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Clinical application of a phenotype-based NGS panel for differential diagnosis of inherited kidney disease and beyond

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Abstract

Understanding the genetic causes of kidney disease is essential for accurate diagnosis and could lead to improved therapeutic strategies and prognosis. To accurately and promptly identify the genetic background of kidney diseases, we applied a targeted next-generation sequencing gene panel including 203 genes associated with kidney disease, as well as diseases originating in other organs with mimicking symptoms of kidney disease, to analyze 51 patients with nonspecific nephrogenic symptoms, followed by validation of its efficacy as a diagnostic tool. We simultaneously screened for copy number variants (CNVs) in each patient to obtain a higher diagnostic yield (molecular diagnostic rate: 39.2%). Notably, one patient suspected of having Bartter syndrome presented with chloride-secreting diarrhea attributable to homozygous *SLC26A3* variants. Additionally, in eight patients, NGS confirmed the genetic causes of undefined kidney diseases (8/20, 40%), and initial clinical impression and molecular diagnosis were matched in 11 patients (11/20, 55%). Moreover, we found seven novel pathogenic/likely pathogenic variants in *PKD1*, *PKHD1*, *COL4A3*, and *SLC12A1* genes, with a possible pathogenic variant in *COL4A3* (c.1229G>A) identified in two unrelated patients. These results suggest that targeted NGS-panel testing performed with CNV analysis might be advantageous for noninvasive and comprehensive diagnosis of suspected genetic kidney diseases.

KEYWORDS

Kidney disease, Renal disease, NGS panel, Genetic diagnosis, Copy number variant

1 | INTRODUCTION

Kidney diseases represent a heterogeneous group of disorders, including monogenic disorders, such as autosomal dominant/recessive polycystic kidney disease (ADPKD/ARPKD), as well as complex genetic diseases, such as steroid resistance nephrotic syndrome (SRNS), Alport syndrome, and congenital anomalies of the kidney and urinary tract (CAKUT).[1] Kidney diseases, especially those with a chronic course, can lead to permanent and irreversible deterioration of renal function. Therefore, accurate and prompt diagnosis is essential for improving outcomes for patients with these diseases; however, this can be difficult due to either nonspecific symptoms and

signs or clinically silent symptoms in the early stages of disease. Additionally, this is complicated by several systemic diseases or diseases originating from other organs, that can present symptoms similar to those from confined kidney, including various structural abnormalities, electrolyte imbalances, or metabolic acidosis/alkalosis. For example, renal cysts, which are structural abnormalities in the kidney, can also be identified in various multisystemic diseases, such as tuberous sclerosis complex, oral-facial-digital syndrome, and coloboma syndrome, as well as ADPKD or ARPKD.[2,3] Therefore, diagnosing the precise underlying causes of kidney diseases with nonspecific symptoms using conventional laboratory and imaging diagnostic tools can be challenging.

An invasive procedure, such as renal biopsy, could often be performed to identify the underlying etiology of this disease group; however, it is limited by the range of conditions that it can successfully confirm and is associated with a risk of complications, including bleeding.[4-7] Moreover, this diagnostic method often fails to uncover a correct diagnosis in very early or late stages of diseases.[1,8]

According to several reports, 10% of the population with adult chronic kidney disease (CKD) and almost all pediatric patients who progress to end-stage renal disease are identified as having inherited kidney disease.[9-12] Therefore, the importance of genetic testing should not be overlooked during the diagnostic workup of kidney diseases. An accurate genetic diagnosis could provide proper treatment options for the patient in the early phase, leading to prevention of the rapid worsening of the disease and playing a pivotal role in selecting relative kidney donors for transplantation or family planning. However, identifying disease-causing genes is challenging because of the complexity of the genetic background.

The efforts to improve the capabilities of genetic testing have been developed in recent years, and the revolution of next-generation sequencing (NGS) has enabled cost-effective simultaneous sequencing of a broad set of candidate genes. Recently, several studies reported the effectiveness of NGS in identifying various genetic factors in inherited kidney diseases, including glomerular nephropathy, steroid-resistant nephrotic syndrome, and cystic kidney disease.[2,13-17] However, most studies are limited by only analyzing well-known causative genes of inherited kidney disease. Additionally, differential diagnoses of diseases originating in other organs, which could result in symptoms and signs mimicking kidney disease, have not been identified. Moreover, almost all of these studies were performed on patients of mainly European descent; therefore, these results might not represent all ethnic populations.

Here, we applied a targeted NGS panel and simultaneous analysis of copy number variation (CNV) to elucidate the genetic causes of suspected genetic kidney diseases in an Asian population. Additionally, we confirmed the feasibility of this diagnostic method for the differential diagnosis of diseases originating from the kidney or another organ but presenting with overlapping symptoms and signs according to the NGS panel. To validate the diagnostic efficacy of this NGS panel, we tested 51 Korean patients with symptoms of kidney disease and suspected of having inherited kidney disease and referred for evaluation to determine possible genetic causes.

2 | MATERIALS AND METHODS

2.1 | Patient selection and study design

From January 2018 to August 2019, 51 unrelated, genetically undiagnosed patients were enrolled for targeted NGS testing using a comprehensive kidney disease panel developed in the Department of Clinical Genetics at Severance Children's Hospital (Seoul, Korea). All patients had one or more of the following symptoms/signs: proteinuria, hematuria, electrolyte imbalance, metabolic alkalosis/acidosis, or abnormal kidney structure. We retrospectively reviewed the pedigree information, previous medical history, physical examination findings, and any additional investigative results (e.g., ophthalmologic and otology examinations) in the electronic medical records of each patient. Additionally, we collected the available results based on segregation analyses of family members of each patient. This information was collected under anonymity in a routine diagnostic process, and the study protocol was approved by the Institutional Review Board of the Yonsei University Health System (IRB 4-2019-0227). Informed consent for the genetic testing was obtained from each patient or their legal guardians if the patient was aged <19 years.

2.2 | Panel design

First, we searched for symptoms and signs resembling those observed in genetic kidney diseases using PubMed, Embase, and MEDLINE. Accordingly, we searched for the following symptoms and signs: proteinuria, hematuria, electrolyte imbalance, metabolic alkalosis/acidosis, and abnormal kidney structure. Based on data from the Human Genome Mutation Database (HGMD), Online Mendelian Inheritance in Man (OMIM) database (http://www.ncbi.nlm.nih.gov/omim), and an extensive literature review using PubMed, we extracted and optimized 203 disease-causing genes (Table 1). The last search was performed on October 10, 2020.

2.3 | DNA preparation

We collected 3 ml of peripheral blood in EDTA tubes from each patient and extracted the genomic DNA from leukocytes using a DNeasy blood and tissue kit (Qiagen, Hilden, Germany) according to manufacturer instructions. The quality of isolated DNA was checked using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.4 | Library preparation, target capture, and DNA sequencing

We constructed a DNA sequencing library using the TruSeq DNA Library Prep Kits protocol according to manufacturer instructions (TruSeq DNA Library Prep Kits,, FC-121-2003; Illumina, Carlsbad, CA,

TABLE 1 Genes (n = 203) included in the panel of kidney diseases

Gene	Cytogenic location	Inheritance	Gene 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
ARNT2	15q25.1	AR	NM_014862	Webb-Dattani syndrome
ATP6V0A4	7q34	AR	NM_020632	Renal tubular acidosis, distal, autosomal recessive
ATP6V1B1	2p13.3	AR	NM_001692	Renal tubular acidosis with deafness
AVP	20p13	AD	NM_000490	Diabetes insipidus, neurohypophyseal
AVPR2	Xq28	XLR	NM_000054	Diabetes insipidus, nephrogenic; Nephrogenic syndrome of inappropriate antidiuresis
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
BBS1	11q13.2	AR/DR		Bardet-Biedl syndrome 1
BBS2	16q13	AR	NM_031885	Bardet-Biedl syndrome 2
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; Sensorineural deafness with mild renal dysfunction
CA2	8q21.2	AR	NM_000067	Osteopetrosis, autosomal recessive 3, with renal tubular acidosis
CA12	15q22.2	AR	NM_001218	Hyperchlorhidrosis, isolated
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
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 CFHR 5 days eficiency
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

TABLE 1 (Continued)

Gene	Cytogenic location	Inheritance	Gene accession number	Disease association
CLDN10	13q32.1	AR		HELIX syndrome
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
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
DGUOK	2p13.1	AR	NM_080916	Mitochondrial DNA depletion syndrome 3
DMP1	4q22.1	AR	NM_001079911	Hypophosphatemic rickets
DHCR7	11q13.4	AR	NM_001360	Smith-Lemli-Opitz syndrome
EGF	10p13	AR	NM_001178130	Hypomagnesemia 4, renal
EGFR	7p11.2	AR		Inflammatory skin and bowel disease, neonatal, 2
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
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

(Continues)

TABLE 1 (Continued)

Gene	Cytogenic location	Inheritance	Gene accession number	Disease association
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
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; 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
NNT	5p12	AR	NM_012343	Glucocorticoid deficiency 4, with or without mineralocorticoid deficiency
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
NR0B1	Xp21.2	XLR	NM_000475	Adrenal hypoplasia, congenital
NR3C2	4q31.23	AD	NM_000901	Pseudohypoaldosteronism type I, autosomal dominant
NUP214	9q34.13	AR	NM_001318324	Encephalopathy, acute, infection-induced, susceptibility to, 9
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	Propionicacidemia
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
PLVAP	19p13.11	AR	NM_031310	Diarrhea 10, protein-losing enteropathy type
POMC	2p23.3	AR	NM_001035256	Obesity, adrenal insufficiency, and red hair due to POMC deficiency
PTPRO	12p12.3	AR	NM_030667	Nephrotic syndrome, type 6
REN	1q32.1	AR	NM_000537	Renal tubular dysgenesis

TABLE 1 (Continued)

Gene	Cytogenic location	Inheritance	Gene accession number	Disease association
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, and alkalosis
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		NM_175875	Branchiootorenal syndrome 2
SLC12A1	15q21.1	AR	NM_000338	Bartter syndrome, type 1
SLC12A3	16q13	AR	NM_000339	Gitelman syndrome
SLC22A12	11q13.1	AR	NM_144585	Hypouricemia, renal
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
SLC9A3	5p15.33	AR		Diarrhea 8, secretory sodium, congenital
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
SPINK5	5q32	AR	NM_001127698	Netherton syndrome
SPINT2	19q13.2	AR	NM_001166103	Diarrhea 3, secretory sodium, congenital, syndromic
STAR	8p11.23	AR	NM_000349	Lipoid adrenal hyperplasia
TCTN1	12q24.11	AR	NM_024549	Joubert syndrome 13
TMEM216	11q12.2	AR	NM_016499	Joubert syndrome 2
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

(Continues)

TABLE 1 (Continued)

Gene	Cytogenic location	Inheritance	Gene accession number	Disease association
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_000378	Nephrotic syndrome, type 4; Denys–Drash and Frasier syndrome
XPNPEP3	22q13.2	AR	NM_022098	Nephronophthisis-like nephropathy 1
ZMPSTE24	1p34.2	AR	NM_005857	Mandibuloacral dysplasia with type B lipodystrophy
ZNF423	16q12.1	AD/AR	604 557	Joubert syndrome 19; Nephronophthisis 14

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

USA). Briefly, DNA in each sample was sheared to 250bp sequences, tagged, and then purified according to fragment size with magnetic beads (AMPure XP, Beckman Coulter, IN, USA). We subsequently performed repair, phosphorylation, and adenylation of the 3′ ends and isolated the precaptured amplified 300- to 500-bp fragments. We then performed targeted sequence, up to 15 bp from the exon of the target genes based on hg19, capture according to TruSeq Custom Enrichment Kit protocol (FC-123-1096, Illumina). DNA sequencing was performed on a V2 flow cell using MiSeq sequencer (Illumina), generating 150 bp paired-end reads. Image analysis and base calling were performed using the Illumina pipeline. The yield of each DNA sample averaged 2 GB of raw data with a 150-fold mean sequencing depth of the targeted regions.[18,19] Sequenced reads were mapped to the human reference genome (GRCh37), and sequencing alignment was performed using the Burrows-Wheeler Aligner software package.[20]

2.5 | CNV analysis

For CNV detection, we applied ExomeDepth with default parameters.[21] ExomeDepth uses a robust model of the read count data to call CNVs by comparing the test exome data to a matched optimized-aggregate reference set, which is built with combined exomes from the same batch to maximize the power to handle technical variability between samples. This method was applied to the targeted NGS sequencing data sets to detect pathogenic CNVs, and the identified CNVs were confirmed through multiplex ligation-dependent probe amplification or real-time quantitative PCR (RT-qPCR).

2.6 | Sanger sequencing and RT-qPCR

We performed Sanger sequencing for validation and segregation analysis of variants detected by NGS testing. RT-qPCR was performed for segregation analysis of CNVs using primers designed according to the oligonucleotide sequences of the variants.

2.7 | Interpretation and analysis of the detected variants

We examined the NGS data and prioritized DNA variants according to clinical relevance using the following parameters: (1) sequence quality; (2) allele frequency (according to the Exome Aggregation Consortium [ExAC], dbSNP database, and Korean Reference Genome Database [KRGDB; http://coda.nih.go.kr/]); and (3) presence in HGMD, OMIM, dbSNP, or ClinVar. Real or possible damage of variants was predicted using in silico prediction algorithms, including Polymorphism Phenotyping version 2 (PolyPhen-2) and Sorting Tolerant from Intolerant (SIFT; https://sift.bii.a-star.edu.sg/). After compressive analysis of all results, we classified the identified variants into a five-tier system as pathogenic (P), likely pathogenic (LP), variant of unknown significance (VUS), likely benign, or benign according to the American College of Medical Genetics and Genomics (ACMG) guidelines.[22] According to the inheritance pattern of the disease, we considered results positive if one or two P/LP variants in one disease-related gene was identified according to the inheritance patterns of diseases. If only a VUS or one P/LP variant was detected in a gene with an autosomal recessive (AR) inheritance pattern, we considered the result nondiagnostic. We reported cases without any VUS or P/LP as negative results.

3 | RESULTS

3.1 | Patient characteristics

A total of 51 unrelated patients were included in this study (median age: 11.6 years [range: 0–46 years]). Among these, 36 (70.6%) were

male, and 15 (29.4%) were female. Five patients (9.8%) had a family history related to kidney disease, and three (5.9%) had undergone renal biopsy due to hematuria before the NGS panel test. The reasons for referral were as follows: structural abnormalities in kidneys detected by computed tomography or sonogram in 16 patients, urinalysis abnormalities in 21 patients, electrolyte imbalances in 12 patients, and renal failure in two patients (Table 2).

3.2 | Diagnostic yield of NGS

In total, the final diagnostic yield was 39.2% (20/51), which included the diagnostic group when P/LP variants or CNV abnormalities in our NGS panel test matched the symptoms of patients. The final molecular diagnosis confirmed by our targeted NGS panel classified patients into the following diseases: four patients with ADPKD, one patient with ARPKD, three patients with Alport syndrome, one patient with hyperoxaluria type 1, three patients with Bartter syndrome, two patients with Gitelman syndrome, one patient with SLC4A1-associated renal tubular acidosis, and one patient with congenital chloride secretory diarrhea. Moreover, we identified four patients exhibiting dysmorphic features and global delayed development in addition to several renal cysts in both kidneys with CNV abnormalities on chromosome (1p36 [2 patients] and 17q12 [2 patients]). Nondiagnostic results were showed for 31 patients with VUSs only, as well as for four patients with only one P/LP variant in the disease gene with an AR-inheritance trait.

The first clinical symptoms of the diagnosed patients were as follows: urinalysis abnormality, including hematuria or proteinuria (4/21; 19.0%), structural abnormalities in the kidney (9/16; 56.3%), and electrolyte imbalances (7/12; 58.3%). The details of the patients confirmed by molecular diagnosis are summarized in Table 3.

TABLE 2 The clinical causes for using NGS in the renal disease panel test

Reasons for NGS	Patient number (*n*)	
Structural abnormalities in kidney	16	
Polycystic kidney disease		13
Medullary sponge kidney		1
Renal agenesis		1
Bilateral hydronephrosis		1
Urinalysis abnormality	21	
Proteinuria		7
Hematuria		11
Proteinuria and hematuria		3
Electrolyte imbalance	12	
Renal failure	2	
Total	51	

Abbreviation: NGS, next-generation sequencing.

Among five patients with related familial medical histories, three harbored pathogenic variants associated with their symptoms. Their final diagnosis was ADPKD (one patient), Alport syndrome (one patient), and SLC4A1-associated distal renal tubular acidosis (one patient). Among the three patients who had undergone renal biopsy due to hematuria before the NGS panel test, one was determined to have one novel LP variant in COL4A3, and two were found to have the same VUS in COL4A3, which was known to be associated with Alport syndrome. These results were consistent with their histological diagnosis.

3.3 | Detection of genetic variants and CNVs

Targeted NGS analysis identified 169 variants in 84 genes, with every patient having at least one variant. On average, we detected 2.0 variants, with a maximum of 12 per patient. We detected 27 P/LP variants of 10 genes in 24 patients. Of these variants, 18 (66.7%) had been formerly reported as P/LP, whereas 9 (33.3%) had not yet been reported at the time of our investigation. The mutational types of these 27 P/LP variants were as follows: 11 missense variants, six nonsense variants, three frameshift, three small insertion/deletions, two exonal deletions, and two splicing errors. The most frequently detected P/LP variants were observed in *PKD1* (*n* = 4; 14.8%), *CLCNKB* (*n* = 4; 14.8%), and *SLC12A3* (*n* = 4; 14.8%).

Additionally, all patients harbored one or more VUS, with 142 VUSs detected in 70 genes. Among these VUSs, the most frequently involved genes were *PKD1* (*n* = 15; 10.6%), *PKHD1* (*n* = 6; 4.2%), and *ALMS1* (*n* = 6; 4.2%). Additionally, we detected five heterozygous CNVs in five patients, although only two of the CNVs (the 1p36 and 17q12 microdeletions) found in four patients were revealed as pathogenic according to the phenotype of the patients, segregation analysis, and our literature review.

3.4 | Novel variants

Among the 20 patients with confirmed disease according to our molecular diagnosis, seven novel variants in seven patients absent from population databases and our in-house database were identified. Moreover, two novel exonal deletions were identified in *CLCNKB* for two patients (Patient 14 [exon 4 deletions] and Patient 15 [exon 1–14 deletion]).

Among patients with ADPKD with multiple renal cysts, two novel LP variants of *PKD1* were identified, with both were shown to be nonsense variants (Patient 6 [p.Tyr325Ter] and Patient 8 [p.Cys4020Ter]). Further, a 5 day old patient with ARPKD and presenting with polycystic dysplastic kidney disease and hypoplastic lung was found to carry two novel P/LP novel variants in *PKHD1*. One was a paternally inherited missense LP variant (p.Val1627Phe), whereas the other was a maternally inherited frameshift P variant (p.Ile3738SerfsTer19).

In the two patients with Alport syndrome, we detected two different novel P/LP variants of *COL4A3*. Patient 10, a 7-year-old girl,

TABLE 3 Clinical and genetic data of patients in whom disease-causative gene variants were identified

ID	Gender	Age	Fx	Clinical presentation-renal	Clinical presentation-extrarenal	Final diagnosis	Gene	Inheritance	Sequence variant	ACMG class	Zygosity
Patients referred for cystic kidney disease											
01	M	3 m	N	Several renal cysts, both kidney	Sensorineural hearing loss, Rt. Atrial septal defect Umbilical hernia	1p36.32 microdeletion syndrome			1p36.32 microdeletion		Hetero
02	F	11 y	N	Multiple renal cysts, Metabolic acidosis	Delayed development epilepsy	1p36 microdeletion syndrome			1p36 microdeletion		Hetero
03	F	2 y	N	Multiple renal cysts,	Delayed development	17q12 microdeletion syndrome			17q12 microdeletion		Hetero
04	M	6 y	N	Multiple renal cysts, Nephronophthisis	Delayed development	17q12 microdeletion syndrome			17q12 microdeletion		Hetero
05	M	1 m	N	Multiple renal cysts with variable size Decrease kidney size	Atrial septal defect	ADPKD	*PKD1*	AD	c.5303C>A, (p. Thr1768Asn)	4	Hetero
ID	Gender	Age	Fx	Clinical presentation-Renal	Clinical presentation-Extrarenal	Final diagnosis	Gene	Inheritance	Sequence variant	ACMG class	Zygosity
06	M	18 y	Y	A hemorrhagic component in the multiple renal cysts, both kidney		ADPKD	*PKD1*	AD	c.975T>G (p. Tyr325Ter)[a]	4	Hetero
07	F	50 y	N	Multiple renal cysts, both kidney Chronic renal failure	Liver cyst	ADPKD	*PKD1*	AD	c.8056C>T (p. Gln2686Ter)	5	Hetero
08	F	42 y	N	Multiple renal cysts, both kidney	Liver cyst	ADPKD	*PKD1*	AD	c.12060C>A (p. Cys4020Ter)[a]	4	Hetero
09	F	5 d	N	Pulmonary hypoplasia, Polycystic dysplastic kidney		ARPKD	*PKHD1*	AR	c.4879G>T (p. Val1627Phe)(p)[a] c.11212_11213delAT (p. Ile3738SerfsTer19) (m)[a]	4 5	Compound hetero
Patients referred for hematuria +/− proteinuria											
10	F	7 y	Y	Recurrent HU	Asthma, atopic dermatitis	Alport syndrome	*COL4A3*	AD, AR	c.417delG (p. Thr140HisfsTer13)[a]	4	Hetero
11	F	21 y	N	Recurrent HU GBM irregularity, suggestive of hereditary nephritis	Sensorihearing loss, both	Alport syndrome	*COL4A3*	AD, AR	c.1029 + 1G>A[a]	4	Hetero

TABLE 3 (Continued)

ID	Gender	Age	Fx	Clinical presentation-renal	Clinical presentation-extrarenal	Final diagnosis	Gene	Inheritance	Sequence variant	ACMG class	Zygosity
12	M	4 y	N	Hematuria		Alport syndrome	*COL4A4*	AD, AR	c.2084G>A (p. Gly695Asp)(p) c.1327_1344del (p. Pro444-Leu449del) (m)	4 5	Compound hetero
13	F	5 y	N	Hematuria, nephrolithiasis	Short stature	Hyperoxaluria type1	*AGXT*	AR	c.331 T>C (p. Arg111Ter)	5	Homo
ID	Gender	Age	Fx	Clinical presentation-Renal	Clinical presentation-Extrarenal	Final diagnosis	Gene	Inheritance	Sequence variant	ACMG class	Zygosity
Patients referred for electrolyte imbalance											
14	M	4 y	N	polyhydramnios Hx. Hypokalemia		Bartter syndrome	*CLCNKB*	AR	c.371C>T (p.Pro124Leu) (p) Exon 4 del(m)[b]	5	Compound hetero
15	F	27 y	N	Hypokalemia Hypochloremia	Hearing impairment tremor	Bartter syndrome	*CLCNKB*	AR	Exon 1–14 del(p)[b] c.1830G>A (p. Trp610Ter)(m)	5	Compound hetero
16	M	15 y	N	Hypokalemia	Hearing impairment tremor	Bartter syndrome	*SLC12A1*	AR	c.888delG (p)[a] c.1522G>A (p. Ala400Thr) (m)	5 4	Compound hetero
17	F	10 y	N	Hypokalemia Hypomagnesemia		Gitelman syndrome	*SLC12A3*	AR	c.1664C>T (p. Ser555Leu)(p) c. 2186G>A (p. Gly741Arg)(m)	5 5	Compoundhetero
18	M	23 y	N	Hypokalemia	Dystonia, tremor	Gitelman syndrome	*SLC12A3*	AR	c.1919A>G (p. Asn640Ser)(p) c.1868T>C (p. Leu623Pro)(m)	5 5	Compound hetero
19	F	2 y	Y	Renal tubular acidosis		SLC4A1-associated renal tubular acidosis	*SLC4A1*	AD	c.1765C>T (p. Arg589Cys)	5	Hetero
20	M	11 m	N	Hypokalemic alkalosis Diffusely bilateral renal enlargement with increased cortical echogenicity	Colon segmental resection, d/t colon ischemia	Congenital secretory diarrhea, chloride type	*SLC26A3*	AR	c.2063-1G>T (p,m)	4	Homo

Abbreviations: ACMG, American College of Medical Genetics and Genomics; AD, autosomal dominant; AR, autosomal recessive; Fx, family history; F, female; GBM, glomerular basement membrane; Hetero, heterozygous; HU, hematuria; M, male; m, maternal; PU, proteinuria; p, paternal.

[a]Novel pathogenic/likely pathogenic variant.

[b]Novel exonal deletion.

showed recurrent hematuria, and her genetic investigation revealed a paternally inherited P variant (p.Thr140HisfsTer13) in *COL4A3*. Her father also had a history of nephritis and was diagnosed with Alport syndrome after genetic testing. Additionally, Patient 11 presented with recurrent hematuria and sensory hearing loss and was revealed to have a novel LP variant in *COL4A3* that could cause a splicing error (1029 + 1G>A).

Further, we detected a novel P variant (c.888delG) of *SLC12A1* in a 15-year-old boy with recurrent hypokalemia and symptoms of hearing impairment and tremor. His genetic testing showed a paternally inherited novel variant (c.888delG) combined with a maternally inherited missense P variant (p.Ala400Thr) previously reported in *SLC12A1*. Accordingly, he was diagnosed with Bartter syndrome based on these results.

3.5 | Noteworthy VUSs

Notably, we identified a meaningful VUS in the *COL4A3* gene of two unrelated patients (patients 21 and 22). This missense variant was clinically classified as a VUS in a well-defined disease gene of Alport syndrome (c.1229G>A, p.Gly410Glu) according to ACMG guidelines. Patients 21 and 22 were presenting with proteinuria and hematuria since her 20s and the age of 5. Their renal pathologic findings revealed an irregular thickening of the GBM. Based on the clinical symptoms and biopsy findings, they were clinically suspected of having Alport syndrome. Their NGS panel testing identified the same heterozygous VUS (c.1229G>A, p.Gly410Glu) in *COL4A3*, with Sanger sequencing confirming the detected variant. The variant was absent from public genome databases (ExAC, 1000 Genomes data, and our in-house database), and the structure and function of the protein were predicted as likely damaged according to Polyphen2 (PPH2 score: 1.0). The altered residue was revealed to be highly conserved across vertebrate species. Given that this variant was consistently identified in two unrelated patients with highly suspected Alport syndrome from their clinical symptoms and pathological results, along with the results of variant analysis, this suggested a high probability of being reclassified as an LP/P variant. Although further analyses of the genetic consequences based on the family members were recommended to assess the exact pathogenic indication of this VUS, these tests could not be conducted due to lack of agreements by the family members of the patients (Table 4).

3.6 | Correlation between clinically suspected diseases and molecular diagnosis

Of the 20 patients for whom disease was confirmed through molecular diagnosis based on our genetic test, clinically suspected diagnosis and genetic diagnosis were matched in 11 patients (11/20, 55%), including three patients with ADPKD, one patient with ARPKD, two patients with Alport syndrome, three patients with Bartter

TABLE 4 Clinical and genetic data of patients in whom a noteworthy VUS was identified

ID	Gender	Age	Fx	Clinical presentation-Renal	Clinical presentation-Extrarenal	Final diagnosis	Gene	Inheritance	Sequence variant	ACMG class	Zygosity
Patients referred for hematuria +/− proteinuria											
21	F	36 y	N	HU/ PU since 20′ irregular thickening of GBM		Alport syndrome	*COL4A3*	AD, AR	c.1229G>A (p.Gly410Glu)	3	Hetero
22	F	15 y	N	Consistent HU irregular thickening of GBM		Alport syndrome	*COL4A3*	AD, AR	c.1229G>A (p.Gly410Glu)	3	Hetero

Abbreviations: ACMG, American College of Medical Genetics and Genomics; AR, autosomal recessive; Fx, family history; F, female; GBM, glomerular basement membrane; Hetero, heterozygous; HU, hematuria; M, male; m, maternal; PU, proteinuria; p, paternal; VUS, variant of uncertain significance.

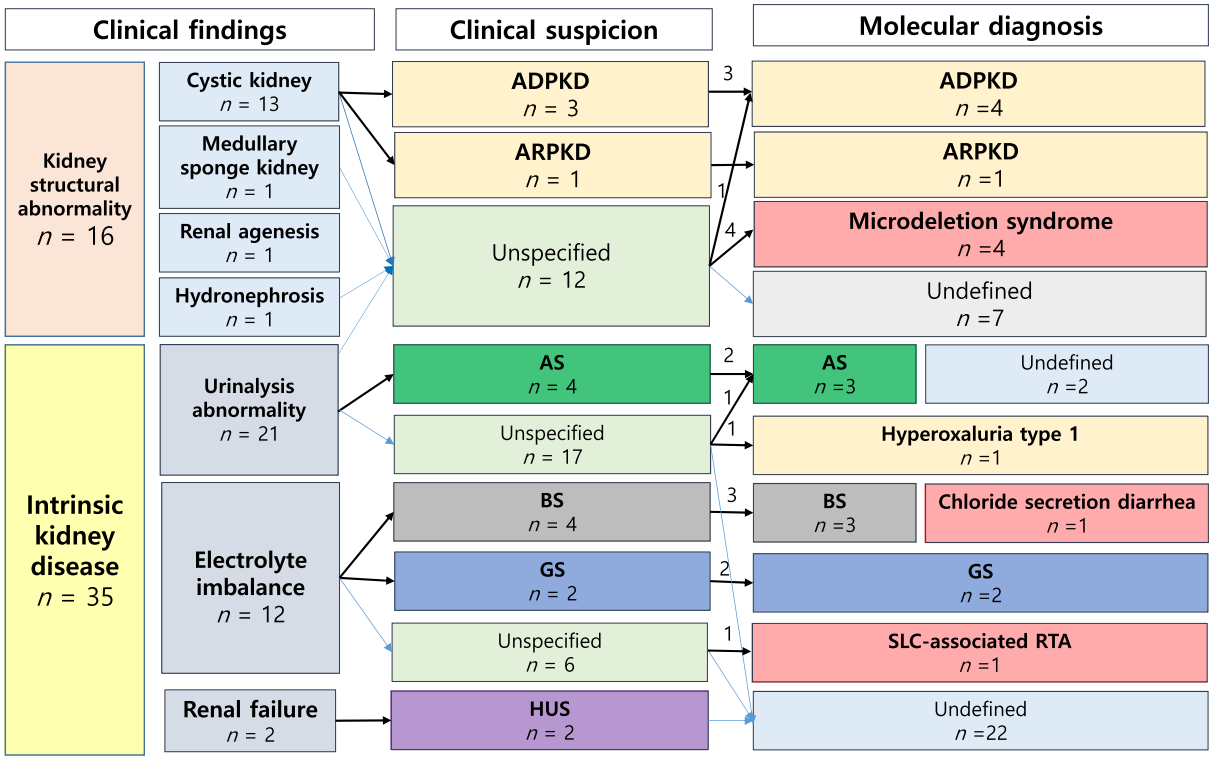

FIGURE 1 Correlations between clinical suspicion and results of molecular analysis. ADPKD, autosomal dominant polycystic kidney disease; AS, Alport syndrome; BS, Bartter syndrome; GS, Gitelman syndrome; HUS, hemolytic uremic syndrome; TBMD, thin basement membrane disease; SRNS, steroid resistance nephrotic syndrome [Colour figure can be viewed at wileyonlinelibrary.com]

syndrome, and two patients with Gitelman syndrome. By contrast, the results of our NGS panel reclassified the final diagnosis from initial clinical diagnosis in one patient (5%). This 11-month-old boy was initially clinically diagnosed with Bartter syndrome before genetic testing and finally diagnosed with chloride-secretion diarrhea originating from the intestine and not from the kidneys (Patient 20).

In eight patients, the NGS panel test played an essential role in confirming the genetic cause of their previously undefined kidney disease (8/20, 40%), including one patient with ADPKD with cystic kidney (Patient 5), one patient with Alport syndrome (Patient 12), one patient with hyperoxaluria type 1 (Patient 13), one patient with SLC4A1-associated renal tubular acidosis (Patient 19) and four patients with CNVs abnormalities (Patients 1, 2, 3 and 4). Detailed results are shown in Figure 1.

4 | DISCUSSION

In this study, we analyzed the genetic diagnosis of 51 unrelated patients with clinically suspected, inherited kidney disease using a customized NGS panel that included genes related to broad symptoms of kidney disease. Consequently, a total of 39.2% (20/51) of patients were confirmed as having a genetic disease.

Several studies analyzed results using targeted NGS panels to diagnose inherited kidney diseases. Sen et al. reported the results of analysis of an SRNS-targeted diagnostic gene panel performed for 302 patients, confirming genetic diagnoses in 26.6% of the patients.[23] Another study of 44 patients with typical PKD who underwent targeted NGS testing with 63 related genes revealed 48 related mutation sites in *PKD1* and *PKD2*.[24]

It should be noted that in the present study, the NGS panel used did not focus on genes limited to kidney diseases but rather included a broad set of 203 genes related to diseases that might mimic the symptoms of inherited kidney diseases, even though diseases originate from other organs. This approach could facilitate the accurate diagnosis of inherited kidney diseases, as well as the prompt differentiation of genetic diseases with overlapping symptoms, whether these might have originated in the confined kidney or other organs.

One of the advantageous characteristics of this NGS panel was evident based on the notable case of patient 20. This patient visited our hospital for the first time exhibiting lethargy. He had been born at 35.4 weeks of gestation from healthy parents and had two healthy older brothers, with no other notable family history. Fifteen days after birth, he developed abdominal distension suggestive of neonatal necrotizing enterocolitis and received ileostomy surgery. After 2 months, he received another surgery for segmental resection of a 7.9-cm ischemic ileum lesion and to repair the ileostomy site. On admission, he showed severe hyponatremia and hypokalemic hypochloremic metabolic alkalosis (serum sodium: 128 mmol/L; potassium: 2.5 mmol/L; and chloride: 67 mmol/L; pH 7.652; pCO2: 32.5 mmHg; pO2: 103.0 mmHg; and HCO3: 36.3 mmol/L). Abdominal sonography showed diffuse renal disease with bilateral renal enlargement. Given

these results, Bartter syndrome was initially suspected as a diagnosis, and he was referred to our study to precisely identify the genetic etiology of his condition. According to the genetic test, we discovered two homozygous splice-site pathogenic mutations in *SLC26A3* (c.2063-1G>T), which encodes a transmembrane glycoprotein that exchanges chloride and bicarbonate ions across the cell membrane. His parents were identified as the asymptomatic carriers of c.2063-1G>T. To confirm the molecular diagnosis, we analyzed the stool of the patient, finding a sodium level of 120 mmol/L. Although there were symptoms that could be mistaken as a disease originating from the kidney, he was finally diagnosed with chloride-secreting diarrhea, which differed from the first clinical impression. This result of the molecular diagnosis offered the chance of appropriate treatment, focusing salt substitution therapy according to the treatment protocol of chloride-secreting diarrhea and he has maintained good condition.

Additionally, our findings emphasized the usefulness of CNV analysis. Recent studies report that most CNVs are likely benign, but that some specific variants might be related to genetic diseases, such as neurodevelopmental diseases and various cancers.[25-28] NGS based CNV detection has a reported sensitivity of up to 92% and specificity of up to 100% for detecting duplications as small as 300 bp and deletions as small as 180 bp in specific genes.[29,30] In some inherited kidney diseases, CNVs could also affect susceptibility, and previous studies have highlighted the need for analyzing CNVs in inherited kidney diseases, such as CAKUT.[29,31,32] In the present study, we detected two known pathogenic CNVs in four patients (Patients 1–4), with both identified as known CNVs that could lead to kidney- related symptoms in addition to systemic manifestations. Details of abnormalities in CNVs and patients are described in Table 3.

Regarding the gene variants detected in this study, we found seven novel P/LP variants and two novel exonal deletions among our patient group. Further, there was a noticeable VUS identified in *COL4A3*, which was found in two unrelated patients. Evidence suggested that this VUS should be reclassified as P/LP, even though it is yet assumed to be a VUS according to ACMG guidelines. Further functional research and segregation analysis of the family of these patients would help define the pathogenicity of this variant.

The present study had a major advantage (ie, using a targeted NGS panel focusing on the phenotype of kidney diseases) that allowed diagnosis of inherited kidney diseases along with differential diagnoses of diseases based on their origin (kidney or other organs), despite their presenting symptoms similar to those of kidney disease. Moreover, we were able to simultaneously obtain relatively high diagnostic performance for CNV analysis with the NGS panel. However, a limitation of the present study is its single-center research design and inclusion of a small number of patients. Further studies with a larger number of patients might aid verification of these results in the future.

Diagnostic approaches using NGS technology could enable accurate and early detection of genetic diseases and minimize the need for invasive diagnostic procedures, as well as optimize outcomes by broadening therapeutic options. Moreover, presymptomatic testing based on family history could be used to detect the genetic causes of diseases prior to the appearance of overt symptoms, which might allow application of genetic results for prenatal genetic testing and counseling in order to prevent the disease.

This study confirmed the efficacy of NGS with CNV analysis as a diagnostic tool for patients with suspected inherited kidney disease based on their symptoms. The rapid development of NGS technology would enable further clinical applicability of this approach for the diagnosis of inherited kidney disease.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

All relevant data are found within the paper and the Supporting information files.

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