



Comprehensive genetic analysis using next-generation sequencing for the diagnosis of nephronophthisis-related ciliopathies in the Japanese population

Sakakibara, Nana ; Nozu, Kandai ; Yamamura, Tomohiko ; Horinouchi, Tomoko ; Nagano, China ; Ye, Ming Juan ; Ishiko, Shinya ; Aoto, Yuya ;...

(Citation)

Journal of Human Genetics, 67(7):427-440

(Issue Date)

2022-07-01

(Resource Type)

journal article

(Version)

Accepted Manuscript

(Rights)

© 2022, The Author(s), under exclusive licence to The Japan Society of Human Genetics. This version of the article has been accepted for publication, after peer review (when applicable) and is subject to Springer Nature's AM terms of use, but is not the Version of Record and does not reflect post-acceptance improvements, or any...

(URL)

<https://hdl.handle.net/20.500.14094/90009374>



[Article]

Comprehensive genetic analysis using next-generation sequencing for the diagnosis of nephronophthisis-related ciliopathies in the Japanese population

Nana Sakakibara¹, Kandai Nozu¹, Tomohiko Yamamura¹, Tomoko Horinouchi¹, China Nagano¹, Ming Juan Ye¹, Shinya Ishiko¹, Yuya Aoto¹, Rini Rossanti¹, Riku Hamada², Nobuhiko Okamoto³, Yuko Shima⁴, Koichi Nakanishi⁵, Masafumi Matsuo⁶, Kazumoto Iijima^{1,7}, Naoya Morisada^{*1,8}

¹Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan

²Department of Nephrology, Tokyo Metropolitan Children's Medical Center, Tokyo, Japan

³Department of Medical Genetics, Osaka Women's and Children's Hospital, Osaka, Japan

⁴Department of Pediatrics, Wakayama Medical University, Wakayama, Japan

⁵Department of Child Health and Welfare (Pediatrics), Graduate School of Medicine, University of the Ryukyus, Nishihara, Japan

⁶KNC Department of Nucleic Acid Drug Discovery, Faculty of Rehabilitation, Kobe Gakuin University, Kobe, Japan

⁷Hospital Director, Hyogo Prefectural Kobe Children's Hospital, Kobe, Japan

⁸Department of Clinical Genetics, Hyogo Prefectural Kobe Children's Hospital, Kobe, Japan

***Corresponding author**

Naoya Morisada, M.D., Ph.D.

Department of Pediatrics

Kobe University Graduate School of Medicine

7-5-1 Kusunoki-cho, Chuo, Kobe, Hyogo

6500017, Japan

Fax: +81-78-382-6099

Tel.:+81-78-382-6090

E-mail: morisada@med.kobe-u.ac.jp

Conflict of Interest Statement

M. Matsuo reports the following:

Current Employer: Kobe Gakuin University which is financially supported by KNC

Laboratories Co. Ltd. Inc. Japan

Consultancy Agreements: JCR Pharma Co., Ltd., Japan ; Daiichi Sankyo Co., Ltd.,

Japan.

Research Funding: Daiichi Sankyo Co., Ltd., Japan

Abstract

Nephronophthisis is an autosomal-recessive kidney disease that is caused by abnormalities in primary cilia. Nephronophthisis-related ciliopathies (NPHP-RCs) are a common cause of end-stage kidney disease (ESKD) in children and adolescents. NPHP-RCs are often accompanied by extrarenal manifestations, including intellectual disability, retinitis pigmentosa, or polydactyly. Although more than 100 causative genes have been identified, its diagnosis is difficult because the clinical features of each mutation often overlap. From September 2010 to August 2021, we performed genetic analysis, including next-generation sequencing (NGS), in 574 probands with kidney dysfunction and retrospectively studied cases genetically diagnosed with NPHP-RCs. Results: We detected mutations related to NPHP-RCs in 93 patients from 83 families. Members of 60 families were diagnosed using NGS, and the mutations and the corresponding number of families are as follows: *NPHP1* (24), *NPHP3* (10), *OFD1* (7), *WDR35* (5), *SDCCAG8* (4), *BBS10* (3), *TMEM67* (3), *WDR19* (3), *BBS1* (2), *BBS2* (2), *IFT122* (2), *IFT140* (2), *IQCB1* (2), *MKKS* (2), *SCLT1* (2), *TTC21B* (2), *ALMS1* (1), *ANKS6* (1), *BBS4* (1), *BBS12* (1), *CC2D2A* (1), *DYNC2H1* (1), *IFT172* (1), and *MAPKBPI* (1). A total of 39 cases (41.9%) progressed to ESKD at the time of genetic analysis, whereas 58 cases (62.3%) showed extrarenal manifestations, the most common being developmental delay, intellectual disability, and autism spectrum disorder in 44 patients. Comprehensive genetic analysis using NGS is useful for diagnosing patients with NPHP-RCs.

Keywords: nephronophthisis-related ciliopathy, primary cilia, retinopathy, skeletal anomaly, next-generation sequencing

1 Introduction

2 Nephronophthisis (NPHP) is a kidney disease characterized by cyst formation in the
3 kidney medulla and extensive tubulointerstitial damage [1,2]. NPHP often presents as a
4 minor urinary abnormality and ultimately leads to end-stage kidney disease (ESKD). It
5 is characterized by dilute urine and low molecular proteinuria and is often consistent
6 with the occurrence of polydipsia, polyuria, and secondary nocturia due to lowered
7 urine-concentrating ability [3,4]. Although the time for NPHP to progress to ESKD
8 varies, it fundamentally has a poor prognosis with no effective therapy so far.

9 NPHP is caused by mutations in genes related to primary cilia; therefore, it is
10 categorized as a ciliopathy. NPHP is often accompanied by extrarenal manifestations,
11 including developmental delay (DD), intellectual disability (ID), autism spectrum
12 disorder (ASD), retinitis pigmentosa (RP), or skeletal anomalies such as polydactyly,
13 and can be classified into disease entities such as Bardet-Biedl syndrome (BBS), Joubert
14 syndrome (JBTS), Senior-Løken syndrome (SLSN), Meckel syndrome (MS), oral-
15 facial-digital syndrome (OFD), and any type of skeletal ciliopathy (SKC), including
16 cranioectodermal dysplasia (CED) or short-rib thoracic dysplasia (SRTD) [5]. The term
17 nephronophthisis-related ciliopathies (NPHP-RCs) is used to describe these clinical
18 conditions.

19 NPHP-RCs mainly show autosomal recessive inheritance, except for *OFD1*;
20 however, it may occur sporadically. Approximately 20%–25% of NPHP cases are
21 caused by *NPHP1* abnormalities [6]. **Nonetheless**, numerous causative genes have been
22 identified in NPHP-RCs. It is often difficult to assume causal genes by clinical
23 symptoms alone since extrarenal manifestations such as DD/ID and RP overlap in each
24 NPHP-RC causative gene. Because of the complexity of this disease, an accurate

genetic diagnosis is difficult. In practice, the identification rate of gene mutations is only approximately 30% in patients with suspected NPHP-RCs [7]. In addition, to diagnose such diseases with high locus heterogeneity, conventional genetic analysis methods that are laborious, such as the Sanger method, are required. However, in recent years, with the advent of next-generation sequencing (NGS), comprehensive genetic analysis has become possible, and screening numerous causative genes has become easier [7–11]. Here, we report 93 patients from 83 families in Japan with mutations associated with NPHP-RCs.

Materials and methods

Subjects

We performed genetic analyses in 574 probands with unexplained kidney dysfunction without apparent urinary abnormalities between September 2010 and August 2021 (Fig. 1). The patients included those with suspected NPHP, congenital anomalies of the kidney and urinary tract (CAKUT), polycystic kidney disease (PKD), and autosomal dominant tubulointerstitial kidney disease (ADTKD) from all over Japan. In addition, we performed genetic analyses in patients with suspected NPHP-RCs, including those without any kidney diseases. Of these, we studied patients genetically diagnosed with NPHP-RCs. Details of the gene mutation, extrarenal manifestations, kidney findings, and age at onset of ESKD were obtained. Clinical data were retrospectively obtained from the referring clinician or the patient's hospital medical records.

Genetic analysis

Genetic analysis was approved by the institutional review board of Kobe University

School of Medicine and performed after informed consent was obtained (approval numbers are 86, 301, and 1210) from all study subjects. Genomic DNA was isolated from peripheral blood leukocytes of patients and their families using the QuickGene whole blood kit S (Kurabo, Osaka, Japan).

Direct or targeted NGS was conducted using HaloPlex HS, SureSelect (Agilent Technologies, Santa Clara, CA, USA), and TruSight One (Illumina, San Diego, CA, USA). The TruSight One Sequencing Panel (<http://www.illumina.com/products/trusight-onesequencing-panel.html>) is a commercially available targeted resequencing kit in which 4813 target genes associated with human genetic disorders can be analyzed. HaloPlex HS and SureSelect are customizable panel kits that were used to sequence genes associated with CAKUT or NPHP-RC as cataloged in the OMIM database (<http://www.omim.org/>) or PubMed (<http://www.ncbi.nlm.nih.gov/pubmed>) (Supplementary Tables 1-8).

NGS samples were prepared according to the manufacturers' instructions. Briefly, 225 ng of genomic DNA was restriction digested and hybridized at 54 °C for 16 h with NGS probes. All indexed as well as captured DNA samples were amplified through polymerase chain reaction (PCR) and sequenced using the MiSeq platform (Illumina, San Diego, CA). Analysis of NGS data was performed using the SureCall software v4.2 (Agilent Technologies). The mutations detected through NGS were confirmed by direct Sanger sequencing using the 3130 Genetic Analyzer (Thermo Fisher Scientific, Tokyo, Japan). Patients suspected with *OFDI* abnormalities were not subjected to NGS, and *OFDI* mutations were directly detected using standard Sanger sequencing. The direct sequencing data were analyzed using CLC Main Workbench version 6.7.1 (CLC bio, Aarhus, Denmark). Pathogenicity predictions were performed

in accordance with the American College of Medical Genetics guidelines. For prioritized candidate novel exonic variants, wANNOVAR (<http://wannovar.wglab.org/>), PROVEAN (<http://provean.jcvi.org/>), SIFT (<https://sift.bii.a-star.edu.sg/>), and PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>) were used to predict the pathogenicity of the variants.

Multiplex ligation-dependent probe amplification

We performed multiplex ligation-dependent probe amplification (MLPA) for *NPHP1*, as per the manufacturer's instructions (P387, MRC-Holland, Amsterdam, Netherlands). The MLPA test was performed twice to confirm the abnormal changes. Briefly, 50–100 ng of genomic DNA in 5 µL of deionized water was denatured and hybridized overnight with the probe mix. Ligation was performed with the SALSA Ligase 65 enzyme, and PCR amplification was performed using the SALSA PCR Primer Mix. Amplification products and Size Standard 600 (Thermo Fisher, Waltham, MA, USA) were mixed thoroughly and subjected to capillary electrophoresis on Gene Mapper v.3.7 (Thermo Fisher).

Array comparative genomic hybridization

Comparative genomic hybridization (CGH) was performed using the Human Genome CGH Microarray (Agilent Technologies) and SurePrint G3 Human CNV Microarray 400K Kit or SurePrint G3 Human CGH Microarray 180K Kit (Agilent Technologies), according to the manufacturer's instructions. Briefly, 1 µg of genomic DNA corresponding to either a human reference control (Promega) or test samples was fragmented by heating at 95 °C for 10 min. The fragmented DNA was then labeled with

Cy3 (reference DNA) and Cy5 (test samples) fluorescent dUTP using the SureTag Complete Labeling Kit (Agilent Technologies). Purification columns (Agilent) were used to remove unincorporated nucleotides and dyes. The labeled samples, along with human Cot-1 DNA, were added together and hybridized on array slides. Hybridization of labeled DNAs to SurePrint G3 Human CGH Arrays (400K) or SurePrint G3 Human CGH Arrays (180K) (Agilent Technologies) was performed in a hybridization oven at 65 °C and 20 rpm for 24 h. The slide was scanned at a resolution of 3 μm using an Agilent Microarray Scanner System (Agilent Technologies). The Agilent CytoGenomics software (Agilent Technologies) was used to visualize, detect, and analyze chromosomal patterns within the microarray profiles.

Minigene assay

We conducted an *in vitro* minigene assay for detecting the splice site mutation c.3201+5G>T in SC737. Patient details are described in Table 2, Table 4, and Supplementary Table 10. To create hybrid minigene constructs, we used the previously developed H492 vector based on the pcDNA 3.0 mammalian expression vector (Invitrogen, Carlsbad, CA, USA) [13]. We cloned DNA fragments from both wild-type and patient peripheral leukocytes containing exons and introns around the target variants in the *NPHP3* gene using In-Fusion cloning methods with the HD Cloning Kit (Takara Bio Inc., Kusatsu, Japan) according to the manufacturer's instructions. The following primers were used for cloning in the minigene assay for each mutation: forward, CCGTGCTTTGTTAGCTGACCAGAAATTAGCCTGTGC; reverse, TCGATGTAAACGCTAGCTGCTTCTGTGAGTCTTATGC. The hybrid minigenes were confirmed by sequencing and then transfected into HEK293T cells using

Lipofectamine® 3000 (Thermo Fisher Scientific, Waltham, MA, USA). Total RNA was extracted from cells after 24 h using the RNeasy Plus Mini Kit (QIAGEN, Hilden, Germany). Total RNA was reverse-transcribed using RNA to cDNA EcoDry Premix (Takara Bio, Kusatsu, Japan). PCR was performed using the aforementioned primers. PCR products were analyzed via electrophoresis on a 1.5% agarose gel using a DNA ladder and confirmed by direct sequencing.

Definitive diagnoses

In this study, the patients diagnosed with ciliopathy were evaluated based on clinical symptoms and the results of the genetic analysis. Definitive diagnoses were performed according to Supplementary Table 9.

Results

Genetic diagnoses and clinical phenotypes

Mutations related to NPHP-RCs were detected in 24 genes in 93 patients, including 37 males and 56 females, from 83 families (Table 1, Supplementary Table 10). The median age at the time of genetic testing was 14.6 years, with the age of the patients ranging from 0 to 69 years. Mutations in *NPHP1* were the most common with 24 cases, followed by mutations in *NPHP3* with 10 cases. *OFD1* mutations were observed in 9 patients from 7 families, including males from 3 families. *WDR35* mutations were observed in 7 patients from 5 families. Other mutations were identified in the following genes: *SDCCAG8*, *BBS10*, *TMEM67*, *WDR19*, *BBS1*, *BBS2*, *IFT122*, *IFT140*, *IQCB1*, *MKKS*, *SCLT1*, *TTC21B*, *ALMS1*, *ANKS6*, *BBS4*, *BBS12*, *CC2D2A*, *DYNC2H1*, *IFT172*, and *MAPKBPI*.

Kidney abnormalities

We detected mutations in the causative genes of NPHP-RCs in 93 patients. Kidney diseases were identified in 86 patients (92.4%) using imaging tests such as ultrasound, CT, and MRI or kidney dysfunction, whereas 7 patients did not show any kidney abnormalities despite carrying mutations (Table 2). Kidney biopsy results were available for 34 patients (36.5%), of which most showed tubulointerstitial damage and 27 patients showed glomerulosclerosis (79.4% of the patients with biopsy). Of the 86 patients with kidney disease, stage 1 chronic kidney disease (CKD) was seen in 1 patient (approximately 2%), stage 2 in 14 patients (15.0%), stage 3 in 15 patients (16.1%), and stage 4 in 12 patients (12.9%); 41 patients progressed to CKD stage 5. Mutation in the *NPHP1* gene was most frequently observed with the occurrence of ESKD (Table 3).

Extrarenal manifestations

Of the 93 patients identified with mutations, 58 showed extrarenal manifestations (Table 4). The most common extrarenal manifestations were ID, DD, and ASD in 44 patients (50.0%); however, most of them were mild. Other extrarenal manifestations included retinopathies, including retinitis pigmentosa, macular dystrophy in 23 patients (26.7%), and polydactyly in 20 patients (22.2%). Definitive diagnoses considering both clinical manifestations and genetic analysis were as follows: NPHP, BBS, SKS, SLSN, and JBTS were identified in 34, 22, 13, 7, and 6 patients, respectively (Table 1).

Genotypes in *NPHP1* and *NPHP3* mutations

We detected *NPHP1* mutations in 24 patients in 24 families, of which 21 (87.5%) were homozygous for whole gene deletion. ESKD was detected in 9 patients (37.5%), and the median age of ESKD was 15.1 years (range 9–32 years). Ocular abnormalities, including retinopathies, were detected in 8 patients, and mild ID or ASD was observed in 8 patients. Additionally, we detected *NPHP3* mutations in 10 patients from 10 families, whereas a splicing mutation (c.3201+5G>A), which indicated exon 22 skipping by minigene assay, was detected in 1 patient (SC737, [Supplementary Fig. 1](#)). During the study, 2 patients **died**, while 1 patient was diagnosed with stage 2 CKD.

Discussion

NPHP-RC manifests as various clinical phenotypes, including kidney disease, ocular abnormalities, hepatic fibrosis, and psychomotor disorders, and affects the quality of life of patients; therefore, early detection and intervention are crucial. **The symptoms of NPHP-RC are usually mild in the early stages, while being progressive, which highlights the need to understand the characteristics of ciliopathies, perform comprehensive genetic analyses, and assess the symptoms that may be caused by the mutations in each causative gene.** In this study, we detected mutations in 24 causative genes of NPHP-RCs in 93 patients from 83 families, wherein 63 probands were identified by NGS (75.9%).

We diagnosed few cases of early-stage CKD in patients with NPHP-RC; therefore, NPHP-RC mutations should be screened in children with CKD. Early diagnosis of NPHP-RC is difficult because urinary abnormalities are uncommon, and in some cases, chronic kidney dysfunction had already progressed. Kidney imaging studies showed renal cysts in many NPHP cases, as well as high echogenicity and abnormal

corticomedullary differentiation, which are typical features of NPHP [12,13]. Kidney size is either slightly decreased or normal [13]. In this study, the kidney size varied from enlarged to hypoplastic. Some NPHP patients do not develop cysts, and this condition makes it difficult to distinguish NPHP from CAKUT. Kidney biopsies were performed in 36.5% of the patients, and although a recent report suggested that the diagnosis of NPHP based on pathological findings is limited [14], we found that they reflect findings typical of NPHP, such as changes in the renal tubules. We diagnosed two patients with focal segmental glomerular sclerosis (SC476, SC703), wherein diagnosis based on pathological findings may be difficult, especially in the early stage of the disease. Therefore, considering the limitations of diagnosis through routine methods, comprehensive genetic analysis is useful for precise diagnosis.

In this study, the most common extrarenal manifestation was DD/ID/ASD, with a frequency of 50%, followed by retinopathies including RP, which is a symptom of NPHP-RC. According to previous reports, approximately 10%–15% of patients with NPHP-RC have retinal abnormalities [15,16]. Skeletal abnormalities were also common symptoms of NPHP-RC, especially polydactyly was an important manifestation, occurring in 21.7% cases. We diagnosed patients with NPHP-RC, based on these extrarenal symptoms and genetic tests, and identified nine types of NPHP-RCs, of which two were unclassified. NPHP, defined without any extrarenal manifestations, was the most common, followed by BBS, which is characterized by NPHP with polydactyly, retinopathy, and ID.

NPHP1 mutations are the most common cause of NPHP, accounting for approximately 20%–25% of all cases [6]. Moreover, approximately 85% of *NPHP1* mutations are large homozygous deletions [17]. In our study, *NPHP1* mutations were

the most common, and 87.5% of the mutations were homozygous whole gene deletions. NPHP caused by *NPHP1* mutations is classically referred to as juvenile nephronophthisis, wherein kidney function is maintained until a relatively older age (Table 4) [12,18]. In our study, among patients with mutations in *NPHP1*, only 37.5% had ESKD, even though the median age at diagnosis was 15.1 years. In addition, 10 patients with mutations in *NPHP1* had no extrarenal manifestations. A previous study showed that 77% of patients with *NPHP1* had no extrarenal manifestations [12]. On the other hand, a case has been previously reported of a 16-year-old male with a homozygous deletion of *NPHP1* and central nervous system abnormalities [19]. It remains unclear how the homozygous deletion of *NPHP1* can result in different manifestations.

The second most frequent mutation detected in this study was in *NPHP3*, a causative gene of adolescent nephronophthisis. In our study, 6 patients with *NPHP3* mutations had no extrarenal manifestations, and some had relatively good kidney function. The two patients (SC263 and SC402) who died at a young age carried homozygous mutations in *NPHP3* [20].

Mutations in *OFDI* are responsible for oral-facial-digital syndrome type 1 (MIM#311200). This syndrome is generally thought to cause lethality in males during the fetal period, but recently, some living males with *OFDI* mutations have been reported [21]. Of the known NPHP-RC causative genes, only *OFDI* is X-linked. Thus, in patients with an X-linked form of inheritance and suspected NPHP-RCs, *OFDI* mutations should be suspected first.

Mutations in *WDR35* cause CED and SRTD. As the symptoms of these diseases frequently overlap, recently, the use of the comprehensive term SKC has been

recommended. In this study, SKC was found in 13 cases with mutations in *DYNC2H1*,
IFT122, *IFT140*, *WDR19*, and *WDR35*. The most evident skeletal abnormality in SKC
 was polydactyly. The other symptoms were narrow thorax, short limb, and short stature.
 Therefore, we suggest that when these features are observed in patients with CKD,
 NPHP-RCs should be considered.

Notably, the types and presence of extrarenal manifestations differ despite the
 presence of mutations in the same causative gene. For example, some patients with a
 homozygous whole gene deletion in *NPHP1* present with retinitis pigmentosa or SLSN,
 while others show no extrarenal manifestations. Thus, mutations in a single causative
 gene can have different phenotypes. In such cases, genetic analysis will greatly aid
 clinical diagnosis.

In this study, comprehensive genetic analysis using NGS revealed causative
 gene mutations associated with various NPHP-RCs in patients who were suspected for
 not only NPHP-RC but also CAKUT and cases of unknown kidney dysfunction. While
 NPHP-RCs usually follow autosomal recessive inheritance, CAKUT follows autosomal
 dominant inheritance. Therefore, inaccurate diagnosis of NPHP-RC as CAKUT may
 also mislead the patient and their family during genetic counseling. Considering the
 costs of identifying mutations against the low rate of identification in NPHP-RCs [7–
 11], it is controversial whether genetic analysis should be performed to confirm
 suspected NPHP or NPHP-RC. However, in cases where NPHP-RC is suspected based
 on abnormal findings on kidney ultrasound, the identification rate of gene mutations has
 been reported to be close to 70% [8]. Therefore, when NPHP-RC is suspected based on
 the evaluation of ultrasound and pathology findings, or when the cause of kidney
 dysfunction is not clear but extrarenal manifestations such as RP and polydactyly are

present, genetic tests should be performed. Considering the invasiveness of kidney biopsies, diagnosis through genetic analysis is advantageous; however, in the absence of extrarenal manifestations, a renal histological examination is still required. In cases of suspected NPHP-RC without any extrarenal manifestations, but based on clinical or pathologic findings, biallelic deletions of *NPHP1* are particularly common and should be considered first. Even if no abnormality is detected, NGS should be performed because mutations in *NPHP1* or *NPHP3* are likely to reflect. Limitations may arise owing to the high costs; however, with decreasing costs, comprehensive genetic analysis will undoubtedly become more accessible in the future.

Furthermore, we found that 7 patients (SC672, SC752's sister, KC138, KC179, KC196 and her sister, KC461) carrying mutations related to NPHP-RCs (*BBS10*, *CC2D2A*, *OFD1*, *TMEM67*, and *WDR35*) did not show kidney abnormalities, and detection of the disease was possible before its onset because of genetic analysis. Diagnosis in the early stages is possible by considering family history or extrarenal manifestations, and we postulate that comprehensive genetic analyses using NGS would further facilitate it.

This study is limited in that the evaluation of clinical symptoms, such as extrarenal manifestations, relies entirely on the clinicians' decisions; hence, symptoms in some patients might have gone undetected. Additionally, segregation analysis was not performed for all cases.

In conclusion, this study showed the clinical characteristics of genetically proven NPHP-RC cases in the Japanese population for the first time and introduced many novel mutations. We postulate that comprehensive genetic analysis using NGS is an indispensable tool for diagnosing patients with kidney dysfunction without urine

abnormalities accompanied by extrarenal manifestations, including DD/ID, RP, or polydactyly. The identification of new causative genes in NPHP-RC would provide in-depth insight into genotype–phenotype relationship of each NPHP-RC causative gene.

Acknowledgments

The authors thank all the patients, their social guardians, and primary doctors. We are grateful to Ms. Yoshimi Nozu, Ms. Yuko Noguchi, and Ms. Tetsuko Yamanouchi (Kobe University) for their excellent technical assistance.

The following doctors provided patient samples for the study: Takayuki Okamoto (Hokkaido University), Tomomi Aizawa (Hirosaki University), Akira Ashida (Osaka Medical University), Shunsuke Goto, Hideki Fujii (Kobe University Graduate School of Medicine), Kouki Tomari (Tokyo Metropolitan Children’s Hospital), Satoshi Hibino (Aichi Children’s Health and Medical Center), Takashi Iijima, Yoshifumi Ubara (Toranomon Hospital), Marie Ito, Shunsuke Sakurai (Showa University Fujigaoka Hospital), Hidefumi Kishikawa (Hyogo Prefectural Nishinomiya Hospital), Koichi Kamei, Mika Okutsu, Ryutaro Suzuki, Mai Sato, Toru Kanamori (National Center for Child Health and Development), Rie Kawakita, Rika Fujimaru, Ichiro Kuki (Osaka City General Hospital), Takayuki Miyai (Okayama University), Kazuetsu Mori (Seirei Sakura Citizen Hospital), Masayuki Nakazawa (Sasebo Chuo Hospital), Ayumi Nogi (Ome Municipal Hospital), Katsusuke Yamamoto (Osaka Women’s and Children’s Hospital), Ken Saida (Yokohama City University), Takayuki Shibano (Hyogo College of Medicine), Junya Shimizu (NHO Okayama Medical Center), Ryojiro Tanaka (Hyogo Prefectural Kobe Children’s Hospital), Emi Utsuno (Chiba University), Takuzo Wada

(Kinan Hospital), Masato Yasui (Fukuyama City Hospital), Shuichiro Fujinaga, Yoshitaka Watanabe, Shota Endo (Saitama Prefectural Children's Medical Center), Tetsuji Inagaki (Miyagi Children's Hospital), Masaki Takahashi (Tokyo Metropolitan Bokutoh Hospital), Tadashi Sofue (Kagawa University), Takeshi Yamada, Keisuke Nagasaki, Hiroya Hasegawa (Niigata University), Ayako Saito (Ibaraki Children's Hopital), Ichiro Hada, Eriko Tanaka (Kyorin University), Yoshinobu Nagaoka (Sapporo Medical University), Tomo Suzuki (Kameda Medical Center), Takashi Uzu (Nippon Life Hospital), Masayuki Iwano (Fukui University), Toshihiko Shirakawa (Nagasaki University), Tomoko Nakamura (Odawara Municipal Hospital), Ryoichi Kitagata (Hamamatsu University School of Medicine), Daisuke Ichikawa (St. Marianna University School of Medicine), Masaki Yamamoto (Seirei Hamamatsu Hospital), Hisatsugu Takahara (Juntendo University Urayasu Hospital), Takashi Ohmae (Nara Prefectural Medical University), Kenichiro Miura (Tokyo Women's Medical University).

Conflict of Interest Statement

M. Matsuo reports the following:

Current Employer: Kobe Gakuin University which is financially supported by KNC Laboratories Co. Ltd. Inc. Japan

Consultancy Agreements: JCR Pharma Co., Ltd., Japan ; Daiichi Sankyo Co., Ltd., Japan.

Research Funding: Daiichi Sankyo Co., Ltd., Japan

Funding

1 This work was supported by the Health Labor Sciences Research Grant for the Research
2 on Measures for Intractable Diseases (H24-nanchi-ippa-041 to K.I.; H29-nanchi-ippa-
3 039 to N.M.) and the Japan Society for the Promotion of Science (KAKENHI Grant
4 Numbers JP15K09261 and 18K08243 to N.M.).

5

6

References

1. Wolf MT, Hildebrandt F. Nephronophthisis. *Pediatr Nephrol* 2011; 26(2): 181-194
2. Hildebrandt F, Otto E. Molecular genetics of nephronophthisis and medullary cystic kidney disease. *J Am Soc Nephrol* 2000; 11(9): 1753–1761
3. Hildebrandt F, Waldherr R, Kutt R, Brandis M. The nephronophthisis complex: clinical and genetic aspects. *Clin Investig* 1992; 70(9): 802–808
4. Simms RJ, Hynes AM, Eley L, Sayer JA. Nephronophthisis: a genetically diverse ciliopathy. *Int J Nephrol* 2011; 2011: 527137
5. Srivastava S, Sayer JA. Nephronophthisis. *J Pediatr Genet* 2014; 3(2):103-114
6. Stokman M, Lilien M, Knoers N. Nephronophthisis. In: Adam MP, Ardinger HH, Pagon RA, et al., ed. *GeneReviews®* [Internet]. Seattle (WA): University of Washington, Seattle; 2016; 1993–2021. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK368475/>
7. 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; 132(8): 865–884
8. Braun DA, Schueler M, Halbritter J, Gee HY, Porath JD, Lawson JA, et al. Whole exome sequencing identifies causative mutations in the majority of consanguineous or familial cases with childhood-onset increased renal echogenicity. *Kidney Int* 2016; 89(2): 468–475
9. Gee HY, Otto EA, Hurd TW, Ashraf S, Chaki M, Cluckey A, et al. Whole-exome resequencing distinguishes cystic kidney diseases from phenocopies in renal ciliopathies. *Kidney Int* 2014; 85(4): 880–887
10. Kang HG, Lee HK, Ahn YH, Joung JG, Nam J, Kim NDK, et al. Targeted exome

sequencing resolves allelic and the genetic heterogeneity in the genetic diagnosis of
nephronophthisis-related ciliopathy. *Exp Mol Med* 2016; 48: e251

11. Schueler M, Halbritter J, Phelps IG, Braun DA, Otto EA, Porath JD, et al. Large-
scale targeted sequencing comparison highlights extreme genetic heterogeneity in
nephronophthisis-related ciliopathies. *J Med Genet* 2016; 53(3): 208–214

12. König J, Kranz B, König S, Schlingmann KP, Titieni A, Tönshoff B, et al.
Phenotypic Spectrum of Children with Nephronophthisis and Related Ciliopathies.
Clin J Am Soc Nephrol 2017; 12(12): 1974-1983

13. Blowey DL, Querfeld U, Geary D, Warady BA, Alon U. Ultrasound findings in
juvenile nephronophthisis. *Pediatr Nephrol* 1996; 10(1): 22–24

14. Stokman MF, van der Zwaag B, van de Kar NCA, van Haelst MM, van Eerde AM,
van der Heijden JW, et al. Clinical and genetic analyses of a Dutch cohort of 40
patients with a nephronophthisis-related ciliopathy. *Pediatr Nephrol* 2018; 33(10):
1701–1712

15. Srivastava S, Molinari E, Raman S, Sayer JA. Many Genes-One Disease? Genetics
of Nephronophthisis (NPHP) and NPHP-Associated Disorders. *Front Pediatr* 2017;
5: 287

16. Wolf MT. Nephronophthisis and related syndromes. *Curr Opin Pediatr* 2015; 27(2):
201–211

17. Hildebrandt F, Rensing C, Betz RC, Sommer U, Brinbaum S, Imm A, et al.
Establishing an algorithm for molecular genetic diagnostics in 127 families with
juvenile nephronophthisis. *Kidney Int* 2001; 59(2): 434–445

18. Snoek R, Van Setten J, Keating BJ, Israni AK, Jacobsen PA, Oetting WS et al.
NPHP1 (Nephrocystin-1) gene deletions cause adult-onset ESRD. *J Am Soc*

Nephrol 2018; 29(6):1772-1779

19. Macferran KM, Buchmann RF, Ramakrishnaiah R, Griebel ML, Sanger WG, Saronwala A, Schaefer GB, et al. Pontine tegmental cap dysplasia with a 2q13 microdeletion involving the NPHP1 gene: insights into malformations of the mid-hindbrain. *Semin Pediatr Neurol* 2010; 17(1): 69-74.

20. Inaguma Y, Kaito H, Morisada N, Iijima K, Tanaka R. Renal-hepatic-pancreatic dysplasia-1 diagnosed on comprehensive gene analysis. *Pediatr Int* 2019; 61(2): 210–212

21. Sakakibara N, Morisada N, Nozu K, Nagatani K, Ohta T, Shimizu J, et al. Clinical spectrum of male patients with OFD1 mutations. *J Hum Genet* 2019; 64(1): 3–9

1 **Figure Legends**

2 Figure 1. Overview of the study.

3 aCGH, array comparative genomic hybridization; ADTKD, autosomal dominant
4 polycystic kidney disease; CAKUT, congenital anomalies of the kidney and urinary
5 tract; CKD, chronic kidney disease; MLPA, multiplex ligation-dependent amplification;
6 NGS, next generation sequencing; NPHP-RCs, nephronophthisis-related ciliopathy,
7 PCR, polymerase chain reaction; PKD, polycystic kidney

574 probands with kidney disorders without apparent urinary abnormalities;
including NPHP-RCs, PKD, ADTKD or CAKUT.

79 probands were clinically suspected NPHP-RCs by primary doctors

Genetic analysis using direct sequencing, MLPA, aCGH or NGS

- 8 probands were diagnosed as other genetic disorders
- No causal genes were identified in 17 probands

54 probands were diagnosed
as NPHP-RCs

495 probands were suspected as other disorders or CKD
of unknown cause, diagnosed by primary doctors

NGS was performed for 489 probands

- 151 probands were diagnosed as other genetic kidney disorders
- No causal genes were identified in 365 probands

6 probands diagnosed
as NPHP-RCs by direct
sequence, PCR

23 probands were diagnosed
as NPHP-RCs

83 probands diagnosed as NPHP-RCs (including this study)

Table 1: summary of the study

Gene	No. of families (patients)	gender (M: F)	average age of diagnosis (y)	No. of ESKD	Clinical diagnoses (No. of patients)
<i>NPHP1</i>	24 (24)	10: 14	18.4 (6-41)	9	NPHP (20), SLSN (3), JBTS (1)
<i>NPHP3</i>	10 (10)	4: 6	12.9 (0-39)	5	NPHP (7), RHPD (2), UCPC (1)
<i>OFD1</i>	7 (9)	4: 5	20.9 (1-69)	5	OFD (5), BBS (2), PKD (1), UCPC (1)
<i>WDR35</i>	5 (7)	2: 5	8.4 (1-15)	4	SKC (7)
<i>SDCCAG8</i>	4 (5)	3: 2	16.2 (2-30)	3	BBS (4), SLSN (1)
<i>BBS10</i>	3 (5)	2: 3	13.2 (8-17)	0	BBS (5)
<i>TMEM67</i>	3 (4)	1: 3	4.25 (0-15)	0	JBTS (4)
<i>WDR19</i>	3 (3)	0: 3	1 (0-3)	2	SKC (2), SLSN1 (1)
<i>BBS1</i>	2 (2)	0: 2	1.5 (0, 3)	0	BBS (2)
<i>BBS2</i>	2 (2)	0: 2	6 (3, 9)	0	BBS (2)
<i>IFT122</i>	2 (2)	1: 1	21 (18, 24)	1	SKC (2)
<i>IFT140</i>	2 (2)	0: 2	8.5 (6, 11)	1	NPHP (1), SKC (1)
<i>IQCB1</i>	2 (2)	1: 1	19.5 (9, 30)	2	SLSN (2)
<i>MKKS</i>	2 (3)	2: 1	11 (0-27)	1	BBS (3)
<i>SCLT1</i>	2 (2)	1: 1	16.5 (10, 23)	2	BBS (2)
<i>TTC21B</i>	2 (2)	2: 0	1, 9	2	NPHP (2)
<i>ALMS1</i>	1 (1)	0: 1	16	0	ALMS (1)
<i>ANKS6</i>	1 (2)	2: 0	50 (47, 53)	1	NPHP (2)
<i>BBS4</i>	1 (1)	1: 0	0	0	BBS (1)
<i>BBS12</i>	1 (1)	0: 1	5	0	BBS (1)
<i>CC2D2A</i>	1(1)	0: 1	7	0	JBTS (1)
<i>DYNC2H1</i>	1 (1)	0: 1	19	0	SKC (1)
<i>IFT172</i>	1 (1)	1: 0	21	1	NPHP (1)
<i>MAPKBPI</i>	1 (1)	0: 1	12	0	NPHP (1)
	83 (93)	37:56		39	NPHP (34), BBS (22), SKC (13), SLSN (7), JBTS (6), OFD (5), RHPD (2), ALMS (1), PKD (1), UCPC (2)

Abbreviations; ALMS, Alström syndrome; BBS, Bardet-Biedl syndrome; JBTS, Joubert syndrome; NPHP, nephronophthisis; OFD, oral-facial-digital syndrome; PKD, polycystic kidney disease; RHPD; renal hepatic pancreatic syndrome; SKC, skeletal ciliopathy; SLSN, Senior-Løken syndrome; UCPC, unclassified primary ciliopathy.

Fami ly	Patie nt	Gene	RefSeq	Nucleotide change (zygosity)	Amino acid change	gend er	Age at diagno sis (yrs)	eGFR at diagno sis	CKD stage at diagno sis	Age at ESKD (yrs)	Renal findings					
											cys ts	high echogene city	abnormal corticomedul lary differentiation	kidney enlargem ent	Kidney biopsy	others
1	SC63 5	ALMS1	NM_0013784 54.1	c.6957_6960del	p.(Arg2319SerfsTer23)	F	16	38.3	Stage 3b	-	-	+	-	-	glomeruloscrel osis, tubulointerstiti al damage	-
				c.10817G>A	p.(Arg3606Gln)											
2	SC56 3	ANKS6	NM_173551.5	c.854C>G (homo)	p.(Pro285Arg)	M	53	47.0	Stage 2T	49	+	NA	NA	+	tubulointerstiti al damage	-
	Broth er					M	47	70.4	Stage 2	-	+	NA	NA	-	NA	-
3	SC58 2	BBS1	NM_024649.5	c.830+350_1110+217de l	-	F	3	88.0	Stage 2	-	NA	+	+	+	NA	dysplastic kidney
				c.1285C>T	p.(Arg429Ter)											
4	SC58 4	BBS1		c.830+1G>A(homo)	-	F	0	45.0	Stage 3a	-	-	+	-	+	NA	-
5	SC47 3	BBS2	NM_031885.5	c.1237C>T	p.(Arg413Ter)	F	3	88.6	Stage 2	-	+	+	+	-	NA	-
				c.1438C>T	p.(Arg480Ter)											
6	SC47 4	BBS2		c.700C>T	p.(Arg234Ter)	F	9	63.4	Stage 2	-	+	+	NA	NA	NA	single kidney
				c.1237C>T	p.(Arg413Ter)											

7	SC37 1	BBS4	NM_033028.5	c.1222_1223insGACA (homo)	p.(Asn408ArgfsTer3)	M	0	NA	NA	-	+	+	+	+	NA	hydronephrosi s
8	SC32 9	BBS10	NM_024685.4	c.203T>G	p.(Ile68Arg)	F	8	90.2	Stage 1	-	+	+	+	+	NA	hypoplastic kidney, nephrocarno sis
	Broth er			c.235dup	p.(Thr79AsnfsTer17)	M	12	89.3	Stage 2	-	+	-	+	-	NA	kidney stones
9	SC75 2	BBS10		c.431T>C	p.(Ile144Thr)	F	15	17.0	Stage 4	-	-	+	-	-	NA	hydoronephros is, hydroureter
	Sister			c.1391C>G	p.(Ser464Ter)	F	14	121.6	-	-	-	-	-	-	NA	-
10	KC48 4	BBS10		c.409C>T	p.(Gln137Ter)	M	17	62.6	Stage 2	-	+	+	+	-	NA	-
		c.719T>C	p.(Val240Ala)													
11	SC39 1	BBS12	NM_152618.3	c.1785del	p.(Trp595CysfsTer43)	F	5	66.1	Stage 2	-	+	-	NA	+	NA	-
				c.2131T>C	p.(*711GlnextTer17)											
12	KC17 9	CC2D2 A	NM_0010805 22.2	c.1465C>T	p.(Arg489Ter)	F	7	NA	-	-	-	-	-	-	NA	-
				c.3352G>A	p.(Glu1118Lys)											
13	SC66 8	DYNC2 HI	NM_0010804 63.2	c.8559dupA	p.(Ser2854IlefsTer7)	F	19	70.8	Stage 2	-	-	+	-	-	NA	hydronephrosi s
				c.12460C>T	p.(Arg4154Cys)											
14	SC69 2	IFT122	NM_052985.4	c.1178A>T	p.(Asp393Val)	M	18	20-25	Stage 4	-	NA	NA	NA	NA	NA	hypoplastic kidney, FSGS
				c.2192_2194del	p.(Ser731del)											
15	SC75 9	IFT122		c.2117_2119del	p.(Glu706del)	F	24	NA	Stage 5D	6	NA	NA	NA	NA	NA	tubular atrophy
				c.3097dupC	p.(Leu1033ProfsTer14)											

16	SC438	IFT140	NM_014714.4	c.3214C>T	p.(Arg1072Ter)	F	6	71.0	Stage 2	-	-	+	+	-	NA	right duplication of renal pelvis	
				c.3454-2A>G	-												
17	SC727	IFT140	NM_014714.4	c.874G>A	p.(Val292Met)	F	11	NA	Stage 5T	4	-	+	+	-	NA	-	
				c.903-142_1359+78delinsGA	-												
18	SC721	IFT172	NM_015662.2	c.112C>T	p.(Arg38Ter)	M	21	NA	Stage 5T	14	NA	NA	NA	NA	NA	NA	
				c.4108G>A	p.(Glu1370Lys)												
19	SC519	IQCB1	NM_001023570.4	c.1090C>T (homo)	p.(Arg364Ter)	M	9	8.0	Stage 5T	9	+	+	NA	-	NA	-	
20	SC533	IQCB1		70.4	c.1194G>A	p.(Trp398Ter)	F	30	NA	Stage 5T	29	NA	NA	NA	NA	NA	-
					c.1522_1523dup	p.(Ala509LysfsTer3)											
21	SC645	MAPKBPI	NM_001128608.2	c.2620C>T	p.(Gln874Ter)	F	12	83.0	Stage 2	-	+	+	+	-	glomerulosclerosis, tubulointerstitial damage	-	
				c.3332+1G>A	-												
22	SC246	MKKS	NM_018848.3	c.968-1G>A(homo)	-	F	27	NA	Stage 5T	3	NA	NA	NA	NA	NA	-	
23	SC421	MKKS		c.986-1G>A (homo)	-	M	0	54.9	Stage 3a	-	NA	NA	NA	NA	NA	NA	hydronephrosis, hypoplastic kidney
	Brother					M	6	64.9	Stage 2	-	NA	NA	NA	-	NA	hydronephrosis	
24	SC181	NPHPI	NM_000272.5	whole gene deletion (homo)	-	M	24	48.0	Stage 3a	-	+	-	-	-	NA	-	

25	SC199	<i>NPHP1</i>	whole gene deletion (homo)	-	F	21	37.0	Stage 3b	-	+	NA	+	-	glomerulosclerosis, tubulointstitial damage	-
26	SC217	<i>NPHP1</i>	whole gene deletion (homo)	-	F	23	24.0	Stage 4	-	-	-	-	-	NA	-
27	SC222	<i>NPHP1</i>	whole gene deletion (homo)	-	F	26	NA	Stage 5T	23	+	-	+	-	glomerulosclerosis, tubulointstitial damage	-
28	SC278	<i>NPHP1</i>	whole gene deletion (homo)	-	F	15	30-40	Stage 3b	-	NA	NA	NA	NA	glomerulosclerosis, tubulointstitial damage	-
29	SC306	<i>NPHP1</i>	c.1810+2insT	-	M	16	NA	Stage 5D	15	+	+	NA	-	NA	-
			whole gene deletion	-											
30	SC419	<i>NPHP1</i>	whole gene deletion (homo)	-	F	9	39.4	Stage 3b	-	-	NA	NA	+	glomerulosclerosis, tubulointstitial damage	-
31	SC455	<i>NPHP1</i>	whole gene deletion (homo)	-	M	15	15.5	Stage 5D	15	+	+	NA	-	NA	-
32	SC476	<i>NPHP1</i>	whole gene deletion (homo)	-	M	7	27.8	Stage 4	-	NA	NA	-	NA	glomerulosclerosis, tubulointstitial damage	-
33	SC495	<i>NPHP1</i>	whole gene deletion (homo)	-	M	10	NA	Stage 5D	9	+	NA	+	-	glomerulosclerosis	-

34	SC530	<i>NPHP1</i>	exon2 deletion (homo)	-	M	25	38.8	Stage 3b	-	-	-	-	-	glomerulosclerosis, tubulointerstitial damage	-
35	SC546	<i>NPHP1</i>	whole gene deletion (homo)	-	F	6	69.0	Stage 2	-	NA	NA	+	NA	glomerulosclerosis, tubulointerstitial damage	-
36	SC571	<i>NPHP1</i>	whole gene deletion (homo)	-	M	10	NA	Stage 5D	10	NA	+	+	+	NA	-
37	SC575	<i>NPHP1</i>	whole gene deletion (homo)	-	F	15	19.0	Stage 4	-	+	+	NA	-	NA	hypoplastic kidney
38	SC580	<i>NPHP1</i>	whole gene deletion (homo)	-	F	25	20.0	Stage 4	-	NA	NA	NA	-	glomerulosclerosis, tubulointerstitial damage	-
39	SC613	<i>NPHP1</i>	whole gene deletion (homo)	-	F	18	NA	Stage 5D	14	-	NA	NA	-	glomerulosclerosis, tubulointerstitial damage	-
40	SC628	<i>NPHP1</i>	whole gene deletion (homo)	-	M	41	NA	Stage 5D	23	NA	NA	NA	NA	glomerulosclerosis, tubulointerstitial damage	-
41	SC657	<i>NPHP1</i>	whole gene deletion (homo)	-	F	28	38.7	Stage 3b	-	NA	NA	NA	NA	glomerulosclerosis, tubulointerstitial damage	-

42	SC67 5	<i>NPHP1</i>		whole gene deletion (homo)	-	F	16	22.5	Stage 4	-	-	+	-	-	tubulointerstiti al damage	-
43	SC70 0	<i>NPHP1</i>		whole gene deletion (homo)	-	F	32	10.1	Stage 5	-	+	NA	+	-	glomeruloscler osis, tubulointerstiti al damage	-
44	SC76 5	<i>NPHP1</i>		whole gene deletion (homo)	-	F	9	8.8	Stage 5D	9	-	+	+	-	-	-
45	SC80 5	<i>NPHP1</i>		whole gene deletion (homo)	-	M	8	36.6	Stage 3b	-	-	+	-	-	glomeruloscler osis, tubulointerstiti al damage	-
46	SC81 7	<i>NPHP1</i>		exon2 deletion (homo)	-	F	21	24.2	Stage 4	-	-	-	-	+	tubulointerstiti al damage	-
47	KC15 5	<i>NPHP1</i>		whole gene deletion (homo)	-	M	21	NA	Stage 5T	18	NA	NA	NA	NA	NA	NA
48	SC26 3	<i>NPHP3</i>	NM_153240.5	c.1817G>A	p.(Trp606Ter)	F	1†	NA	Stage 5	0	-	+	+	+	NA	dysplastic kidney
				c.3402_3403del	p.(Ala1135SerfsTer5)											
49	SC30 5	<i>NPHP3</i>		c.2591G>C	p.(Arg864Thr)	M	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
				c.3757C>G	p.(Leu1253Val)											
50	SC40 2	<i>NPHP3</i>		c.2475+1G>A	-	F	0 †	NA	Stage 5	potter sequen ce	+	+	NA	+	NA	-
				c.2694-2_2694-1del	-											
51	SC54 7	<i>NPHP3</i>		c.3757C>G	p.(Leu1253Val)	M	39	77.5	Stage 2	-	+	+	-	-	glomeruloscler osis, tubulointerstiti al damage	-

52	SC558	NPHP3		c.2694-2_2694-1del	-	F	11	25.2	Stage 4	-	NA	+	NA	+	tubulointerstitial damage	-
				c.3757C>G	p.(Leu1253Val)											
53	SC623	NPHP3		c.1577_1581del	p.(Leu526ArgfsTer49)	M	17	NA	Stage 5T	9	-	-	NA	-	glomerulosclerosis, tubulointerstitial damage	hydronephrosis
				c.3757C>G	p.(Leu1253Val)											
54	SC644	NPHP3		c.924delT	p.(Asp308GlufsTer36)	F	6	NA	Stage 5T	6	+	+	+	-	glomerulosclerosis, tubulointerstitial damage	-
				c.3757C>G	p.(Leu1253Val)											
55	SC659	NPHP3		c.2104C>T	p.(Arg702Ter)	M	14	14.3	Stage 5	-	+	-	NA	+	glomerulosclerosis, tubulointerstitial damage	-
				c.3757C>G	p.(Leu1253Val)											
56	SC737	NPHP3		c.1280T>C	p.(Ile427Thr)	F	12	NA	Stage 5T	1	NA	NA	NA	NA	tubulointerstitial damage	NA
				c.3201+5G>A	-											
57	SC802	NPHP3		c.424C>T	p.(Arg142Ter)	F	16	51.4	Stage 3a	-	+	+	-	-	-	-
				c.1474G>A	p.(Glu492Lys)											
58	SC119	OFD1	NM_003611.2	c.840_844del	p.(Lys280AsnfsTer27)	F	35	5.1	Stage 5D	35	+	-	-	+	glomerulosclerosis, tubulointerstitial damage	-
	Mother					F	69	8.1	Stage 5	-	+	+	-	-	glomerulosclerosis, tubulointerstitial damage	-

59	SC218	OFDI		c.1323_1326del	p.(Glu44ArgfsTer27)	F	37	NA	Stage 5T	19	+	NA	NA	+	NA	-
60	SC234	OFDI		c.2600-18_2600delinsACCT	p.(Ser867_Asp869delinsAsn)	M	8	NA	Stage 5T	8	+	-	-	-	NA	hypoplastic kidney, hydronephrosis
61	SC296	OFDI		c.539A>T (hemi)	p.(Asp180Val)	M	4	NA	Stage 5D	2	+	+	-	+	NA	-
62	SC454	OFDI		c.2260+2T>G	-	M	3	62.2	Stage 2	-	-	+	+	+	NA	-
	M					29	NA	Stage 5T	14	NA	NA	NA	-	NA	atrophic kidney	
63	KC138	OFDI		c.1117_1121del	p.(Arg373GlufsTer2)	F	1	130.7	-	-	-	-	-	-	NA	-
64	KC461	OFDI	c.1265_1266del	p.(Lys422ThrfsTer7)	F	2	115.2	-	-	-	-	-	-	NA	-	
65	SC446	SCLT1	NM_144643.4	c.1218G>A	p.(Met406Ile)	F	10	NA	Stage 5T	10	+	+	+	-	NA	-
				c.1631A>G	p.(Lys544Arg)											
66	KC213	SCLT1		c.1218G>A	p.(Met406Ile)	M	23	NA	Stage 5T	10	NA	NA	NA	NA	NA	NA
				c.1631A>G	p.(Lys544Arg)											
67	SC232	SDCCA G8	NM_006642.5	c.849_852del (homo)	p.(Cys283Ter)	F	4	NA	Stage 5D	3	-	+	+	+	NA	-
68	SC303	SDCCA G8		c.1357-9G>A (homo)	-	F	21	NA	Stage 5T	8	+	NA	NA	NA	NA	NA
	Brother					M	24	NA	Stage 3a	-	NA	NA	NA	NA	NA	NA

69	SC54 1	SDCCA G8		c.849_852del (homo)	p.(Cys283Ter)	M	2	23.6	Stage 4	-	NA	+	+	-	glomerulosclerosis, tubulointerstitial damage	-	
70	SC72 5	SDCCA G8		c.849_852del	p.(Cys283Ter)	M	30	NA	Stage 5T	10	NA	NA	NA	NA	NA	NA	
				c.1068+1G>T	-												
71	SC25 7	TMEM6 7	NM_153704.5	c.2440-2A>G	-	M	15	32.0	Stage 3b	-	+	NA	NA	-	NA	giant bladder	
				c.2662G>T	p.(Val888Phe)												
72	SC65 3	TMEM6 7		c.329A>G	p.(Asp110Gly)	F	0	NA	NA	-	+	+	NA	+	NA	-	
				c.507G>T	p.(Arg169Ser)												
73	KC19 6	TMEM6 7		c.329A>G	p.(Asp110Gly)	F	2	169.6	-	-	-	-	-	-	NA	-	
	Sister			c.511G>T	p.(Val171Phe)	F	0	NA	NA	-	-	-	-	-	NA	-	
74	SC32 8	TTC21B		NM_024753.5	c.1685A>G	p.(Try562Cys)	M	9	NA	Stage 5T	8	+	NA	NA	NA	polycysts, tubulointerstitial damage	-
					c.2569G>A	p.(Ala857Thr)											
75	SC70 3	TTC21B			c.379G>A	p.(Ala127Thr)	M	1	NA	Stage 5D	1	NA	NA	NA	NA	glomerulosclerosis, tubulointerstitial damage	
					c.2992_2994del	p.(Arg998del)											
76	SC23 7	WDR19	NM_025132.4		c.956delA	p.(Asn319IlefsTer16)	F	0	NA	Stage 5D	0	+	+	+	-	glomerulosclerosis, tubulointerstitial damage	-
					c.3533G>A	p.(Arg1178Gln)											
77	SC25 5	WDR19			c.2465+1G>T	-	F	3	NA	Stage 5D	2	+	+	+	+	NA	-
					c.3533G>A	p.(Arg1178Gln)											

78	SC825	WDR19	NM_001006657.2	c.3262-2A>G	-	F	0	16	Stage 4	-	+	NA	-	-	-	-
				c.3533G>A	p.(ARg1178Gln)											
79	SC152	WDR35		c.1292C>G	p.(Pro431Arg)	F	4	NA	Stage 5T	0	-	+	NA	-	NA	hypoplastic kidney
				c.2408_2416del	p.(Asn803_Ala805del)											
80	SC254	WDR35		c.622G>C	p.(Ala208Pro)	F	15	NA	Stage 4	18	+	+	NA	NA	NA	-
				c.2134delC	p.(Leu712TyrfsTer20)											
81	SC460	WDR35		c.994C>T	p.(Arg332Ter)	M	12	45.1	Stage 3a	-	NA	+	+	-	glomerulosclerosis, tubulointerstitial damage	-
	Brother			c.2408_2416del	p.(Asn803_Ala805del)	M	15	NA	Stage 5T	6	NA	NA	NA	NA	NA	hypoplastic kidney
82	SC531	WDR35		c.2134delC	p.(Leu712TyrfsTer20)	F	3	NA	Stage 5D	3	NA	NA	NA	-	NA	-
	Sister			c.2408_2416del	p.(Asn803_Ala805del)	F	1	34.2	Stage 3b	-	NA	NA	NA	-	glomerulosclerosis, tubulointerstitial damage	-
83	SC672	WDR35		c.1255G>A (homo)	p.(Gly419Ser)	F	9	111.8	-	-	-	-	-	-	NA	-

Abbreviations; CKD, chronic kidney disease; eGFR, estimated glomerular filtration; ESKD, end stage kidney disease; F, female; M, Male; NA, not available; †, deceased case

Table 3: the generation of end-stage kidney disease in the study

Genes	neonate, infant (0y)	preschool children (1-5y)	School children (6-12y)	Adolescent (13-19y)	Adult (20y-)	Number of Patients
<i>NPHP1</i>			3	4	2	9
<i>NPHP3</i>	2	0	2	0		4*
<i>OFD1</i>		1	1	2	1	5
<i>WDR35</i>	1	1	1	1		4
<i>SDCCAG8</i>		1	2			3
<i>IQCB1</i>			1		1	2
<i>SCLT1</i>			2			2
<i>TTC21B</i>		1	1			2
<i>WDR19</i>	1	1				2
<i>ANKS6</i>					1	1
<i>IFT122</i>			1			1
<i>IFT140</i>		1				1
<i>IFT172</i>				1		1
<i>MKKS</i>		1				1

*One patient was unknown to the age of end-stage kidney disease, but it seems in childhood.

Table 4: Extrarenal phenotypes and definitive diagnosis in the present study

Family	Patient	Extrarenal phenotypes									definitive diagnosis
		eye abnormality		skeletal anomaly		ID/DD	ASD	liver abnormalities	obesity	others	
		retinopathy	another eye abnormality	polydactyly	other skeletal anomaly						
1	SC635	+	-	-	short stature	+	-	+	+	hearing loss, polycystic ovary type 2 diabetes	ALMS
2	SC563	-	-	-	-	-	-	-	-	-	NPHP
	Brother	-	-	-	-	-	-	-	-	-	NPHP
3	SC582	-	-	+	-	mild	-	-	+	imperforate anus	BBS
4	SC584	NA	NA	+ (postaxial)	-	NA	-	+	-	-	BBS
5	SC473	-	-	+ (postaxial)	-	mild	-	-	-	-	BBS
6	SC474	+	-	+ (postaxial)	-	mild	-	-	+	single atrium, vaginal atresia urethral vaginal fistula	BBS
7	SC371	NA	NA	+ (preaxial)	-	NA	-	-	-	-	BBS
8	SC329	-	+	+	-	mild	-	-	-	-	BBS
	Brother	+	-	+	-	-	-	-	+	-	BBS
9	SC752	+	-	+	-	mild	-	-	+	cleft lip, Hirschsprung disease	BBS
	Sister	+	-	+	-	mild	-	-	-	cleft lip, Hirschsprung disease	BBS

[illegible]

		+									
		total blindness									
21	SC645	-	-	-	-	-	-	NA	-	hypokalemia	NPHP
22	SC246	+	-	+(postaxial)	-	mild	-	-	-	ovarian cyst	BBS
23	SC421	-	-	+(postaxial)	-	+	-	-	-	bilateral clubfoot	BBS
	Brother	+	myopia	-	-	+	-	-	+	-	BBS
24	SC181	-	-	-	-	-	+	-	-	-	NPHP
25	SC199	-	-	-	-	-	-	-	-	-	NPHP
26	SC217	-	-	-	-	-	-	-	-	-	NPHP
27	SC222	-	-	-	-	-	-	-	-	-	NPHP
28	SC278	-	+	-	-	-	+	-	-	cleft lip and palate	NPHP
29	SC306	-	-	-	-	mild	-	-	-	-	NPHP
30	SC419	-	+	-	-	-	-	-	-	-	NPHP
31	SC455	-	myopia	-	-	-	-	-	-	-	NPHP
32	SC476	-	-	-	-	-	+	-	-	primary aldosteronism	NPHP
33	SC495	+	-	-	-	-	+	-	-	-	SLSN
34	SC530	-	-	-	-	-	-	-	-	-	NPHP
35	SC546	-	strabismus, nystagmus	-	-	-	-	-	-	cerebellar vermis hypoplasia, epilepsy	NPHP
36	SC571	+	-	-	-	mild	-	-	-	-	SLSN

[illegible]

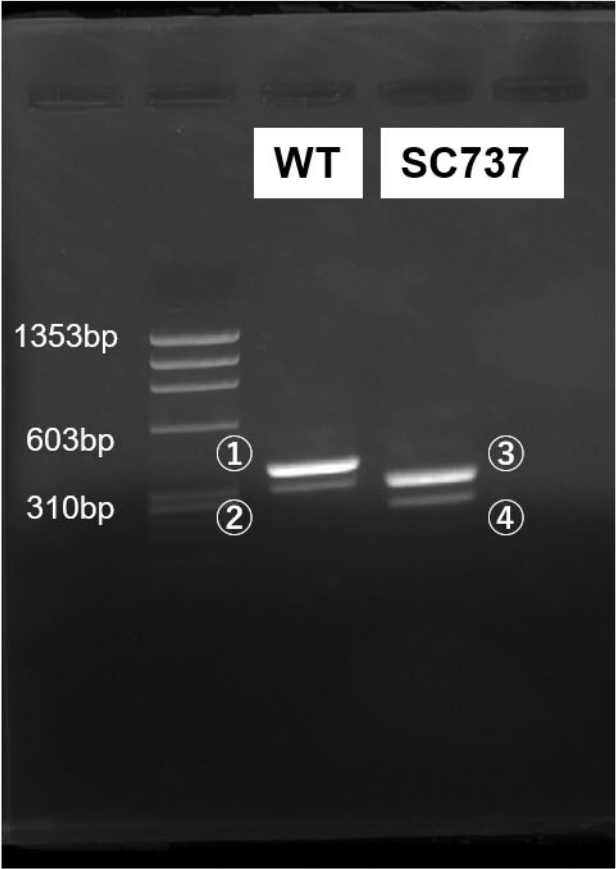
54	SC644	-	-	-	-	-	-	-	-	-	NPHP
55	SC659	-	-	-	-	-	-	NA	-	-	NPHP
56	SC737	-	-	-	-	-	-	+ (post liver transplantation)	-	-	RHPD
57	SC802	-	-	-	-	-	-	-	-	-	NPHP
58	SC119	-	-	-	-	-	-	+	-	oral tumor	OFD
	Mother	-	-	-	-	-	-	+	-	-	OFD?
59	SC218	-	-	+ (preaxial)	+	mild	-	-	-	cleft palate, lobulated tongue, peribiliary cysts	OFD
60	SC234	-	-	-	-	-	-	-	-	hearing loss	PKD
61	SC296	-	+	+ (preaxial)	-	severe	-	-	-	high-arched palate, micropenis	UCPC
62	SC454	-	-	-	-	mild	-	-	-	-	BBS
	Uncle	-	-	-	-	mild	-	-	+	micro penis	BBS
63	KC138	-	-	-	trident hand	mild to moderate	-	-	-	cleft palate, sparse hair	OFD
64	KC461	-	-	+ (preaxial)	trident hand	-	-	-	-	sparse hair	OFD
65	SC446	+	-	-	-	mild	-	-	-	-	BBS
66	KC213	+	-	-	-	mild	-	+	+	-	BBS
67	SC232	+	-	-	-	+	-	-	-	-	BBS

68	SC303	+	-	+	-	-	-	+	+	epilepsy	SLSN
	Brother	+	-	NA	NA	+	-	+	NA	polymicrogyria, HL	BBS
69	SC541	-	opsoclonus	-	-	mild	-	-	-	-	BBS
70	SC725	+	-	-	-	mild	-	-	+	-	BBS
71	SC257	-	+	-	-	mild	-	-	-	MTS	JBTS
72	SC653	NA	NA	+	NA	NA	-	NA	-	MTS, occipital encephalocele	JBTS
73	KC196	-	-	-	-	mild	-	-	-	MTS	JBTS
	Sister	-	-	-	-	mild	-	-	-	MTS	JBTS
74	SC328	-	-	-	-	-	-	+	-	cholangiectasis	NPHP
75	SC703	-	-	NA	+	-	-	+	-	ductal plate malformation	NPHP
76	SC237	-	+	-	dolichocephaly, narrow thorax, brachydactyly	+	-	+	-	-	SKC
77	SC255	+	-	+	dolichocephaly, narrow thorax, polydactyly, short stature	+	-	+	-	hearing loss, pancreatic cyst	SKC
78	SC825	+	-	-	-	-	-	-	-	cholangiectasis	SLSN
79	SC152	NA	NA	-	trigonocephaly	mild	-	-	-	-	SKC

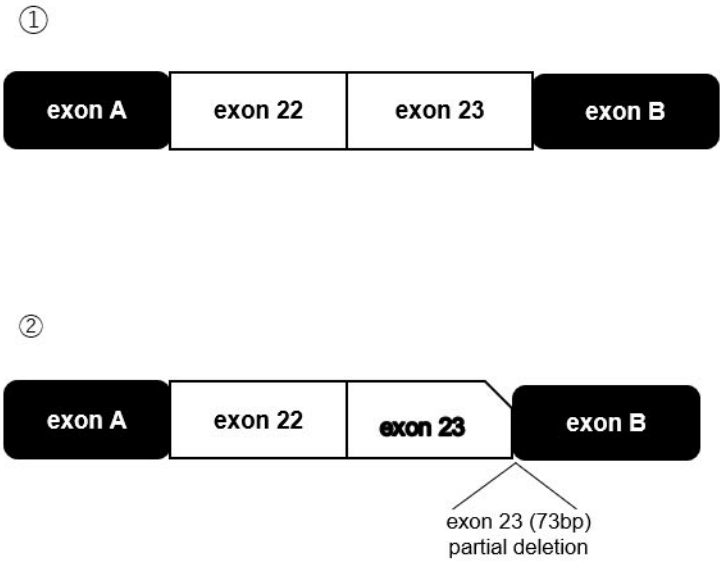
80	SC254	-	optic disc edema	-	narrow thorax	mild to moderate	-	-	-	-	SKC
81	SC460	-	-	-	narrow thorax	mild	-	-	-	microdentism	SKC
	Brother	-	-	-	narrow thorax	mild	-	-	-	-	SKC
82	SC531	-	-	-	cranyosinostosis	-	-	hepatic fibrosis	-	-	SKC
	Sister	-	-	-	cranyosinostosis	-	-	+	-	-	SKC
83	SC672	NA	NA	+ (postaxial)	narrow thorax, short limb, short stature	mild	+	NA	-	bronchiectasis	SKC

Abbreviations; ALMS, Alström syndrome; ASD, autism spectrum disorder; BBS, Bardet-Biedl syndrome; DD, developmental delay; HL, hearing loss; ID, intellectual disability; JBTS, Joubert syndrome; MTS, molar tooth sign; NA, not available; NPHP, nephronophthisis; OFD, oral-facial-digital syndrome; PKD, polycystic kidney disease; RHPD; renal hepatic pancreatic syndrome; SKC, skeletal ciliopathy; SLSN, Senior-Løken syndrome; UCPC, unclassified primary ciliopathy.

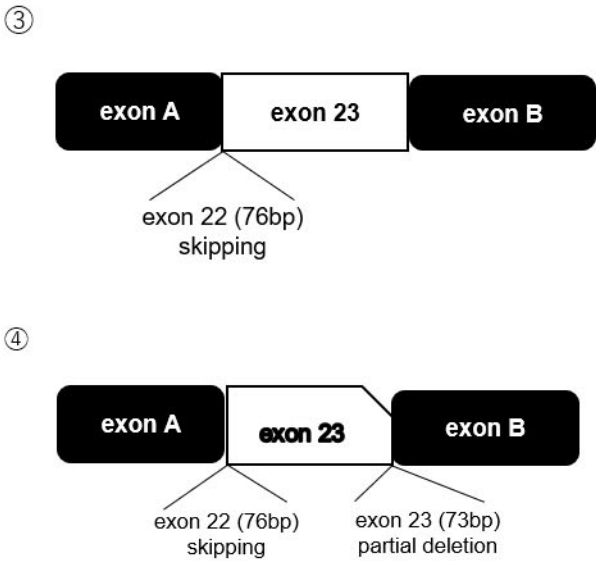
NPHP3 c.3201+5G>A



WT



SC737



Supplementary Table 1: gene list of HaloPlex HS version 1

<i>ACE</i>	<i>CITED1</i>	<i>GATA3</i>	<i>NPHP4</i>	<i>PAX2</i>	<i>SPRY1</i>
<i>ACTN4</i>	<i>CTDNEP1</i>	<i>GDNF</i>	<i>IQCB1</i>	<i>PAX8</i>	<i>TNXB</i>
<i>AGT</i>	<i>CXCL12</i>	<i>HOXA13</i>	<i>CEP290</i>	<i>PKD1</i>	<i>TP53</i>
<i>AGTR1</i>	<i>CXCR4</i>	<i>HNF1B</i>	<i>GLIS2</i>	<i>PKD2</i>	<i>TRAP1</i>
<i>AHI1</i>	<i>DSTYK</i>	<i>HOXD11</i>	<i>RPGLIP1L</i>	<i>PKHD1</i>	<i>TRPC6</i>
<i>ANLN</i>	<i>EP300</i>	<i>IFN2</i>	<i>NEK8</i>	<i>REN</i>	<i>UMOD</i>
<i>APOL1</i>	<i>EYA1</i>	<i>KAL1</i>	<i>SDCCAG8</i>	<i>RET</i>	<i>UPK3A</i>
<i>BMP2</i>	<i>FAT3</i>	<i>LMX1B</i>	<i>TMEM67</i>	<i>ROBO2</i>	<i>VANGL2</i>
<i>BMP4</i>	<i>FAT4</i>	<i>MDM2</i>	<i>TTC21B</i>	<i>ROR1</i>	<i>WNT4</i>
<i>BMP7</i>	<i>FOXD1</i>	<i>MI2B(CHD4)</i>	<i>WDR19</i>	<i>ROR2</i>	<i>WNT5A</i>
<i>CC2D2A</i>	<i>FGF2</i>	<i>MUC1</i>	<i>XNF423</i>	<i>SALL1</i>	<i>WNT7A</i>
<i>CD2AP</i>	<i>FGF9</i>	<i>MYO1E</i>	<i>CEP164</i>	<i>SIX1</i>	<i>WNT7B</i>
<i>CDC5L</i>	<i>FGF20</i>	<i>NPHP1</i>	<i>ANKS6</i>	<i>SIX2</i>	<i>WNT9B</i>
<i>CHD1L</i>	<i>FZD4</i>	<i>INVS</i>	<i>IFT172</i>	<i>SIX5</i>	<i>WNT11</i>
<i>CHRM3</i>	<i>FZD8</i>	<i>NPHP3</i>	<i>OFD1</i>	<i>SOX17</i>	<i>WT1</i>
					<i>ZEB2</i>

Supplementary Table 2: gene list of HaloPlex HS version 2

<i>ACE</i>	<i>BMP7</i>	<i>CXCL12</i>	<i>FZD4</i>	<i>IQCB1</i>	<i>NPHS2</i>	<i>RET</i>	<i>TBX18</i>	<i>WNT4</i>
<i>ACTN4</i>	<i>CC2D2A</i>	<i>CXCR4</i>	<i>FZD8</i>	<i>ITGA8</i>	<i>NUP107</i>	<i>ROBO2</i>	<i>TMEM138</i>	<i>WNT5A</i>
<i>ADCK4</i>	<i>CD2AP</i>	<i>DCDC2</i>	<i>GATA3</i>	<i>KAL1</i>	<i>NUP133</i>	<i>ROR1</i>	<i>TMEM231</i>	<i>WNT7A</i>
<i>AGT</i>	<i>CDC5L</i>	<i>DSTYK</i>	<i>GDNF</i>	<i>KIAA0586</i>	<i>NXF5</i>	<i>ROR2</i>	<i>TMEM237</i>	<i>WNT7B</i>
<i>AGTR1</i>	<i>CEP164</i>	<i>EP300</i>	<i>GLIS2</i>	<i>LAMB2</i>	<i>OFD1</i>	<i>RPGLIP1L</i>	<i>TMEM67</i>	<i>WNT9B</i>
<i>AGTR2</i>	<i>CEP290</i>	<i>EYA1</i>	<i>GREM1</i>	<i>LMNA</i>	<i>OSR1</i>	<i>SALL1</i>	<i>TNXB</i>	<i>WT1</i>
<i>AHI1</i>	<i>CEP41</i>	<i>FAT3</i>	<i>GRIP1</i>	<i>LMX1B</i>	<i>PAX2</i>	<i>SDCCAG8</i>	<i>TP53</i>	<i>ZEB2</i>
<i>ALG13</i>	<i>CEP83</i>	<i>FAT4</i>	<i>HNF1B</i>	<i>MDM2</i>	<i>PAX8</i>	<i>SIX1</i>	<i>TRAP1</i>	<i>ZNF423</i>
<i>ANKS3</i>	<i>CHD1L</i>	<i>FGF2</i>	<i>HOXA13</i>	<i>MKKS</i>	<i>PDE6D</i>	<i>SIX2</i>	<i>TRPC6</i>	
<i>ANKS6</i>	<i>CHD4</i>	<i>FGF20</i>	<i>HOXD11</i>	<i>MUC1</i>	<i>PKD1</i>	<i>SIX5</i>	<i>TTC21B</i>	
<i>ANLN</i>	<i>CHRM3</i>	<i>FGF9</i>	<i>IFN2</i>	<i>MYO1E</i>	<i>PKD2</i>	<i>SLIT2</i>	<i>UMOD</i>	
<i>APOL1</i>	<i>CITED1</i>	<i>FOXD1</i>	<i>IFT172</i>	<i>NEK8</i>	<i>PKHD1</i>	<i>SOX17</i>	<i>UPK3A</i>	
<i>ARL13B</i>	<i>COQ6</i>	<i>FRAS1</i>	<i>IFT81</i>	<i>NPHP1</i>	<i>PLCE1</i>	<i>SPRY1</i>	<i>VANGL2</i>	
<i>BMP2</i>	<i>CSPP1</i>	<i>FREM1</i>	<i>INPP5E</i>	<i>NPHP3</i>	<i>PODXL</i>	<i>SRGAP1</i>	<i>WDR19</i>	
<i>BMP4</i>	<i>CTDNEP1</i>	<i>FREM2</i>	<i>INVS</i>	<i>NPHP4</i>	<i>REN</i>	<i>TBX1</i>	<i>WNT11</i>	

Supplementary Table 3: gene list of HaloPlex HS version 4

<i>ACE</i>	<i>B9D1</i>	<i>CD2AP</i>	<i>CTDNEP1</i>	<i>FOXD1</i>	<i>IFN2</i>	<i>LMNA</i>	<i>NUP107</i>	<i>RET</i>	<i>TBX1</i>	<i>TSC1</i>	<i>WNT7B</i>
<i>ACTN4</i>	<i>B9D2</i>	<i>CDC5L</i>	<i>CXCL12</i>	<i>FRAS1</i>	<i>IFT27</i>	<i>LMX1B</i>	<i>NUP133</i>	<i>ROBO2</i>	<i>TBX18</i>	<i>TSC2</i>	<i>WNT9B</i>
<i>ADCK4</i>	<i>BBIP1</i>	<i>CENPF</i>	<i>CXCR4</i>	<i>FREM1</i>	<i>IFT43</i>	<i>LZTFL1</i>	<i>NXF5</i>	<i>ROR1</i>	<i>TCTN2</i>	<i>TTC8</i>	<i>WNT11</i>
<i>AGT</i>	<i>BBS1</i>	<i>CEP41</i>	<i>DCDC2</i>	<i>FREM2</i>	<i>IFT81</i>	<i>MDM2</i>	<i>OFD1</i>	<i>ROR2</i>	<i>TCTN3</i>	<i>TTC21B</i>	<i>WT1</i>
<i>AGTR1</i>	<i>BBS2</i>	<i>CEP83</i>	<i>DDX59</i>	<i>FZD4</i>	<i>IFT122</i>	<i>MKKS</i>	<i>OSR1</i>	<i>RPGLIP1L</i>	<i>TMEM67</i>	<i>UMOD</i>	<i>XPNPEP3</i>
<i>AGTR2</i>	<i>BBS4</i>	<i>CEP104</i>	<i>DSTYK</i>	<i>FZD8</i>	<i>IFT140</i>	<i>MKS1</i>	<i>PAX2</i>	<i>SALL1</i>	<i>TMEM138</i>	<i>UPK3A</i>	<i>ZEB2</i>
<i>AHI1</i>	<i>BBS5</i>	<i>CEP120</i>	<i>DYNC2H1</i>	<i>GANAB</i>	<i>IFT172</i>	<i>MUC1</i>	<i>PAX8</i>	<i>SDCCAG8</i>	<i>TMEM216</i>	<i>VANGL2</i>	<i>ZNF423</i>
<i>ALG13</i>	<i>BBS7</i>	<i>CEP164</i>	<i>EP300</i>	<i>GATA3</i>	<i>INPP5E</i>	<i>MYO1E</i>	<i>PDE6D</i>	<i>SIX1</i>	<i>TMEM231</i>	<i>WDPCP</i>	
<i>ALMS1</i>	<i>BBS10</i>	<i>CEP290</i>	<i>EYA1</i>	<i>GDNF</i>	<i>INVS</i>	<i>NEK1</i>	<i>PKD1</i>	<i>SIX2</i>	<i>TMEM237</i>	<i>WDR19</i>	
<i>ANKS3</i>	<i>BBS12</i>	<i>CHD1L</i>	<i>FAT3</i>	<i>GLIS2</i>	<i>IQCB1</i>	<i>NEK8</i>	<i>PKD2</i>	<i>SIX5</i>	<i>TNXB</i>	<i>WDR34</i>	
<i>ANKS6</i>	<i>BMP2</i>	<i>CHD4</i>	<i>FAT4</i>	<i>GREM1</i>	<i>ITGA8</i>	<i>NODAL</i>	<i>PKHD1</i>	<i>SLIT2</i>	<i>TP53</i>	<i>WDR35</i>	
<i>ANLN</i>	<i>BMP4</i>	<i>CHRM3</i>	<i>FGF2</i>	<i>GRIP1</i>	<i>KAL1</i>	<i>NPHP1</i>	<i>PLCE1</i>	<i>SOX17</i>	<i>TRAF3IP1</i>	<i>WDR60</i>	
<i>APOL1</i>	<i>BMP7</i>	<i>CITED1</i>	<i>FGF9</i>	<i>HOXA13</i>	<i>KIAA0586</i>	<i>NPHP3</i>	<i>PODXL</i>	<i>SPRY1</i>	<i>TRAP1</i>	<i>WNT4</i>	
<i>ARL6</i>	<i>C5orf42</i>	<i>COQ6</i>	<i>FGF20</i>	<i>HOXD11</i>	<i>KIF14</i>	<i>NPHP4</i>	<i>PTHB1</i>	<i>SRGAP1</i>	<i>TRIM32</i>	<i>WNT5A</i>	
<i>ARL13B</i>	<i>CC2D2A</i>	<i>CSPP1</i>	<i>FGFR2</i>	<i>HNF1B</i>	<i>LAMB2</i>	<i>NPHS2</i>	<i>REN</i>	<i>TBC1D1</i>	<i>TRPC6</i>	<i>WNT7A</i>	

Supplementary Table 4: gene list of HaloPlex HS version 5

<i>ACE</i>	<i>BBS1</i>	<i>CEP41</i>	<i>DYNC2H1</i>	<i>GANAB</i>	<i>IFT80</i>	<i>LMNA</i>	<i>PDE6D</i>	<i>SIX1</i>	<i>TMEM107</i>	<i>WDPCP</i>
<i>AGT</i>	<i>BBS2</i>	<i>CEP83</i>	<i>DZIP1L</i>	<i>GATA3</i>	<i>IFT81</i>	<i>LMX1B</i>	<i>PIBF1</i>	<i>SIX2</i>	<i>TMEM138</i>	<i>WDR19</i>
<i>AGTR1</i>	<i>BBS4</i>	<i>CEP104</i>	<i>EP300</i>	<i>GNDF</i>	<i>IFT122</i>	<i>LZTFL1</i>	<i>PKD1</i>	<i>SIX5</i>	<i>TMEM216</i>	<i>WDR34</i>
<i>AGTR2</i>	<i>BBS5</i>	<i>CEP120</i>	<i>EVC</i>	<i>GLIS2</i>	<i>IFT140</i>	<i>MKKS</i>	<i>PKD2</i>	<i>SLIT2</i>	<i>TMEM231</i>	<i>WDR35</i>
<i>AHI1</i>	<i>BBS7</i>	<i>CEP164</i>	<i>EVC2</i>	<i>GLIS3</i>	<i>IFT172</i>	<i>MKS1</i>	<i>PKHD1</i>	<i>SOX17</i>	<i>TMEM237</i>	<i>WDR60</i>
<i>ALG9</i>	<i>BBS10</i>	<i>CEP290</i>	<i>EXOC4</i>	<i>GRIP1</i>	<i>INPP5E</i>	<i>MUC1</i>	<i>PTHB1</i>	<i>SPRY1</i>	<i>TNXB</i>	<i>WNT4</i>
<i>ALMS1</i>	<i>BBS12</i>	<i>CHD1L</i>	<i>EXOC8</i>	<i>GRLF1</i>	<i>INVS</i>	<i>NEK1</i>	<i>REN</i>	<i>SRGAP1</i>	<i>TRAF3IP1</i>	<i>WT1</i>
<i>ANKS3</i>	<i>C2CD3</i>	<i>CHD4</i>	<i>EYA1</i>	<i>HNF1B</i>	<i>IQCB1</i>	<i>NEK8</i>	<i>RET</i>	<i>TBC1D1</i>	<i>TRIM32</i>	<i>XPNPEP3</i>
<i>ANKS6</i>	<i>C5orf42</i>	<i>CHRM3</i>	<i>FAN1</i>	<i>HOXA13</i>	<i>ITGA8</i>	<i>NPHP1</i>	<i>ROBO2</i>	<i>TBC1D32</i>	<i>TSC1</i>	<i>ZNF423</i>
<i>ARL6</i>	<i>C21orf2</i>	<i>CITED1</i>	<i>FGF9</i>	<i>HPRT1</i>	<i>JAG1</i>	<i>NPHP3</i>	<i>RPGLIP1L</i>	<i>TBX1</i>	<i>TSC2</i>	
<i>ARL13B</i>	<i>CC2D2A</i>	<i>CSPP1</i>	<i>FGF20</i>	<i>HYLS1</i>	<i>KAL1</i>	<i>NPHP4</i>	<i>SALL1</i>	<i>TBX18</i>	<i>TTBK2</i>	
<i>ATXN10</i>	<i>CCDC28B</i>	<i>CTDNEP1</i>	<i>FGFR2</i>	<i>ICK</i>	<i>KIAA0586</i>	<i>OFD1</i>	<i>SARS2</i>	<i>TCTN1</i>	<i>TTC8</i>	
<i>B9D1</i>	<i>CDC5L</i>	<i>DCDC2</i>	<i>FRAS1</i>	<i>IFN2</i>	<i>KIF7</i>	<i>PAX2</i>	<i>SCLT1</i>	<i>TCTN2</i>	<i>TTC21B</i>	
<i>B9D2</i>	<i>CENPF</i>	<i>DDX59</i>	<i>FREM1</i>	<i>IFT27</i>	<i>KIF14</i>	<i>PAX8</i>	<i>SDCCAG8</i>	<i>TCTN3</i>	<i>UMOD</i>	
<i>BBIP1</i>	<i>CEP19</i>	<i>DSTYK</i>	<i>FREM2</i>	<i>IFT43</i>	<i>LIFR</i>	<i>PBX1</i>	<i>SEC61A1</i>	<i>TMEM67</i>	<i>VANGL2</i>	

Supplementary Table 5: gene list of HaloPlex HS version 6

<i>ACE</i>	<i>BBS1</i>	<i>CEP41</i>	<i>DYNC2H1</i>	<i>GANAB</i>	<i>IFT80</i>	<i>LMNA</i>	<i>PDE6D</i>	<i>SIX1</i>	<i>TMEM107</i>	<i>WDPCP</i>
<i>AGT</i>	<i>BBS2</i>	<i>CEP83</i>	<i>DZIP1L</i>	<i>GATA3</i>	<i>IFT81</i>	<i>LMX1B</i>	<i>PIBF1</i>	<i>SIX2</i>	<i>TMEM138</i>	<i>WDR19</i>
<i>AGTR1</i>	<i>BBS4</i>	<i>CEP104</i>	<i>EP300</i>	<i>GDNF</i>	<i>IFT122</i>	<i>LZTFL1</i>	<i>PKD1</i>	<i>SIX5</i>	<i>TMEM216</i>	<i>WDR34</i>
<i>AGTR2</i>	<i>BBS5</i>	<i>CEP120</i>	<i>EVC</i>	<i>GLIS2</i>	<i>IFT140</i>	<i>MKKS</i>	<i>PKD2</i>	<i>SLIT2</i>	<i>TMEM231</i>	<i>WDR35</i>
<i>AHI1</i>	<i>BBS7</i>	<i>CEP164</i>	<i>EVC2</i>	<i>GLIS3</i>	<i>IFT172</i>	<i>MKS1</i>	<i>PKHD1</i>	<i>SOX17</i>	<i>TMEM237</i>	<i>WDR60</i>
<i>ALG9</i>	<i>BBS10</i>	<i>CEP290</i>	<i>EXOC4</i>	<i>GRIP1</i>	<i>INPP5E</i>	<i>MUC1</i>	<i>PTHB1</i>	<i>SPRY1</i>	<i>TNXB</i>	<i>WNT4</i>
<i>ALMS1</i>	<i>BBS12</i>	<i>CHD1L</i>	<i>EXOC8</i>	<i>GRLF1</i>	<i>INVS</i>	<i>NEK1</i>	<i>REN</i>	<i>SRGAP1</i>	<i>TRAF3IP1</i>	<i>WT1</i>
<i>ANKS3</i>	<i>C2CD3</i>	<i>CHD4</i>	<i>EYA1</i>	<i>HNF1B</i>	<i>IQCB1</i>	<i>NEK8</i>	<i>RET</i>	<i>TBC1D1</i>	<i>TRIM32</i>	<i>XPNPEP3</i>
<i>ANKS6</i>	<i>C5orf42</i>	<i>CHRM3</i>	<i>FAN1</i>	<i>HOXA13</i>	<i>ITGA8</i>	<i>NPHP1</i>	<i>ROBO2</i>	<i>TBC1D32</i>	<i>TSC1</i>	<i>ZNF423</i>
<i>ARL6</i>	<i>C21orf2</i>	<i>CITED1</i>	<i>FGF9</i>	<i>HPRT1</i>	<i>JAG1</i>	<i>NPHP3</i>	<i>RPGLIP1L</i>	<i>TBX1</i>	<i>TSC2</i>	
<i>ARL13B</i>	<i>CC2D2A</i>	<i>CSPP1</i>	<i>FGF20</i>	<i>HYLS1</i>	<i>KAL1</i>	<i>NPHP4</i>	<i>SALL1</i>	<i>TBX18</i>	<i>TTBK2</i>	
<i>ATXN10</i>	<i>CCDC28B</i>	<i>CTDNEP1</i>	<i>FGFR2</i>	<i>ICK</i>	<i>KIAA0586</i>	<i>OFD1</i>	<i>SARS2</i>	<i>TCTN1</i>	<i>TTC8</i>	
<i>B9D1</i>	<i>CDC5L</i>	<i>DCDC2</i>	<i>FRAS1</i>	<i>IFN2</i>	<i>KIF7</i>	<i>PAX2</i>	<i>SCLT1</i>	<i>TCTN2</i>	<i>TTC21B</i>	
<i>B9D2</i>	<i>CENPF</i>	<i>DDX59</i>	<i>FREM1</i>	<i>IFT27</i>	<i>KIF14</i>	<i>PAX8</i>	<i>SDCCAG8</i>	<i>TCTN3</i>	<i>UMOD</i>	
<i>BBIP1</i>	<i>CEP19</i>	<i>DSTYK</i>	<i>FREM2</i>	<i>IFT43</i>	<i>LIFR</i>	<i>PBX1</i>	<i>SEC61A1</i>	<i>TMEM67</i>	<i>VANGL2</i>	

Supplementary Table 7: gene list of HaloPlex HS version 8

<i>ACE</i>	<i>ATXN10</i>	<i>C8orf37</i>	<i>CHRM3</i>	<i>EYA1</i>	<i>GLIS3</i>	<i>IFT74</i>	<i>KIAA0753</i>	<i>NPHP3</i>	<i>SALL1</i>	<i>SUFU</i>	<i>TRAF3IP1</i>	<i>WT1</i>
<i>ACTG2</i>	<i>B9D1</i>	<i>CC2D2A</i>	<i>CITED1</i>	<i>FAN1</i>	<i>GPC3</i>	<i>IFT80</i>	<i>KIF7</i>	<i>NPHP4</i>	<i>SARS2</i>	<i>TBC1D32</i>	<i>TRIM32</i>	<i>XPNPEP3</i>
<i>ADAMTS9</i>	<i>B9D2</i>	<i>CCDC28B</i>	<i>CRB2</i>	<i>FGF20</i>	<i>GREB1L</i>	<i>IFT81</i>	<i>KIF14</i>	<i>OFD1</i>	<i>SCLT1</i>	<i>TBX1</i>	<i>TSC1</i>	<i>ZNF423</i>
<i>AGT</i>	<i>BBIP1</i>	<i>CDC5L</i>	<i>CSPP1</i>	<i>FGFR1</i>	<i>GREM1</i>	<i>IFT122</i>	<i>LMX1B</i>	<i>PAX2</i>	<i>SDCCAG8</i>	<i>TBX18</i>	<i>TSC2</i>	
<i>AGTR1</i>	<i>BBS1</i>	<i>CDKN1C</i>	<i>DCDC2</i>	<i>FGFR2</i>	<i>GRIP1</i>	<i>IFT140</i>	<i>LRIG2</i>	<i>PAX8</i>	<i>SEC61A1</i>	<i>TCTEX1D2</i>	<i>TTC8</i>	
<i>AGTR2</i>	<i>BBS2</i>	<i>CENPF</i>	<i>DDX59</i>	<i>FRAS1</i>	<i>HNF1B</i>	<i>IFT172</i>	<i>LRP5</i>	<i>PBX1</i>	<i>SIX1</i>	<i>TCTN1</i>	<i>TTC21B</i>	
<i>AHI1</i>	<i>BBS4</i>	<i>CEP41</i>	<i>DNAJB11</i>	<i>FREM1</i>	<i>HOXA13</i>	<i>INPP5E</i>	<i>LZTFL1</i>	<i>PDE6D</i>	<i>SIX2</i>	<i>TCTN2</i>	<i>UMOD</i>	
<i>ALG8</i>	<i>BBS5</i>	<i>CEP83</i>	<i>DSTYK</i>	<i>FREM2</i>	<i>HPRT1</i>	<i>INTU</i>	<i>MAPKBP1</i>	<i>PIBF1</i>	<i>SIX5</i>	<i>TCTN3</i>	<i>UPK3A</i>	
<i>ALG9</i>	<i>BBS7</i>	<i>CEP104</i>	<i>DYNC2H1</i>	<i>GANAB</i>	<i>HPSE2</i>	<i>INVS</i>	<i>MKKS</i>	<i>PKD1</i>	<i>SLIT2</i>	<i>TMEM67</i>	<i>VANGL2</i>	
<i>ALMS1</i>	<i>BBS9</i>	<i>CEP120</i>	<i>DZIP1L</i>	<i>GATA3</i>	<i>HYLS1</i>	<i>IQCB1</i>	<i>MKS1</i>	<i>PKD2</i>	<i>SON</i>	<i>TMEM107</i>	<i>WDPCP</i>	
<i>ANKS6</i>	<i>BBS10</i>	<i>CEP164</i>	<i>EP300</i>	<i>GDF11</i>	<i>INF2</i>	<i>ITGA8</i>	<i>MUC1</i>	<i>PKHD1</i>	<i>SOX9</i>	<i>TMEM138</i>	<i>WDR19</i>	
<i>ARL3</i>	<i>BBS12</i>	<i>CEP290</i>	<i>EVC</i>	<i>GDNF</i>	<i>IFT27</i>	<i>JAG1</i>	<i>NEK1</i>	<i>REN</i>	<i>SOX11</i>	<i>TMEM216</i>	<i>WDR34</i>	
<i>ARL6</i>	<i>BICC1</i>	<i>CHD1L</i>	<i>EVC2</i>	<i>GEN1</i>	<i>IFT43</i>	<i>KAL1</i>	<i>NEK8</i>	<i>RET</i>	<i>SOX17</i>	<i>TMEM231</i>	<i>WDR35</i>	
<i>ARL13B</i>	<i>C2CD3</i>	<i>CHD4</i>	<i>EXOC4</i>	<i>GFRA1</i>	<i>IFT52</i>	<i>KIAA0556</i>	<i>NOTCH2</i>	<i>ROBO2</i>	<i>SPRY1</i>	<i>TMEM237</i>	<i>WDR60</i>	
<i>ARMC9</i>	<i>C5orf42</i>	<i>CHD7</i>	<i>EXOC8</i>	<i>GLIS2</i>	<i>IFT57</i>	<i>KIAA0586</i>	<i>NPHP1</i>	<i>RPGRIP1L</i>	<i>SRGAP1</i>	<i>TNXB</i>	<i>WNT4</i>	

Supplementary Table 8: gene list of HaloPlex HS version 9

<i>ACE</i>	<i>BBS2</i>	<i>CEP164</i>	<i>EVC2</i>	<i>GPC3</i>	<i>IFT172</i>	<i>MKS1</i>	<i>RET</i>	<i>TBC1D32</i>	<i>TTC21B</i>
<i>ACTG2</i>	<i>BBS4</i>	<i>CEP290</i>	<i>EXOC4</i>	<i>GREB1L</i>	<i>INPP5E</i>	<i>MUC1</i>	<i>ROBO2</i>	<i>TBX1</i>	<i>UMOD</i>
<i>ADAMTS9</i>	<i>BBS5</i>	<i>CHD1L</i>	<i>EXOC8</i>	<i>GREM1</i>	<i>INTU</i>	<i>NEK1</i>	<i>RPGRIP1L</i>	<i>TBX6</i>	<i>UPK3A</i>
<i>AGT</i>	<i>BBS7</i>	<i>CHD4</i>	<i>EYA1</i>	<i>GRIP1</i>	<i>INVS</i>	<i>NEK8</i>	<i>SALL1</i>	<i>TBX18</i>	<i>VANGL2</i>
<i>AGTR1</i>	<i>BBS9</i>	<i>CHD7</i>	<i>FAM149B1</i>	<i>HNF1B</i>	<i>IQCB1</i>	<i>NOTCH2</i>	<i>SARS2</i>	<i>TCTEX1D2</i>	<i>WDPCP</i>
<i>AGTR2</i>	<i>BBS10</i>	<i>CHRM3</i>	<i>FAN1</i>	<i>HOXA11</i>	<i>ITGA8</i>	<i>NPHP1</i>	<i>SCLT1</i>	<i>TCTN1</i>	<i>WDR19</i>
<i>AHI1</i>	<i>BBS12</i>	<i>CHRNA3</i>	<i>FGF20</i>	<i>HOXA13</i>	<i>JAG1</i>	<i>NPHP3</i>	<i>SDCCAG8</i>	<i>TCTN2</i>	<i>WDR34</i>
<i>ALG8</i>	<i>BICC1</i>	<i>CLCN5</i>	<i>FGFR1</i>	<i>HPRT1</i>	<i>KAL1</i>	<i>NPHP4</i>	<i>SEC61A1</i>	<i>TCTN3</i>	<i>WDR35</i>
<i>ALG9</i>	<i>C2CD3</i>	<i>CITED1</i>	<i>FGFR2</i>	<i>HPSE2</i>	<i>KIAA0556</i>	<i>NRIP1</i>	<i>SIX1</i>	<i>TMEM67</i>	<i>WDR60</i>
<i>ALMS1</i>	<i>C5orf42</i>	<i>CRB2</i>	<i>FRAS1</i>	<i>HYLS1</i>	<i>KIAA0586</i>	<i>OCRL</i>	<i>SIX2</i>	<i>TMEM107</i>	<i>WNT4</i>
<i>ANKS6</i>	<i>C8orf37</i>	<i>CSPP1</i>	<i>FREM1</i>	<i>INF2</i>	<i>KIAA0753</i>	<i>OFD1</i>	<i>SIX5</i>	<i>TMEM138</i>	<i>WT1</i>
<i>ARL3</i>	<i>CC2D2A</i>	<i>DCDC2</i>	<i>FREM2</i>	<i>IFT27</i>	<i>KIF7</i>	<i>PAX2</i>	<i>SLC20A1</i>	<i>TMEM216</i>	<i>XPNPEP3</i>
<i>ARL6</i>	<i>CCDC28B</i>	<i>DDX59</i>	<i>GANAB</i>	<i>IFT43</i>	<i>KIF14</i>	<i>PAX8</i>	<i>SLIT2</i>	<i>TMEM231</i>	<i>ZNF423</i>
<i>ARL13B</i>	<i>CDC5L</i>	<i>DNAJB11</i>	<i>GATA3</i>	<i>IFT52</i>	<i>LMX1B</i>	<i>PBX1</i>	<i>SON</i>	<i>TMEM237</i>	
<i>ARMC9</i>	<i>CDKN1C</i>	<i>DSTYK</i>	<i>GDF11</i>	<i>IFT57</i>	<i>LRIG2</i>	<i>PDE6D</i>	<i>SOX9</i>	<i>TNXB</i>	
<i>ATXN10</i>	<i>CENPF</i>	<i>DYNC2H1</i>	<i>GDNF</i>	<i>IFT74</i>	<i>LRP5</i>	<i>PIBF1</i>	<i>SOX11</i>	<i>TRAF3IP1</i>	
<i>B9D1</i>	<i>CEP41</i>	<i>DYNC2LI1</i>	<i>GEN1</i>	<i>IFT80</i>	<i>LZTFL1</i>	<i>PKD1</i>	<i>SOX17</i>	<i>TRIM32</i>	
<i>B9D2</i>	<i>CEP83</i>	<i>DZIP1L</i>	<i>GFRA1</i>	<i>IFT81</i>	<i>MAFB</i>	<i>PKD2</i>	<i>SPRY1</i>	<i>TSC1</i>	
<i>BBIP1</i>	<i>CEP104</i>	<i>EP300</i>	<i>GLIS2</i>	<i>IFT122</i>	<i>MAPKBP1</i>	<i>PKHD1</i>	<i>SRGAP1</i>	<i>TSC2</i>	
<i>BBS1</i>	<i>CEP120</i>	<i>EVC</i>	<i>GLIS3</i>	<i>IFT140</i>	<i>MKKS</i>	<i>REN</i>	<i>SUFU</i>	<i>TTC8</i>	

Supplementary Table 9: clinical diagnosis considering phenotypes and genotypes in this study.

	short name	Kidney diseases	ID, DD	retinopathy	MTS	polydactyly	skeletal abnormalities	hepatic disease	Other findings	Related genes
Alström syndrome	ALMS	+	+ or -	+	-	+	-	+	type 2 DM, hearing loss	<i>ALMS1</i>
Bardet-Biedl syndrome	BBS	+	+	+	-	+ or -	-	+ or -	-	<i>BBS1, BBS2, ARL6, BBS4, BBS5, MKKS, BBS7, TTC8, PTHB1, BBS10, TRIM32, BBS12, MKS1, CEP290, WDPCP, SDCCAG8, LZTFL1, BBIP1, IFT27, IFT172, C8orf37, IFT74, SCLT1</i>
Joubert syndrome	JBTS	+ or -	+	+ or -	+	+ or -	-	-	-	<i>INPP5E, TMEM216, AH11, NPHP1, CEP290, TMEM67, RPGLIP1L, ARL13B, CC2D2A, OFD1, TTC21B, TCTN1, TMEM237, CEP41, TMEM138, C5orf42, TCTN3, ZNF423, TMEM231, CSPP1, PDE6D, KIAA0586, TCTN2, CEP104, KIAA0556, B9D1, MKS1</i>
Meckel syndrome	MS	+ or -	+	+ or -	-	+	-	-	meningocele	<i>MKS1, TMEM216, TMEM67, CEP290, RPGLIP1L, WDPCP, NPHP3, TCTN2, B9D1, B9D2, TMEM231, KIF14, TMEM107</i>
Nephronophthisis	NPHP	+	- (some patients may have ASD)	-	-	-	-	-	-	<i>NPHP1, INVS, NPHP3, NPHP4, IQCB1, CEP290, GLIS2, RPGRIP1L, NEK8, SDCCAG8, TMEM67, TTC21B, WDR19, ZNF423, CEP164, ANKS6, IFT172, CEP83, DCDC2, MAPKBP1</i>
Oral-facial-digital syndrome	OFD	+ or -	- (mild ID may be present)	-	-	+ or -	+	-	oral abnormalities	<i>OFD1, TCTN3, DDX59, C5orf42, SCLT1, C2CD3, KIAA0753, TMEM107, INTU, IFT57</i>
Renal hepatic pancreatic dysplasia	RHPD	+	-	-	-	-	-	+	pancreatic fibrosis	<i>NPHP3, NEK8</i>
Senior-Løken syndrome	SLSN	+	-	+	-	-	-	-	-	<i>NPHP1, NPHP4, IQCB1, CEP290, SDCCAG8, WDR19, TRAF3IP1</i>
Skeletal ciliopathy	SKC	+ or -	+ or -	-	-	+ or -	+	+ or -	-	<i>IFT122, IFT80, DYNC2H1, TTC21B, WDR19, NEK1, WDR35, WDR60, IFT140, IFT172, WDR34, CEP120, KIAA0586, IFT52, TCTEX1D2, IFT43, IFT81, INTU, EVC, EVC2</i>
Unclassifiable primary ciliopathies	UCPC	+ or -	+ or -	+ or -	-	+ or -	+ or -	+ or -	+ or -	not specified

Abbreviations: ASD, autism spectrum disorder; DD, developmental delay; DM, diabetes mellitus; ID, intellectual disability; MTS, molar tooth sign; +, present; -, not present.

Supplementary Table 10: The genotypes of the present study.

Family	Patient	Gene	RefSeq	Nucleotide change(zygosity)	Amino acid change	Inheritance	Diagnostic tool	HGMD	dbSNP ClinVar	gnomAD (%)	jMorp (%)	CADD	PROVEAN	SIFT	PP2	ACMG pathogenicity	Category	Reference
1	SC635	ALMS1	NM_001378454.1	c.6957_6960del	p.(Arg2319SerfsTer23)	paternal	SS v8	-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM4,	
				c.10817G>A	p.(Arg3606Gln)	maternal		-	rs752052259	0.013	-	32	NA	T	D	uncertain significance	PM2, PM3, PP4, BP1	
2	SC563	ANKS6	NM_173551.5	c.854C>G (homo)	p.(Pro285Arg)	NA	HP v7	-	-	-	-	23.1	D	D	D	likely pathogenic	PM1, PM2, PP1, PP4	
	Brother																	
3	SC582	BBS1	NM_024649.5	c.830+350_1110+217del	-	paternal	HP v7	-	RCV001257323.1	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4,	
				c.1285C>T	p.(Arg429Ter)	maternal		CM031135	rs768443448	0.0012	0.02	ND	ND	ND	ND	pathogenic	PVS1, PM2,PM3, PP3, PP4, PP5	
4	SC584	BBS1		c.830+1G>A(homo)	-	parental	HP v7	CS033489	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	
5	SC473	BBS2	NM_031885.5	c.1237C>T	p.(Arg413Ter)	NA	HP v5	CM033336	rs147030232	0.0021	0.01	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP3, PP4, PP5	
				c.1438C>T	p.(Arg480Ter)	NA		CM111288	rs778090540	0.0024	0.01	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP3, PP4, PP5	
6	SC474	BBS2		c.700C>T	p.(Arg234Ter)	NA	HP v5	CM051012	rs779690256	0.0028	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP3, PP4, PP5	
				c.1237C>T	p.(Arg413Ter)	NA		CM033336	rs147030232	0.0021	0.01	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP3, PP4, PP5	
7	SC371	BBS4	NM_033028.5	c.1222_1223insGACA (homo)	p.(Asn408ArgfsTer3)	NA	HP v4	-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM6, PP4	
8	SC329	BBS10		c.203T>G	p.(Ile68Arg)	paternal	HP v4	-	-	-	-	24.6	D	D	P	likely pathogenic	PM1, PM2, PM3, PP3, PP4, BP1	
	Brother			c.235dup	p.(Thr79AsnfsTer17)	maternal		CI171093	rs760693838	0.00083	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	
9	SC752	BBS10	NM_024685.4	c.431T>C	p.(Ile144Thr)	NA	SS v9	-	-	-	-	23.2	N	D	B	likely pathogenic	PM1, PM2, PP1, PP4, BP1	
	Sister			c.1391C>G	p.(Ser464Ter)	NA		-	rs759682922	0.0012	0.0001	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP1, Pp3, PP4, PP5	
10	KC484	BBS10		c.409C>T	p.(Gln137Ter)	paternal	SS v8	-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP3, PP4	
				c.719T>C	p.(Val240Ala)	maternal		-	-	-	-	24.7	D	D	P	likely pathogenic	PM2, PM3, PP3, PP4	
11	SC391	BBS12	NM_152618.3	c.1785del	p.(Trp595CysfsTer43)	paternal	HP v4	-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP4	
				c.2131T>C	p.(*711GlnextTer17)	maternal		-	-	-	-	ND	ND	ND	ND	likely pathogenic	PM2, PM3, PM4, PP3, PP4	
12	KC179	CC2D2A	NM_001080522.2	c.1465C>T	p.(Arg489Ter)	NA	HP v6	-	rs145678228	0.0012	0.01	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP3, PP4	
				c.3352G>A	p.(Glu1118Lys)	NA		-	rs199759274	0.00042	0.04	26.1	D	D	D	likely pathogenic	PM1, PM2, PP3, PP4	
13	SC668	DYNC2H1	NM_001080463.2	c.8559dupA	p.(Ser2854Ilefs*7)	maternal	SS v8	-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	
				c.12460C>T	p.(Arg4154Cys)	paternal		CM182036	rs755441612	0.0016	-	34	D	D	D	likely pathogenic	PM1, PM2, PM3, PP3, PP4	
14	SC692	IFT122	NM_052985.4	c.1178A>T	p.(Asp393Val)	NA	SS v8	-	rs1195894937	0.0008	0.08	28.5	D	D	D	likely pathogenic	PM1, PM2, PP3, PP4	
				c.2192_2194del	p.(Ser731del)	NA		-	-	-	-	ND	ND	ND	ND	uncertain significance	PM2, PM4, PP4	
15	SC759	IFT122		c.2117_2119del	p.(Glu706del)	NA	SS v9	-	rs3832258	0.0064	0.11	ND	D (-12.982)	ND	ND	uncertain significance	PM2, PM4, PP4	

				c.3097dupC	p.(Leu1033ProfsTer14)	NA		-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP4	
16	SC438	IFT140	NM_014714.4	c.3214C>T	p.(Arg1072Ter)	maternal	HP v5	-	rs1432688490	0.0012	0.02	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	
				c.3454-2A>G	-	paternal		-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	
17	SC727	IFT140		c.874G>A	p.(Val292Met)	NA	SS v8	CM123480	rs431905521	0.0004	-	23.9	N	D	D	likely pathogenic	PM1, PM2, PP3, PP4, PP5	
				c.903-142_1359+78delinsGA	-	NA		-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP4	
18	SC721	IFT172	NM_015662.2	c.112C>T	p.(Arg38Ter)	NA	SS v8	-	rs139021548	0.0024	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP3, PP4, PP5	
				c.4108G>A	p.(Glu1370Lys)	NA		-	rs779981992	0.0025	0.01	22.9	N	T	B	uncertain significance	PM1, PM2, PP4	
19	SC519	IQCB1	NM_001023570.4	c.1090C>T (homo)	p.(Arg364Ter)	parental	HP v6	CM093302	rs727503968	0.0078	0.01	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP3, PP4, PP5	
20	SC533	IQCB1		c.1194G>A	p.(Trp398Ter)	NA	HP v6	-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP3, PP4	
				c.1522_1523dup	p.(Ala509LysfsTer3)	NA		CI150233	rs587783011	0.0008	0.02	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP4	
21	SC645	MAPKBPI	NM_001128608.2	c.2620C>T	p.(Gln874Ter)	paternal	SS v8	-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP3, PP4	
				c.3332+1G>A	-	maternal		-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	
22	SC246	MKKS	NM_018848.3	c.986-1G>A(homo)	-	NA	TS1	CS163335	rs200633158	0.0008	0.01	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP3, PP4	
23	SC421	MKKS		c.986-1G>A (homo)	-	NA	TS1	CS163335	rs200633158	0.0008	0.01	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP3, PP4	Aizawa et al.
	Brother																	
24	SC181	NPHP1	NM_000272.5	whole gene deletion (homo)	-	NA	aCGH	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
25	SC199	NPHP1		whole gene deletion (homo)	-	NA	PCR/MLPA	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
26	SC217	NPHP1		whole gene deletion (homo)	-	NA	PCR/MLPA	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
27	SC222	NPHP1		whole gene deletion (homo)	-	NA	PCR/MLPA	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
28	SC278	NPHP1		whole gene deletion (homo)	-	NA	TS1, PCR	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
29	SC306	NPHP1		c.1810+2insT	-	paternal	HP v4	-	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PM3, PP4	
				whole gene deletion	-	maternal		CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
30	SC419	NPHP1		whole gene deletion (homo)	-	NA	HP v4	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
31	SC455	NPHP1		whole gene deletion (homo)	-	NA	PCR	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
32	SC476	NPHP1		whole gene deletion (homo)	-	NA	HP v5	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
33	SC495	NPHP1		whole gene deletion (homo)	-	NA	PCR	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
34	SC530	NPHP1		exon2 deletion* (homo)	-	NA	HP v6	-	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PM3, PP4	
35	SC546	NPHP1		whole gene deletion (homo)	-	parental	HP v6	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
36	SC571	NPHP1		whole gene deletion (homo)	-	NA	HP v7	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
37	SC575	NPHP1		whole gene deletion (homo)	-	NA	HP v7	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
38	SC580	NPHP1		whole gene deletion (homo)	-	NA	PCR/MLPA	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	Akira et al.
39	SC613	NPHP1		whole gene deletion (homo)	-	NA	PCR	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
40	SC628	NPHP1		whole gene deletion (homo)	-	NA	PCR	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
41	SC657	NPHP1		whole gene deletion (homo)	-	NA	PCR	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
42	SC675	NPHP1		whole gene deletion (homo)	-	parental	MLPA	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
43	SC700	NPHP1		whole gene deletion (homo)	-	NA	MLPA	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	Hirai et al.
44	SC765	NPHP1		whole gene deletion (homo)	-	NA	PCR, MLPA	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
45	SC805	NPHP1		whole gene deletion (homo)	-	NA	MLPA	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
46	SC817	NPHP1		exon2 deletion* (homo)	-	NA	SS v9	-	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PM3, PP4	

47	KC155	<i>NPHP1</i>	NM_153240.5	whole gene deletion (homo)	-	NA	HP v6	CG2024136 etc.	NA	NA	NA	ND	ND	ND	ND	pathogenic	PVS1, PS3, PM3, PP4	
48	SC263	<i>NPHP3</i>		c.1817G>A	p.(Trp606Ter)	paternal	TS1	CM193286	rs182135982	0.06	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP3, PP4, PP5	Inaguma et al.
				c.3402_3403del	p.(Ala1135SerfsTer5)	maternal		CD193287	-		-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4,	
49	SC305	<i>NPHP3</i>		c.2591G>C	p.(Arg864Thr)	unknown	HP v4	-	rs754237446	0.0012	-	29.6	N	D	D	likely pathogenic	PM2, PM3, PP1, PP3, PP4	
						(not paternal)											likely pathogenic	
50	SC402	<i>NPHP3</i>		c.2475+1G>A	-	maternal	HP v4	-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	
				c.2694-2_2694-1del	-	paternal		CD082161	rs751527253	0.028	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	
51	SC547	<i>NPHP3</i>		c.3757C>G	p.(Leu1253Val)	NA	HP v6	CM1611232	rs775281384	0.0028	0.02	24	N	D	P	likely pathogenic	PM1, PM2, PM3, PP1, PP4	
52	SC558	<i>NPHP3</i>		c.2694-2_2694-1del	-	paternal	HP v7	CD082161	rs751527253	0.028	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	
				c.3757C>G	p.(Leu1253Val)	maternal		CM1611232	rs775281384	0.0028	0.02	24	N	D	P	likely pathogenic	PM1, PM2, PM3, PP1, PP4	
53	SC623	<i>NPHP3</i>		c.1577_1581del	p.(Leu526ArgfsTer49)	paternal	SS v8	-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	
				c.3757C>G	p.(Leu1253Val)	maternal		CM1611232	rs775281384	0.0028	0.02	24	N	D	P	likely pathogenic	PM1, PM2, PM3, PP1, PP4	
54	SC644	<i>NPHP3</i>		c.924delT	p.(Asp308GlufsTer36)	maternal	SS v8	-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	
				c.3757C>G	p.(Leu1253Val)	paternal		CM1611232	rs775281384	0.0028	0.02	24	N	D	P	likely pathogenic	PM1, PM2, PM3, PP1, PP4	
55	SC659	<i>NPHP3</i>		c.2104C>T	p.(Arg702Ter)	maternal	SS v8	CM094106	rs267606916	0.0053	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP3, PP4, PP5	
				c.3757C>G	p.(Leu1253Val)	paternal		CM1611232	rs775281384	0.0028	0.02	24	N	D	P	likely pathogenic	PM1, PM2, PM3, PP1, PP4	
56	SC737	<i>NPHP3</i>		c.1280T>C	p.(Ile427Thr)	NA	SS v8	-	rs774834753	-	-	23.5	N	D	B	uncertain significance	PM2, PP3, PP4	
				c.3201+5G>A	-	NA		-	-	-	ND	ND	ND	ND	likely pathogenic	PS3, PM2, PP4		
57	SC802	<i>NPHP3</i>		c.424C>T	p.(Arg142Ter)	NA	SS v9	CM1611240	rs771742823	0.0014	0.01	37	NA	NA	NA	Pathogenic	PVS1, PM2, PP3, PP4, PP5	
			c.1474G>A	p.(Glu492Lys)	NA	-		-	-	-	29.8	N	D	P	Uncertain significance	PM2, PP3, PP4		
58	SC119	<i>OFD1</i>	NM_003611.2	c.840_844del	p.(Lys280AsnfsTer27)	maternal	Sanger	CD066689	rs312262853	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP4	Iijima et al.
	NA																	
59	SC218	<i>OFD1</i>		c.1323_1326del	p.(Glu44ArgfsTer27)	maternal	Sanger	-	rs312262875	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP4	
60	SC234	<i>OFD1</i>		c.2600-18_2600delinsACCT	p.(Ser867_Asp869delinsAsn)	maternal	HP v1	CX1912845	-	-	-	ND	ND	ND	ND	uncertain significance	PM2, PP4	Sakakibara et al.
61	SC296	<i>OFD1</i>		c.539A>T (hemi)	p.(Asp180Val)	de novo	Sanger	CM1912846	-	-	-	27.7	D	D	D	likely pathogenic	PS2, PM2, PP3, PP4, BP1	Sakakibara et al.
62	SC454	<i>OFD1</i>		c.2260+2T>G	-	maternal	Sanger	CS1912847	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP4	Sakakibara et al.
	maternal																	
63	KC138	<i>OFD1</i>		c.1117_1121del	p.(Arg373GlufsTer2)	NA	Sanger	-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP4	

64	KC461	<i>OFD1</i>		c.1265_1266del	p.(Lys422ThrfsTer7)	de novo	Sanger	-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PS2, PM2, PP4	
65	SC446	<i>SCLT1</i>	NM_144643.4	c.1218G>A	p.(Met406Ile)	maternal	HP v5	CS2011718	-	-	-	24.9	N	D	B	likely pathogenic	PM2, PM3, PP3, PP4	Morisada et al.
				c.1631A>G	p.(Lys544Arg)	paternal		CS1817511	rs762215370	0.00082	0.12	24.2	N	T	D	likely pathogenic	PM2, PM3, PP3, PP4	
66	KC213	<i>SCLT1</i>		c.1218G>A	p.(Met406Ile)	paternal	HP v5	CS2011718	-	-	-	24.9	N	D	B	likely pathogenic	PM2, PM3, PP3, PP4	Morisada et al., Horiuchi et al.
				c.1631A>G	p.(Lys544Arg)	maternal		CS1817511	rs762215370	0.00082	0.12	24.2	N	T	D	likely pathogenic	PM2, PM3, PP3, PP4	
67	SC232	<i>SDCCAG8</i>	NM_006642.5	c.849_852del (homo)	p.(Cys283Ter)	parental	TS1	CD163328	rs1283378694	-	0.05	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	Yamamura et al.
68	SC303	<i>SDCCAG8</i>		c.1357-9A>G (homo)	-	NA	HP v2	-	rs748049587	0.0016	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP1, PP4	Fujii et al.
	Brother			c.849_852del (homo)	p.(Cys283Ter)	parental	HP v6	CD163328	rs1283378694	-	0.05	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	Watanabe et al.
69	SC541	<i>SDCCAG8</i>		c.849_852del	p.(Cys283Ter)	NA	SS v8	CD163328	rs1283378694	-	0.05	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	
	70			SC725	<i>SDCCAG8</i>	c.1068+1G>T		-	NA	CS106176	-	-	0.01	ND	ND	ND	ND	
71	SC257	<i>TMEM67</i>	NM_153704.5	c.2440-2A>G	-	NA	HP v2	-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP4	
				c.2662G>T	p.(Val888Phe)	NA		-	RCV000809240.1	-	0.01	32	N	D	D	uncertain significance	PM2, PP3, PP4	
72	SC653	<i>TMEM67</i>		c.329A>G	p.(Asp110Gly)	paternal	SS v8	CM131897	rs1014876003	0.0004	0.05	28.1	D	D	D	likely pathogenic	PM1, PM2, PP3, PP4	
				c.507G>T	p.(Arg169Ser)	maternal		-	-	-	-	21.1	N	T	B	likely pathogenic	PM1, PM2, PM3, PP3, PP4	
73	KC196	<i>TMEM67</i>		c.329A>G	p.(Asp110Gly)	paternal	TS1	CM131897	rs1014876003	0.0004	0.05	28.1	D	D	D	likely pathogenic	PM1, PM2, PP3, PP4	
	Sister			c.511G>T	p.(Val171Phe)	maternal		-	-	-	-	24.3	N	T	D	likely pathogenic	PM1, PM2, PM3, PM5, PP1, PP4	
74	SC328	<i>TTC21B</i>	NM_024753.5	c.1685A>G	p.(Try562Cys)	maternal	HP v4	CM2013300	-	-	-	23.1	D	D	D	likely pathogenic	PM1, PM2, PM3, PP4	Hibino et al.
				c.2569G>A	p.(Ala857Thr)	paternal		CM1824418	rs190101048	0.023	0.63	34	D	D	D	likely pathogenic	PM1, PM2, PM3, PP3, PP4	
75	SC703	<i>TTC21B</i>		c.379G>A	p.(Ala127Thr)	maternal	SS v8	CM1611234	rs769548518	0.0008	0.03	29.3	D	D	D	likely pathogenic	PM1, PM2, PM3, PP3, PP4	
				c.2992_2994del	p.(Arg998del)	paternal		-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	
76	SC237	<i>WDR19</i>	NM_025132.4	c.956delA	p.(Asn319Ilefs*16)	paternal	HP v1	CD1719158	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP4	Yoshikawa et al.
				c.3533G>A	p.(Arg1178Gln)	maternal		CM139117	rs79436363	0.0063	0.01	32	D	T	D	uncertain significance	PM2, PP3, PP4, PP5	
77	SC255	<i>WDR19</i>		c.2645+1G>T	-	paternal	TS1	CS1719159	rs1237821935	0.0004	0.01	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	
				c.3533G>A	p.(Arg1178Gln)	maternal		CM139117	rs79436363	0.0063	0.01	32	D	T	D	likely pathogenic	PM2, PM3, PP3, PP4, PP5	
78	SC825	<i>WDR19</i>		c.3262-2A>G	-	NA	SS v9	-	rs753291151	0.04	0.04	NA	NA	NA	NA	uncertain significance	PVS1, PP4	

				c.3533G>A	p.(ARg1178Gln)	NA		CM139117	rs79436363	0.04	0.04	32	D	T	D	uncertain significance	PM2, PP3, PP4		
79	SC152	WDR35	NM_001006657.2	c.1292C>G	p.(Pro431Arg)	NA	TS1	-	-	-	-	25.6	D	D	D	uncertain significance	PM2, PP3, PP4		
				c.2408_2416del	p.(Asn803_Ala805del)	NA		-	rs771220566	0.0008	0.02	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP4		
80	SC254	WDR35		c.622G>C	p.(Ala208Pro)	paternal	TS1	CM163336	-	-	-	31	D	D	D	likely pathogenic	PM2, PM3, PP3, PP4	Yamamura et al.	
				c.2134delC	p.(Leu712TyrfsTer20)	maternal		CD163337	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4		
81	SC460	WDR35		c.994C>T	p.(Arg332Ter)	maternal	HP v5	-	rs199840434	0.0018	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP1, PP3, PP4, PP5	
	Brother			c.2408_2416del	p.(Asn803_Ala805del)	paternal		-	rs771220566	0.0008	0.02	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP1, PP4		
82	SC531	WDR35		c.2134delC	p.(Leu712TyrfsTer20)	paternal	HP v6	CD163337	-	-	-	-	ND	ND	ND	ND	pathogenic	PVS1, PM2, PM3, PP4	
	Sister			c.2408_2416del	p.(Asn803_Ala805del)	maternal		-	rs771220566	0.0008	0.02	ND	ND	ND	ND	pathogenic	PVS1, PM2, PP4		
83	SC672	WDR35			c.1255G>A (homo)	p.(Gly419Ser)	NA	SS v8	-	-	-	0.02	28.1	D	T	D	likely pathogenic	PM2, PM3, PP3, PP4	

Abbreviations; CADD combined annotation dependent depletion; D, damaging; HP, HaloPlex HS; MLPA, multiplex ligation polymerase assay; NA, not available; ND, not done; P, possibly pathogenic; PCR, polymerase chain reaction; PP2, PolyPhen2; SS, SureSelect; T, tolerated; TS1, TruSight One; v, version

Supplementary Table 11: References of Case Reports in the study.

1. Aizawa T, Morisada N, Nozu K, Tandai S, Tanaka H. Expanding the phenotype of Bardet-Biedl syndrome: Newly diagnosed sibling cases. *Pediatr Int.* 2020 Jan;62(1):101-103. doi: 10.1111/ped.14066.
2. Akira M, Suzuki H, Ikeda A, Iwasaki M, Honda D, Takahara H, Rinno H, Tomita S, Suzuki Y. Atypical histological abnormalities in an adult patient with nephronophthisis harboring NPHP1 deletion: a case report. *BMC Nephrol.* 2021 Jul 10;22(1):261. doi: 10.1186/s12882-021-02466-z.
3. Hirai Y, Mizumoto A, Mitsumoto K, Uzu T. Senior-Loken syndrome misdiagnosed as nephrosclerosis related to hypertensive disorders of pregnancy. *BMJ Case Rep.* 2020 Oct 27;13(10):e236137. doi: 10.1136/bcr-2020-236137.
4. Inaguma Y, Kaito H, Morisada N, Iijima K, Tanaka R. Renal-hepatic-pancreatic dysplasia-1 diagnosed on comprehensive gene analysis. *Pediatr Int.* 2019 Feb;61(2):210-212. doi: 10.1111/ped.13758.
5. Iijima T, Hayami N, Takaichi K, Morisada N, Nozu K, Iijima K, Sawa N, Hoshino J, Ubara Y. An Orofaciodigital Syndrome 1 Patient and Her Mother Carry the Same OFD1 Mutation but Have Different X Chromosome Inactivation Patterns. *Intern Med.* 2019 Oct 15;58(20):2989-2992. doi: 10.2169/internalmedicine.2571-18.
6. Sakakibara N, Morisada N, Nozu K, Nagatani K, Ohta T, Shimizu J, Wada T, Shima Y, Yamamura T, Minamikawa S, Fujimura J, Horinouchi T, Nagano C, Shono A, Ye MJ, Nozu Y, Nakanishi K, Iijima K. Clinical spectrum of male patients with OFD1 mutations. *J Hum Genet.* 2019 Jan;64(1):3-9. doi: 10.1038/s10038-018-0532-x.
7. Morisada N, Hamada R, Miura K, Ye MJ, Nozu K, Hattori M, Iijima K. Bardet-Biedl syndrome in two unrelated patients with identical compound heterozygous SCLT1 mutations. *CEN Case Rep.* 2020 Aug;9(3):260-265. doi: 10.1007/s13730-020-00472-y.
8. Horiuchi K, Kogiso T, Sagawa T, Ito T, Taniai M, Miura K, Hattori M, Morisada N, Hashimoto E, Tokushige K. Bardet-Biedl Syndrome Caused by Skipping of SCLT1 Complicated by Microvesicular Steatohepatitis. *Intern Med.* 2020;59(21):2719-2724. doi: 10.2169/internalmedicine.5045-20. Epub 2020 Nov 1.

9. Yamamura T, Morisada N, Nozu K, Minamikawa S, Ishimori S, Toyoshima D, Ninchoji T, Yasui M, Taniguchi-Ikeda M, Morioka I, Nakanishi K, Nishio H, Iijima K. Rare renal ciliopathies in non-consanguineous families that were identified by targeted resequencing. *Clin Exp Nephrol*. 2017 Feb;21(1):136-142. doi: 10.1007/s10157-016-1256-x.
10. Fujii Y, Matsumura H, Shirasu A, Nakakura H, Yamazaki S, Morisada N, Iijima K, Ashida A. Genetic analysis diagnosed Bardet–Biedl syndrome in a patient with a clinical diagnosis of Senior–Løken syndrome. *Renal Replacement Therapy* 2020 6:46
11. Watanabe Y, Fujinaga S, Sakuraya K, Morisada N, Nozu K, Iijima K. Rapidly Progressive Nephronophthisis in a 2-Year-Old Boy with a Homozygous SDCCAG8 Mutation. *Tohoku J Exp Med*. 2019 Sep;249(1):29-32. doi: 10.1620/tjem.249.29.
12. Hibino S, Morisada N, Takeda A, Tanaka K, Nozu K, Yamakawa S, Iijima K, Fujita N. Medullary Cystic Kidney Disease and Focal Segmental Glomerulosclerosis Caused by a Compound Heterozygous Mutation in TTC21B. *Intern Med*. 2020 Jul 15;59(14):1735-1738. doi: 10.2169/internalmedicine.4266-19.
13. Yoshikawa T, Kamei K, Nagata H, Saida K, Sato M, Ogura M, Ito S, Miyazaki O, Urushihara M, Kondo S, Sugawara N, Ishizuka K, Hamasaki Y, Shishido S, Morisada N, Iijima K, Nagata M, Yoshioka T, Ogata K, Ishikura K. Diversity of renal phenotypes in patients with WDR19 mutations: Two case reports. *Nephrology (Carlton)*. 2017 Jul;22(7):566-571. doi: 10.1111/nep.12996.