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ORIGINAL ARTICLE

Clinical and genetic characteristics of Japanese nephronophthisis patients

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Abstract
Background Nephronophthisis (NPH) accounts for 4–5 % of end-stage renal disease occurring in childhood.
Method We investigated the clinical context and characteristics of renal and extrarenal symptoms, as well as the *NPHP* genes, in 35 Japanese patients with clinical and histologic features suggesting NPH.
Results NPH occurred fairly uniformly throughout Japan irrespective of region or gender. In three families, NPH affected siblings. The median age of patients was 12.5 years. Renal abnormalities attributable to NPH discovered through mass screening, such as urine tests in school. However, NPH accounted for less than 50 % of children with abnormal findings, including incidentally discovered renal dysfunction during evaluation of extrarenal symptoms or during routine check-ups. Typical extrarenal manifestations leaded to discovery including anemia and delayed physical development. The urine often showed low gravity specific density and low molecular weight proteinuria. Frequent renal histologic findings included cystic dilation of tubules, mainly in the medulla, and irregularity of tubular basement membranes. Genetically abnormalities of *NPHP1* were not common, with large deletions frequently noted. Compound heterozygotes showing single abnormalities in each of *NPHP1*, *NPHP3*, and *NPHP4* were observed.
Conclusions Our findings resemble those reported in Western populations.

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Keywords End-stage renal disease · Renal cysts · *NPHP* genes · Children · Renal tubules

Introduction

Nephronophthisis (NPH) is a disease characterized by renal medullary cyst formation. Additional histologic findings include tubulointerstitial nephritis accompanied by progressive sclerosis and hyaline glomeruli. Although NPH characteristically shows autosomal recessive inheritance, it may occur sporadically [1]. NPH accounts for approximately 4–5 % of end-stage renal disease (ESRD) in childhood. Disease subtypes include: infantile NPH (NPH2), which progresses to ESRD around the age of 5 years; juvenile NPH (NPH1), which develops from early childhood to school age and usually progresses to ESRD by an age of about 13 or 14 years; and adolescent NPH (NPH3), with development of ESRD at an average age of 19 years. Juvenile NPH is reported to be the most common subtype [1].

NPHP1, the gene most often responsible for juvenile nephronophthisis, encodes the nephrocystin-1 molecule. This gene has an extent of approximately 11 kbp, and is located on chromosome 2q12-13 [2]. The nephrocystin-1 protein consists of 677 amino acids and includes three coiled domains; two highly acidic negatively charged glutamic acid-rich domains; and an Src-homology 3 domain. Nephrocystin-1 has a molecular weight of 83 kD. As this protein is located in the transition zone of primary cilia of renal tubular epithelial cells, its abnormalities typically cause dysfunction of these primary cilia (ciliopathy) [1, 2].

NPHP4, whose abnormalities cause a second form of NPH1, is located on chromosome 1p36 and encodes the nephrocystin-4 (nephroretinin) molecule. Nephrocystin-4

Primers for *NPHP1*

exon	F primer	5′-nucleotide sequence -3′	R primer	5′-nucleotide sequence -3′	Amprified fragment length (bp)
exon1	NPHP1E01F010	GACCACCGCAAGAGAACATTT	NPHP1E01R010	AAGCTCCCAGGATTAGGTGGG	319
exon2	NPHP1E02F010	GGTATATGGGTTTTCACTGTA	NPHP1E02R010	TTCCATTTGATTCCAAAGGAC	319
exon3	NPHP1E03F010	TAATTGCCTTGCCTGCTCAAC	NPHP1E03R010	CAGACTTAGCAAGCCTGTTCG	320
exon4	NPHP1E04F010	GATAGGTGTAATGTCACACTG	NPHP1E04R010	CATGGGATCTAACACCTTCTA	418
exon5	NPHP1E05F010	CCAGCTCCAAATATGGGATAT	NPHP1E05R010	CAGGTGTACAGGCAGAGTTTTC	380
exon6	NPHP1E06F010	GGGAAGCTTTTGATAAACCTT	NPHP1E06R010	GTCATTCACTAGTCAACTGAC	349
exon7	NPHP1E07F010	GTTTTTGTTTTTACTGGAGGG	NPHP1E07R010	GTTGTCTCCATTTCAAGAAAG	306
exon8	NPHP1E08F010	CTCGTTTTCATCTGGAAACTG	NPHP1E08R010	GGAAAGCAGGATCAATGAGAA	443
exon9	NPHP1E09F010	CTTCCACTAAAGTCTGTATGT	NPHP1E09R010	GTGAGATTCAACATCTTCTTC	322
exon10	NPHP1E10F010	TTTGGAAGTGCCTGTACTCTA	NPHP1E10R010	GTCCAAATTCTGCCTTAGTTA	360
exon11	NPHP1E11F010	GCCTGCCAATATTTATTGTTCC	NPHP1E11R010	TACTCTCTTGGGAATTGGGGA	494
exon12	NPHP1E12F010	TCCTCACTTAGTGTAGCCACT	NPHP1E12R010	GTCCTCAAAGAACACCAAAGA	302
exon13	NPHP1E13F010	CACCTTCAACATTGGGATTAC	NPHP1E13R010	CATTCTCATTCCTCAAGGGAT	365
exon14	NPHP1E14F010	GCAAAATGAGATTCTACTGTG	NPHP1E14R010	AGTTATTGGCATGCTCATAGA	342
exon15	NPHP1E15F010	GGCATAAATGAAATGTCTGAG	NPHP1E15R010	GTCTCATATGTGTTACCAAGA	374
exon16	NPHP1E16F010	GCACTACTGGGTGGTATATTT	NPHP1E16R010	GGGAAGAATTAAAGAGGACAA	330
exon17	NPHP1E17F010	GAAGCAAAATTTGGGACTGTT	NPHP1E17R010	AAAGTCACAACCAGAAACAGA	316
exon18	NPHP1E18F010	CCTAGAAGTCAAAGTGTGTAG	NPHP1E18R010	GGAGACATCATCCTAGTAACA	326
exon19	NPHP1E19F010	CAGCATTTTTAACCCTGTCCA	NPHP1E19R010	GGGATTATGACTATGGCTACT	261
exon20	NPHP1E20F010	CCCTCCATCCTACCTCTTAGG	NPHP1E20R010	CTAAGTTGTAAAGTGACAGTG	478

Primers for *NPHP2*

exon	F primer	5′-nucleotide sequence -3′	R primer	5′-nucleotide sequence -3′	Amprified fragment length (bp)
5UTR	I5Uf1	TTTCCCATTGGGCTCTCGGCC	I5Ur1	TGAGTCTGCAGCAGGGGCCAA	366
exon1	IEx1F	CCCACTTGGAACTGATGAGAC	IEx1R	AACAAACTTCCTCAGGACAAAC	265
exon2	IEx2F	ATAATAAACAGCGAATATAGTTCCTAC	IEx2R	TGTCCATTGCATAGTTCCAC	327
exon3	IEx3F	GTGGAATTACAAGCATTTTTCC	IEx3R	AATTCAGGCCTTCTTCCTTG	411
exon4	IEx4F	TTGTTACTGTTGTTATTTCGAGAACC	IEx4R	ACTTCTTGGGGGATGAGTCC	356
exon5	IEx5F	CACCAAATGTAATTTATTGAGGATTC	IEx5R	AGTGGAAGGGAAGGCACAG	317
exon6	IEx6F	CTGCTGTTCAGAAACCGTTG	IEx6R	GGTGTAGGAGTGCAAAAAGC	421
exon7	IEx7F	AGGGGAAAATGCTTTGCTTC	IEx7R	AATTTTATAGCAACATCTACACTTTGG	351
exon8	IEx8F	GATGGGGAAATCAAGAGAGG	IEx8R	TGTGCAGCTTTCTGCTAAGG	348
exon9	IEx9F	CCATAAGAATAAAGCATTAAAGGAAC	IEx9R	TGTGGGTGATCTCTTCATCTTG	494
exon10	IEx10F	CCACATATCCAAAAATACTTACTCC	IEx10R	AGAAAGGATGTATGATAAAGAGCAC	528
exon11	IEx11F	TTCCACATCTTAGAATGAAGTTTCC	IEx11R	CTCATCTGTTTCCCTCTCCTG	427
exon12	IEx12F	CACACAGAGACTTGAGGAGGTG	IEx12R	CGGCAGAAGATGACAAAGG	382
exon13	IEx13F	TGTAACTGCCACTATTATGGTGATG	IEx13R	CACCACATGGAACTCACTGG	939
exon14	IEx14F	AATGGGAGCTTGAATGAACC	IEx14R	TGGTACTCTGGGGGTACTTG	410
exon15	IEx15F	CACACACCTGCAAGCTCAAG	IEx15R	TCTTTGGGGATGAAACAAAGG	255
exon16	IEx16F	CCAATGAACTATTCCCTTCAGC	IEx16R	GCAGAAAATCTGAACTCTGCAC	242

Fig. 1 Genomic DNA extraction, PCR, and determination of *NPHP1, 2, 3* and *4* gene sequence. PCR primers were prepared to amplify approximately 200–300 bp fragments based on *NPHP 1–4* gene sequences registered in GenBank, the following primers were used as shown

has been shown to carry out signal transmission between renal tubular epithelial cells, in cooperation with nephrocystin-1 [3].

NPHP2, the gene responsible for infantile NPH (NPH2), is located on 9q22-31 [4]. *NPHP2* encodes a protein termed inversin (INVS). An abnormality in INVS can cause situs inversus, pancreatic islet-cell dysplasia, cardiovascular abnormalities, and hepato-biliary disorders. In addition, INVS abnormalities can cause cyst formation resembling that in juvenile nephronophthisis. However, the renal prognosis is worse progression to ESRD in early childhood.

The gene responsible for adolescent NPH (NPH3), *NPHP3* is located on chromosome 3q21-22 [5]. *NPHP3* is believed to encode a protein involved in signal

Primers for *NPHP3*

exon	F primer	5′-nucleotide sequence -3′	R primer	5′-nucleotide sequence -3′	Amprified fragment length (bp)
exon1	NPHP4E01F010	ATGCAAATCAGGATGGGCCCG	NPHP4E01R010	AACCCACGTAGCCAAACGGCA	598
exon2	NPHP4E02F010	AGGTTCCTCTGGGATTTAGTG	NPHP4E02R010	AATCAAAGCATCGTAAGCCAG	373
exon3	NPHP4E03F010	TGATATCTGAGCGAGGTGGCC	NPHP4E03R010	AAGTCTGAGACGCGCTGTGAG	368
exon4	NPHP4E04F010	TGCTGTGGCACGTGTAGGAAG	NPHP4E04R010	ACTGCACTCTAGCCTGGTTGA	379
exon5	NPHP4E05F010	AAAGCTCTAGTGGGCGTGGTG	NPHP4E05R010	CAGATAGCAGTTTACACTGAG	273
exon6	NPHP4E06F010	CCTGTTTGTGGTGTTTCTAAC	NPHP4E06R010	TTCCATCCTCCTCCACTGTCC	426
exon7	NPHP4E07F030	TGGAGGAGGTTTGGGGTAGAT	NPHP4E07R020	AGGGGAAAAGACAGAACTACA	569
exon8	NPHP4E08F010	CTGCTCCAGTTTCTTCTCTCT	NPHP4E08R010	TCCCACGTGGGTGAGTCAACA	383
exon9	NPHP4E09F010	ACTTGCTGTTGCAGCAGCACC	NPHP4E09R010	CCATCTCATCTGTATCCTTTG	446
exon10	NPHP4E10F010	CACTGAGCTCTCGTTGAATTT	NPHP4E10R010	GGCATACCCATGACATGAAAA	420
exon11	NPHP4E11F010	GACTTTGTTTTAGGGCAGAGC	NPHP4E11R010	ATGTGGTGATTACCGTACTAG	339
exon12	NPHP4E12F010	AGACAAGGTGGTGAGGCCTGT	NPHP4E12R010	AAGCACGCAGGGATCCACTGT	274
exon13	NPHP4E13F010	TTGAGAAGCGGTCCCAGGTTC	NPHP4E13R010	TGCCACCTAACTAAGGACAGG	384
exon14	NPHP4E14F010	CCAGAGGCAATTAATCGATGA	NPHP4E14R010	ATTGATGCACCTCCCTGTGGA	354
exon15	NPHP4E15F010	CAGACTGTTGGACCTTGTGAA	NPHP4E15R010	TCAGCACAGACAGTTCTGCCA	392
exon16	NPHP4E16F010	GACTAAGGTGCCTGGACCATC	NPHP4E16R010	GGTCACCGTATGATTCTAATG	419
exon17	NPHP4E17F010	GTAGCTATGACAGAAGCAGAA	NPHP4E17R010	ACAAGTCCTGTGCGGGATAGC	392
exon18	NPHP4E18F010	AGGGTCTTATCCTGCGCACAC	NPHP4E18R010	ATTCTCCCGGTTTCCTCCTGG	441
exon19	NPHP4E19F010	AGGCCATTTGAAAGCCACAGC	NPHP4E19R010	CACATGCACACACGCATGCAC	326
exon20	NPHP4E20F010	CCCTCCCTATATAGGTGGTCC	NPHP4E20R010	AGGTAAGAGAGAATCATGTGG	404
exon21	NPHP4E21F010	AATGTCCTCTTCTGAGATCGC	NPHP4E21R010	AGAGAAGTCAAATCGCCCCGG	444
exon22	NPHP4E22F010	TCTCTCCCACTCCTCTGAGCA	NPHP4E22R010	TGCACAGGTAAGGGAGGGACA	391
exon23	NPHP4E23F010	TCAGTGTGAGAGGAGGCTGGT	NPHP4E23R010	AAAAGGCCATTCCCAGGCCCA	346
exon24	NPHP4E24F010	GTCTTGGCAACAGTGGAGATA	NPHP4E24R010	ACCAGGGCATGAAGCCATGAG	360
exon25	NPHP4E25F010	TGACGAGCCTGTCCTGTCCTA	NPHP4E25R010	CCTAAAATGAAGAGGATCCCA	286
exon26	NPHP4E26F010	AGATGCGTTCTGGGAGGGACT	NPHP4E26R010	TTTAGGAAGGGGCCAAGCCCA	308
exon27	NPHP4E27F010	TTTCCCTGCACAGCCTCCTGT	NPHP4E27R010	AAAAGCTGCTGTCAGGGCCAC	390
exon28	NPHP4E28F010	AACCACCCATGACCTTGGGCT	NPHP4E28R010	TGTATCCAGTGGTCCGAGTCA	392
exon29	NPHP4E29F010	TCTTATCTCTGTGGGGGTCCC	NPHP4E29R010	GCTGGTGATTTGAGGAACTCG	364
exon30	NPHP4E30F010	CAGCTCCCCTTGGAAATAAAC	NPHP4E30R020	AAACTGCCAAGGGAAGACGTG	768

Primers for *NPHP4*

exon	F primer	5′-nucleotide sequence -3′	R primer	5′-nucleotide sequence -3′	Amprified fragment length (bp)
exon1	NPHP3E01F020	TGCTCCGCCCAGTCCTGCTCT	NPHP3E01R020	GAGAATATGGCCTCTCAAATT	694
exon2	NPHP3E02F010	CATGAAGTTCCTGATAATTGG	NPHP3E02R010	GAATCCTACATGACTTACTTC	387
exon3	NPHP3E03F010	GAGGACCAAATGAATATTGGT	NPHP3E03R020	GCAGCTGACAGAGAGAACACA	420
exon4	NPHP3E04F020	CAGTATCTTTGAACTTTGCCA	NPHP3E04R020	GATGGTTTGTCAATGGAAAGC	459
exon5	NPHP3E05F020	GGTATGGCAGTATTAACATGT	NPHP3E05R020	GCTTCCTGTTCTTTAAGACAT	391
exon6	NPHP3E06F020	GTATTTGAGAGAAACTTGCCT	NPHP3E06R020	GCTATATTTGCCAAACTCTGA	595
exon7	NPHP3E07F020	GTTGGACCTTTTCTGGCCACT	NPHP3E07R020	GTTCCAGCCACACTGGTTTCT	401
exon8	NPHP3E08F010	CCTAAGGTTGTTGTGAAGATA	NPHP3E08R010	TTCAAAAAGACAAGCAAGTGG	320
exon9	NPHP3E09F020	AAGGCTGTATGTTTGAACTTG	NPHP3E09R020	CACATCTCAACATGGATAATC	440
exon10	NPHP3E10F010	CAGCTTTTCTCCAGTATTTTC	NPHP3E10R020	GGGCATGAACCTATTGTTTTA	350
exon11	NPHP3E11F020	AGTAACTGACCACCTGATTGC	NPHP3E11R020	GACCCGATTGTATCGAATATT	390
exon12	NPHP3E12F020	ATATTCGATACAATCGGGTCC	NPHP3E12R020	CTGTGGGCATACGATATATTT	458
exon13	NPHP3E13F010	CAGAGTTCAGATTGGTGATAA	NPHP3E13R010	CCTCACTGCAAGTTACATAAA	406
exon14	NPHP3E14F010	GTTGTGATTCATTGCTCAAAG	NPHP3E14R010	CCTTATAACAGATCCCCTATA	410
exon15	NPHP3E15F010	TTTCTGTCGGGGTACTTGTTG	NPHP3E15R010	CAGACTGGTGTAGTGATCAGT	283
exon16	NPHP3E16F020	TGACTCTAGCAGCCCCATAAA	NPHP3E16R020	GGCTATCAGCATTCCTGCATA	435
exon17	NPHP3E17F020	GTTATCTTTGGTGTGCTAGAT	NPHP3E17R010	CTTTGGCAGAAATAATCTTGC	487
exon18	NPHP3E18F010	CATTCCACACTTCTGAGATTT	NPHP3E18R010	GAATAGGGAGAGGATTTAATC	496
exon19	NPHP3E19F020	GGTTCTGCATATCACTGAATT	NPHP3E19R020	GGAAAAGCAGATCTAATAGAG	492
exon20	NPHP3E20F010	CAGTACTCGCCTACTAATAAA	NPHP3E20R020	GCAAGATCTGTCATCTGATTA	440
exon21	NPHP3E21F020	CTCTTCTCTTTTTCCAAGATG	NPHP3E21R020	CCACATGAAGACTAGGCACAG	497
exon22	NPHP3E22F020	CTAGACTTGCTTTGTTTTGTC	NPHP3E22R020	CTTTAAAGAACTGAGGTAGCT	614
exon23	NPHP3E23F010	GTTGCCATGTGGAAATATTTG	NPHP3E23R010	CATACATGAAATTTTGCGTGG	436
exon24	NPHP3E24F010	GGAAAGTAAGATTTTGAGCTG	NPHP3E24R020	GTTCTGCTCAGTTACTTGTTA	536
exon25	NPHP3E25F020	GCTTTTCTATACAGTGTAGCT	NPHP3E25R010	CCTTCATACAAGTCTAACTTC	485
exon26	NPHP3E26F010	CCCATCTTTTAGGAGGATATT	NPHP3E26R010	CCCCACTCTAAGAAAAAACAT	341
exon27	NPHP3E27F010	AGGGGAAATGGGCAAATATTT	NPHP3E27R020	CCTTGGATACCATATAATAGG	512

Fig. 1 continued

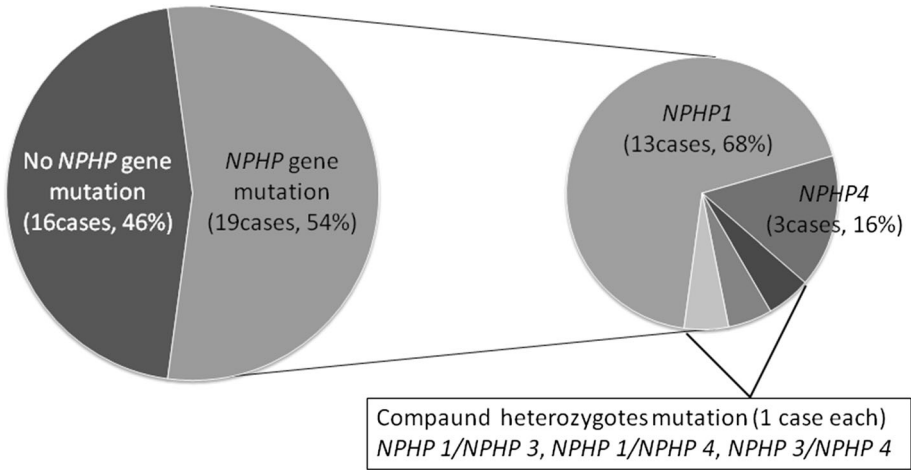

Fig. 2 Percentage of NPH patients with *NPHP* gene mutation. *NPHP* gene mutation was detected in 19 patients. No *NPHP* gene aberration detected within the sequences analyzed in the other 16 patients with suspicion of NPH clinicopathologically

transmission in renal tubular epithelial cells, such as signaling involving diacylglycerol kinase-zeta and receptor-like tyrosine kinase. Abnormalities of the protein disrupt urinary concentrating ability and the structure of cilia of renal tubules, as in the other types of NPH.

Previous reports describe occurrence of *NPHP1* mutations in approximately 30–50 % of juvenile nephronophthisis patients in Western countries [1, 6], where genetic analysis of *NPHP1* is performed initially when juvenile NPH is suspected. If mutation is detected, kidney biopsy usually is deferred [7]. Genetic diagnosis is made less frequently in Japan; so kidney biopsy often is performed to obtain a definitive diagnosis. Not infrequently, NPH is discovered in the advanced or end stage in many Japanese patients, in whom treatment no longer can slow progression. Unfortunately, symptoms typically seen in early stages are incompletely characterized.

In the present study, we investigated clinical, histologic, and genetic features in 35 Japanese patients clinically and histologically suspected to have NPH, aiming to promote early diagnosis. We studied many exons as many as 13 *NPHP* genes. Since such genetic analysis involves significant cost and time, we also screened biopsy specimens by immunohistologic methods employing antibodies against relevant peptides.

Methods

Patient registration and informed consent

Our subjects included 35 patients with clinicopathologic findings suggestive of NPH who were referred to our department from various regions of Japan. The study was performed following approval by the Ethics Committee of Kinki University Faculty of Medicine and acquisition of written informed consent from patients or their parents (Actual state of Japanese juvenile nephronophthisis patients and identification of gene aberrations; approval number 20–99).

Genomic DNA extraction, polymerase-chain reaction (PCR), and determination of *NPHP* gene sequence.

After approximately 5 mL of peripheral blood was collected from patients into tubes containing Na-EDTA, genomic DNA was extracted using NucleoSpin for Blood (TaKaRa Bio Inc, Shiga, Japan). Human genomic DNA (TaKaRa Clontech, code 636401; Shiga, Japan) was used as a control. Patient samples and control genomic DNA were diluted with sterile water to prepare 10 ng/μL solutions. PCR was performed using these as templates and TaKaRa PCR Thermal Cycler Dice Gradient (TaKaRa Bio Inc, Shiga, Japan). To determine extent of deletions and identify break points, PCR primers were prepared to amplify approximately 200–300 bp fragments based on *NPHP* gene sequences registered in GenBank (Fig. 1). For PCR, annealing temperatures and times were 63 °C and 15 s for *NPHP1* and *NPHP3*; 60 °C and 15 s for *NPHP2*; and 60 °C and 20 s for *NPHP4*, respectively. For sequence analysis, PCR products were purified by an enzyme reaction, and templates for sequencing were prepared. The sequencing reaction was carried out using the prepared template DNA and a BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, CA, USA), employing the dye terminator method. Reaction products were purified by gel filtration, and sequence analysis was performed using a capillary-type sequencer, ABI3730xl (Applied Biosystems, CA, USA). The algorithm established by Salomon et al. [8]. was adapted for use in our analytical procedure. In children with renal dysfunction

Table 1 Characteristics of patients found to have NPHP gene mutations

Age/gender	Motive of discovery	BUN/Orn (mg/dL)[a]	UP	Urinary LMP	Low gravity urine	Extrarenal symptom	Diagnosis at first biopsy	*NPHP*/mutation	The other *NPHP* mutation	Consenguineous marriage	Family history of renal disease
14 years/M	Anemia	49/3.3	(−)	(−)	(−)	n.f	n.d	Large delation (>2.0kbp)		(−)	(−)
13 years/F	Nocturnal enuresis	27/1.3	(−)	(−)	(−)	n.f	n.d	Large delation		(−)	NPH (younger brother)
11 years/M	Sibling with NPH	17/0.6	(−)	(+)	(+)	n.f	n.d	Large delation		(−)	NPH (elder sister)
15 years/F	Protein uria (school urinalysis)	21.3/1.3	(±)	(−)	(−)	n.f	TIN	(−)	D1980G (*NPHP4*, hetero)	(−)	(−)
15 years/F	Protein uria (school urinalysis)	89.6/11.6	(−)	(+)	(+)	RP	NPH	Partial delation (=300bp)		(−)	Acute glomerulonephritis (mother)
14 years/M	Chance discovery of the RD (heatstroke)	17/0.9	(±)	(+)	(+)	n.f	n.d	n.d	L939 (*NPHP4*, hetero)	(−)	(−)
11 years/F	Enuresis, polyuria	74/5.2	(1+)	(+)	(+)	SS(-2.5SD)	NPH	E677Q (hetero)	E642L (*NPHP4*, hetero)	(−)	(−)
18 years/F	Enuresis, polydipsia	82/8.1	(±)	(+)	(+)	SS(-1.8SD)	NPH	Gln547 (hetero)	S80L (*NPHP3*, hetero)	(−)	Protein uria (father)
8 years/M	Glycosuria (school urinalysis)	159/11.1	(2+)	(+)	(+)	n.f	Similar NPH	E677Q (hetero)	*NPHP4*(−)	(−)	(−)
14 years/M	Glycosuria (school urinalysis	48/5.0	(±)	(+)	(+)	RP	NPH	Large delation		(−)	NPH (younger sister)
13 years/F	Sibling with NPH	58/2.7	(1+)	(+)	(+)	RP	NPH	Large delation		(−)	NPH (elder brother)
20 years/F	Chance discovery of the RD (medical examination)	44.6/2.4	(1+)	(+)	(+)	n.f	n.d	(−)	L939Q (*NPHP4*, homo)	(−)	(−)
8 years/F	Chance discovery of the RD	32.2/1.4	(−)	(+)	(+)	n.f	NPH	Large delation		(−)	(−)
15 years/F	Protein uria (school urinalysis)	37/2.6	(−)	(+)	(+)	n.f	Tubular enlargement medullary cysts	Large delation		(−)	(−)
7 years/F	Chance discovery of the RD (urine tract infection)	40/3.0	(1+)	(+)	(+)	Joubert syndrome	n.d	(−)	AA/OO→AG/OT (exon 26/exon 20)	(−)	(−)
19 years/F	Chance discovery of the RD (bronchitis)	90.3/8.4	(1+)	(+)	(+)	n.f	n.d	(−)	A150V (*NPHP3*, hetero)	(−)	(−)

Table 1 continued

Age/gender	Motive of discovery	BUN/Orn (mg/dL)[a]	UP	Urinary LMP	Low gravity urine	Extrarenal symptom	Diagnosis at first biopsy	*NPHP*/mutation	The other *NPHP* mutation	Consenguineous marriage	Family history of renal disease
8 years/M	Visual impairment	46.5/1.9	(−)	(+)	(+)	RP	NPH	Large delation	D1980G (*NPHP4*, hetero)	(−)	(−)
16 years/F	Protein uria (school urinalysis)	43.3/2.6	(1+)	(+)	(+)	n.f	NPH	Large delation		(−)	(−)
13 years/F	Anemia, fatigue	39/2.2	(−)	(+)	(−)	n.f	TIN, tubular enlargement	Large delation		(−)	(−)

RD renal dysfunction, *n.f.* not found, *n.d* not done, *UP* urinary protein, *LMP* low molecule protein, *SS* short stature, *RP* retinitis pigmentosa, *TIN* tubulo interstitial nephritis

[a] Renal function at the time of the discovery

who were 5 years old or younger, the gene responsible for infantile NPHP (*NPHP2)* was analyzed first. In patients older than 5 years, *NPHP1* was analyzed first; if no mutation was detected, *NPHP4* was examined. *NPHP3* analysis was added when no mutation was detected in other genes in patients whose disease progressed to end-stage renal disease at an age of 16 years or older.

Clinical data

Data originally collected at our department as well as data provided by other institutions were surveyed using a questionnaire. Questionnaire consists of personal data including the patient's age, motive of discovery, urinary abnormality and renal dysfunction, detailed clinical data, extrarenal symptom, renal tissue diagnosis at the first biopsy, consanguineous marriage, and family history of renal disease.

Results

NPHP gene analysis

Among 35 patients, an *NPHP* gene mutation was identified in 19 patients. Although NPH was suspected clinicopathologically in the other 16 patients, no *NPHP* gene aberration was detected within the sequences analyzed (Fig. 2). Characteristics of patients with *NPHP1* gene mutations (Table 1) and without *NPHP* gene mutations (Table 2) were shown. A mutation was detected only in *NPHP1* in 13 patients; deletion was extensive in 10 (Fig. 3a) and partial in 1. Two other patients had a point mutation (E677Q and K334 N, both heterozygous). In all, these mutations accounted for 37.1 % (13/35) of patients. In another candidate gene responsible for the juvenile type, *NPHP4*, the mutation L939* was detected in 2 patients (Fig. 3b), while a D1980G mutation was detected in 1, accounting for 8.6 % (3/35) of all patients. Compound heterozygotes containing 1 mutation each in *NPHP1* G547* and *NPHP3* S80L (Fig. 4a), 1 mutation each in *NPHP1* E677Q and *NPHP4* E642L (Fig. 4b), and 1 mutation each in *NPHP3* A150 V and *NPHP4* D1089G (Fig. 4c) also were observed. The disease progressed to ESRD before 20 years of age in these patients, similar to the course of other patients with a single-gene mutation. No *NPHP2* mutation was detected in any patient.

Clinical and demographic features of patients

Patient background

Patients were reported from 46 prefectures without evident selection bias, and with no important regional

Table 2 Characteristics of patients without apparent NPHP gene mutations

Age/gender	Motive of discovery	BUN/Orn (mg/dL)[a]	UP	Urinary LMP	Low gravity urine	Extrarenal symptom	Diagnosis at first biopsy	NPHP1 mutation	NPHP3 mutation	NPHP4 mutation	Consanguineous marriage	Family history of renal disease
6 years/M	Lagging physical development	34/1.2	(−)	(−)	(−)	SS (−1.3SD)	NPH	(−)	n.d	(−)	(−)	(−)
12 years/M	SS, fatigue	48/1.3	(−)	(+)	(−)	Sensory deafness	Chronic interstitial nephritis, glomerulosclerosis	(−)	n.d	(−)	(−)	(−)
26 years/M	Chance discovery of the RD (medical examination)	21/1.6	(−)	(+)	(−)	n.f	Interstitial nephritis	(−)	(−)	(−)	(−)	Renal dysfunction (father, young sister)
11 years/M	Pallor, anemia	27.4/1.5	(−)	(+)	(+)	SS (−2.2SD)	n.d	(−)	n.d	(−)	(−)	(−)
17 years/M	SS	34/1.5	(±)	(+)	(+)	SS (−3.8SD)	n.d	(−)	n.d	(−)	(−)	(−)
22 years/M	Hypertention	32.5/1.5	(1+)	(+)	(−)	RP	TIN	(−)	(−)	(−)	(−)	(−)
11 years/F	polydipsia, polyuria	15.4/0.7	(−)	(+)	(+)	n.f	n.d	(−)	n.d	(−)	(−)	(−)
12 years/F	Chance discovery of the RD (protein uria at 3 years old)	32/1.6	(2+)	(+)	(+)	n.f	TIN, tubular enlargement	(−)	(−)	(−)	(−)	(−)
14 years/M	Protein uria (school urinalysis)	58/4.2	(1+)	(+)	(+)	n.f	TIN, tubular enlargement	(−)	(−)	(−)	(−)	(−)
26 years/M	Hypertention (medical examination)	38.7/1.5	(−)	(+)	(+)	n.f	TIN, glomerulosclerosis	(−)	(−)	(−)	(−)	(−)
10 years/M	Pallor, anemia	53.6/1.0	(1+)	(+)	(+)	n.f	NPH	(−)	n.d	(−)	(−)	(−)
28 years/F	Chance discovery of the RD anemia	47.5/2.9	(1+)	n.d	(+)	n.f	TIN, similar NPH	(−)	(−)	(−)	(−)	(−)
11 years/F	Pallor, polydipsia, polyuria	27.4/1.5	(−)	(+)	(+)	SS(-2.2SD)	n.d	(−)	n.d	(−)	(−)	(−)
18 years/M	Crud, fatigability	49.6/4	(±)	(+)	(+)	Specific complexion	Similar NPH	(−)	(−)	(−)	(−)	NPH (young sister)
16 years/F	Sibling with NPH	38.3/1.8	(−)	(+)	(−)	Specific complexion	Similar NPH	(−)	(−)	(−)	(−)	NPH (elder brother)
46 years/F	Protein uria, hematuria (at 30 years old)	33/1.8	(1+)	(+)	(−)	Sensory deafness	Chronic interstitial nephritis, glomerulosclerosis	(−)	(−)	(−)	(−)	NPH (elder brother)

RD renal dysfunction, *n.f* not found, *n.d* not done, *UP* urinary protein, *LMP* low molecule protein, *SS* short stature, *RP* retinitis pigmentosa, *TIN* tubular interstitial nephritis

[a] Renal function at the time of the discovery

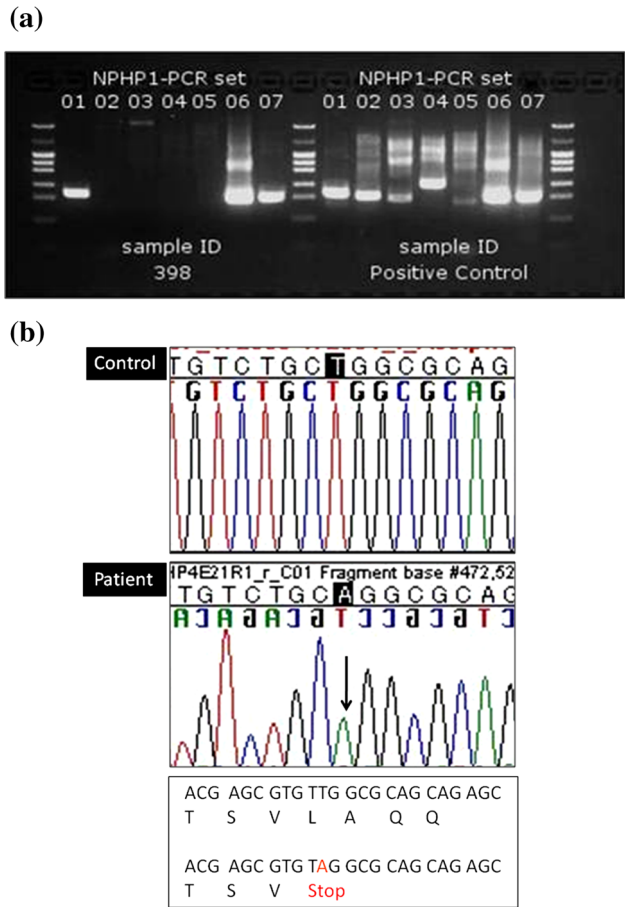

Fig. 3 Analysis of deletion in *NPHP1* (**a**) and analysis of *NPHP4* (**b**). In **a** *lane 1* and *lanes 6 and 7*, contain PCR products of regions within and outside *NPHP1*, respectively; *Lane 2* contains PCR products from the junction between *NPHP1* and the adjacent *MALL* gene. *Lanes 3–5* show the PCR products of *NPHP1* obtained with primers amplifying fragments of approximately 300 bp. *NPHP1* was nearly completely deleted (1.2 kbp deletion). In **b**, substitution of TAG for TTG formed a stop codon, prematurely terminating peptide synthesis

differences (Fig. 5). The male:female ratio was 16:19, with evident gender difference. Ages of patients ranged from 2 to 38 years (median; 12.5). Familial occurrence was noted in 3 families. Other occurrences were solitary, with no family member showing a urinary abnormality, a diagnosis of NPH, or any renal dysfunction of unknown cause.

Initial abnormality deletion

NPH sometimes was discovered following an abnormal urinary finding by mass screening, such as proteinuria detected in a urine test at school (18 %), or renal dysfunction discovered incidentally in working up other medical symptoms, or during medical check-ups (23 %). Approximately 20 % of cases were discovered because of urinary tract symptoms such as polyuria with or without polydipsia, enuresis (often nocturnal), or mellituria. Some 38 % were discovered because of either extrarenal manifestations such as lagging physical development, dwarfism, anemia, pallor, hypertension, or visual disturbance arising from pigmentary retinal degeneration; a prior diagnosis of NPH in a sibling; or both (Fig. 6).

Urinary findings

Urine specific gravity frequently was low (not greater than 1.010); approximately 75 % of cases. Low molecular weight proteinuria, such as β2-microgloburinuria, also was common (85 %), even though inclusion of renal function shown such as between blood urea nitrogen and serum creatinine was relatively mild at that time.

Renal histologic findings

Renal biopsy was performed in 25 patients (71 %). These included 13 patients demonstrated to have an *NPHP* gene mutation and in 12 with no *NPHP* gene mutation identified (suspected cases). Histologic findings included suspected NPH; interstitial nephritis, renal tubular dilation, and glomerulosclerosis. Cystic dilation of renal tubules and irregular contours of tubular basement membranes were observed in most patients, mainly in the renal medulla (Fig. 7a). Sclerotic glomeruli, inflammatory cell infiltration in the renal tubules and interstitium, and fibrosis were frequent, although not seen in all patients (Fig. 7b).

Discussion

Renal tubular epithelial cells are attached to the basement membrane through integrin cross-linking, which transmits extracellular signals to the cell nucleus [2]. Nephrocystin acts importantly in signal transmission between tubular epithelial cells and between these epithelial cells and the extracellular matrix functioning as a docking protein. Nephrocystin also is involved in cell adhesion, together with *N*-cadherin, catenin, and β-catenin [2, 8]. Furthermore, nephrocystin influences actin cytoskeleton structure together with β-tubulin, contributing to maintenance of the cytoskeleton and determination of cell polarity. Nephrocystin forms a complex with Crk-associated substrate, which promotes phosphorylation of Pyk2 and transmits intracellular information through a Pyk2-dependent pathway [2]. Furthermore, nephrocystin is present on primary cilia, where it functions in cooperation with α-tubulin; nephrocystin also is involved in signal transmission in organelles [9]. Accordingly, abnormalities in the nephrocystin molecule disrupt signal transmission between cells

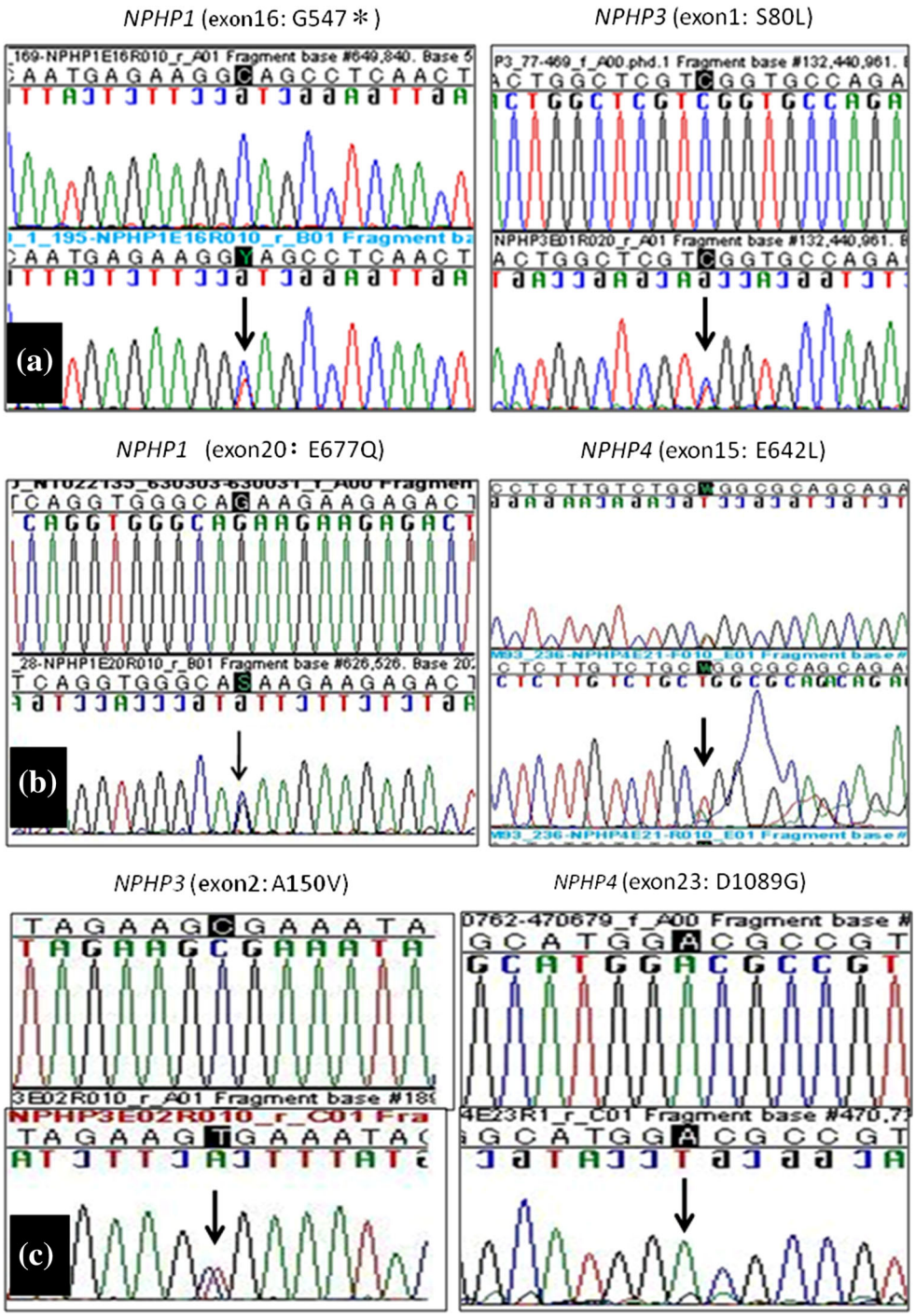

Fig. 4 Compound heterozygotes with heterozygous mutations in different *NPHP* genes. In **a**, a compound heterozygote has one heterozygous mutation involving each of *NPHP1* (G547*) and *NPHP3* (S80L). In **b**, a compound heterozygote has one heterozygous mutation involving each of *NPHP1* (E677Q) and *NPHP4* (E642L). In **c**, a compound heterozygote has one heterozygous mutation involving each of *NPHP3* (A150 V) and *NPHP4* (D1089G)

and the extracellular matrix, intercellular adhesion, cytoskeletal integrity, cell polarity, primary cilia function, and intracellular signal transmission to the nucleus. Structural and functional disorders involving the renal tubular epithelium result.

An *NPHP* gene mutation was detected in about 54 % of all patients, but no mutation was noted within the sequences analyzed in the other 46 %. However, nephronophthisis was suspected clinically and histologically, suggesting possible mutation in some other *NPHP* gene. An *NPHP1* mutation was most frequent among our Japanese patients, most often representing a large deletion rather than a point mutation. Frequency of an *NPHP1* mutation was similar to that reported in Western populations [10].

On the other hand, mutation in the gene responsible for the infantile type, *NPHP2*, a patient in a compound heterozygous stable with another abnormal *NPHP* gene such as that responsible for NPH3 recently has been

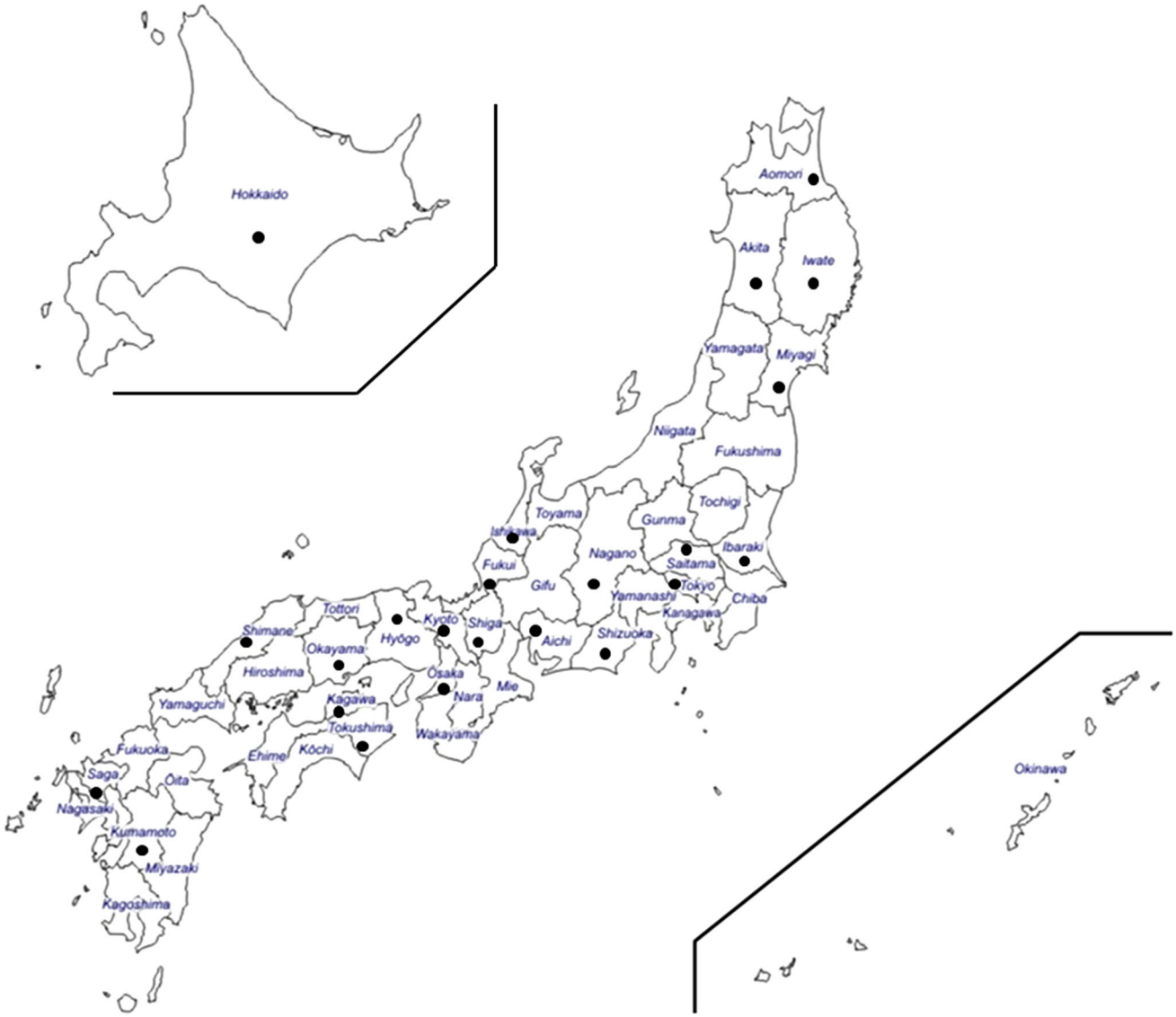

Fig. 5 Demographic features of patients in Japan. Regional distribution of study subjects within Japan, show as a *dot* for each patient

reported [11, 12]. We also found compound heterozygosity across multiple *NPHP* genes in some of our Japanese nephronophthisis patients. In *NPHP4*, L939* (IVS-20T > A) was detected in two geographically distant patients who were not consanguineous. The mutation formed a stop codon by substituting TAG for TTG in exon 21, terminating peptide synthesis. This might prove to be a 'hot spot' among Japanese patients.

We detected in three patients with two mutations in either *NPHP1*, *NPHP3*, or *NPHP4* in this study. As similar to the results of the other studies [13, 14], the age of the initial discovery of this disease and the course of progression to end-stage renal disease were not significantly different from those of the patients having mutation in single *NPHP* gene. An analysis of patient backgrounds revealed that NPH was distributed fairly evenly Japan, including the suspected cases where no causative mutation was identified. Heterozygotes carrying NPHP gene mutations also were rather evenly distributed nationwide. No gender difference was evident from our analysis. Although the median age at time of disease discovery was 12.5 years, individual presentation ranged from infantry to adulthood.

Frequency of disease discovery in mass screening programs, such as school urine tests, was low, as previously reported [15]. Incidental discovery of renal dysfunction during diagnostic workup of possibly unrelated symptoms, or during routine check-ups, accounted for less than 50 % of cases. Often symptoms that led to the discovery of NPH represented extrarenal manifestations such as incomplete physical development reported previously [15]. In particular, currently used urine test strips, intended mainly to detect albuminuria, are insensitive to this disease.

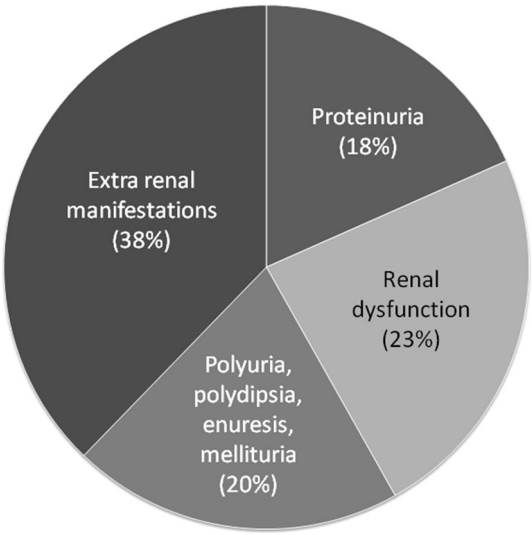

Fig. 6 Clinical suspicion and motivation to discover for NPH. Proteinuria is detected in a urine test at school (18 %), renal dysfunction discovered incidentally (23 %), urinary tract symptoms such as polyuria with or without polydipsia, enuresis, or mellituria (approximately 20 %). Some 38 % were discovered because of either extrarenal manifestations such as lagging physical development, dwarfism, anemia, pallor, hypertension, or visual disturbance arising from pigmentary retinal degeneration

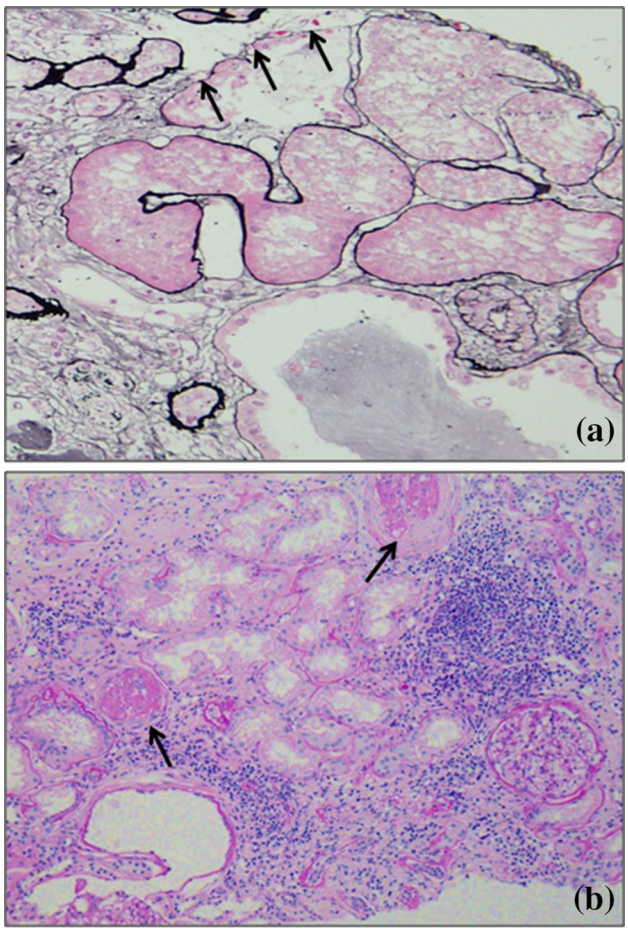

Fig. 7 Pathologic findings in the kidney in nephronophthisis patients. In **a** irregularity (*arrow*) of the renal tubular basement membrane was evident (methenamine silver stain, ×200). In **b**, Inflammatory cell infiltration involved the renal tubular interstitium, and sclerotic glomeruli (*arrow*) were present (periodic acid-Schiff stain, ×100)

Development in siblings was noted in three families, suggesting autosomal recessive inheritance. However, many cases appear to be sporadic. Familial genetic analysis centering on patients, parents is needed.

In contract to albuminuria, urinary findings such as low specific gravity and low-molecular-weight proteinuria are relatively helpful in early discovery. According to the results of this study, we suggest that the findings of the low-molecular weight proteinuria and hypotonic urine reflecting renal tubular disorder coupled with the histologic abnormalities involving cystic dilation of renal tubules and the irregularity of tubular basement membrane could be a convincing diagnostic criterion of this disease. Extrarenal manifestations, such as short stature, delayed physical development, and anemia also were frequent. Unfortunately, these tended to coincide with were progression of renal dysfunction rather than early NPH. Nonetheless, NPH needs to be considered in children with such presentations. Some patients have been reported to show somewhat distinctive extrarenal manifestations [13] such as pigmentary retinal degeneration (Senior-Loken syndrome), ocular dysmetria (Cogan's syndrome), cerebral ataxia, hepatic fibrosis, and skeletal and facial abnormalities [13, 16, 17]. Even the most frequent of these extrarenal manifestations, pigmentary retinal degeneration, was present only in some patients and not in others, even among children showing the same *NPHP1* deletion. Similar lesions also have been reported in Jeune, Joubert, oro-facial-digital (OFD1), and Meckel syndromes [13, 18, 19]. *NPHP1* mRNA is expressed predominantly in a wide range of extrarenal tissues including pituitary gland, spine, testis, lymph nodes, and thyroid [14]. Expression also is high in the central nervous system, which could account for associated cerebellar ataxia. However, associated symptoms may develop in organs with low *NPHP1* expression, such as hepatic fibrosis. The role of nephrocystin in extrarenal manifestations remains poorly understood. The 11 kb interval between the 3′ end of *NPHP1* and an inverted repeat containing the distal deletion breakpoint was found to contain the first exon of a second gene, *MALL* [20]. Although the detail of the *MALL* gene function has not been clarified, recent report suggested the involvement of the age-related macular degeneration (AMD) [21]. Interestingly, associations have also been reported between AMD and chronic kidney disease [22]. Since pigmentary retinal degeneration is the most common extrarenal

manifestation of NPH, similar to AMD, *MALL* gene may involve the pathogenesis of this eye disorder found in NPH patients as the contiguous gene syndrome.

No truly effective treatment currently is available for NPH. Dietary therapy and administration of ion exchange resins and bicarbonate are carried out to manage hyponatremia, hyperkalemia, or metabolic acidosis. Studies possibly relevant to drug therapy have been conducted in various animals, even protozoa [23, 24]. Previous studies reported that renal cyst expression was inhibited by stimulating the G-protein-coupled calcium sensing receptor and elevating Ca^{2+} and cAMP in the renal tubular epithelial cells of pcy mice. Morphology and function of cilia in zebrafish with ciliopathy may be improved by the administration of rapamycin and rescovitine [25, 26]; however, applicability to human NPHP is unknown. Living-donor kidney transplantation was found to have favorable outcome in many reports including the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) [27].

Acknowledgments This study was performed after approval by the Ethics Committee of Kinki University Faculty of Medicine. Written informed consent was obtained from the patient's guardian for genetic examination. We thank Ai Itoh for technical support in tissue staining and manuscript preparation.

Compliance with ethical standards

Conflicts of interest This study was partly supported by a Grant-in-Aid for Scientific Research from Morinaga Hoshikai to Tsukasa Takemura (2013–2014) and from Ministry of Health, Labour and Welfare Japan (grant number: 26070201, Representative investigator: Kazumoto Iijima, Pediatrics, Kobe University School of Medicine). The authors declare that they have no competing interests involving this work.

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