KI REPORTS
KIReports.org
CLINICAL RESEARCH

Genetic Background and Clinicopathologic Features of Adult-onset Nephronophthisis

Takuya Fujimaru[1], Kunio Kawanishi[2], Takayasu Mori[1], Eikan Mishima[3], Akinari Sekine[4,5], Motoko Chiga[1], Masayuki Mizui[6], Noriaki Sato[7], Motoko Yanagita[7,8], Yuki Ooki[9], Kiyotaka Nagahama[10], Yuko Ohnuki[11], Naoto Hamano[12], Saki Watanabe[13], Toshio Mochizuki[13], Katsushi Nagatsuji[14], Kenichi Tanaka[15], Tatsuo Tsukamoto[16], Hideo Tsushima[17], Mamiko Shimamoto[18], Takahiro Tsuji[19], Tamaki Kuyama[20], Shinya Kawamoto[21], Kenji Maki[22], Ai Katsuma[23], Mariko Oishi[24], Kouhei Yamamoto[25], Shintaro Mandai[1], Hiroaki Kikuchi[1], Fumiaki Ando[1], Yutaro Mori[1], Koichiro Susa[1], Soichiro Iimori[1], Shotaro Naito[1], Tatemitsu Rai[1], Junichi Hoshino[4,5], Yoshifumi Ubara[4,5], Mariko Miyazaki[3], Michio Nagata[2], Shinichi Uchida[1] and Eisei Sohara[1]

[1]Department of Nephrology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan; [2]Kidney and Vascular Pathology, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan; [3]Division of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan; [4]Nephrology Center, Toranomon Hospital, Tokyo, Japan; [5]Okinaka Memorial Institute for Medical Research, Toranomon Hospital, Tokyo, Japan; [6]Department of Nephrology, Osaka University Graduate School of Medicine, Osaka, Japan; [7]Department of Nephrology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; [8]Institute for the Advanced Study of Human Biology, Kyoto University, Kyoto, Japan; [9]Devision of Nephrology and Hypertension, Yokohama City University Medical Center, Yokohama, Japan; [10]Department of Pathology, Kyorin University School of Medicine, Tokyo, Japan; [11]Department of Clinical Genetics, Tokai University School of Medicine, Isehara, Japan; [12]Division of Nephrology, Endocrinology and Metabolism, Tokai University School of Medicine, Isehara, Japan; [13]Department of Nephrology, Tokyo Women's Medical University, Tokyo, Japan; [14]Department of Nephrology and Hypertension, Osaka City General Hospital, Osaka, Japan; [15]Department of Nephrology and Hypertension, Fukushima Medical University, Fukushima, Japan; [16]Department of Nephrology and Dialysis, Kitano Hospital, The Tazuke Kofukai Medical Research Institute, Osaka, Japan; [17]Department of Nephrology, Nara Medical University, Nara, Japan; [18]Department of Nephrology, Sapporo City General Hospital, Sapporo, Japan; [19]Department of Pathology, Sapporo City General Hospital, Sapporo, Japan; [20]Department of Nephrology, Japanese Red Cross Musashino Hospital, Tokyo, Japan; [21]Department of Nephrology, Dokkyo Medical University Saitama Medical Center, Saitama, Japan; [22]Department of Nephrology, Kyushu Central Hospital of the Mutual Aid Association of Public School Teachers, Fukuoka, Japan; [23]Division of Nephrology and Hypertension, Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan; [24]Department of Nephrology, Hiratsuka Kyosai Hospital, Kanagawa, Japan; and [25]Department of Comprehensive Pathology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Tokyo, Japan

Introduction: Recently, nephronophthisis (NPH) has been considered a monogenic cause of end-stage renal disease (ESRD) in adults. However, adult-onset NPH is difficult to accurately diagnose and has not been reported in a cohort study. In this study, we assessed the genetic background and clinicopathologic features of adult NPH.

Methods: We investigated 18 sporadic adult patients who were suspected as having NPH by renal biopsy. We analyzed 69 genes that cause hereditary cystic kidney disease and compared clinicopathologic findings between patients with and without pathogenic mutations in NPH-causing genes.

Results: Seven of 18 patients had pathogenic NPH-causing mutations in *NPHP1*, *NPHP3*, *NPHP4*, or *CEP164*. Compared with patients without pathogenic mutations, those with pathogenic mutations were significantly younger but did not significantly differ in the classic NPH pathologic findings, such as tubular cysts. On the other hand, the number of tubules with thick tubular basement membrane (TBM) duplication, which was defined as $>$10-μm thickness, was significantly higher in patients with genetically proven adult NPH than in those without pathogenic mutations. α-Smooth muscle actin (α-SMA)-positive myofibroblasts were detected inside thick TBM duplication.

Conclusions: In adult patients with NPH, thick TBM duplication was the specific finding. Our analysis also suggested that older patients tended to have no pathogenic mutations, even when they were suspected to have NPH by renal biopsy. These findings could be the novel clinical clue for the diagnosis of NPH in adult patients.

Kidney Int Rep (2021) **6,** 1346–1354; https://doi.org/10.1016/j.ekir.2021.02.005

Correspondence: Eisei Sohara, Department of Nephrology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan. E-mail: esohara.kid@tmd.ac.jp

Received 24 November 2020; revised 29 January 2021; accepted 1 February 2021; published online 4 March 2021

KEYWORDS: adult-onset kidney disease; chronic kidney disease; human genetics; nephronophthisis; renal cystic disease; renal pathology

NPH is an autosomal recessive kidney disease and is the most frequent genetic cause of pediatric ESRD.[1–3] In principle, molecular genetic analysis is currently the only method for the accurate clinical diagnosis of NPH.[3,4] To date, more than 25 different genes have been found to be associated with NPH.[3] Because of the increasing number of NPH genes identified, Sanger sequencing has become more tedious and costly, and comprehensive mutation analysis using next-generation sequencing is now required.[5] Recently, we have developed a target panel of genes that are related with inherited renal cystic diseases, including NPH.[6] This system enables us to perform comprehensive mutation analysis of NPH. However, in the real clinical setting, genetic testing is not performed routinely because of the limited availability of sequencing platforms and the associated high cost.

Recently, advances in genetics have revealed the importance of adult-onset NPH. In 2018, a Dutch study on 5 international cohorts reported that 26 of 5606 patients (0.5%) with adult-onset ESRD showed homozygous *NPHP1* deletions,[7] suggesting that NPH is a relatively frequent monogenic cause of adult-onset ESRD. Although the importance of NPH in adult patients has been increasing, only a few case reports are available, and there had been no clinical cohort studies on adult NPH, probably because of the difficulty in the diagnosis.

In a real clinical setting around adult-onset NPH patients, a major clinical problem is the difficulty in an accurate diagnosis. In the Dutch study mentioned previously, only 3 (12%) patients were correctly diagnosed as NPH, and the other 88% were misdiagnosed as other kidney diseases or were defined as having chronic kidney disease with unknown etiology. Several factors make the diagnosis of NPH very difficult in adult patients. First, the extrarenal abnormalities in NPH are fewer in adult patients than in pediatric cases.[7–11] In addition, the clinical and radiologic symptoms of NPH are unspecific compared with those in the more common causes of chronic kidney disease.[9–11] Therefore, most cases of adult-onset NPH are suspected only after renal biopsy.[11,12] However, although the renal histology exhibits a characteristic triad of corticomedullary cysts, TBM disruption, and tubulointerstitial nephropathy,[1,13] it is not disease specific and is commonly seen in any chronic tubulointerstitial disorder.[4,14] This absence of specific histologic findings makes the correct diagnosis of adult NPH more difficult.

Even in adult patients, accurate diagnosis of NPH is important because management options, such as kidney transplantation and appropriate genetic counseling, are available.[12] Therefore, knowledge on the specific clinical and histologic findings in adult NPH is highly required. Furthermore, the Dutch study only investigated complete *NPHP1* deletion in adult NPH using genome wide association study data.[7] Although complete *NPHP1* deletion is responsible for 20% of pediatric NPH cases,[2–4] more than 25 genes have been reported to cause NPH.[3] Therefore, adult NPH cases may be caused by other kinds of mutations in different genes. However, because only a few case reports are available, the genetic background of adult patients with NPH remains unknown.

The present study aimed to assess the genetic background and clinicopathologic features of adult NPH wherein adult patients who were suspected to have NPH on renal biopsy were analyzed.

METHODS

Patients

We investigated 18 adult patients who were suspected to have NPH by renal biopsy. After the pathologists in each institution suspected NPH based on the presence of tubular dilatation or TBM thickening and lamellation, the clinicians consulted us for genetic testing of NPH. All patients had no extrarenal findings, such as retinitis pigmentosa or liver function disorder, and no family history of autosomal dominant chronic kidney disease. Patients younger than 17 years were excluded. The patients were recruited at 16 institutions in Japan between 2015 and 2019. This study was approved by the research ethics committee of each institution.

The clinical data of the patients at the time of renal biopsy were collected from the medical records. The estimated glomerular filtration rate was calculated using the Japanese glomerular filtration rate equation.[15] A liver or renal cyst was defined as the presence of at least 1 cyst detected by computed tomography or magnetic resonance imaging.

Genetic Analysis

Comprehensive genetic testing was performed using capture-based next-generation sequencing of 69 genes that cause 9 types of hereditary cystic kidney disease, including NPH, NPH-related ciliopathies (Joubert

syndrome, Meckel syndrome, Senior-Løken syndrome, Bardet-Biedl syndrome, and skeletal ciliopathies), autosomal dominant polycystic kidney disease, autosomal recessive polycystic kidney disease, and autosomal dominant tubulointerstitial kidney disease[6] (Supplementary Table S1). The detailed methods are described in Supplementary Methods, as well as in our previous reports.[6,16]

To detect large genomic rearrangements, such as gross deletions or duplications, copy number variation analysis was conducted using Copy Number Analysis for Targeted Resequencing (http://contra-cnv. sourceforge.net/).[17] If homozygous entire deletion of *NPHP1* was detected by copy number variation analysis, we performed polymerase chain reaction for exons 1, 10, and 20 of *NPHP1*. The primer sequences are shown in Supplementary Table S2.

Pathologic Assessment

For each patient, the tissue slides that were stained with hematoxylin-eosin, periodic acid-Schiff, and periodic acid–methenamine silver were digitized using the NanoZoomer HT Scan system (Hamamatsu Photonics, Hamamatsu, Japan). Whole standard glass slides were scanned at 40× magnification (0.23 μm/pixel).

We defined 3 types of pathologic findings that were classically known to be specific for NPH; these included tubular diverticulum, tubular floret, and tubular cyst (Supplementary Figure S1A–C).[13,14] For tubular diverticulum, tubular lumens that extended through the long axis of the tubule were excluded. A tubular floret was defined as branching in at least 4 directions (Supplementary Figure S1A).[14] A tubular cyst was defined as having >200 μm in diameter (Supplementary Figure S1C).[14] Atrophic tubules, which were defined according to the Banff working classification,[18] were excluded. In addition, we defined thick TBM duplication as thickness >10 μm. Tubules with >50% fibrosis within the thick TBM duplication were excluded.

A nephrologist and a pathologist blindly assessed the pathologic findings using the tissue slides with periodic acid–methenamine silver stain. First, they counted the number of the 3 types of tubules (i.e., tubular diverticulum, tubular floret, and tubular cyst) in the specimen of each patient. Thereafter, they assessed and calculated the number of tubules that had thick TBM duplication as follows: number of tubules with thick TBM duplication = (number of tubules with thick TBM duplication/total number of counted tubules) × 10. The cumulative number of the 3 types of counted tubules was noted.

Table 1. Characteristics of patients at the time of renal biopsy

Characteristics	N = 18
Age, years	52 (25.8–74.5)
Male	6 (33)
Hypertension	11 (61)
Proteinuria	14 (78)
Serum Cr, mg/dl	2.4 (1.60–3.66)
eGFR, ml/min per 1.73 m^2	18.4 (10.4–29.0)
CKD stage	
3	3 (17)
4	8 (44)
5	7 (39)
Liver cyst	2 (11)
Renal cyst	9 (50)

CKD, chronic kidney disease; Cr, creatine; eGFR, estimated glomerular filtration rate. Values are reported as medians (25th–75th percentile) or numbers (%).

Low-vacuum Scanning Electron Microscopic and Immunofluorescence Analysis

To investigate what the essence of thick TBM duplication was, we performed low-vacuum scanning electron microscopic (Hitachi, Tokyo, Japan) analysis and immunofluorescence (IF) analysis in the representative cases with or without pathogenic mutations. For low-vacuum scanning electron microscopic analysis, 5-μm formalin-fixed paraffin-embedded sections were stained with periodic acid–methenamine silver to evaluate the TBM as described previously.[19,20] For IF analysis, monoclonal antibody against α-SMA (Merck KGaA, Darmstadt, Germany) for myofibroblast and *Phaseolus vulgaris* (Vector Laboratories, Inc., Burlingame, CA) for proximal tubules, respectively, were used in formalin-fixed paraffin-embedded sections. Streptavidin, Alexa Fluor 488 conjugate and goat anti-mouse IgG (H+L; Thermo Fisher Scientific Inc, Waltham, MA), and Alexa Fluor 568 (Thermo Fisher Scientific Inc) were used, and sections were counterstained with 4′,6-diamidino-2-phenylindole. IF images were captured using BZ-X800 (Keyence, Osaka, Japan).

Statistical Analysis

All statistical analyses were performed using JMP version 15 (SAS Institute, Cary, NC). Nonnormally distributed variables were expressed as the median with interquartile range. The Mann-Whitney *U* test was used to compare the medians of continuous variables, such as age, and the chi-square test was used to compare the percentages of categoric variables, such as sex, between patients with pathogenic mutations and those without pathogenic mutations. A P value < 0.05 was considered statistically significant.

RESULTS

Patient Characteristics

The patient characteristics are presented in Table 1. The median age at renal biopsy was 52 years; 6 patients

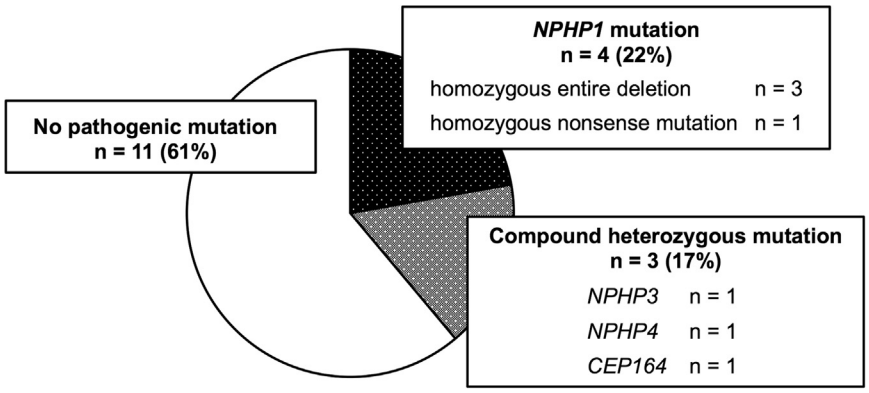

Figure 1. Disease-causing mutations in adults with suspected nephronophthisis (NPH). Of the 7 patients (39%) who had pathogenic mutations related with NPH, 4 patients had a mutation in *NPHP1*, and the other 3 patients had compound heterozygous mutations in *NPHP3*, *NPHP4*, and *CEP164*, respectively.

(33%) were men, and 11 patients (61%) had hypertension. For the renal manifestations, 14 patients (78%) had proteinuria. The median estimated glomerular filtration rate was 18.4 ml/min per 1.73 m^2. Chronic kidney disease was stage 3 in 3 patients (17%), stage 4 in 8 patients (44%), and stage 5 in 7 patients (39%). No patient received dialysis. Nine patients had at least 1 renal cyst identified on imaging, such as computed tomography or magnetic resonance imaging.

Panel-based Genetic Diagnosis of NPH

A summary of the results of our comprehensive genetic testing is presented in Figure 1. Of the 7 patients (39%) who had pathogenic mutations related with NPH; 4 patients had a mutation in *NPHP1*; and the other 3 patients had compound heterozygous mutations in *NPHP3*, *NPHP4*, and *CEP164*, respectively. Notably, to the best of our knowledge, the patient with the *CEP164* mutation was the first case in an adult. Of the patients with an *NPHP1* mutation, 3 patients (patient numbers 883, 896, and 1207) were detected to have homozygous entire deletion of *NPHP1* by copy number variation analysis. The regions that were expected to be identified by copy number variation analysis are shown in Supplementary Table S3. In all 3 patients, no polymerase chain reaction products were detected in exons 1, 10, and 20 of *NPHP1*; this confirmed complete gene deletion of *NPHP1*. The other patient (patient number 1107) had homozygous nonsense mutation in *NPHP1* (Supplementary Table S4). The details of the mutations in the patients with compound heterozygous mutations in *NPHP3*, *NPHP4*, and *CEP164* are shown in Supplementary Table S4.

Clinical Characteristics of Adult NPH Patients

As shown in Table 2, the patients with pathogenic mutations were significantly younger compared with those who had no pathogenic mutations (median age, 26 vs. 63 years; $P = 0.01$). Interestingly, no patient who was >50 years old at the time of renal biopsy had pathogenic mutations (Figure 2). In addition, compared with patients who had no pathogenic mutations, there was a significantly higher proportion of men for those with pathogenic mutations (71% vs. 9%, $P = 0.01$) and a significantly lower incidence of hypertension (29% vs. 82%, $P = 0.049$) but a similar estimated glomerular filtration rate, level of proteinuria, and number of renal or liver cysts (at least 1 for each).

Pathologic Findings Specific for Adult-onset NPH

The 3 pathologic findings that are known to be specific for NPH were not significantly different between the adult patients with NPH and those who did not have a mutation causing NPH (Table 2). Therefore, we further searched for novel pathologic findings that were specific to adult NPH cases. We focused on TBM duplication, which was reported to be another specific finding in NPH.[13,14] Although TBM duplication was reported in noninherited renal diseases,[14] we noticed that the TBMs were quite thick and reduplicated in genetically proven adult NPH cases. Therefore, we focused on the thickness of TBM duplication; we defined thick TBM duplication as >10 μm (Figure 3). Interestingly, the number of tubules with thick TBM duplication was significantly higher in genetically proven adult NPH than in those who did not have pathogenic mutation (Table 2).

For investigating the ultrastructure and components of thick TBM duplication, we observed renal tissues of representative cases using low-vacuum scanning electron microscopy and IF. In low-vacuum scanning electron microscopic observations, thick TBM duplications were detected in the patient with homozygous entire deletion of *NPHP1* (patient number 896) (Figure 4a and 4b). Furthermore, from immunofluorescence results, α-SMA–positive myofibroblasts were

Table 2. Phenotypic characterization of the patients according to the presence of pathogenic mutation

Phenotype	Pathogenic mutation n = 7	No pathogenic mutation n = 11	*P* value
Clinical findings at renal biopsy			
Age, years	26 (22–35)	63 (55–77)	0.01
Male	5 (71)	1 (9)	0.01
Hypertension	2 (29)	9 (82)	0.049
Proteinuria	5 (71)	9 (82)	1.00
eGFR, ml/min per 1.73 m^2	28.8 (5.2–39.5)	15.2 (10.4–25.9)	0.53
Liver cyst	1 (14)	1 (9)	1.00
Renal cyst	3 (43)	6 (55)	1.00
Pathologic findings			
Tubule with thick TBM duplication, /10 counted tubules[a]	4.5 (1.5–5.4)	0 (0–0.4)	<0.001
Tubular diverticulum	8 (5–9)	5.5 (5–7)	0.22
Tubular floret[b]	1 (0.5–2.5)	2.5 (1–3.5)	0.12
Cyst[c]	0 (0–0)	1 (0–1.5)	0.15
Total counted tubules[d]	9 (6–11.5)	9.5 (8.5–11)	0.56

eGFR, estimated glomerular filtration rate; TBM, tubular basement membrane.
Values are reported as median (25th–75th percentile) or numbers (%).
[a]Number of tubules with thick TBM duplication divided by the total number of counted tubules and then multiplied by 10.
[b]Branching in at least 4 directions.
[c]Diameter >200 μm.
[d]Sum of the number of tubular diverticula, tubular florets, and cysts.

detected inside thick TBM duplication, in addition to vascular smooth muscle cells (Figure 5a and b). On the other hand, in the patient who had no pathogenic mutations related with NPH (patient number 669), thick TBM duplications were not detected in low-vacuum scanning electron microscopic analysis (Figure 4c). Additionally, α-SMA–positive myofibroblasts were detected only in vascular smooth muscle cells by IF observation (Figure 5c).

In general, IgA nephropathy, diabetic nephropathy, and tubulointerstitial nephritis are known to have tubular interstitial lesions. Therefore, we assessed for the presence of tubules with thick TBM duplication in these noninherited renal diseases (Supplementary Table S5). Among the 9 patients, the diagnoses by renal biopsy were IgA nephropathy in 4 patients, diabetic nephropathy in 3 patients, and tubulointerstitial nephritis in 2 patients. Although most patients had >30% of tubulointerstitial fibrosis, we could not find any tubule that had thick TBM duplication in these samples. Therefore, thick TBM duplication could be specific for NPH.

DISCUSSION

To the best of our knowledge, this was the first cohort study to investigate the clinicopathologic findings and genetic background of adult patients with NPH. Through genetic analysis of 18 adult patients who were suspected to have NPH by renal biopsy, we found pathogenic mutations related with NPH in 7 patients. Compared with patients who had no pathogenic mutations, adult patients who had genetically proven NPH were significantly younger and had a significantly higher proportion of men, a significantly lower incidence of hypertension, and a significantly higher number of tubules with thick TBM duplication.

In this study, the pathogenic mutations in the 7 patients with NPH were in the genes *NPHP1*, *NPHP3*, *NPHP4*, and *CEP164*. In a genome-wide association study on adult-onset ESRD patients, Snoek *et al.*[7] analyzed only homozygous *NPHP1* full gene deletions using generated genomic data. However, based on our results, 4 of 7 genetically confirmed NPH cases had pathogenic mutations other than homozygous *NPHP1* full gene deletions. Moreover, mutations in *CEP164* were reported to cause Senior-Løken syndrome or Joubert syndrome.[21,22] In this study, patient

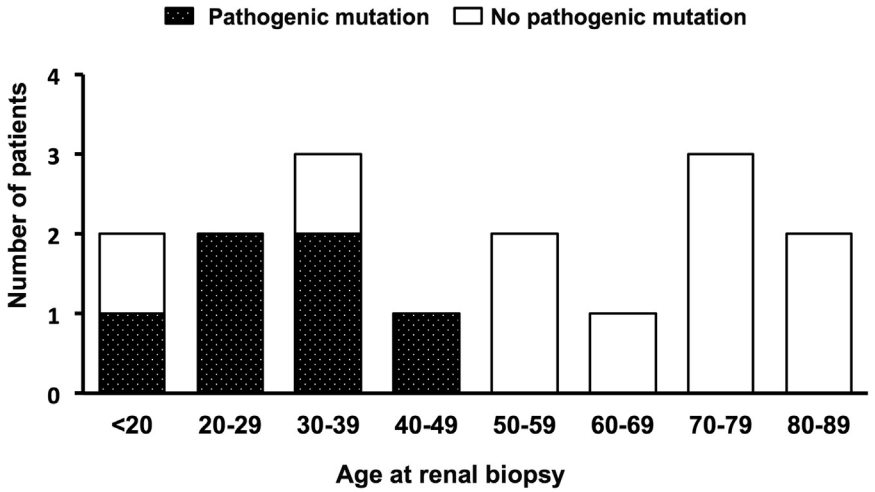

Figure 2. The age distribution of patients according to the presence of pathogenic mutations. No pathogenic mutations in the known genes are detected in patients >50 years old.

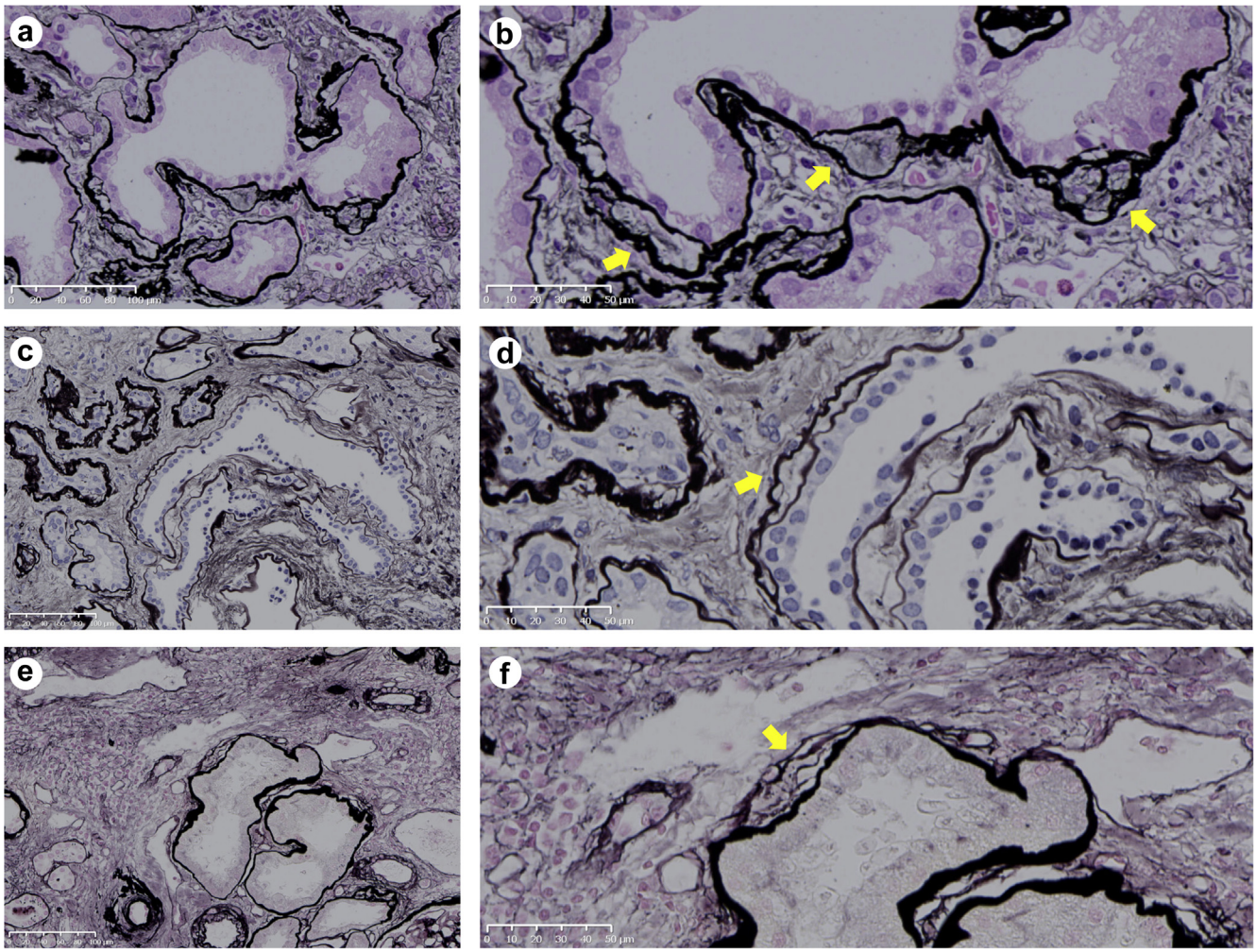

Figure 3. Thick tubular basement membrane duplication. Thick tubular basement membrane duplication was defined as thickness of >10 μm (yellow arrows). (a and b) Patient number 883, periodic acid–methenamine silver (PAM) stain, 20× and 40× magnification, respectively. (c and d) Patient number 478, PAM stain, 20× and 40× magnification, respectively. (e and f) Patient number 896, PAM stain, 20× and 40× magnification, respectively. All slides were scanned on the NanoZoomer NDP system with 40× resolution (0.23 μm/pixel) (Hama-matsu Photonics, Hamamatsu-City, Japan).

number 930, who was a 26-year-old man, had compound heterozygous mutations in *CEP164*. To the best of our knowledge, this was the first reported case of adult NPH with a *CEP164* mutation. These findings strongly indicated that comprehensive genetic testing may be useful for adult patients suspected to have NPH.

In our study, we detected α-SMA–positive cells in the thick TBM duplication. α-SMA is commonly used as a marker for myofibroblasts and the resulting fibrosis.[23] Interestingly, through the study of primary cilia in cultured epithelial cells, primary cilia undergo a dynamic biphasic change during epithelial-myofibroblast transition as well as fibroblast-to-myofibroblast transition induced by transforming growth factor-β.[24] Furthermore, the study of inhibition of ciliogenesis demonstrated that deficiency of primary cilia induces epithelial-to-mesenchymal transition.[25] Therefore, because it is well-known that products of genes causing NPH are localized at segments associated with primary cilia, it could be possible that abnormal primary cilia in NPH patients result in increased α-SMA–positive myofibroblasts and fibrosis, resulting in thick TBM duplication.

In this study, adult patients with genetically proven NPH were significantly younger compared with those who had no pathogenic mutations at the time of renal biopsy. Snoek *et al.*[7] analyzed 5606 patients with adult-onset ESRD and revealed that 26 (0.5%) of the patients had homozygous *NPHP1* full gene deletions. Of the patients who were >50 years old at the time of the initiation of renal replacement therapy, only 2 had homozygous *NPHP1* full gene deletions. Similar to the report by Snoek *et al.*, our study showed that no patient who was >50 years old at the time of renal biopsy had pathogenic NPH mutations. These findings

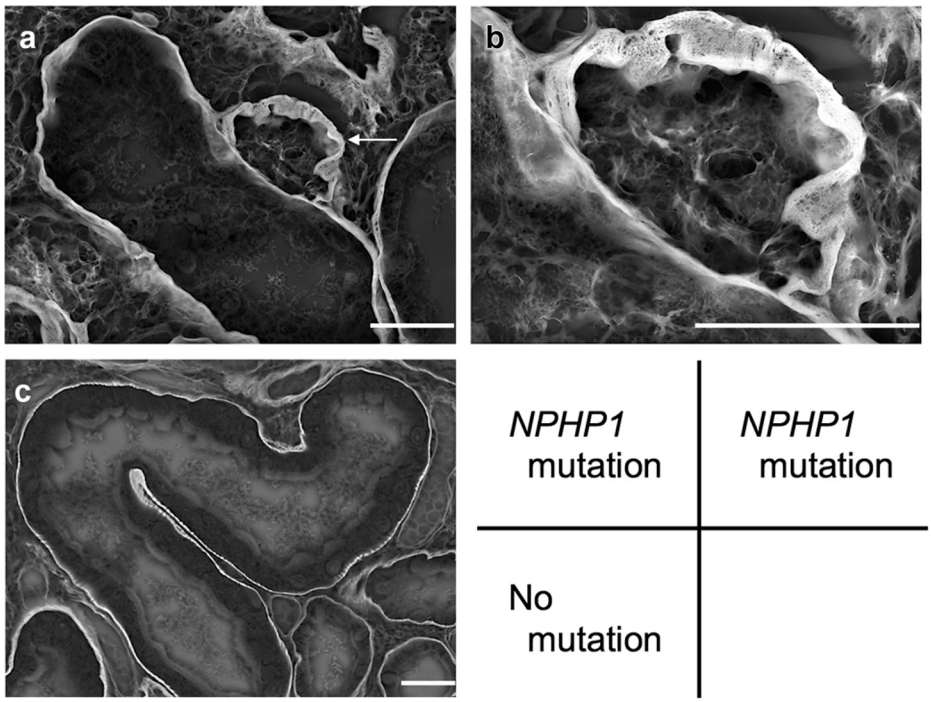

Figure 4. Low-vacuum scanning electron microscopic imaging of the patient with pathogenic mutation in *NPHP1* (patient number 896) and without pathogenic mutations (patient number 669). Low-vacuum scanning electron microscope images for periodic acid–methenamine silver using formalin-fixed paraffin-embedded samples (5-μm section). (a and b) Imaging of the patient with pathogenic mutation in *NPHP1*; b presents higher magnification images of a. The white arrow shows thick tubular basement membrane duplication. (c) imaging of the patient without pathogenic mutations. White bars = 20 μm.

suggested that older patients tended to have no pathogenic mutations, even if they were suspected to have NPH by renal biopsy.

In this study, the incidence of hypertension was significantly higher in patients without pathogenic mutations than in those who had pathogenic mutations. Hypertensive nephrosclerosis is a disorder that is usually associated with disease chronicity. The renal pathologic features of arteriolar nephrosclerosis are characterized by the involvement of arteries, arterioles, glomeruli, and the tubulointerstitium[26]; the presence of chronic tubular and interstitial lesions in the form of tubular atrophy and interstitial fibrosis[26,27]; and lamellated TBMs in atrophic tubules.[28] In this study, the patients who had no pathogenic mutation tended to have a relatively high number of tubular cysts. In aging kidneys, tubular diverticulum is often observed and is a probable source of renal cysts.[29–31] Therefore, in patients without pathogenic mutations, tubular disorders can be caused by secondary factors, such as hypertension and aging. Moreover, we found that patients with pathogenic mutations included a relatively high proportion of men. However, in the Dutch study about adult-onset ESRD, 12 of 26 patients who had homozygous *NPHP1* deletion were men,[7] demonstrating no sex difference. Further study will be required to confirm this observation.

This study had several limitations. First, the sample size of our study was small. Nevertheless, only a few adult cases of suspected NPH by renal biopsy have been reported,[7–9,11] and no cohort studies on adult NPH cases have been available. Considering that there were only 26 patients with homozygous *NPHP1* deletions even in the study using nationwide data from 5 countries,[7] the number of patients in our study could be reasonable. Therefore, although we collected samples from all over Japan, this number of study patients was inevitable. Furthermore, to the best of our knowledge, this was the first study to investigate the clinicopathologic findings and genetic background of adult patients who were suspected to have NPH. We expect an increase in the number of cases in the future. Second, in our study, 2 of 7 patients with mutations in the genes related with nephronophthisis had only novel missense mutations. Therefore, we could not exclude the possibility that these missense mutations were not disease-causing mutations. Third, we could not analyze other genes that had been identified very recently as rare causal genes for NPH-related ciliopathies, such as *C8orf37*,[32] *KIAA0586*,[33] and *MAPKBP1*.[34] However, the phenotypes of the patients with mutations in these genes were Bardet-Biedl syndrome, Joubert syndrome, or NPH with extrarenal findings. Therefore, considering that all patients in this study do not exhibit any extrarenal findings, it is unlikely that

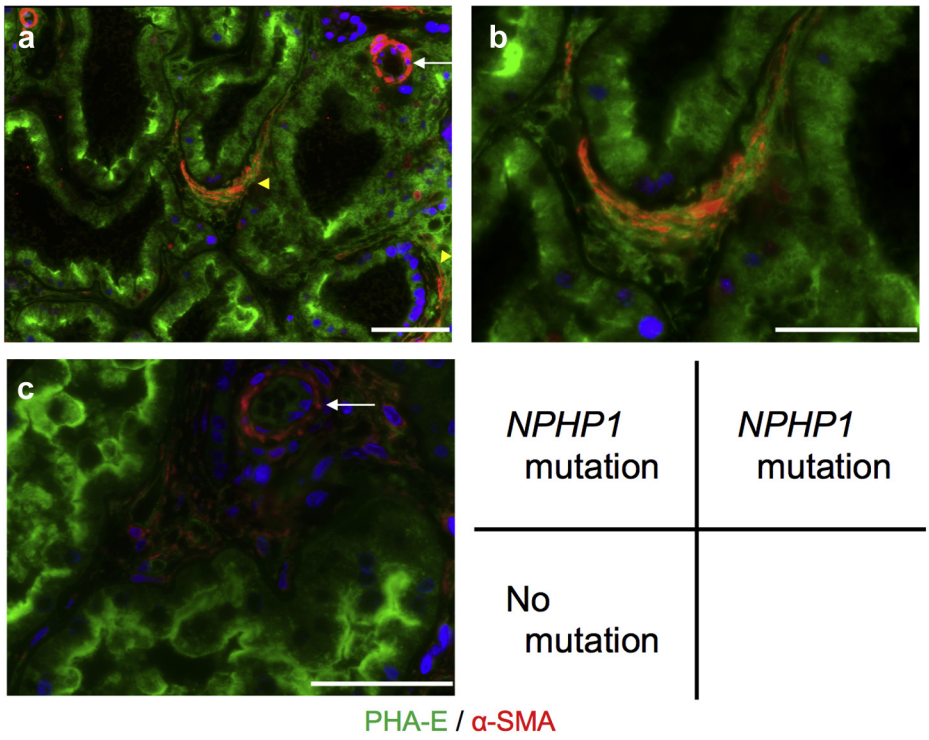

Figure 5. Immunofluorescence imaging of the patient with pathogenic mutations in *NPHP1* (patient number 896) and without pathogenic mutations (patient number 669). (a and b) Imaging of the patient with pathogenic mutation in *NPHP1*. Alpha-smooth muscle action (α-SMA) (red) positive myofibroblasts were detected inside thick tubular basement membrane duplication (yellow arrowhead), in addition to vascular smooth muscle cells in the artery (white arrow); b presents higher magnification images of a. (c) Imaging of the patient without pathogenic mutations. α-SMA (red) was detected only in vascular smooth muscle cells (white arrow). *Phaseolus vulgaris* (PHA-E) (green) was used to identify proximal tubules. Nuclear counterstain with DAPI (blue). White bars = 100 μm.

our 11 patients without identified pathogenic mutations have mutations in these genes.

In conclusion, our analysis showed that older patients tended to have no pathogenic mutations, even if they were suspected to have NPH by renal biopsy. On pathology, the number of tubules with thick TBM duplication could be an effective measure to diagnose NPH in adult patients. In addition, comprehensive genetic testing with a panel system could be useful for adult patients suspected to have NPH.

DISCLOSURE

TM reports personal fees from honoraria for lectures and personal fees from employment outside the submitted work. JH has received a research grant from Otsuka Pharmaceutical Co. All the other authors declared no competing interests.

ACKNOWLEDGMENTS

Supported in part by the Grants-in-Aid for Scientific Research (25221306, 16H05314, 19H01049, and 19H03672), the Challenging Exploratory Research (15K15327, 16K15467, and 18K19534), the Research Activity Start-up (17H06657, 20K22926), the Young Scientists (19K17733), and the Early-Career Scientists (19K17730) from the Japan Society of the Promotion of Science; the Health Labor Science Research Grant from the Ministry of Health Labor and Welfare, AMED under Grant Number JP18ek0109304; grants from the Yukiko Ishibashi Foundation; the Salt Science Research Foundation (1925, 2030); and low-vacuum scanning electron microscope device support from the LVSEM Study Group of Renal Biopsy and Hitachi High-Technologies Corporation in Japan Grant Number 007.

SUPPLEMENTARY MATERIAL

Supplementary Material (PDF)

Supplementary Methods.

Supplementary References.

Table S1. Disease categories and the targeted genes included in this panel

Table S2. Primer sequences of *NPHP1*

Table S3. Detected copy number variants in *NPHP1*

Table S4. Mutations in the genes related with nephronophthisis

Table S5. Clinical characteristics of the pathologic control group

Figure S1. Representative pathologic findings.

REFERENCES

1. Hildebrandt F, Attanasio M, Otto E. Nephronophthisis: disease mechanisms of a ciliopathy. *J Am Soc Nephrol.* 2009;20:23–35.

2. Srivastava S, Molinari E, Raman S, et al. Many genes-one disease? Genetics of nephronophthisis (NPHP) and NPHP-associated disorders. *Front Pediatr*. 2017;5:287.
3. Luo F, Tao Y-H. Nephronophthisis: A review of genotype-phenotype correlation. *Nephrology (Carlton)*. 2018;23:904–911.
4. Wolf MTF. Nephronophthisis and related syndromes. *Curr Opin Pediatr*. 2015;27:201–211.
5. Halbritter J, Porath JD, Diaz KA, et al. Identification of 99 novel mutations in a worldwide cohort of 1,056 patients with a nephronophthisis-related ciliopathy. *Hum Genet*. 2013;132:865–884.
6. Fujimaru T, Mori T, Sekine A, et al. Kidney enlargement and multiple liver cyst formation implicate mutations in PKD1/2 in adult sporadic polycystic kidney disease. *Clin Genet*. 2018;94:125–131.
7. Snoek R, van Setten J, Keating BJ, et al. NPHP1 (nephrocystin-1) gene deletions cause adult-onset ESRD. *J Am Soc Nephrol*. 2018;29:1772–1779.
8. Haghighi A, Savaj S, Haghighi-Kakhki H, et al. Identification of an NPHP1 deletion causing adult form of nephronophthisis. *Ir J Med Sci*. 2016;185:589–595.
9. Hoefele J, Nayir A, Chaki M, et al. Pseudodominant inheritance of nephronophthisis caused by a homozygous NPHP1 deletion. *Pediatr Nephrol*. 2011;26:967–971.
10. Wang Y, Chen F, Wang J, et al. Two novel homozygous mutations in NPHP1 lead to late onset end-stage renal disease: a case report of an adult nephronophthisis in a Chinese intermarriage family. *BMC Nephrol*. 2019;20:173.
11. Bollée G, Fakhouri F, Karras A, et al. Nephronophthisis related to homozygous NPHP1 gene deletion as a cause of chronic renal failure in adults. *Nephrol Dial Transplant*. 2006;21:2660–2663.
12. Hudson R, Patel C, Hawley CM, et al. Adult-diagnosed non-syndromic nephronophthisis in Australian families caused by biallelic NPHP4 variants. *Am J Kidney Dis*. 2020;76:282–287.
13. Waldherr R, Lennert T, Weber HP, et al. The nephronophthisis complex. A clinicopathologic study in children. *Virchows Arch A Pathol Anat Histol*. 1982;394:235–254.
14. Larsen CP, Bonsib SM, Beggs ML, et al. Fluorescence in situ hybridization for the diagnosis of NPHP1 deletion-related nephronophthisis on renal biopsy. *Hum Pathol*. 2018;81:71–77.
15. Matsuo S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis*. 2009;53:982–992.
16. Mori T, Hosomichi K, Chiga M, et al. Comprehensive genetic testing approach for major inherited kidney diseases, using next-generation sequencing with a custom panel. *Clin Exp Nephrol*. 2017;21:63–75.
17. Li J, Lupat R, Amarasinghe KC, et al. CONTRA: copy number analysis for targeted resequencing. *Bioinformatics*. 2012;28:1307–1313.
18. Roufosse C, Simmonds N, Clahsen-van Groningen M, et al. 2018 reference guide to the Banff classification of renal allograft pathology. *Transplantation*. 2018;102:1795–1814.
19. Inaga S, Kato M, Hirashima S, et al. Rapid three-dimensional analysis of renal biopsy sections by low vacuum scanning electron microscopy. *Arch Histol Cytol*. 2010;73:113–125.
20. Okada S, Inaga S, Kawaba Y, et al. A novel approach to the histological diagnosis of pediatric nephrotic syndrome by low vacuum scanning electron microscopy. *Biomed Res*. 2014;35:227–236.
21. Chaki M, Airik R, Ghosh AK, et al. Exome capture reveals ZNF423 and CEP164 mutations, linking renal ciliopathies to DNA damage response signaling. *Cell*. 2012;150:533–548.
22. Vilboux T, Doherty DA, Glass IA, et al. Molecular genetic findings and clinical correlations in 100 patients with Joubert syndrome and related disorders prospectively evaluated at a single center. *Genet Med*. 2017;19:875–882.
23. Gewin L, Zent R, Pozzi A. Progression of chronic kidney disease: too much cellular talk causes damage. *Kidney Int*. 2017;91:552–560.
24. Rozycki M, Lodyga M, Lam J, et al. The fate of the primary cilium during myofibroblast transition. *Mol Biol Cell*. 2014;25:643–657.
25. Han SJ, Jung JK, Im S-S, et al. Deficiency of primary cilia in kidney epithelial cells induces epithelial to mesenchymal transition. *Biochem Biophys Res Commun*. 2018;496:450–454.
26. Freedman BI, Cohen AH. Hypertension-attributed nephropathy: what's in a name? *Nat Rev Nephrol*. 2016;12:27–36.
27. Meyrier A. Nephrosclerosis: update on a centenarian. *Nephrol Dial Transplant*. 2015;30:1833–1841.
28. Lusco MA, Fogo AB, Najafian B, et al. AJKD atlas of renal pathology: tubular atrophy. *Am J Kidney Dis*. 2016;67:e33–e34.
29. Kanasaki K, Kitada M, Koya D. Pathophysiology of the aging kidney and therapeutic interventions. *Hypertens Res*. 2012;35:1121–1128.
30. Denic A, Glassock RJ, Rule AD. Structural and functional changes with the aging kidney. *Adv Chronic Kidney Dis*. 2016;23:19–28.
31. Darmady EM, Offer J, Woodhouse MA. The parameters of the ageing kidney. *J Pathol*. 1973;109:195–207.
32. Heon E, Kim G, Qin S, et al. Mutations in C8ORF37 cause Bardet Biedl syndrome (BBS21). *Hum Mol Genet*. 2016;25:2283–2294.
33. Bachmann-Gagescu R, Phelps IG, Dempsey JC, et al. KIAA0586 is mutated in Joubert syndrome. *Hum Mutat*. 2015;36:831–835.
34. Macia MS, Halbritter J, Delous M, et al. Mutations in MAPKBP1 cause juvenile or late-onset cilia-independent nephronophthisis. *Am J Hum Genet*. 2017;100:323–333.