Diverse phenotypic expression of NPHP4 mutations in four siblings

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Nephronophthisis (NPHP) is an autosomal recessive disease characterized by renal tubular basement membrane disruption, interstitial fibrosis and tubular cysts that progresses to end-stage kidney disease (ESKD). There are also characteristic extrarenal manifestations. Mutations of more than thirteen genes that can cause NPHP have been identified. We herein report four siblings from a consanguineous family, who carried the same NPHP4 mutations but presented with different disease phenotypes ranging from enuresis nocturna to ESKD. Diluted urine and echogenic kidneys in ultrasound examination were consistent, which is typical for 100% of the NPHP cases that have been described. Chronic kidney disease developed in the older two brothers. The observed phenotypic differences are likely to be related to environmental and epigenetic factors, oligogenic inheritance and modifier genes affecting the age of presentation of signs and symptoms.

Key words: hereditary nephropathy, nephronophthisis, NPHP4

Nephronophthisis (NPHP) is an autosomal recessive chronic tubulointerstitial nephritis that constitutes the most frequent genetic cause of ESKD in children¹. Thirteen causative genes have been identified. They encode proteins expressed in the primary cilia or centrosomes of renal epithelial cells. The NPHP1 gene encodes nephrocystin-1; NPHP2 inversin; NPHP3 nephrocystin-3; NPHP4 nephrocystin-4 (nephroretinin); NPHP7 GLIS family zinc finger 2; NPHP11 meckelin; etc.² Three clinical variants have been described: infantile, juvenile and adolescent forms³. Juvenile NPHP is the most common form, which accounts for nearly 5-10% of sporadic cases of ESKD in children⁴. The first symptoms, polyuria and polydipsia, generally develop around 4–6 years of age⁵. Late symptoms are related to progressive renal insufficiency; these include anemia, metabolic acidosis, nausea, anorexia and growth retardation. ESKD develops around age 13 but can also occur much later⁶.⁷.

The gene product of NPHP1, nephrocystin, encodes a docking protein that interacts with components of cell-cell and cell-matrix signaling⁸. It also interacts with the gene product of NPHP4, nephroretinin-4. The mutations of NPHP1 or NPHP4 are associated with the juvenile type of NPHP⁹. NPHP4, located on chromosome 1p36, encodes a 1,426 amino acid protein called nephroretinin-4/ nephroretinin¹⁰.

We herein report a family (F1270) with four children who carry the same homozygous NPHP4 mutation but exhibit heterogenous phenotypes. The first case was referred to our clinic with mild renal insufficiency at the age of 13. His brother had chronic kidney disease (CKD) stage IV when he presented. Two siblings have only enuresis at the ages of 13 and 10, respectively.

Case 1

This is a 13-year-old boy who was referred...
to our clinic with complaints of polyuria, polydipsia, enuresis and growth retardation. Past medical history was unremarkable except for one year of growth hormone (GH) therapy two years before. There was parental consanguinity.

On admission, physical and eye examinations were normal. Blood pressure (BP) was 100/60 mmHg. Body weight (BW) was 34.5 kg (5th-10th percentile); height, 151 cm (25th-50th percentile). Complete blood count (CBC) and peripheral smear were normal. He had mild metabolic acidosis (pH: 7.25, HCO₃: 19.6, BE: -4.0). Urinalysis showed a specific gravity (SG) of 1005, pH of 7, 3-5 erythrocytes and 1-2 leukocytes/HPF. He had no proteinuria. Serum creatinine (Cr) was 1.1 mg/dl and creatinine clearance (CCr) 75 ml/min/1.73 m². Serum complement component C3 and C4 levels were normal, and anti-double-stranded DNA (anti-dsDNA) and anti-nuclear antibody (ANA) were negative. His parathormone level was 316 pg/ml (N: 12-75); ferritin, 48.52 ng/ml (12-200). Abdominal ultrasonography (US) revealed normal-sized, moderately echogenic kidneys (grade 2-3). There was no vesicoureteral reflux.

Since he had stage II CKD, a renal biopsy was performed for investigating underlying renal disease. The biopsy revealed chronic tubulointerstitial damage and periglomerular fibrosis, which can be suggestive of NPHP (Fig-1).

His genetic examination gave a negative result for NPHP1 mutation, but a homozygous mutation on NPHP4 (c. 2368 G>T (p.E790X)) was detected⁹. He has been on symptomatic treatment for CKD (calcitriol, calcium acetate and bicarbonate). At the last examination of his serum (at age 20), Cr level was 3.4 mg/dl and CCr was 27.5 ml/min/m². The pedigree is shown in Fig 2. The patient had 3 affected siblings: 2 younger sisters (Cases 2 and 3) and 1 older brother (Case 4).

Case 2

Patient 2 was a younger sister of the patient (13 years old). She was evaluated for the complaints of polyuria, polydipsia, enuresis and growth retardation. Past medical history was unremarkable except for one year of GH therapy, two years ago. On admission, her BP was 110/70 mmHg. BW was 35 kg (5th-10th percentile) and height 157 cm (50th-75th percentile). Laboratory tests, including CBC, renal function tests and CCr, were normal. Urine analysis was normal except for a decreased SG (1006). Abdominal ultrasonography showed increased renal echogenicity (grade 1). The same mutation of NPHP4 was detected.

Case 3

Patient 3, the youngest sibling, was a 10-year-old girl, with the same complaints as case 2 except for growth retardation. Past medical history was unremarkable. On admission, her physical and eye examinations and BP were normal. BW was 40 kg (75th-90th percentile) and height 145 cm (75th-90th percentile). There was no anemia or uremia. 24-h urine protein excretion and CCr were normal. Abdominal ultrasonography showed increased echogenicity up to grade 1. Mutation analysis for NPHP4 revealed the same homozygous mutation.

Case 4

Patient 4 was the oldest sibling in the family (24 years old). He was diagnosed with CKD stage IV with unknown etiology in another center when he was 15. He had been enuretic. He had no growth retardation, but had bilateral small and echogenic kidneys. On admission, he had hypertension, diluted urine, mild proteinuria and anemia. Serum complement C3 and C4 levels were normal, and ANA and anti-dsDNA were negative. He had been biopsied when his creatinine level was 3.3 mg/dl. The biopsy revealed 30 glomeruli, 75% of which showed global sclerosis. Mesangial proliferation was present in 8 nonsclerotic glomeruli. Interstitial fibrosis and tubular atrophy were present in large areas. These findings were interpreted as chronic renal damage possibly secondary to chronic tubulointerstitial nephritis. Due to the progression to ESKD in 18 months, a preemptive kidney transplantation (with his father as the donor) was performed in another center. His mutation analysis for NPHP4 revealed the same homozygous mutation as in the other siblings.

Discussion

Nephronophthisis is a recessive cystic kidney disease leading to ESKD in the first three decades of life. The corresponding proteins
encoded by the NPHP genes, the nephrocystins, are expressed in the kidney, brain and eye, and it has been suggested that they participate in the formation of complex protein networks. Nephrocystins are found in cell–cell junctions, centrosomes and primary cilia of renal epithelial cells and may have different functions at various sites as suggested by the variety of extrarenal symptoms, such as retinal dystrophy, cerebellar hypoplasia, mental retardation, situs inversus, polydactyly and hepatic cysts.

Positional cloning and candidate gene approaches led to the identification of the causative 13 genes, NPHP1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13. Clinical and histological features of patients with NPHP4 mutation were first reported by Hoefele et al. After a 20-year follow-up of an index family with three affected siblings, mapping of NPHP4 was possible. Our index patient had CKD stage II when he was 13 years old and now, at the age of 21, has stage IV CKD, which is a typical clinical course of juvenile NPHP. However, his older brother developed ESKD at a younger age, 16.5 years. This may be partly explained by the success in medical management strategies for CKD (management of anemia, hyperparathyroidism and metabolic acidosis, ensuring patient adherence to medication and diet instructions, maintaining good volume control, etc.) and close follow-up of our index patient, which may delay the progression of CKD to ESKD. The other two siblings had normal renal function at the ages of 10 and 13. Diverse phenotypic expression of the disease among siblings who carry the same mutation in NPHP4 (Fig. 2) can partly be explained by oligogenic inheritance, which is shown in polycystic kidney disease and NPHP. Oligogenic inheritance in NPHP was confirmed by Hoefele et al. in 2007 by detecting two mutations in one of the NPHP genes in combination with a third mutation in another NPHP gene in six different families with NPHP. In our study, NPHP1 mutation analysis was performed only in Case 1. After obtaining a negative result for NPHP1, NPHP4 was studied and mutation was detected in the index case first and then in three siblings. Since mutation analysis was not performed for all known NPHP genes, the possibility of oligogenicity can not be ruled out. Therefore, a new mutation screening for all identified NPHP genes might be helpful in explaining the intrafamilial variability in
our cases\textsuperscript{2}. Environmental factors might be another cause for this variability, since Case 1 and Case 4 grew up in a location distant from their sisters. Alternatively, modifier genes segregating independently among the siblings may be postulated to explain the variable age of onset, as previously proposed by Hoefele\textsuperscript{13}.

The common initial complaints of our four siblings were urination symptoms like polyuria, polydipsia and enuresis, while anemia was not a prominent finding. Similarly, growth retardation was not detected in any of the siblings. This may be partly explained by the GH therapy recorded in the medical history of the two siblings. Echogenic kidneys in differing degrees of severity, even if in the absence of cysts in US examination, and diluted urine were the only consistent findings in all of our four siblings. If a child presents with polyuria, enuresis, growth failure, renal insufficiency without hematuria or proteinuria and normal BP, NPHP could be a possible diagnosis, and, if possible, all known genes should be screened for disease-causing mutations, even if a full-blown clinical picture is absent. On the other hand, it should be kept in mind that the frequency of homozygous or compound heterozygous \textit{NPHP4} mutations was quite low, 2.4%, in a study of 250 patients with NPHP\textsuperscript{17}. However, genetic analysis is the only way to make a definitive diagnosis in this rare disease group.

In children with recessive mutations in \textit{NPHP1}, 2, 3 and 4, retinitis pigmentosa occurs in approximately 10% of all affected families. Since there is no genotype/phenotype correlation for extrarenal manifestations\textsuperscript{17} in cases of \textit{NPHP4} mutation, such a manifestation may be absent, as in our patients. There was no retinal finding in the ophthalmologic examination of any of the siblings.

In conclusion, NPHP is an important cause of CKD in children, which insidiously progresses to ESKD. Since there is no specific therapy for the disease, early diagnosis, close follow-up, and prevention and treatment of the complications of progressive renal insufficiency are the mainstays of its management.

REFERENCES