Gene Mutation Analysis in Iranian Children With Nephronophthisis

A Two-Center Study

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Keywords. familial juvenile nephronophthisis, mutation, chronic kidney disease, child

Introduction. Nephronophthisis is of the most commonly inherited ciliopathies that leads to end-stage renal disease in children. The *NPHP1* gene is the first identified gene responsible for nephronophthisis and related diseases. This study assessed mutations of the *NPHP1* gene in 16 Iranian families with at least one member presenting features of nephronophthisis.

Materials and Methods. Fifty-seven patients diagnosed with chronic kidney disease or end-stage renal disease were referred to Imam Hossein Children Hospital, in Isfahan, Iran. The gene analysis study was carried on 16 patients and their first-degree relatives (40 DNA samples) suspicious of having nephronophthisis. The *NPHP1* deletion analysis was performed for exons 5, 7, and 20 of the *NPHP1* gene.

Results. The patients' median age was 15 years. The mean and median age of the first presentation was 10.06 ± 2.59 years and 10.5 years, respectively. A homozygous deletion was identified in the *NPHP1* gene spanning at least from exon 5 to exon 20 in two families. High-throughput mutation analysis identified a homozygous truncating mutation (c.1504C > T, p.R502*) in the *NPHP5* in 5 families.

Conclusions. By combining *NPHP1* deletion analysis with multiplex-polymerase-chain-reaction-based high-throughput mutation analysis we could identify the molecular disease-cause in 7 of 15 families from Iran. In 8 families, the molecular disease cause remained unknown.

IJKD 2015;9:119-25
www.ijkd.org

Original Paper

INTRODUCTION

Nephronophthisis is the most inherited ciliopathy, manifested as a cystic kidney disease that leads to end-stage renal disease (ESRD) in the early 2 decades of life.[1-4] Three distinctive clinical presentations of nephronophthisis have been reported by age of onset of ESRD: infantile, juvenile, and adolescent.[5-8] Extrarenal manifestations have been reported in more than 10% of the patients, including cerebellar vermis aplasia (Joubert syndrome), retinal degeneration (Senior-Loken syndrome), cone-shaped epiphyses, and liver fibrosis.[9-13] Homozygous single-gene mutations or deletions or compound heterozygous mutations may lead to different clinical presentations of nephronophthisis and related ciliopathies.[14] The *NPHP1* gene is the first identified gene among at least 12 genes responsible for nephronophthisis and

related diseases. The gene has been detected by positional cloning.[15] *NPHP1* homozygous deletion has been identified in nephronophthisis and related syndromes such as Senior-Loken syndrome.

Limited studies have been reported cases of Senior-Loken syndrome and Joubert syndrome in Iranian families. However, there is no study on children and adolescents with chronic kidney disease (CKD) in Iran to evaluate *NPHP1* mutations. In this study, we assessed the mutations of *NPHP1* gene as the most frequent gene responsible for nephronophthisis in 16 families with at least 1 member presenting features of nephronophthisis. Since most patients from Qeshm Island had blindness and almost all of them had a history of consanguinity between parents, a genetic mutation was suspected. Therefore, we compared the genetic results between the two groups.

MATERIALS AND METHODS

Participants

From December 2011 to January 2013, a total of 57 patients diagnosed as either CKD or ESRD were referred to Imam Hossein Children Hospital, Isfahan, Iran. Among them, we identified 16 patients suspicious of having nephronophthisis. The gene analysis study was carried on 16 patients and their first-degree relatives (40 DNA samples). Nine families were native people of the Qeshm Island in the Persian Gulf and 7 families were native to Isfahan province in the central part of Iran. All participants were examined for any possible ophthalmic abnormalities by an expert ophthalmologist. Possible diagnosis of nephronophthisis was based upon meeting the following criteria[14,16,17]: (1) median age of onset of CKD or ESRD within the 1st or 2nd decade of life; (2) polyuria and polydipsia (and salt wasting) in early childhood; (3) urinary concentration defect (less than 400 mOsm/kg for fasting urine); (4) growth retardation (secondary to salt wasting, dehydration, and renal insufficiency); (5) absence of (or minimal) hematuria and proteinuria; (6) renal ultrasonography findings including renal cortical hyperechogenicity, loss of corticomedullary differentiation, and corticomedullary cysts; (7) renal biopsy before reaching ESRD showing interstitial nephritis, tubular atrophy, and tubular dilatations; and (8) presence of extra-renal disorder mentioned in the introduction.

Sampling

DNA samples were extracted from whole blood. Genomic DNA was extracted from peripheral ethylenediaminetetraacetic acid-treated samples using Qiagen DNA Mini kit (cat No, 51304; Qiagen, Dusseldorf, Germany), and then was subjected to the polymerase chain reaction (PCR) amplification.

NPHP1 Deletion Analysis

NPHP1 deletion analysis was performed using specific primers for exon 5, 7, and 20 of the *NPHP1* gene. Exon 4 and 6 of the *LHX9* gene served as controls. These 5 primer pairs were used in a multiplex PCR setting with a final concentration of 10 pMol. Exonic DNA was amplified from purified genomic DNA using a 2-step PCR protocol with an initial annealing temperature of 72°C for 24 cycles and a decreased annealing temperature of 55°C for the second part of 24 cycles. The PCR products were analyzed on a 1.5% agarose gel. Two control samples with either known presence or absence of an *NPHP1* deletion were included as reference.

High-throughput Mutation Analysis

Using the Fluidigm 48.48-Access Arrays system (South San Francisco, CA, USA), the patient samples were screened for mutations in 15 known *NPHP* genes, as previously described by Halbritter and colleagues, namely *NPHP1*, *INVS*, *NPHP3*, *NPHP4*, *IQCB1*, *CEP290*, *GLIS2*, *RPGRIP1L*, *NEK8*, *SDCCAG8*, *TMEM67*, *TTC21B*, *WDR19*, *ANKS6*, and *IFT172*.[18] With the use of barcoded multiplex PCR, this system allows the simultaneous screening of about 600 amplicons in 48 patients in each run. After amplification, samples were pooled and sequenced on an Ilumina next-generation sequencing instrument (MiSeq, San Diego, CA, USA). Subsequently, sequence reads were aligned to normal reference sequence using CLC Genomics Workbench (CLC-bio, Aarhus, Denmark).[18] Synonymous variants and single nucleotide polymorphisms with a minor allele frequency above 1% in a heathy control population (NHLBI Exome Sequencing Project-Exome Variant Server) were excluded. The remaining variants were validated for their likelihood to explain the disease phenotype considering the severity of the allele (protein-truncating, or splice versus missense), web-based prediction score systems (PolyPhen-2, SIFT, Mutation Taster), and previous

descriptions in the human gene mutational database (Biobase HGMD, Qiagen, Dusseldorf, Germany). All variants were confirmed by Sanger sequencing. With regards to the autosomal-recessive mode of inheritance of nephronophthisis, variants were only considered as disease-causing when they occurred in biallelic state.

RESULTS

Among the patients, 7 were from Isfahan and 8 were from the Qeshm Island. There was a female preponderance, with female-male ratio of 1.6:1 (6 males and 10 females). The patients' median age was 15 years. The mean age of patients was 15.81 ± 2.85 years. The mean and median age of the first presentation was 10.06 ± 2.59 years and 10.5 years, respectively. For the patients from Isfahan and Qeshm Island, the median age of the first presentation was not significantly different (median age of 11 years and 10 years, respectively). The mean values of height and weight were 139.18 ± 24.99 cm and 40.37 ± 17.61 kg, respectively. The mean values of systolic and diastolic blood pressure were 124.06 ± 19.08 mm Hg and 87.81 ± 11.87 mm Hg, respectively. Approximately, 62.5% (10 patients) and 75% (12 patients) had systolic and diastolic hypertension, respectively. Demographic data of the participants is demonstrated in Table 1.

In 4 families, at least 1 other member was affected. Seven patients (43.8%) were diagnosed with symptoms of acute on chronic kidney failure. Among the remaining patients, 6 had been evaluated because of poor weight gain or polyuria and polydipsia. Three patients had been diagnosed during routine school examination. However, most of the patients had polyuria and polydipsia in their past history. Only 1 patient had edema while demonstrating symptoms of acute on chronic kidney failure. None of the patients had mental retardation or liver fibrosis. Five of 17 patients (30%) had no ophthalmic involvement. Ocular findings are given in Table 2. Significant proteinuria and microscopic hematuria were not reported. Laboratory parameters of the participants are tabulated in Table 3. Ultrasonographic imaging showed kidney hyperechogenicity in almost all the patients. However, small-sized kidneys were found in 15 patients (88%). Corticomedullary cysts were reported in 7 patients (41%). Histopathologic findings were evaluated in 6 patients who had been referred to the hospital before developing ESRD (Table 4).

NPHP1 deletion analysis identified a homozygous deletion in the *NPHP1* gene spanning at least from

Table 1. Demographic Data of the 16 Participants

Parameter	Mean Value
Height, cm	139.19 ± 24.99
Weight, kg	40.38 ± 17.62
Systolic blood pressure, mm Hg	124.06 ± 19.08
Diastolic blood pressure, mm Hg	87.81 ± 11.69
Age, y	15.81 ± 2.86

Table 3. Laboratory Findings of the Participants

Laboratory Parameter	Mean (Range)
Blood	
Creatinine, mg/dL	3.20 ± 2.37 (1.3 to 6.7)
Albumin, mg/dL	4.01 ± 0.29 (3.6 to 4.5)
Hemoglobin mg/dL	8.58 ± 1.46 (6.5 to 11.0)
Calcium mg/dL	8.56 ± 0.46 (7.8 to 9.5)
Phosphorus mg/dL	4.96 ± 1.42 (2.5 to 8.0)
Bicarbonate mEq/L	15.06 ± 1.56 (13.0 to 19.0)
Aspartate aminotransferase, mg/dL	21.50 ± 6.58 (10.0 to 33.0)
Alanine aminotransferase, mg/dL	23.75 ± 8.20 (11.0 to 40.0)
Glomerular filtration rate mL/min/1.73 m^2	40.06 ± 25.52 (15.0 to 80.0)
Urine	
24-h urine protein, mg/L	138.31 ± 75.33 (45.0 to 350.0)
Fasting urine specific gravity	230.00 ± 74.38 (120.0 to 360.0)

Table 2. Ophthalmic Findings of the Participants

Ocular Finding	Frequency (%) in Qeshm	Frequency (%) in Isfahan	Total Percentage
Optic atrophy	6 (37.5)	5 (31.2)	68.8
Narrowed retinal vessels	9 (56.2)	7 (43.8)	100
Pigmented spot	9 (56.2)	5 (31.2)	87.5
Choroidal atrophy	7 (43.7)	5 (31.2)	75.0
Generalized depigmentation	6 (37.5)	4 (25.0)	62.5
Decreased visual acuity	2 (12.5)	3 (18.8)	31.2
Blindness	7 (43.7)	0	43.7
Refractory error	0	3 (18.8)	18.7

Table 4. Clinical and genetic characteristics of the participants.

Participant	Family	Sex	Age, y	Age at onset, y	Initial Presentation	Ophthalmic Signs	Modality	Cysts on Ultrasonography	Histopathology†	*NPHP1* Homozygous Deletion
A	A5225	Female	12	4	AOC, PP	RP	HD	Yes	ND	No
B	A5226	Female	15	12	AOC	RP, RD	HD	No	ND	No
C	A5227	Male	20	10	PP, DP, GD	RD	KT	Yes	ND	No
D1	A5228-111	Female	15	8	PP	RP	MRRT	Yes	ND	No
D2	A5228-112	Female	22	7	AOC	RP	KT	Yes	ND	No
E	A5229	Female	14	11	PP, GR	RD	HD	No	ND	No
F	A5230	Male	16	12	AOC	RP, RD	HD	Yes	ND	No
G	A5231	Female	20	10	AOC	RD	KT	No	ND	No
006	A5232	Female	16	11	PP, GD	RP	KT	Yes	Yes	No
007	A5233	Female	17	12	PP, DP, GD	RFD	MRRT	Yes	Yes	No
008	A5234	Male	12	7	PP	RFD	MRRT	Yes	Yes	Yes
009	A5235	Male	15	14	AOC	RFD	MRRT	Yes	Yes	No
011	A5236	Male	14	10	PP, DP	RP, RD	MRRT	Yes	Yes	No
012	A5237	Female	17	14	AOC	RFD	KT	Yes	ND	Yes
013	A5238	Male	12	11	AOC	RP	HD	No	ND	No
014	A5239	Female	15	6	PP, GD	RFD	PD	Yes	Yes	No

*A to G are families from Qeshm and 006 to 014 are families from Isfahan province.
AOC indicates acute on chronic renal failure; PP, polyuria and polydipsia; RP, retinitis pigmentosa; HD, hemodialysis; ND, not done; RD, retinal dystrophy; DP, delayed puberty, GD, growth delay; KT, kidney transplantation; MRRT, medical renal replacement therapy; GR, growth retardation; RFD, refractory disturbances; and PD, peritoneal dialysis.
†Histopathological triad included tubular basement membrane thickening and disruption, interstitial infiltration and fibrosis, and tubular atrophy and dilatation with or without cyst formation.

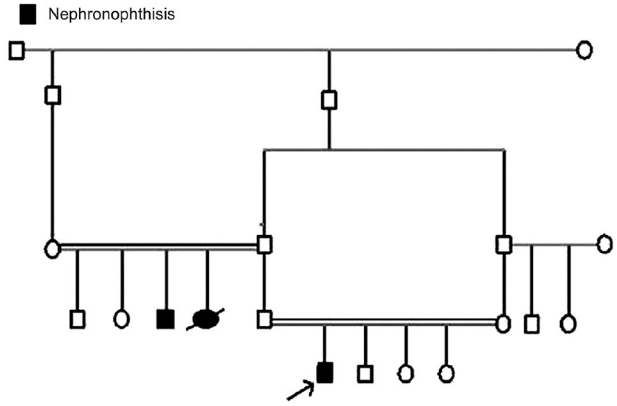

Figure 1. Pedigree of patient number 008.

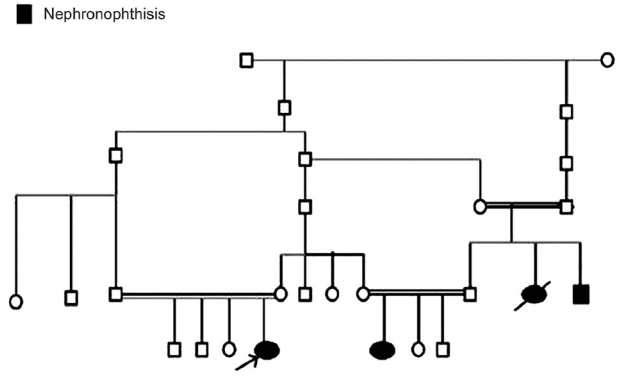

Figure 2. Pedigree of patient number 012.

exon 5 to exon 20 (Table 4; families 008 and 012). Pedigree of both patients is shown in Figures 1 and 2. High-throughput mutation analysis identified a homozygous truncating mutation (c.1504C > T, p.R502*) in *NPHP5* (IQCB1) in 5 families (B, C, D, F, and G). Interestingly, the exact same allele occurred in 2 additional families (A and E) in heterozygous state. Since a second allele could not be identified in these two families, the molecular disease cause remained unsolved.

DISCUSSION

In this study, we evaluated the incidence of *NPHP1* gene mutations in children with CKD who met the criteria of nephronophthisis from two different regions of Iran. To the best of our knowledge, it is the first study in Iran that evaluated *NPHP1* mutations in this group of patients. Nephronophthisis is an uncommon but worldwide disease. The incidence of diseases has been reported differently from 1.12 per million populations in the United States to 20 per million populations in Canada.[4,19,20] Unfortunately, the

incidence of the disease has not been determined in Iran.

Nephronophthisis-related ciliopathies include heterogeneous diseases that have similar phenotypes irrespective of various gene mutations. The phenotypes include renal cyst, retinal degeneration, and cerebellar agenesis.[21] Up to now, mutations in 8 genes (*NPHP1* to *NPHP8*) have been identified.[22] Nephrocystin that is encoded by *NPHP1* has interrelation with components of cell-cell and cell-matrix signaling comprising p130Cas, tensin, and filamin.[23-25] Recently, the ciliary theory has been proposed. This theory explains defects in signaling mechanism involving 2 pathways; the non-canonical Wnt and sonic hedgehog signaling pathways, which lead to failure in cell polarity and cell preservation. In addition, this theory describes multiple organ involvement in nephronophthisis.[26] Mutation in *NPHP1* gene is the most prevalent mutation causes for juvenile type of nephronophthisis, in which kidney failure symptoms are the dominant feature.[27] About 10% of patients show extrarenal manifestations including eye and cerebellar involvement.[28] However, mutation in *NPHP5* gene has been defined as the gene responsible for Senior-Loken syndrome, in which retinal degeneration and blindness occur even before the symptoms of kidney failure.[29]

Although nephronophthisis has been reported at the median age of 1 year (infantile form), most patients present the features of ESRD at a median age of 13 to 19 years (juvenile and adolescent forms).[5-8,26] The median age of our patients at the time of the diagnosis was 10.5 years. Most patients from Qeshm met the criteria of Senior-Loken syndrome. All of them had either blindness or retinitis pigmentosa and retinal dystrophy. Although juvenile nephronophthisis equally affects both sexes, we observed a female predominance.[4]

Hypertension is not a common feature of nephronophthisis.[30] We found either systolic or diastolic hypertension in more than half of our patients. Hypertension was commonly detected in the patients who underwent dialysis. However, most of these patients did not have the symptoms of fluid overload.

Considering gene deletion, Otto and colleagues carried out a worldwide cohort study on patients who met the criteria of nephronophthisis. According to their study, the most frequent mutations in nephronophthisis patients were homozygous *NPHP1* deletion followed by *NPHP5* deletion.[22] Since 20% to 40% of nephronophthisis patients have *NPHP1* mutation, it has been recommended to evaluate suspicious patients firstly for homozygous or heterozygous *NPHP1* deletion.[30] The patients with ophthalmic involvement but not having *NPHP1* deletion are candidates for analysis for *NPHP5* gene mutation.[30]

We assessed all patients suspicious for *NPHP1* mutations. Nevertheless, we did not find *NPHP1* deletion in any patients from Qeshm. By using high-throughput mutation analysis a homozygous truncating mutation (c.1504C > T, p.R502*) in *NPHP5* (*IQCB1*) in 5 families (B, C, D, F, and G) were identified. Furthermore, in 2 additional families (A and E) the exact same allele occurred in heterozygous state. Since a second allele could not be identified in these two families the molecular disease cause remained unsolved.

Interestingly, almost all patients from Qeshm had blindness or retinal pigmentation. However, 2 patients from Isfahan had homozygous *NPHP1* mutation (approximately 28% of Isfahanian patients). In a similar study, Soleiman and coworkers evaluated 20 children from 17 unrelated families and showed *NPHP1* deletion in 29.4% of the patients.[31] Using combined approach of DNA pooling followed by massively parallel re-sequencing ascertained gene mutations in approximately 25% of nephronophthisis patients.[32] Irrespective of sensitivity of this method, mutation was not found in 75% of patients; declaring wide heterogeneity in nephronophthisis.[32]

Although 3 published studies in Iran have assessed gene mutations in familial cases with ciliopathy-related diseases (Senior-Loken and Jubert syndromes), no one has evaluated *NPHP1* gene deletion in unrelated patients with nephronophthisis phenotype and CKD.[33-35] Our study demonstrated a frequency of 28% for *NPHP1* gene deletion in patients with nephronophthisis phenotype without ophthalmic involvement.

CONCLUSIONS

By combining *NPHP1* deletion analysis with multiplex-PCR based high-throughput mutation analysis we could identify the molecular disease-cause in 7 out of 15 families from Iran. In 8 families, the molecular disease cause remained unknown.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Hildebrandt F, Otto E. Cilia and centrosomes: a unifying pathogenic concept for cystic kidney disease? Nat Rev Genet. 2005;6:928-40.
2. Smith C, Graham J. Congenital medullary cysts of the kidneys with severe refractory anemia. Am J Dis Child. 1945;69:369-77.
3. Fanconi G, Hanhart E, Albertini A. Die familia¨re juvenile Nephronophthise. Helv Pediatr Acta. 1951;6:1-49.
4. Hildebrandt F, Zhou W. Nephronophthisis-associated ciliopathies. J Am Soc Nephrol. 2007;18:1855-71.
5. Gagnadoux MF, Bacri JL, Broyer M, Habib R. Infantile chronic tubulo-interstitial nephritis with cortical microcysts: variant of nephronophthisis or new disease entity? Pediatr Nephrol. 1989;3:50-5.
6. Otto EA, Schermer B, Obara T, et al. Mutations in INVS encoding inversin cause nephronophthisis type 2, linking renal cystic disease to the function of primary cilia and left-right axis determination. Nat Genet. 2003;34:413-20.
7. Hildebrandt F, Waldherr R, Kutt R, Brandis M. The nephronophthisis complex: clinical and genetic aspects. Clin Investig. 1992;70:802-8.
8. Omran H, Fernandez C, Jung M, et al. Identification of a new gene locus for adolescent nephronophthisis, on chromosome 3q22 in a large Venezuelan pedigree. Am J Hum Genet. 2000;66:118-27.
9. Loken AC, Hanssen O, Halvorsen S, Jolster NJ. Hereditary renal dysplasia and blindness. Acta Paediatr. 1961;50:177-84.
10. Saraiva JM, Baraitser M. Joubert syndrome: a review. Am J Med Genet. 1992;43:726-31.
11. Valente EM, Marsh SE, Castori M, et al. Distinguishing the four genetic causes of Jouberts syndrome-related disorders. Ann Neurol. 2005;57:513-9.
12. Boichis H, Passwell J, David R, Miller H. Congenital hepatic fibrosis and nephronophthisis. A family study. Q J Med. 1973;42:221-33.
13. Mainzer F, Saldino RM, Ozonoff MB, Minagi H. Familial nephropathy associatdd with retinitis pigmentosa, cerebellar ataxia and skeletal abnormalities. Am J Med. 1970;49:556-62.
14. Simms RJ, Eley L, Sayer JA. Nephronophthisis. Eur J Hum Genet. 2009;17:406-16.
15. Hurd WT, Hildebrandt F. Mechanisms of nephronophthisis and related ciliopathies. Nephron Exp Nephrol. 2011;118:e9-14.
16. Bollee 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;21:2660-3.
17. Aguilera A, Rivera M, Gallego N, Nogueira J, Ortuno J. Sonographic appearance of the juvenile nephronophthisis-cystic renal medulla complex. Nephrol Dial Transplant. 1997;12:625-6.
18. Halbritter J, Diaz K, Chaki M, et al. High-throughput mutation analysis in patients with a nephronophthisis-associated ciliopathy applying multiplexed barcoded array-based PCR amplification and next-generation sequencing. J Med Genet. 2012;49:756-67.
19. Pistor K, Olbing H, Scharer K. Children with chronic renal failure in the Federal Republic of Germany: I. Epidemiology, modes of treatment, survival. Arbeits-gemeinschaft fur Padiatrische Nephrologie. Clin Nephrol. 1985;23:272-7.
20. Potter DE, Holliday MA, Piel CF, Feduska NJ, Belzer FO, Salvatierra O, Jr. Treatment of end-stage renal disease in children: a 15-year experience. Kidney Int. 1980;18:103-9.
21. Hildebrandt F, Benzing T, Katsanis N. Ciliopathies. N Engl J Med. 2011;364:1533-43.
22. Otto EA, Helou J, Allen SJ, et al. Mutation analysis in nephronophthisis using a combined approach of homozygosity mapping, CEL I endonuclease cleavage, and direct sequencing. Hum Mutat. 2008;29:418-26.
23. Benzing T, Gerke P, Hopker K, Hildebrandt F, Kim E, Walz G. Nephrocystin interacts with Pyk2, p130(Cas), and tensin and triggers phosphorylation of Pyk2. Proc Natl Acad Sci U S A. 2001;98:9784-9.
24. Donaldson JC, Dempsey PJ, Reddy S, Bouton AH, Coffey RJ, Hanks SK. Crk-associated substrate p130(Cas) interacts with nephrocystin and both proteins localize to cell-cell contacts of polarized epithelial cells. Exp Cell Res. 2000;256:168-78.
25. Donaldson JC, Dise RS, Ritchie MD, Hanks SK. Nephrocystin-conserved domains involved in targeting to epithelial cell-cell junctions, interaction with filamins, and establishing cell polarity. J Biol Chem. 2002;277:29028-35.
26. Hildebrandt F, Attanasio M, Otto E. Nephronophthisis: disease mechanisms of a ciliopathy. J Am Soc Nephrol. 2009;20:23-35.
27. Hildebrandt F, Otto E, Rensing C, et al. A novel gene encoding an SH3 domain protein is mutated in nephronophthisis type 1. Nat Genet. 1997;17:149-53.
28. SENIOR B, FRIEDMANN AI, BRAUDO JL. Juvenile familial nephropathy with tapetoretinal degeneration. A new oculorenal dystrophy. Am J Ophthalmol. 1961;52:625-33.
29. Otto EA, Loeys B, Khanna H, et al. Nephrocystin-5, a ciliary IQ domain protein, is mutated in Senior-Loken syndrome and interacts with RPGR and calmodulin. Nat Genet. 2005;37:282-8.
30. Salomon R, Saunier S, Niaudet P. Nephronophthisis. Pediatr Nephrol. 2009;24:2333-44.
31. Soliman NA, Hildebrandt F, Otto EA, et al. Clinical characterization and NPHP1 mutations in nephronophthisis and associated ciliopathies: a single center experience. Saudi J Kidney Dis Transpl. 2012;23:1090-8.
32. Otto EA, Ramaswami G, Janssen S, et al. Mutation analysis of 18 nephronophthisis associated ciliopathy disease genes using a DNA pooling and next generation sequencing strategy. J Med Genet. 2011;48:105-16.
33. Haghighi A, Al-Hamed M, Al-Hissi S, et al. Senior-Loken syndrome secondary to NPHP5/IQCB1 mutation in an

Iranian family. Nephrology Dialysis Transplant Plus. 2011;4:421-3.

34. Roozbeh J, Sharifian M, Hosseini H, et al. Senior-Loken syndrome in an Iranian family. Saudi J Kidney Dis Transpl. 2010;21:735-7.

35. Barzegar M, Malaki M, Sadegi-Hokmabadi E. Joubert syndrome with variable features: presentation of two cases. Iran J Child Neurol. 2013;7:43-6.

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Received May 2014
Revised August 2014
Accepted September 2014