as research, enabling simultaneous detection of genetic variants in a large set of candidate genes at once. Several studies have reported the effectiveness of NGS for identifying various inherited kidney diseases, including glomerular nephropathy or cystic kidney disease [2,9-11]. However, previous studies have based their analyses only on well-known causative genes of inherited kidney disease and could not provide a differential diagnosis for diseases originating in other organs but may present with common symptoms of kidney

disease.

Here, we developed an NGS panel of 167 genes for the differential diagnosis of kidney diseases and diseases originating in other organs with overlapping symptoms of kidney disease. In order to validate the diagnostic efficacy of this panel, we investigated 30 patients with suspected inherited kidney disease who were referred to be evaluated possible genetic causes of different renal symptoms.

II. MATERIALS AND METHODS

1. Patient selection and study design

Thirty unrelated, genetically undiagnosed patients with suspected kidney disease were identified in a department of clinical genetics in Severance Children's Hospital from January 2017 to August 2018. All patients had one or more symptoms/signs of these; proteinuria, hematuria, electrolyte imbalance, metabolic alkalosis/acidosis, abnormal kidney imaging findings, or a combination of these. Pedigree information, previous medical history of each subject, physical examination findings, and any additional investigative results (e.g., ophthalmologic and otology examination) in patients' electronic medical records (EMR) in Severance Hospital were reviewed retrospectively. Although this information was collected under anonymity in a routine diagnostic process, the study protocol was approved by the Institutional Review Board of the Yonsei University Health System (IRB). Informed consent for genetic testing was

obtained from each patient or his or her parents if a subject was under 19 years old.

2. DNA preparation

Three milliliters of blood was collected in EDTA tubes from subjects, and genomic DNA was extracted from leukocytes using a DNeasy Blood and Tissue Kit (Qiagen, Hilden, DE) according to manufacturer's instructions. The DNA quality was checked using Nanodrop spectrophotometry (Thermo Fisher, Massachusetts).

3. Library preparation and sequencing

A DNA library was prepared using Nextera Rapid Capture Enrichment protocol according to manufacturer's instructions (TruSight One Sequencing Panel, FC-141-1007, Illumina, California). Briefly, DNA of each sample was fragmented into 250 bp sequences and tagged, then purified according to fragment size. Repair, phosphorylation, and adenylation were performed on the 3' ends. The pre-capture amplification of 300-500 bp fragments was isolated. Finally, targeted sequence capture was performed according to the manufacturer's instructions (TruSight One Sequencing Panel, FC-141-1007, Illumina, California). The DNA sequencing was conducted using a MiSeq sequencer (Illumina, USA) in paired-end 100 bp reads. The yield of each DNA sample gave an average of 2 GB raw data with a 150-fold mean sequencing depth of targeted regions [12,13]. The sequenced reads were mapped to the human reference (GRCh37) with Burrows-Wheeler Aligner (BWA), and variants were identified with the Genome Analysis Toolkit (GATK). Sequence variants were filtered according to

various quality parameters. Chromosomal copy number variations (CNVs) were detected using a robust model for the read count data to detect CNVs by building an optimized reference set, which considers technical variability well. This method is applied to whole-genome sequencing data sets to detect pathogenic CNVs and identified CNVs are confirmed though multiplex ligation-dependent probe amplification MLPA or real-time PCR.

4. Panel design

To develop an efficient and feasible NGS panel for molecular diagnosis, 167 candidate genes were manually optimized based on the Human Genome Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk), Online Mendelian Inheritance in Man (OMIM) database (http://www.ncbi.nlm.nih.gov/omim), and extensive literature review using PubMed. The list of genes included those associated with kidney disease, as well as diseases originating in other organs that may present with common symptoms of kidney disease (Table 1).

5. NGS data analysis and annotation/interpretation of variants

NGS data was interpreted using the HGMD, OMIM, dbSNP, ClinVar, Exome Aggregation Consortium (ExAC), and Korean Reference Genome Database (KRGDB) resources. Pathogenicity of detected variants was predicted using *in silico* prediction algorithms, including Polymorphism Phenotyping v2 (PolyPhen-2) and Sorting Tolerant from Intolerant (SIFT). Identified variants were reported by the Human

Genome Variation Society (http://www.hgvs.org/mutnomen), and were classified into a five-tier system as a pathogenic/likely pathogenic/variant of unknown significance (VUS)/likely benign/benign according to American College of Medical Genetics and Genomics (ACMG) guidelines. This analysis took 32.6 days (range: 29-34 days) on average from blood sample collection to final NGS result.

Table 1. Gene list for NGS kidney disease Panel

Gene	Cytogenic location	Inheritance	Accession number	Disease association
ACTN4	19q13.2	AD	NM_004924	Glomerulosclerosis, focal segmental, 1
ADAMTS13	9q34.2	AR	NM_139025	Thrombotic thrombocytopenic purpura, familial
AGTR1	3q24	AR	NM_000685	Renal tubular dysgenesis
AGXT	2q37.3	AR	NM_000030	Hyperoxaluria, primary, type 1
AHI1	6q23.3	AR	NM_017651	Joubert syndrome 3
ALG8	11q14.1	AR	NM_019109	Polycystic liver disease 3 with or without kidney cysts
ALMS1	2p13.1	AR	NM_015120	Alström syndrome, retinitis pigmentosa, sensorineural hearing loss
ANKS6	9q22.33	AR	NM_173551	Nephronophthisis 16
AP2S1	19q13.32	AD	NM_001301076	Hypocalciuric hypercalcemia, type III
APRT	16q24.3	AR	NM_000485	Adenine phosphoribosyltransferase deficiency
AQP2	12q13.12	AD/AR	NM_000486	Diabetes insipidus, nephrogenic
ARHGDIA	17q25.3	AR	NM_001185077	Nephrotic syndrome, type 8
ARL13B	3q11.1-q11.2	AR	NM_182896	Joubert syndrome 8
ATP6V0A4	7q34	AR	NM_020632	Renal tubular acidosis, distal, autosomal recessive
ATP6V1B1	2p13.3	AR	NM_001692	Renal tubular acidosis with deafness

Gene	Cytogenic location	Inheritance	Accession number	Disease association
AVPR2	Xq28	XLR	NM_000054	Diabetes insipidus, nephrogenic;
B9D2	19q13.2	AR	NM_030578	Joubert syndrome 34
BBS10	12q21.2	AR	NM_024685	Bardet-Biedl syndrome 10
BBS12	4q27	AR	NM_152618	Bardet-Biedl syndrome 12
BBS2	16q13	AR	NM_031885	Bardet-Biedl syndrome 2
BBS4	15q24.1	AR	NM_033028	Bardet-Biedl syndrome 4
BBS4	15q24.1	AR	NM_033028	Bardet-Biedl syndrome 4
BBS9	7p14.3	AR	NM_001033604	Bardet-Biedl syndrome 9
BCS1L	2q35	AR	NM_004328	Mitochondrial complex III deficiency, nuclear type 1
BICC1	10q21.1	AD	NM_025044	Renal dysplasia, cystic, susceptibility to
BSND	1p32.3	AR	NM_057176	Bartter syndrome, type 4a;
CA2	8q21.2	AR	NM_000067	Osteopetrosis, autosomal recessive 3, with renal tubular acidosis
CASR	3q13.3-q21.1	AD	NM_000388	Hypocalcemia, autosomal dominant, with Bartter syndrome
CC2D2A	4p15.32	AR	NM_001080522	Joubert syndrome 9
CD151	11p15.5	AR	NM_004357	Nephropathy with pretibial epidermolysis bullosa and deafness
CD2AP	6p12.3	AD/AR	NM_012120	Glomerulosclerosis, focal segmental, 3
CEP164	11q23.3	AR	NM_014956	Nephronophthisis 15

Gene	Cytogenic location	Inheritance	Accession number	Disease association
CEP290	12q21.32	AR	NM_025114	Bardet-Biedl syndrome 14; Joubert syndrome 5
CEP41	7q32.2	AR	NM_018718	Joubert syndrome 15
CFH	1q31.3	AD/AR	NM_000186	Hemolytic uremic syndrome, atypical, susceptibility to, 1
CFHR5	1q31.3	AD	NM_030787	Nephropathy due to CFHR5 deficiency
CLCN5	Xp11.23	XLR	NM_000084	Dent disease; Nephrolithiasis, type I; Proteinuria, low molecular weight, with hypercalciuric nephrocalcinosis
CLCNKB	1p36.13	AR DR	NM_000085	Bartter syndrome, type 3 Bartter syndrome, type 4b, digenic
CLDN16	3q28	AR	NM_006580	Hypomagnesemia 3, renal
CLDN19	1p34.2	AR	NM_148960	Hypomagnesemia 5, renal, with ocular involvement
CNNM2	10q24.32	AD	NM_017649	Hypomagnesemia 6, renal
COL4A1	13q34	AD	NM_001303110	Angiopathy, hereditary, with nephropathy, aneurysms, and muscle cramps
COL4A3	2q36.3	AD/AR	NM_012120	Alport syndrome
COL4A4	2q36.3	AR	NM_000091	Alport syndrome, familiar hematuria
COL4A5	Xq22.3	X-linked	NM_000092	Alport syndrome
COQ2	4q21.22-q21.23	AR	NM_015697	Mitochondrial disease, encephalopathy/isolated nephropathy
COQ6	14q24.3	AR	NM_182476	Nephrotic syndrome ± sensorineural deafness
CTNS	17p13.2	AR	NM_004937	Cystinosis, nephropathic

Gene	Cytogenic location	Inheritance	Accession number	Disease association
CUBN	10p13	AR	NM_001081	Imerslund-Grasbeck syndrome
CYP11B2	3q24.3	AR	NM-000498	Hypoaldosteronism, congenital, due to CMO I deficiency
DGKE	17q22	AR	NM_003647	Nephrotic syndrome, type 7
DMP1	4q22.1	AR	NM_001079911	Hypophosphatemic rickets
EGF	10p13	AR	NM_001178130	Hypomagnesemia 4, renal
EHHADH	3q27.2	AD	NM_001166415	Fanconi renotubular syndrome 3
EYA1	8q13.3	AD	NM_000503	Branchiootorenal syndrome 1, with or without cataracts
FAM58A	Xq28	XLD	NM_152274	STAR syndrome
FAN1	15q13.3	AR	NM_014967	Interstitial nephritis, karyomegalic
FGF23	12p13.32	AD	NM_020638	Hypophosphatemic rickets
FN1	2q35	AD	NM_212476	Glomerulopathy with fibronectin deposits 2
FRAS1	4q21.21	AR	NM_001166133	Fraser syndrome 1
FREM1	9p22.3	AD/AR	NM_144966	Bifid nose with or without anorectal and renal anomalies
FREM2	13q13.3	AR	NM_207361	Fraser syndrome 2
FXYD2	11q23.3	AD	NM_021603	Hypomagnesemia 2, renal
GATA3	10p14	AD	NM_001002295	Hypoparathyroidism, sensorineural deafness, and renal dysplasia
GLA	Xq22.1	XLR	NM_000169	Fabry disease

Gene	Cytogenic location	Inheritance	Accession number	Disease association
GLB1	3p22.3	AR	NM_000404	Mucopolysaccharidosis type IVB (Morquio)
GLIS2	16p13.3	AR	NM_032575	Nephronophthisis 7
GLIS3	9p24.2	AR	NM_152629	Diabetes mellitus, neonatal
GNA11	19p13.3	AD	NM_002067	Hypocalciuric hypercalcemia, type II
HNF1B	17q12	AD	NM_000458	Renal cysts and diabetes syndrome
HPRT1	Xq26.2-q26.3	XLR	NM_000194	HPRT-related gout, Lesch-Nyhan syndrome
HSD11B2	16q22.1	AR	NM_000196	Apparent mineralocorticoid excess
IFT122	3q21.3-q22.1	AR	NM_018262	Cranioectodermal dysplasia 1
IFT140	16p13.3	AR	NM_014714	Short-rib thoracic dysplasia 9 with or without polydactyly
IFT172	2p23.3	AR	NM_015662	Short-rib thoracic dysplasia 10 with or without polydactyly
INF2	14q32.33	AD	NM_022489	Glomerulosclerosis, focal segmental, 5
INPP5E	9q34.3	AR	NM_019892	Joubert syndrome 1
INVS	9q31.1	AR	NM_014425	Nephronophthisis 2, infantile
IQCB1	3q13.33	AR	NM_014642	Senior-Loken syndrome 5
ITGB4	17q25.1	AR	NM_000213	Epidermolysis bullosa, junctional, with pyloric atresia
KAL1	Xp22.31	XLR	NM_000216	Hypogonadotropic hypogonadism 1 with or without anosmia (Kallmann syndrome 1)
KANK2	19p13.2	AR	NM_015493	Nephrotic syndrome, type 16

Gene	Cytogenic location	Inheritance	Accession number	Disease association
KCNJ1	11q24.3	AR	NM_000220	Bartter syndrome, type 2
KCNJ10	1q23.2	AR	NM_002241	SESAME syndrome
KIF7	15q26.1	AR	NM_198525	Joubert syndrome 12
LAMB2	3p21.31	AR	NM_002292	Pierson syndrome
LCAT	16q22.1	AR	NM_000229	Norum disease
LMX1B	9q33.3	AD	NM_002316	Nail-patella syndrome; also FSGS without extrarenal involvement
LRP2	2q31.1	AR	NM_004525	Donnai-Barrow syndrome
LYZ	12q15	AD	NM_000239	Amyloidosis, renal
MAFB	20q12	AD	NM_005461	Multicentric carpotarsal osteolysis syndrome
MED28	4p15.32	AR	NM_025205	nephrotic syndrome
MKKS	20p12.2	AR	NM_018848	Bardet-Biedl syndrome 6
MKS1	17q22	AR	NM_017777	Bardet-Biedl syndrome 13, Joubert syndrome 28
MYH9	22q12.3	AD, association	NM_002473	MYH9-related disease; Epstein and Fechtner syndromes
MMACHC	1p34.1	AR	NM_015506	Methylmalonic aciduria and homocystinuria, cblC type
MYO1E	15q22.2	AR	NM_004995	Glomerulosclerosis, focal segmental, 6
NEK1	4q33	AD/AR	NM_001199397	Short-rib thoracic dysplasia 6 with or without polydactyly
NEK8	17q11.2	AR	NM_178170	Renal-hepatic-pancreatic dysplasia 2

Gene	Cytogenic location	Inheritance	Accession number	Disease association
NOTCH2	1p12	AD	NM_024408	Hajdu-Cheney syndrome
NPHP1	2q13	AR	NM_000272	Joubert syndrome 4, Nephronophthisis 1, juvenile
NPHP3	3q22.1	AR	NM_153240	Nephronophthisis 3
NPHP4	1p36.31	AR	NM_001291593	Nephronophthisis 4
NPHS1	19q13.12	AR	NM_004646	Nephrotic syndrome, type 1
NPHS2	1q25.2	AR	NM_014625	Nephrotic syndrome, type 2
NR3C2	4q31.23	AD	NM_000901	Pseudohypoaldosteronism type I, autosomal dominant
OCRL	Xq26.1	XLR	NM_000276	Dent disease 2, Lowe syndrome
OFD1	Xp22.2	XLR	NM_003611	Joubert syndrome 10
PAX2	10q24.31	AD	NM_000278	Glomerulosclerosis, focal segmental, 7
PCCA	13q32.3	AR	NM_000282	Propionic acidemia
PDSS2	6q21	AR	NM_020381	Leigh syndrome
PHEX	Xp22.11	XLD	NM_000444	Hypophosphatemic rickets, X-linked dominant
PKD1	16p13.3	AD	NM_000296	Polycystic kidney disease 1
PKD2	4q22.1	AD	NM_000297	Polycystic kidney disease 2
PKHD1	6p12.3-p12.2	AR	NM_138694	Polycystic kidney disease 4, with or without hepatic disease
PLCE1	10q23.33	AR	NM_016341	Nephrotic syndrome, type 3

Gene	Cytogenic location	Inheritance	Accession number	Disease association
PTPRO	12p12.3	AR	NM_030667	Nephrotic syndrome, type 6
REN	1q32.1	AR	NM_000537	Renal tubular dysgenesis
RPGRIP1L	16q12.2	AR	NM_015272	Joubert syndrome 7
RRM2B	8q22.3	AR	NM_001172477	Mitochondrial DNA depletion syndrome 8A (encephalomyopathic type with renal tubulopathy)
SALL1	16q12.1	AD	NM_002968	Townes-Brocks branchiootorenal-like syndrome
SALL4	20q13.3	AD	NM_001318031	IVIC syndrome
SARS2	19q13.2	AR	NM_017827	Hyperuricemia, pulmonary hypertension, renal failure
SCARB2	4q21.1	AR	NM_005506	Action myoclonus-renal failure syndrome ± hearing loss
SCNN1A	12p13.31	AD	NM_001038	Liddle syndrome 3, Bronchiectasis with or without elevated sweat chloride 2
SCNN1B	16p12.2	AD	NM_000336	Liddle syndrome 1, Bronchiectasis with or without elevated sweat chloride 1
SCNN1G	16p12.2	AD	NM_001039	Liddle syndrome, Bronchiectasis with or without elevated sweat chloride 3
SDCCAG8	1q43-44	AR	NM_006642	Bardet-Biedl syndrome 16
SIX5	19q13.32	AD	NM_175875	Branchiootorenal syndrome 2
SLC12A1	15q21.1	AR	NM_000338	Bartter syndrome, type 1
SLC12A3	16q13	AR	NM_000339	Gitelman's syndrome
SLC22A12	11q13.1	AR	NM_144585	Hypouricemia, renal

Gene	Cytogenic location	Inheritance	Accession number	Disease association
SLC26A3	7q22.3-q31.1	AR	NM_000111	Diarrhea 1, secretory chloride, congenital
SLC2A2	3q26.2	AR	NM_000340	Fanconi-Bickel syndrome
SLC34A1	5q35.3	AR	NM_003052	Fanconi renotubular syndrome 2
SLC34A3	9q34.3	AR	NM_080877	Hypophosphatemic rickets with hypercalciuria
SLC3A1	2p21	AD/AR	NM_000341	Cystinuria
SLC4A1	17q21.31	AD/AR	NM_000342	Renal tubular acidosis, distal
SLC4A4	4q13.3	AR	NM_003759	Renal tubular acidosis, proximal, with ocular abnormalities
SLC5A2	16p11.2	AD/AR	NM_003041	Renal glucosuria
SLC6A19	5p15.33	AD	NM_001003841	Hyperglycinuria
SLC6A20	3p21.31	AD	NM_020208	Hyperglycinuria
SLC7A7	14q11.2	AR	NM_001126105	Lysinuric protein intolerance
SLC7A9	19q13.11	AD/AR	NM_001126335	Cystinuria
SLC9A3R1	17q25.1	AD	NM_004252	Nephrolithiasis/osteoporosis, hypophosphatemic, 2
SMARCAL1	2q35	AR	NM_014140	Schimke immuno-osseous dysplasia
SOX17	8q11.23	AD	NM_022454	Vesicoureteral reflux 3
TCTN1	12q24.11	AR	NM_024549	Joubert syndrome 13
TMEM216	11q12.2	AR	NM_016499	Joubert syndrome 2

Gene	Cytogenic location	Inheritance	Accession number	Disease association
TMEM237	2q33.1	AR	NM_152388	Joubert syndrome 14
TMEM67	8q22.1	AR	NM_153704	Joubert syndrome 6, Nephronophthisis 11
TRIM32	9q33.1	AR	NM_012210	Bardet-Biedl syndrome 11
TRPC6	11q22.1	AD	NM_004621	Glomerulosclerosis, focal segmental, 2
TTC21B	2q24.3	AD/AR	NM_024753	Nephronophthisis 12
TTC8	14q31.3	AR	NM_144596	Bardet-Biedl syndrome 8
UMOD	16p12.3	AD	NM_001008389	Uromodulin-associated kidney disease
UPK3A	22q13.31	UD	NM_006953	Involvement renal dysplasia, possible
VIPAS39	14q24.3	AR	NM_022067	Arthrogryposis, renal dysfunction, and cholestasis 2
VPS33B	15q26.1	AR	NM_018668	Arthrogryposis, renal dysfunction, and cholestasis 1
WDR19	4p14	AR	NM_001317924	Nephronophthisis 13, Senior-Loken syndrome 8
WDR35	2p24.1	AR	NM_020779	Short-rib thoracic dysplasia 7 with or without polydactyly
WNK1	12p13.33	AD	NM_018979	Pseudohypoaldosteronism, type IIC
WNK4	17q21.2	AD	NM_001321299	Pseudohypoaldosteronism, type IIB
WNT4	1p36.12	AD	NM_030761	Mullerian aplasia and hyperandrogenism
WT1	11p13	AD	NM_024426_449AAs.3	Nephrotic syndrome, type 4, Denys-Drash and Frasier syndrome
XPNPEP3	22q13.2	AR	NM_022098	Nephronophthisis-like nephropathy 1

Gene	Cytogenic location	Inheritance	Accession number	Disease association
ZMPSTE24	1p34.2	AR	NM_005857	Mandibuloacral dysplasia with type B lipodystrophy
ZNF423	16Q12.1	AD/AR	NM_015069	Joubert syndrome 19; Nephronophthisis 14

Abbreviations: AD, autosomal dominant; AR, autosomal recessive; UD, undetermined

III. Result

1. Patient characteristics

The median age of the referred patients was 11.6 years old (range 0-46 years of age). Twenty-seven (90%) were male, and three (10 %) were female. Three patients (10 %) had a family history related to kidney disease, and four (13 %) had undergone renal biopsy due to hematuria prior to the NGS panel test. The reasons for referral were as follows: urinalysis abnormalities in 11 patients, steroid-resistant nephrotic syndrome in two patients, renal failure in two, electrolyte imbalance in five, and abnormal kidney imaging finding on CT or sonogram in ten (Table 2).

Table 2. Reason for NGS of renal disease panel test

Reason for NGS test		Patient (n)
Urinalysis abnormality	11	
Proteinuria		1
Hematuria		6
Proteinuria & hematuria		4
Abnormal Imaging finding	10	
Polycystic kidney disease		7
Medullary sponge kidney		1
Renal agenesis		1
Bilateral hydronephrosis		1
Steroid Resistant Nephrotic Syndrome	2	
Electrolyte imbalance	5	
Renal failure	2	
Total	**30**	

2. Detection of genetic variants and CNV abnormality

Targeted NGS analysis identified 164 variants in 84 genes, and every patient had at least one variant . On average, 5.4 variants were identified per patient, with a maximum of 12. Sixteen pathogenic or likely pathogenic variants (P/LP) were detected in 14 genes in nine patients. Among them, eight variants of P/LP were the type of nonsense mutations, and one was splicing error. The most frequently detected P/LP variants were found in *NPHS1* (n = 3, 16.7 %), *PKD1* (n = 2, 11.1 %), and *COL4A3* genes (n = 2, 11.1 %). Additionally, all patients had one or more variants of unknown significance (VUS), and 142 VUS were detected in 70 genes. Among the 142 VUS, the most frequently involved genes were *PKD1* (n = 15, 9.7 %), *PKHD1* (n = 6, 3.9 %), and *ALMS1* (n = 6, 3.9 %). We detected five heterozygous CNVs anomalies in each five patients, but only one of CNV abnormality, 1q36 microdeletion, was revealed to pathogenic CNV abnormality according to the patient's phenotype and a literature review.

3. A novel mutation of *COL4A4*

We found a novel variant of the *COL4A4* gene (C.155G>T, p.Cys52Phe) associated with Alport syndrome in a 5-year-old girl with recurrent hematuria and progressive bilateral sensorineural hearing loss, whose father also had nephritis and hearing loss. We performed additional Sanger sequencing of her father to confirm the mutation, with results demonstrating that this mutation was

paternally inherited. This mutation has not been reported previously in population databases.

4. Rate of molecular diagnosis

Based on our NGS panel test, the total diagnostic yield in the population was shown to be 46.7 % (14/30). NGS analysis results and phenotype were consistent in six patients with P/LP variants, including one patient with ADPKD, two with Alport syndrome, one with Bartter syndrome, one with Gitelman's syndrome, and one with chloride-secreting diarrhea. Among those with VUS, NGS results and phenotypes were consistent in seven patients: three with ADPKD, three with Alport syndrome, and one with Gitelman's syndrome. In one patient with dysmorphic features, sensorial hearing loss, atrial septal defect, and global delayed development in addition to several renal cysts at both kidneys, a heterozygous copy number deletion on chromosome 1q36 was identified. These results are summarized in Table 3.

Ten patients (33.3%) perfectly matched between the initial clinical suspicion and molecular diagnosis (Figure 1). Of the seven patients referred for cystic kidney disease, two were diagnosed as ADPKD, which consistent with the first clinical impression. Among the five patients with undefined renal cysts, two were diagnosed with ADPKD, and one was diagnosed with 1q36 deletion syndrome according to CNV analysis. Of six patients with suspected Alport syndrome or

thin glomerular basement membrane, five were eventually diagnosed with Alport syndrome. Two patients with suspected Gitelman's syndrome was diagnosed with Gitelman's syndrome as expected initially. But among two patients with suspected Bartter syndrome, NGS results unexpectedly showed that one patient had chloride-secreting diarrhea, which originates in the intestine rather than the kidney.

Three patients with similar familial medical histories were found to have pathogenic variants associated with their initial suspicions and were finally diagnosed with ADPKD (one patient) or Alport syndrome (two patients). Among four who had undergone renal biopsy due to hematuria before the NGS panel test, three patients were found to have gene variants of *COL4A3* or *COL4A4*, which are known to be associated with Alport syndrome; as such, these results were consistent with their histological diagnosis.

Table 3. Clinical and genetic data of patients in whom disease causative variants were identified

No.	Sex	Age	Fx	Clinical Presentation -Renal alterations	Clinical Presentation -Extra renal	Diagnosis	Gene	Sequence Variant
Patients referred for cystic kidney disease								
001	M	3mon	N	Several renal cysts, both kidney	Sensorineural hearing loss, Rt. Atrial septal defect Umbilical hernia,	1q36.32 microdeletion syndrome	1q36.3	1q36.32 microdeletion
004	M	1mon	N	Multiple cystic lesions with variable size and no communication in Rt. kidney, suggesting MCDK	ASD 2'	ADPKD	*PKD 1*	c.5303C>A, p(Thr1768Asn)
005	F	10days	N	Decrease kidney size, multiple cortical cysts in the kidney, both	Arrhythmia	ADPKD	*PKD1*	c.5037C>A, p.(Ser1679Arg)(h) c.4810G>A,p.(Val1604Met)(h)
007	M	2ys	N	Tiny cystic lesions in corticomedullary junction, Lt. kidney	Delayed development Dextrocardia, Inguinal hernia	ADPKD	*PKD1*	c.1916C>T, p.(Ala639Val) (h) c.6935C>T. p.(Ala2312Val) (h)
008	M	18ys	Y	Hemorrhagic component in the multiple renal cysts, both kidney		ADPKD	*PKD1*	c.975T>G, p.(Tyr325Ter)¶ (m)

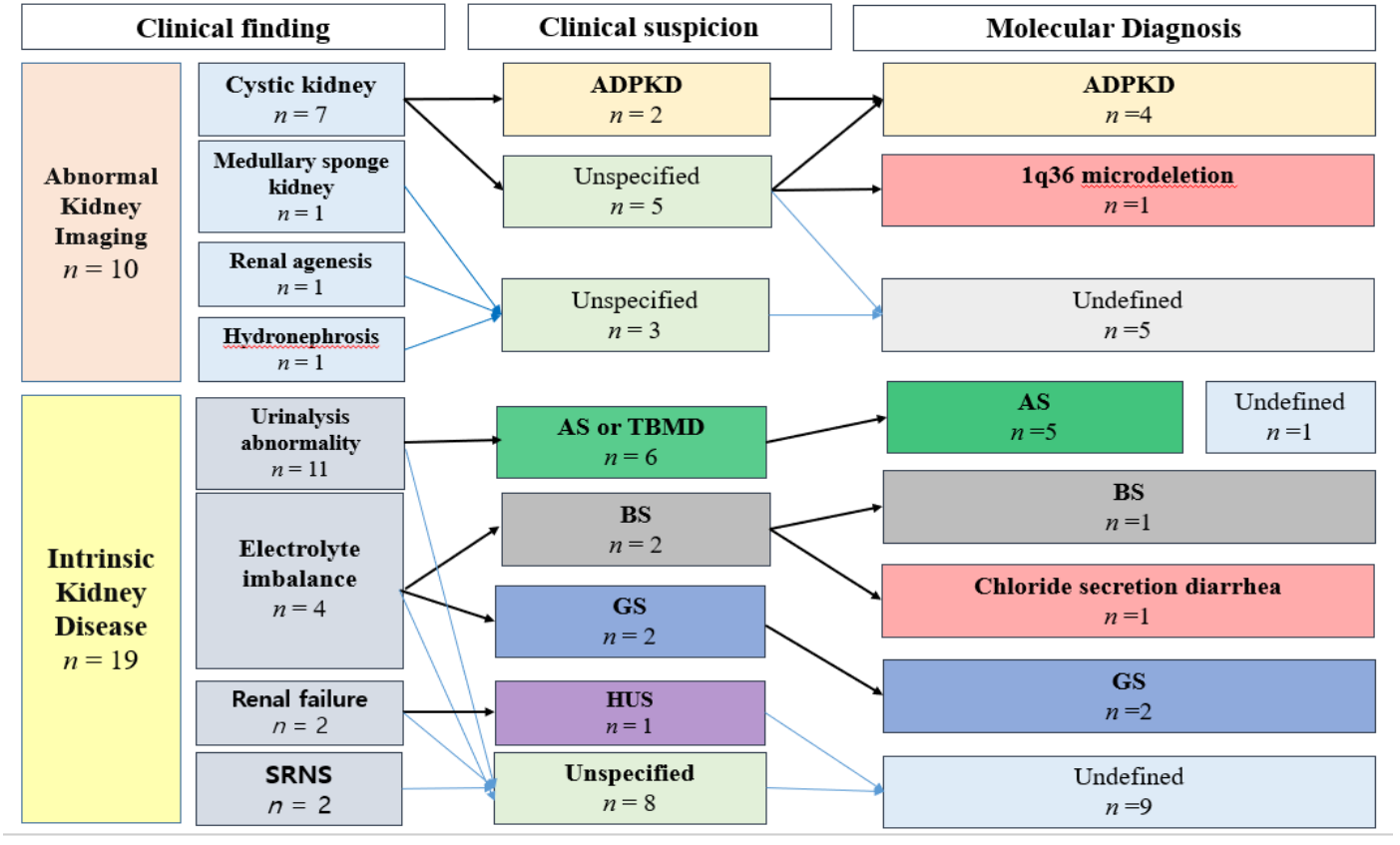
Patients referred for hematuria +/- proteinuria								
015	F	36yrs	N	HU/ PU since 20' irregular thickening of GBM		Alport syndrome	*COL4A3*	c.1229G>A, p.(Gly410Glu) (h)
016	F	15yrs	N	Consistent HU irregular thickening of GBM		Alport syndrome	*COL4A3*	c.1229G>A, p.(Gly410Glu) (h)
019	M	4yrs	Y	Mildly increased cortical echogenicity in both kidneys	Hearing insufficiency	Alport syndrome	*COL4A4*	c.155G>T, p.(Cys52Phe) (m)
020	F	7yrs	Y	Recurrent HU	Asthma, atopic dermatitis	Alport syndrome	*COL4A3*	c.417delG, p.(Thr140HisfsTer13)¶(p)
021	F	21yrs	N	GBM irregularity, suggestive of hereditary nephritis	Sensorineural hearing loss, both	Alport syndrome	*COL4A3*	c.1029+1G>A¶(h)
Patients referred for electrolyte imbalance								
027	M	4yrs	N	polyhydramnios Hx. Hypokalemia		Gitelman's syndrome	*CLCNKB*	c.330C>G p.(Phe110Leu) (p) exon 4. del (m)
028	M	15yrs	N	Hypokalemia	Hearing impairment; tremor	Bartter syndrome, type 1	*SLC12A1*	c.888delG¶(p) c.1199T>A p.(Ile400Asn)(m)

029	M	23yrs	N	Hypokalemia	Dystonia, tremor	Gitelman's syndrome	*SLC12A3*	c.539C>A p.(Thr180Lys) (p) c.1868T>C p.(Leu623Pro)¶(m)
030	M	11mon	N	Hypokalemic alkalosis Diffusely bilateral renal enlargement with increased cortical echogenicity	Colon segmental resection, d/t colon ischemia	Congenital secretory diarrhea, chloride type	*SLC26A3*	c.2063-1G>T ¶ (p,m)

Abbreviations: Fx, family history; M, male; F, female; HU, hematuria; PU, proteinuria; GBM, glomerular basement membrane; ADPKD, autosomal dominant polycystic kidney disease; h, heterozygous; p, paternal; m, maternal

¶ Pathogenic/likely pathogenic variant

Figure 1. Correlations between clinical suspicion and molecular analysis results



IV. Discussion

More than 1800 human genes associated with monogenic or Mendelian diseases have been identified [14]. NGS technology, which facilitates simultaneous screening and analysis of large sets of disease-related genes, will likely yield major changes in diagnostic approaches to identifying inherited genetic diseases. This approach can enable accurate and early detection and minimize the need for invasive diagnostic procedures. In turn, this can help optimize outcomes by broadening therapeutic options. Also, pre-symptomatic testing based on family history can be used to detect disease-causing mutations before the appearance of overt symptoms and can be applied in prenatal genetic testing and counseling.

In recent years, NGS has also been used in patients with inherited kidney diseases to identify genetic causes [15-19]. The use of NGS panel screening in suspected kidney diseases can be useful for analyzing genetic etiologies of diseases with genetic heterogeneity and detecting a variety of causative genes associated with conditions such as Bartter/Gitelman's syndrome, SRNS, and cystic kidney disease simultaneously. As a result, NGS technology has been widely applied in the diagnosis of kidney diseases, and many genes have been known to be associated with disruption of kidney function, structure, or development. [20]. Additionally, this approach can rapidly differentiate between kidney diseases and non-kidney diseases with mimicking symptoms. However, many of the genetic aspects of suspected kidney diseases are still largely undiscovered. Therefore, we aimed to

generate a comprehensive dataset using an NGS panel to investigate 167 genes associated with kidney diseases and diseases with mimicking symptoms to renal diseases.

Based on this approach, we reached a diagnostic yield of 46.7 % in a group of 30 patients with overlapping and non-specific nephrogenic symptoms such as proteinuria, hematuria, electrolyte imbalance, and/or metabolic acidosis/alkalosis. This yield is comparably higher than in similar findings by other NGS studies[16,18] It seems that we designed expanded NGS panel with CNV analysis based on associated symptoms rather than specific diseases followed by in-depth analysis of the NGS data.

Especially in two cases, we demonstrated that NGS is highly useful in differentiating non-kidney-origin diseases with symptoms similar to kidney disease. In such cases, the application of NGS can rapidly make in-depth etiological diagnoses compared to conventional expensive and complicated tests, such as various imaging, chemistry analyses, metabolic analyses, or invasive renal biopsy [21].

In one notable case in our study, an 11-month-old boy, visited our hospital with reported lethargy. He had been born at 35.4 weeks of gestation from healthy parents. Fifteen days after his birth, he developed abdominal distension suggestive of neonatal necrotizing enterocolitis (NEC) and received ileostomy surgery. After two

months, he received another surgery to repair the ileostomy site and for segmental resection of a 7.9 cm ischemic ileum lesion. He had healthy two older brothers, with no other notable family history. Upon admission, he was found to have severe hyponatremia, and hypokalemic hypochloremic metabolic alkalosis with serum sodium of 128 mmol/L, potassium of 2.5 mmol/L, and chloride of 67 mmol/L. His arterial blood gas analysis indicated severe metabolic alkalosis with pH of 7.652, pCO_2 of 32.5 mmHg, pO_2 of 103.0 mmHg, and HCO_3 of 36.3 mmol/L. Abdominal sonography showed diffuse kidney disease with bilateral renal enlargement, and autosomal recessive polycystic kidney disease (ARPKD) could not be excluded. Given all these results, Bartter syndrome was suspected as his clinical diagnosis, and he was referred to our department to identify the genetic etiology of his condition precisely. We detected two homozygous splice site pathogenic mutations in the *SLC26A3* gene (c.2063-1G>T), which encodes the transmembrane glycoprotein exchanging chloride and bicarbonate ions across the cell membrane. Mutation analysis of the patient's parents revealed that they were asymptomatic carriers of the *SLC26A3* gene mutation. To confirm the molecular diagnosis, we analyzed the electrolyte level of the patient's stool; its sodium level was shown to be 120 mmol/L. Therefore, despite his seemingly distinctive presentation mimicking to kidney disease, he was finally diagnosed as having chloride-secreting diarrhea, in contrast to the first clinical expectations.

Our results also indicate the usefulness of NGS in CNV analysis. Recent studies

have reported that CNVs are widespread in the human genome and serve as an important genetic factor explaining disease etiology and population diversity [17,22]. Most CNV variants are probably benign, but some specific variants may be related to Mendelian conditions such as neurodevelopmental diseases and various cancers [23-26]. CNVs can also affect susceptibility to some inherited kidney diseases, and studies have highlighted CNV analysis in inherited kidney diseases such as congenital anomalies of the kidney and urinary tract (CAKUT) [17,27,28]. NGS-based CNV detection method is accurate to 92 % sensitivity and 100 % specificity in detecting duplications as small as 300 bp and deletions as small as 180 bp in specific genes [17,29].

In our dataset, one particular case study emphasizes the importance of CNV detection in this setting. Pathogenic CNV was detected in one 1-year-old boy, referred to our department for evaluation of several renal cysts on both kidneys discovered using sonography. He also showed characteristic facial features, sensorineural hearing loss, atrial septal defect, and developmental delay in addition to the renal anomaly. CNV analysis using identified a heterozygous copy number deletion of 5 Mb on chromosome 1q36. We performed real-time quantitative PCR analysis on samples from him and his parents to confirm this result and found that the deletion was absent in both of his parents, indicating a *de novo* origin of his deletion. By considering both his phenotype and the detected abnormal CNV, we were able to diagnose the patient as 1q36 deletion syndrome conclusively, rather

than as disease with pathology confined to the kidney.

V. Conclusion

We identified six different diseases in 14 patients with 46.7 % of overall diagnostic yield. Based on the analysis of the results so far, we were able to identify relatively promising results from the application of NGS in patients with kidney disease-related symptoms.

But despite the high diagnostic yield of this NGS panel, 16 patients remained undiagnosed genetically. These patients with negative results of NGS panel could be candidates for WES analysis for the identifying novel genes or diseases, and there is room for further analysis after the database has been filed up in the future.

NGS technology still needs further and in-depth research for data interpretation to determine the definite pathogenicity of each variant. It should be kept in mind that accurate identification of clinical symptoms and in-depth physical examination systematically by specialist are preceded and comprehensive collaboration between clinical genetics, nephrologists, and bioinformaticians must be followed. Also, in-depth genetic counseling should be followed to help patients understanding their results and applying it for treatment and prevention of their disease properly.

We look forward that the rapid development of NGS technology will enable further clinical applicability of our approach for diagnosing kidney disease.

Conflict of interest

The authors have no conflict of interest to declare.

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Abstract (in Korean)

Next-generation sequencing (NGS) panel 결과 분석을 통한 신장 질환의 진단

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배경: 신장 질환의 특성상 진단이 늦어질 경우, 영구적이고 비 가역적인 신장 기능의 손상으로 이어져 조기에 정확한 진단을 하는 것이 필수적이다. 하지만 다양한 원인에 의해 신장 질환이 발생되는 것에 비하여, 대부분 비 특이적이고 공통적인 증상을 보이는 경우가 대부분이고 이 또한 early stage에는 무증상 이거나 경한 증상만 나타날 수 있어 진단 자체가 늦어지는 경우가 많다. 이에 본 연구는 신장 질환 관련 증상을 가진 환자들에서 시행된 NGS panel test의 결과를 수집, 분석해 봄으로써, 추후 대상군 환자들에게 NGS panel test의 진단적 tool의 하나로써의 가능성을 확인해 보고자 한다.

연구방법: 신장 관련 증상의 유전적 원인 확인을 위해 시행되었던 30명 환자의 NGS 결과 및 결과와 연관된 임상 증상, 결과를 수집하여 분석하였다. 본 NGS panel에는 신장 질환 환자에서 나타날 수 있는 증상들을 보일 수 있는 질환과 관련된 167개의 유전자가 포함되었다.

결과: 30명의 환자에서 NGS 패널 검사를 시행한 원인으로는 각각 소변검사 결과 이상 (11), 영상 검사 이상 (10), 스테로이드 저항성 신 증후군 (2),

불명확한 원인의 신기능 부전 (2), 전해질 불균형 (5) 이었다. 16명의 환자의 14개의 각각 다른 유전자에서 9개의 pathogenic or likely pathogenic 변이가 발견되었다. 본 검사를 통한 최종 질환 진단율은 46.7 % (14/30) 었으며, 진단된 질환은 각각 전해질 불균형을 보였던 환자군에서는Bartter syndrome(2), Gitelman syndrome(1), chloride secreting diarrhea(1)이었고, 영상 검사상 이상 소견을 보였던 환자군에서는 우성 다낭신(4) 과1q36 미세결실 증후군 (1) 이었으며, 혈뇨를 주소로 검사한 환자군에서는 Alport syndrome (5) 이었다. 총 10명 (33.3%)의 환자에서 NGS검사 시행전의 임상적 의심 질환과 최종 진단이 일치하였다.

결론: 본 연구를 통해 신장 관련 증상을 보이는 환자군에서 추후 진단적 목적의 검사로써의 NGS 검사의 가능성을 확인해 볼 수 있었다.

핵심어: Next-generation sequencing (NGS), Copy number variation (CNV), 신장 질환, 유전자 검사