의학박사 학위논문

Two-step genetic diagnosis of nephronophthisis-related ciliopathy using PCR amplicon sequencing and targeted re-sequencing

-PCR amplicon sequencing과 targeted re-sequencing의 이단계 접근법을 이용한 nephronophthisis-related ciliopathy의 유전 진단-

2014년 8월

서울대학교 대학원

의학과 분자유전체의학 전공

이 현 경

Two-step genetic diagnosis of nephronophthisis-related ciliopathy using PCR amplicon sequencing and targeted re-sequencing

-PCR amplicon sequencing과 targeted re-sequencing의 이단계 접근법을 이용한 nephronophthisis-related ciliopathy의 유전 진단-

2014년 8월

서울대학교 대학원

의학과 분자유전체의학 전공

이 현 경

ABSTRACT

Introduction: Nephronophthisis-related ciliopathy (NPHP-RC) is the most common genetic cause of end-stage renal disease (ESRD) in childhood and adolescence. Although genetic diagnosis is critical to provide optimal care to patients with NPHP-RC, genetic heterogeneity decreases the efficacy of individual gene sequencing in detecting pathogenic mutations.

Methods: A two-step approach (PCR amplicon sequencing followed by targeted re-sequencing) for the screening of NPHP-RC-related genes was designed to make the confirmative genetic diagnostic detect rate high.

Results: In a cohort of 55 NPHP-RC patients, five genes (*NPHP1, INVS/NPHP2, IQCB1/NPHP5*, *CEP290/NPHP6/BBS14* and *TMEM67/ MKS3/NPHP11/JBTS6*) detected with relatively high frequency in mutations were sequenced. Damaging mutations in 12 patients (21.8%) were found by PCR amplicon sequencing: total deletions of *NPHP1* in 4 juvenile NPHP patients, *IQCB1/NPHP5* mutations in 3 Senior-Löken syndrome patients, a *CEP290/NPHP6/BBS14* mutation in 1 Joubert syndrome patient, and *TMEM67/MKS3/NPHP11/JBTS6* mutations in 4 Joubert syndrome patients with retinitis pigmentosa. Subsequently, targeted re-sequencing of 34 NPHP-RC-related genes was done in the remaining 43 patients. Additional candidate pathogenic mutations in 9 NPHP-RC-related genes were found in 13 (30.2%) of 43 patients.

Conclusions: Pathogenic mutations including 20 novel mutations were detected in 12 NPHP-RC-related genes in 25 (45.4%) of 55 NPHP-RC patients using two-step genetic diagnosis. The two-step approach could be a powerful tool for detecting pathogenic mutations in NPHP-RC.

Keywords: nephronophthisis-related ciliopathy, PCR amplicon sequencing, targeted re-sequencing

Student Number: 2010-30585

Contents

List of tables

List of figures

INTRODUCTION

Nephronophthisis (NPHP; literally "disappearance of nephrons") was first described over 50 years ago by Smith and Graham[1] and Fanconi et al[2] who introduced the term familial juvenile nephronophthisis. NPHP is a renal cystic disease that is inherited as an autosomal recessive trait. It is the most frequent genetic cause for end stage renal disease (ESRD) in the first 3 decades of life[3-6]. It should be included in the differential diagnosis of children or adolescents presenting with ESRD of unknown etiology[3-6].

NPHP is classified into two clinical forms according to the age of onset of ESRD[7]. Infantile type manifests with ESRD within 5 years of birth. The juvenile type manifests later. NPHP is clinically characterized by severe anemia, growth retardation due to impaired renal function, polyuria/nocturia and polydipsia due to decreased renal concentrating ability[7]. Urinalysis is often normal[8] and the blood pressure is not high. Ultrasonography reveals increased echogenicity without corticomedullary differentiation and normal sized or relatively small kidneys, which differs from autosomal recessive or autosomal dominant polycystic kidney disease (ARPKD and ADPKD, respectively)[9]. Renal pathology includes tubular basement membrane disruption, tubulointersitial nephropathy, and cysts. NPHP is often accompanied by impairment of other systems, such as retina, brain, hepatobiliary system, and skeletal system, comprising various syndromes, including Senior-Löken syndrome (SLS; retinal degeneration), Joubert syndrome (JBTS; cerebellar vermis hypo/aplasia), Meckel Gruver syndrome

(MKS; occipital meningoencephalocele, postaxial polydactyly, and liver fibrosis) and Jeune asphyxiating thoracic dystrophy (shortening of bones and narrow rib cage).

NPHP is considered a renal ciliopathy. Pazour et al[10] first described the relationship between cilia and cystic kidney disease in 2000. The authors reported shortened cilia in the kidneys of *orpk* mice, a model for polycystic kidney disease (PKD) involving a mutation in the *IFT88* gene encoding intraflagellar transport protein. Because most of the causative genes of cystic kidney diseases are related to the structure and the function of primary cilia[13], all cystic kidney diseases are thought to represent renal ciliopathies. Cystic kidney disease comprises ADPKD, ARPKD, NPHP, medullary cystic kidney disease, and other cystic kidney diseases including Bardet–Biedl syndrome (BBS) and oro-facio-digital syndrome, type1 (OFD1). A collective term, NPHP-related ciliopathy (NPHP-RC), is used to describe this group of diseases, NPHP and NPHP-related syndromes as a whole[11,12].

The cilium is a hair-like structure that extends from the cell surface into the extracellular surface[7]. Virtually all vertebrate cell types can produce cilia. Cilia are divided into motile cilia and non-motile primary cilia. Primary cilia are sensory organelles that connect mechanosensory, visual, osmotic and other stimuli, and maintain cell-cycle control and epithelial cell polarity by signaling mechanisms that involve the non-canonical Wnt signaling pathway and the sonic hedgehog signaling pathway[1]. All cystic kidney diseases result from mutations of these cilia-related genes including *NPHP*, *PKD1/2, JBTS*,

MKS, *BBS*, *OFD1* and *IFT*, and other genes expressed in primary cilia, basal bodies, mitotic spindle, focal adhesions and adherens junctions, BBSome and centrosomes of renal epithelial cells[7]. Because cilia are sensory organelles expressed in the retina, cerebellum, hepatobiliary system, skeletal system and others, the ciliary theory can explain multisystem involvement in NPHP.

A genetic diagnosis of NPHP-RC is required for a definitive diagnosis because the clinical features of patients with NPHP-RC are rather non-specific, and significantly overlap with various symptoms[13]. The most common genetic cause of NPHP is a large deletion of *NPHP1*[14-16], which is located in chromosome 2q13 and covers more than 20% of patients with NPHP, whereas other genes contribute less than 2-3%[12] each. Polymerase chain reaction (PCR) amplicon sequencing against common causative genes of NPHP can detect mutations in less than one-third of patients[13].

The identified NPHP-RC-related genes have been rapidly increasing, especially after the introduction of next-generation sequencing (NGS) techniques[17,18]. NGS technologies allow efficient gene identification by sequencing large parts (targeted exome sequencing) or even full complement of DNA (whole exome sequencing) of a single individual at once. The exome refers to the transcribed region of the genome. It comprises 5 percent of the whole genome. The protein coding region comprises 1 percent of the whole genome. Eighty five percent of the disease-causing mutations can be detected by this method. By applying NGS of robust parallel sequencing of

known and candidate genes, we can obtain a genetic diagnosis for more patients and expand the pool of causative genes. This technology is still evolving, demanding a relatively large cost and advanced bioinformatics support for handling large quantities of data.

The present study explored a two-step diagnostic approach involving the combination of PCR amplicon sequencing and targeted exome re-sequencing in a cohort of the patients. The results indicate that this approach could be the best way to obtain a genetic diagnosis of NPHP-RC with a high rate of accuracy.

MATERIALS AND METHODS

Study population

This study was approved by the independent review board of Seoul National University Hospital (H-0812-002-264), and only those patients from whom written informed consent was obtained were screened and included in this study. Patients who were clinically diagnosed as NPHP[32] were included. Presentation with incidentally found chronic kidney disease (CKD) in the first 3 decades of life, without evidence of previous renal damage, was typical. Other causes of CKD were excluded by past medical history and imaging of the urinary tract in the majority of the cases. Patients with a clinical diagnosis of ADPKD were excluded. Patients received fundus examinations and abdominal sonography to evaluate the intra-abdominal organs. Renal ultrasonographic findings of increased echogenicity, normal or slightly decreased size for age and corticomedullary differentiation loss were considered to be typical for NPHP-RC[8]. For those individuals with developmental delay and neurological problems, brain imaging was requested. Renal pathology additionally determined for several patients showed typical findings of chronic tubular interstitial disease.

The patients were classified according to their age at the time of ESRD as infantile when younger than 5 years old and juvenile when older. Cerebellar vermis hypoplasia or aplasia was designated as Joubert syndrome, retinal

involvement with retinitis pigmentosa (RP) or Leber's congenital amaurosis as Senior-Löken syndrome, and those subjects with multiple problems without cerebellar hypo/aplasia were considered as having Meckel-Gruber Syndrome.

PCR amplicon sequencing

The most probable genes were selected from the known frequencies and phenotypes of their mutations to stratified application of PCR amplicon sequencing (Fig. 1). Peripheral blood mononuclear cells were harvested from the patients, and genomic DNA was obtained using a QIA amp DNA Blood Mini Kit (Qiagen, Hilden, Germany). All patients were screened for large deletions of *NPHP1*, which is the most common mutation in NPHP, as well as in all NPHP-RC-related genes, by amplifying each of the exons using PCR; failure of amplification is considered to be a total homozygous deletion of the gene encompassing the corresponding exons[15, 36]. Among those patients without large deletions of *NPHP1*, infantile NPHP patients were tested for *INVS/NPHP2* mutations[37]. The selection of genes according to phenotype was *IQCB1/NPHP5* for RP[38], *CEP290/NPHP6/BBS14* for Joubert syndrome[39] and *TMEM67/MKS3/NPHP11/JBTS6* for hepatic fibrosis[40,41].

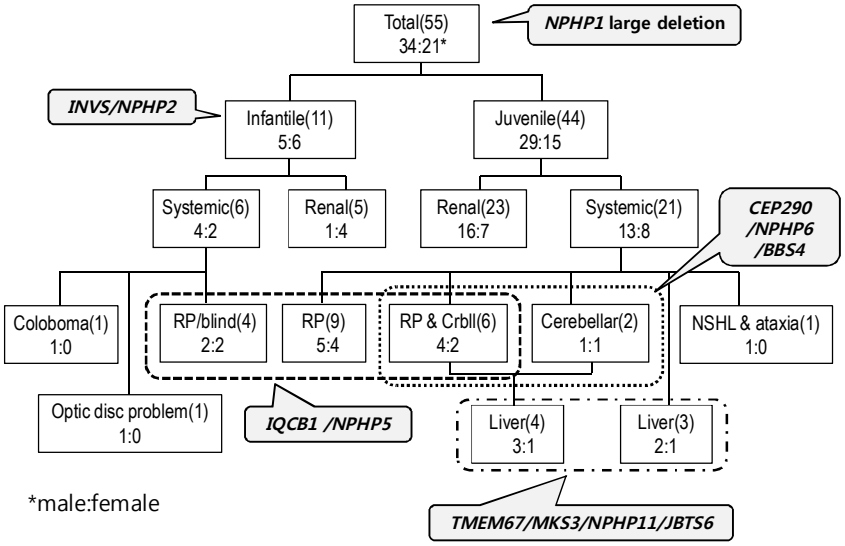

Figure 1. Strategy for PCR amplicon sequencing.

All patients were screened for large deletions of *NPHP1*, which is the most common mutation in NPHP. Among those without large deletions of *NPHP1*, infantile NPHP patients were tested for *INVS/NPHP2* mutations. *IQCB1/NPHP5*, *CEP290/NPHP6/BBS14*, and *TMEM67/MKS3/NPHP11 /JBTS6* were tested according to the phenotypes of the patients.

M, male; F, female; RP: retinitis pigmentosa; Crbll, cerebellar; SNHL, sensory neural hearing loss.

Targeted exome sequencing

Customized targeted exome capture using a SeqCap EZ kit (Roche NimbleGen, Madison, WI, USA) against 34 genes related to NPHP-RC, Bardet-Biedl syndrome, and ARPKD was designed (Table 1). After capturing the target sequences, the DNA libraries were amplified and sequenced using a HiSeq2000 apparatus (Illumina, San Diego, CA, USA).

Table 1. Target genes for sequencing of NPHP-RC patients

Gene	Protein	Chr	Accession No.	Exon count	Coding exon	ORF (bp)	HGNC	OMIM
NPHP1	nephrocystin 1	2q13	NM_000272.3	20	20	2,202	7905	[609583] Joubert syndrome 4 [256100] Nephronophthisis 1, juvenile [266900] Senior-Löken
INVS /NPHP2	inversin	9q31.1	NM_014425.3	17	16	3,198	17870	[602088] Nephronophthisis 2, infantile
NPHP3 /MKS7	nephrocystin 3	3q22.1	NM_153240.4	27	27	3,993	7907	[267010] Meckel syndrome 7 [604387] Nephronophthisis 3
NPHP4	nephroretinin	1p36.31	NM_015102.3	30	29	4,281	19104	[606966] Nephronophthisis 4 [606996] Senior-Löken syndrome 4
IQCB1 /NPHP5	IQ motif containing B1	3q13.33	NM_001023570.2	15	13	1,797	28949	[609254] Senior-Löken syndrome 5
CEP290 /NPHP6 /BBS14	centrosomal protein 290 kDa	12q21.32	NM_025114.3	54	53	7,440	29021	[209900] Bardet-Biedl syndrome 14 [610188] Joubert syndrome 5 [611755] Leber congenital amaurosis 10 [611134] Meckel syndrome 4 [610189] Senior-Löken syndrome 6
GLIS2 /NPHP7	GLIS family zinc finger protein 2	16p13.3	NM_032575.2	6	6	1,575	29450	[611498] Nephronophthisis 7
RPGRIP1L /NPHP8	RPGRIP1-like	16q12.2	NM_015272.2	27	26	3,948	29168	[216360] COACH syndrome [611560] Joubert syndrome 7 [611561] Meckel syndrome 5

NEK8 /NPHP9	serine/threonine-protein kinase Nek8	17q11.2	NM_178170.2	15	15	2,079	13387	[613824] Nephronophthisis 9
SDCCAG8 /NPHP10 /BBS16	serologically defined colon cancer antigen 8	1q43	NM_006642.3	18	18	2,139	10671	[613615] Senior-Löken syndrome 7
TMEM67 /MKS3 /NPHP11 /JBTS6	transmembrane protein 67	8q22.1	NM_153704.5	28	28	2,988	28396	[216360] COACH syndrome [610688] Joubert syndrome 6 [607361] Meckel syndrome 3 [613550] Nephronophthisis 11 [209900] Bardet-Biedl syndrome 14, modifier of
TTC21B /NPHP12	tetratricopeptide repeat domain 21B	2q24.3	NM_024753.4	29	29	3,951	25660	[613820] Nephronophthisis 12
CEP164 /NPHP15	centrosomal protein 164 kDa	11q23.3	NM_014956.4	33	31	4,383	29182	[614845] Nephronophthisis 15
XPNPEP3	probable Xaa-Pro aminopeptidase 3	22q13.2	NM_022098.3	10	10	1,524	28052	[613159]Nephronophthisis-like nephropathy 1
INPP5E	72 kDa inositol polyphosphate 5-phosphatase	9q34.3	NM_019892.4	10	10	1,935	21474	[213300] Joubert syndrome 1 [610156]Mental retardation, truncal obesity, retinal dystrophy, and micropenis
TMEM216 /JBTS2	transmembrane protein 216	11q12.2	NM_016499.5	5	5	270	25018	[608091] Joubert syndrome 2 [603194] Meckel syndrome 2
AHI1	Abelson helper	6q23.3	NM_017651.3	29	26	3,591	21575	[608629] Joubert syndrome 3

/JBTS3	integration site 1							
ARL13B /JBTS8	ADP-ribosylation factor-like 13B	3q11.1	NM_001174151.1	9	8	978	25419	[612291] Joubert syndrome 8
CC2D2A /JBTS9 /MKS6	coiled-coil and C2 domain containing 2A	4p15.32	NM_001080522.2	38	37	4,863	29253	[216360] COACH syndrome [612285] Joubert syndrome 9 [612284] Meckel syndrome 6
OFD1	oral-facial-digital syndrome 1	Xp22.2	NM_003611.2	23	23	3,039	2567	[300804] Joubert syndrome 10 [311200]Oral-facial-digital syndrome 1 [300209]Simpson-Golabi-Behmel syndrome, type 2
C5orf42 /JBTS17	chromosome 5 open reading frame 42	5p13.2	NM_023073.3	52	51	9,594	25801	[614615] Joubert syndrome 17
BBS1	Bardet-Biedl syndrome 1	11q13.2	NM_024649.4	17	17	1,782	966	[209900]Bardet-Biedl syndrome 1
BBS2	Bardet-Biedl syndrome 2	16q12.2	NM_031885.3	17	17	2,166	967	[209900] Bardet-Biedl syndrome 2
ARL6 /BBS3	ADP-ribosylation factor-like protein 6	3q11.2	NM_032146.4	9	7	561	13210	[209900] Bardet-Biedl syndrome 3 [613575] Retinitis pigmentosa 55 [209900]Bardet-Biedl syndrome 1, modifier of
BBS4	Bardet-Biedl syndrome 4	15q24.1	NM_033028.4	16	16	1,560	969	[209900] Bardet-Biedl syndrome 4
BBS5	Bardet-Biedl syndrome 5	2q31.1	NM_152384.2	12	12	1,026	970	[209900] Bardet-Biedl syndrome 5
MKKS	McKusick-Kaufman	20p12.2	NM_018848.3	6	4	1,713	7108	[209900] Bardet-Biedl syndrome 6

/*BBS6*	syndrome							[236700]McKusick-Kaufman syndrome
BBS7	Bardet-Biedl syndrome 7	4q27	NM_176824.2	19	19	2148	18758	[209900] Bardet-Biedl syndrome 7
BBS9 /*PTHB1*	Bardet-Biedl syndrome 9, parathyroid hormone-responsive B1	7p14.3	NM_014451.3	21	20	2,544	30000	[209900] Bardet-Biedl syndrome 9
BBS10	Bardet-Biedl syndrome 10	12q21.2	NM_024685.3	2	2	2,172	26291	[209900] Bardet-Biedl syndrome 10
TRIM32 /*BBS11*	tripartite motif containing 32	9q33.1	NM_001099679.1	2	1	1,962	16380	[209900] Bardet-Biedl syndrome 11
BBS12	Bardet-Biedl syndrome 12	4q27	NM_001178007.1	3	1	2,133	26648	[209900] Bardet-Biedl syndrome 12
MKS1 /*BBS13*	Meckel syndrome, type 1	17q22	NM_017777.3	18	18	1,680	7121	[209900] Bardet-Biedl syndrome 13 [249000] Meckel syndrome 1
PKHD1	polycystic kidney and hepatic disease 1 (autosomal recessive)	6p12.3-p12.2	NM_170724.2	67	66	10,191	9016	[263200] Polycystic kidney and hepatic disease

Alignment, coverage calculation, and variant detection

Reads were aligned to the UCSC hg19 reference genome using BWA-0.6.1 with default settings[41] for SNV/indel detection, duplications were removed, data cleanup was followed by GATK, and variants were identified using Unified Genotyper in GATK-1.3[43]. Perl script and Annovar were used to annotate variants and search the known single nucleotide polymorphsims (SNPs) and indels from dbSNP v135 and the 1000 Genomes project data (drafted February 2012). Coverage and depth were calculated using the GATK DepthOfCoverage analysis. The significance of variants was assessed *in silico* using PolyPhen-2, SIFT, SNP3D, and MutationTaster, and those variants predicted to be disease-causing or not tolerated by two or more programs were considered to be pathogenic mutations[44-47]. Predication of alternative splicing by intronic variation was performed by NetGene2 (http://www.cbs.dtu.dk/services/NetGene2/), Ex-skip (http://ex-skip.img.cas.cz/), and BDGP acceptor site prediction (http://www.fruitfly.org/seq_tools/other.html).

Variant calls were obtained using the following filter parameters: minimum base quality was 17 (default value of GATK Unified Genotyper). A minimum variant count of 10 was applied for potential truncating mutations (nonsense, frameshift, and obligatory splice-site mutations) and non-synonymous missense variants were filtered by a minimum count of 3. Synonymous variants and common dbSNP (v135) with a population allele frequency greater than 1% were excluded.

RESULTS

Clinical features of NPHP-RC patients

Fifty five unrelated patients with a clinical diagnosis of NPHP were recruited (34 males, 21females) after referral to our laboratory for genetic diagnosis. The mean age of the patients at ESRD was 9.0 ± 5.1 years old (median, 8.3 years; range, 0.6-17.9 years). Renal histology from 15 patients showed typical findings of tubulointerstitial nephropathy. Nineteen patients (34%) had RP and Leber's congenital amaurosis, eight patients (14.5%) had the molar tooth sign in brainstem on brain imaging, and seven patients (12.7%) had hepatic fibrosis. Eight patients (14.5%) had siblings with similar symptoms; however, none of their parents had ERSD.

PCR amplicon sequencing for genetic diagnosis

As a first step in the stratified approach to determine genetic diagnosis of NPHP, the patients were selected and screened by PCR amplicon sequencing against candidate genes (Table 2). Homozygous total deletion of *NPHP1* was detected in four patients (Fig. 1, 7% of total patients and 17% of juvenile isolated NPHP without other organ involvement). In infantile type NPHP (N=11), one single nucleotide variation (SNV) in *INVS/NPHP2* was found, c.721A>T or p.Thr241Ser. Three (15.8%) of 19 NPHP patients with RP and Leber's congenital amaurosis carried a common pathogenic homozygous

Table 2. Mutations found in NPHP-RC patients using PCR amplicon sequencing

Pt	ESRD (yrs)	Extra-renal manifestations	Gene	Nucleotide change	Amino acid change	Mut. Taster	Poly-Phen2	SIFT	Ref.
K-1	8.1	RD	*NPHP1*	Total deletion (Hom)					
J-57	13.0	RD, nystagmus							
J-104	14.0	RD							
J-92	CKD	Myopia							
O-508	17.9	Congenital cataract	*IQCB1*	c.1522_1523dupGA	p.Ala509Lysfs*3				20
K-2	11.0	RD, LCA, nystagmus	*/NPHP5*	(Hom)					
K-3	10.8	RD, LCA							
J-86	NA	LCA, cataract, CVA, MR	*CEP290*	c.1666delA	p.Ile556Phefs*17				21
			/NPHP6	/ IVS43-12 T>A	/alt. splicing				22
			/BBS14						
K-4	11.8	Apraxia, MR, CVA, Caroli disease, choledochal cyst	*TMEM67* */MKS3*	c.2758delT /c.A725G	p.Tyr920Thrfs /p.Asn242Ser				25
J-63	6.0	OMA, ONA, CVA, MR, HF, choledochal cyst	*/NPHP11* */JBTS6*	c.579_580delAG /c.G274A	p.Gly195Ilefs*13 /p.Gly92Arg				24 23
J-55	6.8	RD, CVA, MR, CP, HF		c.1353delA /c.G274A	p.Glu452Lysfs*4 /p.Gly92Arg				23
J-61	14.4	ONA, RD, CVP, MR, HF, choledochal cyst		c.1353delA /c.T2096C	p.Glu452Lysfs*4 /p.Leu699Ser	DC	PoD	Damaging	

CP, cerebral palsy; CVA, cerebellar vermis aplasia; CVP, cerebellar vermis hypoplasia; DD, developmental delay; ds., disease; ESRD, end-stage renal disease; HF, hepatic fibrosis; Hom, homozygous mutation; LCA, Leber congenital amaurosis; MR, mental retardation; NA, not applicable; OMA, oculomotor apraxia; ONA, optic nerve anomaly; RD, retinal dystrophy; DC, disease causing; PoD, possibly damaging

mutation (c.1522_1523dupGA, p.Ala509Lysfs*3) in the *IQCB1/NPHP5* genes[20]. Screening NPHP patients for cerebellar vermis hypotrophy/atrophy involvement (N=8) revealed one familial case of Joubert syndrome with a compound heterozygote mutation in *CEP290/NPHP6/BBS14,* a known pathogenic c.1666delA, p.Ile556Phefs*17,[21] and a SNV (c.6011-12T>A) predicted to cause alternative splicing[19]. Four of seven NPHP patients with hepatic fibrosis carried compound heterozygous mutations in *TMEM67/MKS3/NPHP11/JBTS6,* a frameshift truncation, and SNV[23-25]; all four of these patients had Joubert syndrome and congenital hepatic fibrosis, compatible with COACH (cerebellar vermis hypoplasia/aplasia, oligophrenia, ataxia, coloboma, and hepatic fibrosis) syndrome[26,27]. In total, PCR amplicon sequencing detected genetic aberrations in 12 patients (21.8%) with a clinical diagnosis of NPHP-RC.

Targeted re-sequencing in patients with NPHP-RC

Targeted exome sequencing against 34 NPHP-RC-related genes was applied in 43 patients, who did not have mutations in PCR amplicon sequencing. An average of 21.8 Mb of mapped data per individual for approximately 166 kb of targeting and flanking regions was obtained (Table 3). The mean read depth was 131 ± 55, and 97.8% and 95.4% of the captured target exons covered more than 1× and 10×, respectively. The average read depths of seven samples were significantly lower than those of the other samples,

Table 3. Summary of targeted exome sequencing data of NPHP-RC patients

Read	Mean (± standard deviation)
Aligned sequence (Mb)	21.8 (± 9.2)
Total reads	3,118,431 (± 489,391)
Aligned paired reads	2,991,119 (± 468,766)
Aligned singleton reads	15,815 (± 9107)
% of bases covered to	
≥ x1	97.8 (± 1.1)
≥ x10	95.4 (± 1.2)
≥ x25	87.2 (± 10.9)
≥ x50	69.5 (± 30.0)
≥ x75	59.5 (± 30.4)
≥ x100	50.8 (± 27.2)

which might have been related to variant detection failure (Table 3). Pathogenic mutations were found in 13 of 43 cases with various types of mutations in nine genes: *NPHP1, NPHP3/MKS7, NPHP4, SDCCAG8/NPHP10/BBS16, TTC21B/NPHP12, TRIM32/BBS11, CEP164 /NPHP15*, *C5orf42/JBTS17*[28], and *PKHD1* (Table4). In four cases, a heterozygous known pathogenic mutation in *BBS4* or *MKKS/BBS6* was found, which could not explain the genetic pathogenesis but might explain a mild phenotype resembling BBS, as previously reported[29,30]. Heterozygous mutations in 13 patients in at least one of the target NPHP-RC genes were identified, including one truncating mutation of *NPHP3/MKS7* in two patients with isolated juvenile NPHP (Table 5). In the remaining 13 patients, no significant variations of the target genes were found. All of the candidate

mutations were validated by Sanger sequencing, and their co-segregation in the family if available was verified. In total, pathogenic mutations in 25 (45.5%) of 55 NPHP-RC patients with two-step genetic diagnosis could be detected (Fig. 2).

Table 4. Pathogenic mutations found in NPHP-RC patients using targeted re-sequencing

Pt	ESRD (yrs)	Extra-renal manifestations	Gene	Nucleotide change	Amino acid change	Mut. Taster	Poly Phen2	SIFT	Ref.
J-39	12.7	RD, nystagmus	*NPHP1*	total deletion /c.609_610insC	/p.Arg204Glnfs				
J-4	16.7	Elliptocytosis	*NPHP3* */MKS7*	c.1599_1600delGG insCGAGAAGTCTC /c.G1597C	p.Gly533delinsGEVS /p.Gly533Arg	 DC	 PD	 D	
K-5	2.5	ONA	*NPHP4*	c.G2260A (Hom)	p.Gly754Arg				[49]
J-50	14	Amblyopia, strabismus, LCA	*SDCCAG8* */NPNP10* */BBS16*	c.845_848delTTTG /c.1300delA	p.Cys283fs*1 /p.Asn434Ilefs				
J-10	15.6		*TTC21B* */NPHP12*	c.G379A /c.C2572T	p.Ala127Thr /p.Arg858X	DC	PD	NT	
J-13	1.2		*CEP164* */NPHP15*	c.C3716T /c.C1484G	p.Pro1239Leu /p.Pro495Arg	SNP SNP	PD PoD	NT T	
J-70	13		*CEP164* */NPHP15*	c.C3716T /c.C1484G	p.Pro1239Leu /p.Pro495Arg	SNP SNP	PD PoD	NT T	
J-60	1.2	OMA, ONA, CVA, DD, HF, choledochal cyst	*TRIM32* */BBS11*	c.T467C /c.T479G	p.Leu156Pro /p.Met160Arg	DC DC	PD Benign	T T	

J-102	6.5	LCA, MR, brain atrophy, Caroli disease	*C5orf42* */JBTS17*	c.G8539A /c.C4699T	p.Asp2847Asn /p.Pro1567Ser	DC SNP	PD Benign	NT T	
J-94	0.8	OMA, nystagmus, MR, osteochondrodysplasia	*C5orf42* */JBTS17*	c.G8746A /c.A5532T	p.Ala2916Thr /p.Glu1844Asp	DC SNP	PD Benign	T T	
O-463	CKD	Caroli disease	*PKHD1*	c.T2507C /c.T11611C	p.Val836Ala /p.Trp3871Arg	SNP	PD	NT	50
K-6	12.0	RD	*PKHD1*	c.C9629G /c.G325A	p.Ser3210Cys /p.Ala109Thr	DC SNP	PD Benign	NT NT	
K-7	12.9	ONA	*PKHD1*	c.C1690T /c.T1756G	p.Arg564X /p.Phe586Val	DC	NA	T	

AR, aortic regurgitation; compound heterozygous mutation; CVA, cerebellar vermis aplasia; DD, developmental delay; ESRD: end-stage renal disease; Het, heterozygous mutation; HF, hepatic fibrosis; Hom, homozygous mutation; LCA, Leber congenital amaurosis; MR, mental retardation; NA, not applicable; OMA, oculomotor apraxia; ONA, optic nerve anomaly; RD, retinal dystrophy; DC, disease causing; PD: probably damaging; PoD, possibly damaging; D, damaging; NT, not tolerated; T, tolerated; SNV, single nucleotide variation

Table 5. Single mutations found in NPHP-RC patients

Pt	ESRD (yrs)	Extra-renal manifestations	Gene	Nucleotide change	Amino acid change	Mutation Taster	Poly-phen2	SIFT	Ref
J-6	9.9		*NPHP1*	c.G2029C	p.Gluy677Gln	DC	PoD	T	
J-12	1.9		*INVS /NPHP2*	c.A721T	p.Thr241Ser	DC	PD	T	
HNF-38	16.7		*NPHP3*	c.G2852A	p.Arg951Gln	DC	PD	T	
J-46	3.1		*/MKS7*	c.C424T	p.Arg142X				
J-83	8.4			c.C424T	p.Arg142X				
J-7	16.8		*CEP290*	c.A328G	p.Thr110Ala	SNP	PoD	T	
J-35	7.4		*/NPHP6*	c.G5237A	p.Arg746Gln	DC	PoD	T	
J-77	14.4		*/BBS14*	c.C2267T	p.Ser756Leu	DC	benign	T	
J-79	CKD		*GLIS2 /NPHP7*	c.G53A	p.Arg18Gln	DC	PD	T	
K-8	3.2	ONA, ADHD, AR	*BBS4*	c.1548_1549del	p.516_517del				[51]
J-84	5.5	LCA, MR, HF		c.A1414G	p.Met472Val				[29]
K-9	5.2			c.A1414G	p.Met472Val				[29]
J-59	11.8	strabismus, CVA, DD, Sz	*MKKS /BBS6*	c.G416A	p.Arg139Gln	DC	PD	T	
J-14	0.6		*AHI1 /JBTS3*	c.A3257G	p.Glu1086Gly				[52]

K-10	3	RD, HF	*ARL13B /JBTS8*	c.A259G	p.Ile87Val	DC	PD	T
K-11	CKD	Choledochal cyst, HF, Caroli disease	*CC2D2A /JBTS9*	c.C4202G	p.Thr1401Ser	DC	PD	T
K-12	CKD		*/MKS6*	c.G4238A	p.Cys1413Tyr	DC	PD	T

AR, aortic regurgitation; ADHD, attention deficit and hyperactivity disorder; CVA, cerebellar vermis aplasia; DD, developmental delay; ESRD, end-stage renal disease; HA, hemolytic anemia; HF, hepatic fibrosis; MR, mental retardation; NA, not applicable; OMA, oculomotor apraxia; ONA, optic nerve anomaly; RD, retinal dystrop hy; Sz, seizure; DC, disease causing; PD, probably damaging; PoD, possibly damaging; D, damaging; NT, not tolerated; T, tolerated; SNV, single nucleotide variation

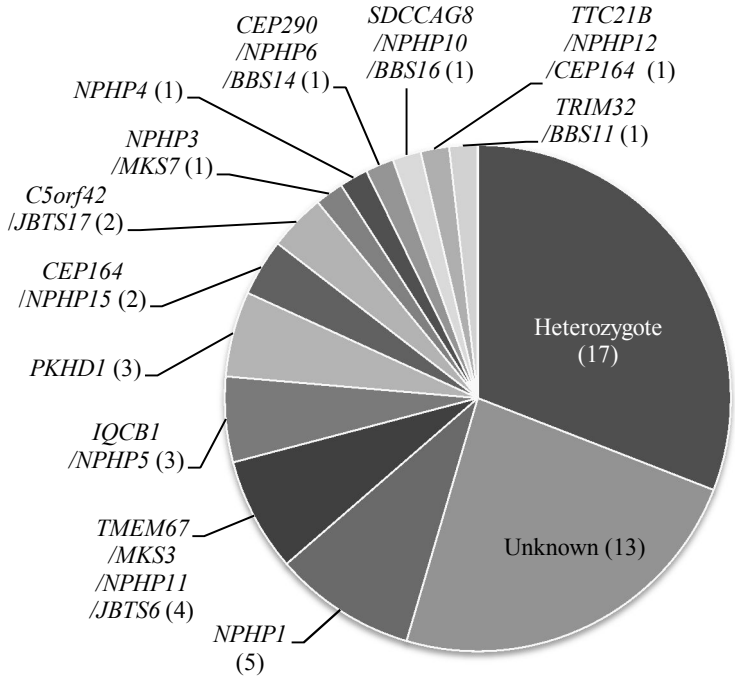

Figure 2. Results of genetic diagnosis for NPHP-RC.

Almost half (n = 25; 45.5%) of the patients with clinical diagnoses of NPHP-RC obtained a genetic diagnosis by two-step genetic diagnosis using PCR amplicon sequencing (12, 21.8%) and targeted re-sequencing (n = 13; 23.7%). Four patients with homozygous total deletion of *NPHP1*, three with *IQCB1/NPHP5*, one with *CEP290/NPHP6/BBS14,* and four with *TMEM67 /MKS3/NPHP11/JBTS6* were detected using PCR amplicon sequencing. Mutations of other genes were detected using targeted re-sequencing. Additionally, heterozygous mutations in NPHP-RC genes in 17 patients (30.9%) were detected.

DISCUSSION

The genetic causes of several Mendelian diseases, such as NPHP-RC, RP, and nonsyndromic hearing loss, are heterogeneous. In these groups of diseases, it is difficult to provide a precise diagnosis until we identify the pathogenic mutations. Therefore, precise mapping of genetic aberrations will help clinicians and patients to delineate the clinical course of the diseases. Although early intervention for NPHP-RC in children might not delay the onset of CKD, genetic counseling can help the patients and their families to cope with the disease. To this end, high-throughput sequence analysis is now available to screen all of the candidate genes[31].

In the present study, genetic diagnosis in almost half (45.5%) of the patients with a clinical diagnosis of NPHP-RC could be obtained by two-step genetic diagnosis using PCR amplicon sequencing and targeted re-sequencing. Heterozygous mutations in NPHP-RC genes were detected in 70 (30.9%) patients using targeted re-sequencing, which will enable future study of additional mutations in candidate genes.

More than two-thirds of patients with NPHP-RC did not obtain genetic diagnoses[12,13, 32,33], implying that there are other genes yet to be discovered for these patients. Indeed, the number of the new genes explaining NPHP-RC is increasing, especially with the introduction of NGS. While awaiting the complete repertoire of NPHP-RC genes, the efficacy of genetic diagnosis might be improved by stepwise application of traditional PCR amplicon sequencing and NGS, as

shown in the present study. For efficient PCR amplicon sequencing, a careful selection of candidate genes is required, based on disease phenotype and frequency. For example, all three patients with congenital blindness due to Leber's congenital amaurosis with incidental CKD were shown to have mutations in *IQCB1/NPHP5*, and four patients with Joubert syndrome, RP, hepatic fibrosis, and developmental delay were found to have *TMEM67/MKS3/NPHP11/JBTS6* mutations. These four patients looked similar to each other, with rectangular faces and square jaws, and had amiable natures despite mental retardation. The patient with the mildest phenotype with preserved speaking ability had a frameshift extension mutation, whereas the others had truncating frameshift mutations. Nonetheless, with PCR amplicon sequencing only, less than one fourth (12/55, 21.9%) of the patients obtained genetic diagnoses. Therefore, NGS technique was introduced to improve the efficacy of genetic diagnosis in these patients.

Although whole genome sequencing can provide the most comprehensive results, this method is less useful in clinical implementation and has low cost-effectiveness. Whole exome sequencing is useful for discovering the novel mutations in candidate genes, such as *SDCCAG8/NPHP10/BBS16*, in retinal-renal ciliopathy[18]. Targeted exome sequencing against disease-related genes with high read depth can enhance clinical utility through multiplex screening of candidate genes[32]. In this study, 43 patients were screened and mutations were detected in 30.2%, which was not inferior to PCR amplicon sequencing of selected genes. Re-sequencing with high read depth demonstrated high accuracy because 43 of 46 variants proved to

be correct by PCR amplicon sequencing. In a recent study on high-throughput mutation screening against 13 NPHP genes in 1056 NPHP-RC patients, the molecular diagnosis was successful only in 127 patients (12.0%), with a single heterozygous truncating mutation in 31 individuals (2.9%)[32]. In contrast, pathogenic mutations were detected more efficiently in 49.1% of NPHP-RC patients in this study. Notably, the compound heterozygous mutations in *CEP164*/*NPHP15*[34] would be the second group of cases ever published, to the best of my knowledge.

Interestingly, the proportion of *NPHP1* mutations in these patients was lower than has been reported[12,13,33]. Additionally, none of the infantile cases were caused by *INVS2/NPHP2* mutations. The three patients with *IQCB1/NPHP5* mutations had identical homozygous indel mutations causing frameshift and truncation, suggesting a founder effect. The majority (four of eight) of patients with Joubert syndrome had mutations in *TMEM67/MKS3/NPHP11/JBTS6*. A patient with a *C5orf42/JBTS17* mutation had Caroli disease. Although the number of patients of this study was not sufficiently large to determine conclusively the distribution of genetic aberration types and loci, this finding might delineate the characteristics of Korean or Asian NPHP.

Applying the NGS technique, a significant number of patients with only single heterozygous mutations in candidate genes was found, which was not sufficient to explain the phenotype. Nonetheless, this group of patients had a relatively mild phenotype, which suggests the possibility of involvement of these heterozygous

mutations in the pathogenesis of NPHP-RC. It might be speculated that those patients might have additional genetic or epigenetic mutations in a compound heterozygous fashion or another candidate gene.

Recently, mutations in *WDR19*/*INF144*/*NPHP13*[32,52,53], *ZNF423*/*NPHP14*[34], *ANKS6*/*NPHP16*[54], and *IFT172*/*NPHP17*[55] gene have been reported. These mutations had various clinical manifestations such as isolated NPHP, skeletal anomalies with RP[53], RP or Senior-Löken syndrome[52], and Caroli disease[32] in *WDR19*/*INF144*/*NPHP13* mutation, Joubert syndrome[34] in *ZNF423/OAZ* /*JBTS19*/*NPHP14* mutation, renal insufficiency, severe cardiovascular abnormalities, liver fibrosis and situs inversus[54] in *ANS6*/*NPHP16* mutation, and Jeune syndrome (asphyxiating thoracic dystrophy), Mainzer-Saldino syndromes (phalangeal cone shape epiphysis) and Joubert syndrome[55] in *IFT172*/*NPHP17* mutation. These newly found mutations might help to find additional mutations to the patients with only single heterozygous mutations in candidate genes or without mutations.

Obtaining genetic diagnosis for patients with obscure phenotypes is necessary for a better understanding and for more appropriate management of diseases. The sequence analysis of candidate genes might be easier with NGS technique, and the collective knowledge on mutations in different ethnic groups will provide a standard operation procedure for the genetic diagnosis of NPHP-RC. If common mutations of the population were known, such as c.1523_1524insGA of *IQCB1*/*NPHP5*, for the NPHP-RC patients with congenital blindness in this study,

genetic diagnosis could be straightforward by testing the particular mutation in a given population. In ethnicities with high proportions of *NPHP1* total deletions, gel-electrophoresis of PCR-products of *NPHP1* would significantly reduce the cost and effort required for molecular diagnosis.

Therefore, two-step diagnostic approaches, the combination of both PCR amplicon sequencing and targeted exome re-sequencing are thought to be a powerful tool to obtain a genetic diagnosis of NPHP-RC, which is cost effective and has high mutation detect rate.

REFERENCES

1. Smith CH, Graham JB. Congenital medullary cysts of the kidneys with severe refractory anemia. Am J Dis Child 1945; 69:369–77.

2. Fanconi G, Hanhart E, von Albertini A, Uhlinger E, Dolivo G, Prader A. Die familiäre juvenile Nephronophthise. Helv Paediatr Acta 1951 Feb; 6(1):1–49.

3. Helin I, Winberg J. Chronic renal failure in Swedish children. Acta Paediatr Scand 1980 Sep; 69(5):607-11.

4. Steele BT, Lirenman DS, Beattie CW. Nephronophthisis. Am J Med 1980 Apr; 68(4):531-8.

5. Pistor K, Scharer K, Olbing H, Tamminen-Möbius T. Children with chronic renal failure in the Federal Republic of Germany: II. Primary renal diseases, age and intervals from early renal failure to renal death. Arbeitsgemeinschaft fur Padiatrische Nephrologie. Clin Nephrol 1985 Jun; 23(6):278-84.

6. Cantani A, Bamonte G, Ceccoli D, Biribicchi G, Farinella F. Familial juvenile

nephronophthisis. A review and differential diagnosis. Clin Pediatr (Phila) 1986 Feb; 25(2):90-5.

7. Avner ED, Harmon WE, Niaudet P, Yoshikawa N. Pediatric nephrology. 6th Ed.2009. 831-848.

8. Hirano D, Fujinaga S, Ohtomo Y, Nishizaki N, Hara S, Murakami H, et al. Nephronophthisis cannot be detected by urinary screening program. Clin Pediatr (Phila) 2013 Aug; 52(8):759-61

9. Blowey DL, Querfeld U, Geary D, Warady BA, Alon U. Ultrasound findings in juvenile nephronophthisis. Pediatr Nephrol 1996 Feb; 10(1):22-4.

10. Bergmann C. Educational paper: ciliopathies. Eur J Pediatr 2012 Sep; 171(9):1285-1300.

11. Hildebrandt F, Otto E. Cilia and centrosomes: a unifying pathogenic concept for cystic kidney disease? Nat Rev Genet 2005 Dec; 6(12):928-40.

12. Chaki M, Hoefele J, Allen SJ, Ramaswami G, Janssen S, Bergmann C, et al. Genotype-phenotype correlation in 440 patients with NPHP-related ciliopathies. Kidney Int 2011 Dec; 80(11):1239-45.

13. Hildebrandt F, Attanasio M, Otto E. Nephronophthisis: disease mechanisms of a ciliopathy. J Am Soc Nephrol 2009 Jan; 20(1):23-35.

14. Nothwang HG, Stubanus M, Adolphs J, Hanusch H, Vossmerbäumer U, Denich D, et al. Construction of a gene map of the nephronophthisis type 1 (NPHP1) region on human chromosome 2q12-q13. Genomics 1998 Jan; 47(2):276-85.

15. Hildebrandt F, Otto E, Rensing C, Nothwang HG, Vollmer M, Adolphs J, et al. A novel gene encoding an SH3 domain protein is mutated in nephronophthisis type 1. Nat Genet 1997 Oct; 17(2):149-53.

16. Soliman NA, Hildebrandt F, Otto EA, Nabhan MM, Allen SJ, Badr AM, et al. Clinical characterization and NPHP1 mutations in nephronophthisis and associated ciliopathies: a single center experience. Saudi J Kidney Dis Transpl 2012 Sep; 23(5):1090-8.

17. Sang L, Miller JJ, Corbit KC, Giles RH, Brauer MJ, Otto EA, et al. Mapping the NPHP-JBTS-MKS protein network reveals ciliopathy disease genes and pathways. Cell 2011 May 13; 145(4):513-28.

18. Otto EA, Hurd TW, Airik R, Chaki M, Zhou W, Stoetzel C, et al. Candidate

exome capture identifies mutation of SDCCAG8 as the cause of a retinal-renal ciliopathy. Nat Genet 2010 Oct; 42(10):840-50.

19. Arts HH, Knoers NV. Current insights into renal ciliopathies: what can genetics teach us? Pediatr Nephrol 2013 Jun; 28(6):863-74.

20. Estrada-Cuzcano A, Koenekoop RK, Coppieters F, Kohl S, Lopez I, Collin RW, et al. IQCB1 mutations in patients with leber congenital amaurosis. Invest Ophthalmol Vis Sci. 2011 Feb; 52(2):834-9.

21. Brancati F, Barrano G, Silhavy JL, Marsh SE, Travaglini L, Bielas SL, et al. CEP290 mutations are frequently identified in the oculo-renal form of Joubert syndrome-related disorders. Am J Hum Genet 2007 Jul; 81(1):104-13.

22. Tsurusaki Y, Kobayashi Y, Hisano M, Ito S, Doi H, Nakashima M, et al. The diagnostic utility of exome sequencing in Joubert syndrome and related disorders. J Hum Genet 2013 Feb; 58(2):113-5.

23. Szymanska K, Berry I, Logan CV, Cousins SR, Lindsay H, Jafri H, et al. Founder mutations and genotype-phenotype correlations in Meckel-Gruber syndrome and associated ciliopathies. Cilia 2012 Oct ; 1(1):18.

24. Brancati F, Iannicelli M, Travaglini L, Mazzotta A, Bertini E, Boltshauser E, et al. MKS3/TMEM67 mutations are a major cause of COACH Syndrome, a Joubert Syndrome related disorder with liver involvement. Hum Mutat 2009 Feb; 30(2):E432-42.

25. Doherty D, Parisi MA, Finn LS, Gunay-Aygun M, Al-Mateen M, Bates D, et al. Mutations in 3 genes (MKS3, CC2D2A and RPGRIP1L) cause COACH syndrome (Joubert syndrome with congenital hepatic fibrosis). J Med Genet 2010 Jan; 47(1):8-21.

26. Gentile M, Di Carlo A, Susca F, Gambotto A, Caruso ML, Panella C, et al. COACH syndrome: report of two brothers with congenital hepatic fibrosis, cerebellar vermis hypoplasia, oligophrenia, ataxia, and mental retardation. Am J Med Genet 1996 Aug; 64(3):514-20.

27. Kumar S, Rankin R. Renal insufficiency is a component of COACH syndrome. Am J Med Genet. 1996 Jan; 61(2):122-6.

28. Parisi M, Glass I. Joubert Syndrome and Related Disorders. In: Pagon RA, Bird TD, Dolan CR, Stephens K, et al. (eds). GeneReviews™. University of Washington, Seattle: Seattle, WA, 1993.

29. Hoskins BE, Thorn A, Scambler PJ, Beales PL. Evaluation of multiplex capillary heteroduplex analysis: a rapid and sensitive mutation screening technique. Hum Mutat 2003 Aug; 22(2):151-7.

30. Karmous-Benailly H, Martinovic J, Gubler MC, Sirot Y, Clech L, Ozilou C, et al. Antenatal presentation of Bardet-Biedl syndrome may mimic Meckel syndrome. Am J Hum Genet 2005 Mar; 76(3):493-504.

31. Bamshad MJ, Ng SB, Bigham AW, Tabor HK, Emond MJ, Nickerson DA, et al. Exome sequencing as a tool for Mendelian disease gene discovery. Nat Rev Genet 2011 Sep; 12(11):745-55.

32. Halbritter J, Porath JD, Diaz KA, Braun DA, Kohl S, Chaki M, et al. Identification of 99 novel mutations in a worldwide cohort of 1,056 patients with a nephronophthisis-related ciliopathy. Hum Genet 2013 Aug; 132(8):865-84.

33. Otto EA, Ramaswami G, Janssen S, Chaki M, Allen SJ, Zhou W, et al. Mutation analysis of 18 nephronophthisis associated ciliopathy disease genes using a DNA pooling and next generation sequencing strategy. J Med Genet 2011 Feb; 48(2):105-16.

34. Chaki M, Airik R, Ghosh AK, Giles RH, Chen R, Slaats GG, et al. Exome capture reveals ZNF423 and CEP164 mutations, linking renal ciliopathies to DNA damage response signaling. Cell 2012 Aug ; 150(3):533-48.

35. Hildebrandt F, Strahm B, Nothwang HG, Gretz N, Schnieders B, Singh-Sawhney I, et al. Molecular genetic identification of families with juvenile nephronophthisis type 1: rate of progression to renal failure. Kidney Int 1997 Jan; 51(1):261-9.

36. Al-Romaih KI, Genovese G, Al-Mojalli H, Al-Othman S, Al-Manea H, Al-Suleiman M, et al. Genetic diagnosis in consanguineous families with kidney disease by homozygosity mapping coupled with whole-exome sequencing. Am J Kidney Dis 2011 Aug; 58(2):186-95.

37. Otto EA, Schermer B, Obara T, O'Toole JF, Hiller KS, Mueller AM, et al. Mutations in INVS encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. Nat Genet 2003 Aug; 34(4):413-20.

38. Otto EA, Loeys B, Khanna H, Hellemans J, Sudbrak R, Fan S, et al. Nephrocystin-5, a ciliary IQ domain protein, is mutated in Senior-Löken

syndrome and interacts with RPGR and calmodulin. Nat Genet 2005 Mar; 37(3):282-8.

39. Sayer JA, Otto EA, O'Toole JF, Nurnberg G, Kennedy MA, Becker C, et al. The centrosomal protein nephrocystin-6 is mutated in Joubert syndrome and activates transcription factor ATF4. Nat Genet 2006 Jun; 38(6):674-81.

40. Baala L, Romano S, Khaddour R, Saunier S, Smith UM, Audollent S, et al. The Meckel-Gruber syndrome gene, MKS3, is mutated in Joubert syndrome. Am J Hum Genet 2007 Jan; 80(1):186-94.

41. Otto EA, Tory K, Attanasio M, Zhou W, Chaki M, Paruchuri Y, et al. Hypomorphic mutations in meckelin (MKS3/TMEM67) cause nephronophthisis with liver fibrosis (NPHP11). J Med Genet 2009 Oct; 46(10):663-70.

42. Min BJ, Kim N, Chung T, Kim OH, Nishimura G, Chung CY, et al. Whole-exome sequencing identifies mutations of KIF22 in spondyloepimetaphyseal dysplasia with joint laxity, leptodactylic type. Am J Hum Genet 2011 Dec; 89(6):760-6.

43. 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 Res 2010 Sep; 20(9):1297-303.

44. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen2. Curr Protoc Hum Genet 2013 Jan; Chapter 7:Unit7.20.

45. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 2009; 4(7):1073-81.

46. Schwarz JM, Rödelsperger C, Schuelke M, Seelow D.MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods 2010 Aug; 7(8):575-6.

47. Yue P, Moult J. Identification and analysis of deleterious human SNPs. J Mol Biol 2006 Mar 10; 356(5):1263-74.

48. Otto E, Hoefele J, Ruf R, Mueller AM, Hiller KS, Wolf MT, et al. A gene mutated in nephronophthisis and retinitis pigmentosa encodes a novel protein, nephroretinin, conserved in evolution. Am J Hum Genet 2002

Nov; 71(5):1161-7.

49. Sharp AM, Messiaen LM, Page G, Antignac C, Gubler MC, Onuchic LF, et al. Comprehensive genomic analysis of PKHD1 mutations in ARPKD cohorts. J Med Genet 2005 Apr; 42(4):336-49.

50. Hichri H, Stoetzel C, Laurier V, Caron S, Sigaudy S, Sarda P, et al. Testing for triallelism: analysis of six BBS genes in a Bardet-Biedl syndrome family cohort. Eur J Hum Genet 2005 May; 13(5):607-16.

51. Kroes HY, van Zon PH, Fransen van de Putte D, Nelen MR, Nievelstein RJ, Wittebol-Post D, et al. DNA analysis of AHI1, NPHP1 and CYCLIN D1 in Joubert syndrome patients from the Netherlands. Eur J Med Genet 2008 Jan-Feb; 51(1):24-34.

52. Coussa RG, Ottoe EA, Gee H-Y, Arthurs P, Ren H, Lopez I, et al. *WDR19*: An ancient, retrograde, intraflagellar ciliary protein is mutated in autosomal recessive retinitis pigmentosa and in Senior-Loken syndrome. *Clin Genet* 2013 August ; 84(2): 150–9.

53. Bredrup C, Saunier S, Oud MM, Fiskerstrand T, Hoischen A, Brackman D, et al. Ciliopathies with skeletal anomalies and renal insufficiency due to

mutations in the IFT-A gene WDR19. Am J Hum Genet 2011 Nov 11; 89(5):634-43.

54. Hoff S, Halbritter J, Epting D, Frank V, Nguyen T-MT, van Reeuwijk J, et al. ANKS6 is a central component of a nephronophthisis module linking NEK8 to INVS and NPHP3.Nat Genet 2013 Aug; 45(8):951-6.

55. Halbritter J, Bizet AA, Schmidts M, Porath JD, Braun DA, Gee HY, et al. Defects in the IFT-B Component IFT172 Cause Jeune and Mainzer-Saldino Syndromes in Humans. Am J Hum Genet 2013 Nov; 93(5):915-25.

초 록

서론: nephronophthisis-related ciliopathy (NPHP-RC)는 소아 및 청소년기 말기 신부전의 유전적 원인 질환 중 가장 많은 부분을 차지한다. 유전적 진단이 NPHP-RC 환자들의 적절한 치료에 중요한 역할을 하지만, 질환의 유전적 이질성(genetic heterpgeneity)으로 인해, 원인 유전자 변이의 발견을 위한 gene sequencing의 효율성이 낮다.

방법: PCR amplicon sequencing과 targeted re-sequencing의 이단계 접근법으로 유전적 진단의 효율성을 높여 NPHP-RC의 유전 진단을 하기로 하였다.

결과: 총 55명의 NPHP-RC 환자에서 가장 흔한 5개 유전자 (*NPHP1, INVS/NPHP2, IQCB1/NPHP5*, *CEP290/NPHP6/BBS4, TMEM67/MKS3/NPHP11 /JBTS6*) 의 변이 여부를 PCR amplicon sequencing으로 확인하였다. 총 12명 (21.8%)의 환자에서 유전자 변이가 발견되었는데, 4명의 juvenile nephronophthisis (NPHP) 환자에서 *NPHP1* 유전자의 total deletion, 3명의 Senior-Löken syndrome 환자에서 *IQCB1/NPHP5* 유전자의 mutation, 1명의 Joubert syndrome 환자에서 *CEP290/NPHP6/BBS14* 유전자의 mutation, 그리고 4명의 retinitis pigmentosa를 보이는 Joubert syndrome 환자에서 *TMEM67/MKS3/NPHP11/JBTS6* 유전자의 mutation이 발견되었다. PCR

amplicon sequencing으로 유전자 변이가 발견되지 않은 나머지 43명의 환자에서 34개의 NPHP-RC related gene의 targeted re-sequencing을 시행하였는데, 13명 (30.2%)의 환자에서 9개의 pathogenic mutation으로 의심되는 유전자 변이가 발견되었다. 종합하면, 본 연구에서는 총 55명의 NPHP-RC 환자를 대상으로 이단계 접근법으로 유전 진단을 시행한 결과, 25명 (45.4%)의 환자에서 20개의 novel mutation을 포함한 12개의 NPHP-RC 관련 유전자의 pathogenic mutation이 발견되었다.

결론: 이단계 접근법은 NPHP-RC 환자에서 원인 유전자 변이를 발견하는데 효과적인 진단법이 될 수 있을 것이다.

주요어: nephronophthisis-related ciliopathy, PCR amplicon sequencing, targeted re-sequencing

학 번: 2010-30585