Pseudodominant inheritance of nephronophthisis caused by a homozygous *NPHP1* deletion

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Abstract

Nephronophthisis (NPHP) is an autosomal recessive kidney disease characterized by tubular basement membrane disruption, interstitial infiltration, and tubular cysts. NPHP leads to end-stage renal failure in the first two decades of life and is the most frequent genetic cause of chronic renal failure in children and young adults. Mutations in eleven genes (*NPHP1-11*) have been identified. Extrarenal manifestations are known, such as retinitis pigmentosa (Senior-Løken syndrome, SLS), brainstem and cerebellar anomalies (Joubert syndrome), liver fibrosis, and ocular motor apraxia type Cogan.

We report on a Turkish family with clinical signs of nephronophthisis. The phenotype occurred in two generations and therefore seemed to be inherited in an autosomal dominant pattern. Nevertheless, a deletion analysis of the *NPHP1* gene on chromosome 2 was performed and showed a homozygous deletion. Analysis of the family pedigree indicated no obvious consanguinity in the last three generations. However, haplotype analysis demonstrated homozygosity on chromosome 2 indicating a common ancestor to the parents of all affected individuals. *NPHP1* deletion analysis should always be considered in patients with apparently dominant nephronophthisis, especially from likely consanguineous families.

INDEX WORDS

Nephronophthisis; *NPHP1*; cystic kidney disease

Introduction

Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease that represents the most common genetic cause for end-stage renal disease in children and young adults [1]. NPHP leads to end-stage renal disease (ESRD) at a median age of 13 years. The

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characteristic histological findings in NPHP are a triad of renal interstitial infiltration with fibrosis, tubular atrophy with basement membrane disruption, and cyst development at the corticomedullary border [2]. There is extensive genetic heterogeneity, with at least eleven different causative genes [3]. The most frequently mutations are homozygous deletions in *NPHP1*, which accounts for approximately 21% of all NPHP cases [4]. The gene for its protein product nephrocystin-1 is localized on chromosome 2q13 and is mutated in NPHP type 1 [1, 5]. Nephrocystin-1 is expressed in collecting duct cells and interacts in a protein complex of nephrocystins [3]. Identification of NPHP genes has contributed to a unifying pathogenic theory that describes cystic kidney diseases as ''ciliopathies'' [6]. This implies that functional defects of primary cilia, basal bodies, and centrosomes are important for the pathogenesis of NPHP. The ubiquitous cilial expression of NPHP proteins might explain why other organs can also be affected in patients with NPHP. The most frequently associated extra-renal involvements are retinopathy described as Senior–Løken syndrome (15% of the cases), cerebellar ataxia known as Joubert syndrome (10–15%), and liver fibrosis (5%) [7].

We here present a clinical and molecular analysis of a Turkish family with a clinical presentation of nephronophthisis which occurred in two generations suggesting autosomal dominant inheritance. Haplotype analysis, however, revealed homozygosity on chromosome 2 indicating a distant relationship between the parents of all affected individuals.

Patients

Case 1

Case 1 (F852, IV-1, born in 1979), the index patient of a Turkish family, is a 17-year-old girl who presented with vomiting and weight loss (57 kg; 50. percentile). Laboratory findings at that time showed severe renal failure (serum creatinine, 10 mg/dL [884 μmol/L]). Renal ultrasound revealed bilateral small kidneys with multiple cysts. Supine blood pressure was reported as 100/70 mm Hg. A percutaneous kidney biopsy was not performed. Hemodialysis was started immediately. Ophthalmologic examination at 18 years of age did not show retinitis pigmentosa. There were no extrarenal manifestations. She is the first child of a healthy mother and a father who also has nephronophthisis. The pedigree is shown in Fig 1. The patient has 2 unaffected brothers.

Case 2

Case 2 (F852, III-2, born in 1955) is the father of case 1. He first presented at the age of 43 years after diagnosis in his daughter with oedema, abdominal pain, pruritus, and breathlessness. Medical history revealed polydipsia and polyuria since childhood. Laboratory findings showed severe renal failure with a serum creatinine of 10 mg/dL (884 μmol/L). Ultrasonography revealed bilateral small kidneys with multiple cysts. Supine blood pressure was 150/100 mm Hg. A percutaneous kidney biopsy was not performed. Hemodialysis was started immediately. Ophthalmologic examination did not show signs of retinitis pigmentosa. He does not have any other extrarenal manifestations. Case 2 has two affected sisters and three healthy siblings (Fig. 1).

Case 3

Case 3 (F852 III-3, born in 1966) is a sister of case 2. At the age of 27 years she was first seen with back pain, dizziness, and nausea. Her serum creatinine was 10 mg/dL (884 μmol/L). Hemodialysis was started. Extrarenal manifestations were not observed.

Case 4

Case 4 (F852 III-5, born in 1973) is the second affected sister of case 2. She presented with weight loss in 2004 and was in end-stage renal failure with a serum creatinine of 10 mg/dL (884 μmol/L). Supine blood pressure was 150/110 mm Hg. She was started on hemodialysis, and a successful kidney transplantation was performed at the age of 35 years. The examination of others organs was unremarkable.

Parents and Unaffected Siblings

A detailed pedigree (Fig. 1) failed to reveal a common ancestor to the affected individuals. The grandparents are healthy, a genetic examination could not be done.

Methods and Results

Blood samples were obtained after informed consent was given by all affected family members, their siblings, and parents. Approval for this study was obtained from the University of Michigan Institutional Review Board.

For the screening of a homozygous deletion in the *NPHP1* gene a multiplex PCR approach was used amplifying five different markers. Two control markers and three *NPHP1* exon markers (exon 5, 7, and 20) were amplified in a single multiplex PCR. Fragments were separated on a 1.5% agarose gel for 3 h at 120 V (Fig. 2). Case III-2, III-3, III-5, and IV-1 lack the bands for the respective *NPHP1* exons indicating a homozygous *NPHP1* deletion and confirming the diagnosis NPHP type 1.

Haplotype analysis on chromosome 2q12.2 to 2q14.2 was performed in individual II-1, II-2, III-2, III-3, III-5, III-6, and IV-1 using the following six consecutive polymorphic microsatellite markers: cen - *D2S176*, *D2S340*, *D2S160*, *D2S308*, *D2S363*, and *D2S283* - q-ter. In the entire region homozygosity was obvious in all affected family members demonstrating that a common ancestor to all four affected individuals must exist (Fig. 3).

Discussion

Nephronophthisis (NPHP) and medullary cystic kidney disease (MCKD) constitute a complex of renal cystic diseases that share the macroscopic feature of cyst development at the corticomedullary border of the kidneys [8]. Both disorders have a characteristic renal histology in common. NPHP, the autosomal recessive form, leads to end-stage renal disease (ESRD) in the first two decades of life, and there is extensive genetic heterogeneity with at least eleven causative genes [9]. MCKD, the autosomal dominant form of cystic kidney disease, has been linked to two loci, MCKD1 and MCKD2. In contrast to MCKD1, patients with MCKD2 show a more severe phenotype regarding hypertension, hyperuricemia, and gout. Moreover, in patients with MCKD2, an earlier median age of onset has been reported (age of onset in MCKD2, 32 years compared with 62 years in MCKD1) [10].

Because of the clinical features in our Turkish family, a new dominant form of NPHP was first considered. Nevertheless, to exclude a genetic defect in one of the *NPHP* genes, deletion analysis of *NPHP1* gene was performed, which showed a homozygous deletion in all affected family members (Fig. 2). Because NPHP is considered to be inherited in a recessive manner, we now had to postulate a pseudodominant inheritance of unknown etiology.

For recessive genetic disorders like NPHP a horizontal pattern of inheritance is typical. On the other hand, vertical patterns of inheritance, in which affected individuals are found in more than one generation, are typical of dominant genetic disorders, i.e. MCKD [11]. In

contrast, pseudodominance is an autosomal recessive condition present in individuals in two or more generations of a family, thereby appearing to follow a dominant inheritance pattern. Common explanations for a pseudodominant appearance are: i) a high carrier frequency for the autosomal recessive disease; ii) birth of an affected child to an individual affected by an autosomal recessive disease and a genetically related (consanguineous) reproductive partner, who is an unsuspecting carrier of a mutation/deletion in the same gene as the affected partner. Pseudodominant inheritance has been described in many autosomal recessive, renal associated disorders including familial Mediterranean fever [12], Cogan syndrome [13], and primary hyperoxaluria type 1 [11], and can easily be distinguished from dominant inheritance with molecular diagnosis and haplotype analysis. Only a few reports are known describing families with NPHP-affected individuals in more than one generation [14]. Because of the supposed low heterozygosity frequency for *NPHP1* deletions, a distant relationship within our Turkish family was assumed. To prove the hypothesis of a common ancestor haplotype analysis on chromosome 2q12.2 to 2q14.2 was performed in several family members revealing a homozygous region in all affected individuals (Fig. 3). We therefore had to postulate, that the family is most likely double consanguineous.

A second important consideration concerning other possibilities that could generate the pseudodominant inheritance pattern in this family would be loss of heterozygosity on chromosome 2q12.2 to 2q14.2 in the father and his affected siblings, and uniparental disomy of the paternal mutant chromosome in the index patient.

It is worth mentioning that the father of the index patient (case 2) and his two sisters (case 3 and 4) suffered from ESRD at the age of 43, 27, 31 years resp. This finding of a very late-onset of ESRD is not consistent with the experience in NPHP reaching ESRD before the age of 20 years [4]. So far, three clinical forms of NPHP have been distinguished by onset of ESRD: infantile, juvenile, and adolescent NPHP, which manifest with ESRD at the median ages of < 4, 13, and 19 years, respectively [3]. Georges et al. described 4 patients with Senior-Løken syndrome leading to ESRD between the ages of 42 and 56 years [15]. A possible explanation for the different age at ESRD could be the influence of yet unknown modifier genes.

A pseudodominant pattern of inheritance in NPHP, although certainly extremely rare, can obscure the correct diagnosis. A thorough clinical assessment is one of the first steps in diagnosing NPHP. Clinical vigilance in combination with a diagnostic strategy that includes the close interaction between clinicians and geneticists is the key to a correct diagnosis in patients with cystic kidney diseases.

Acknowledgments

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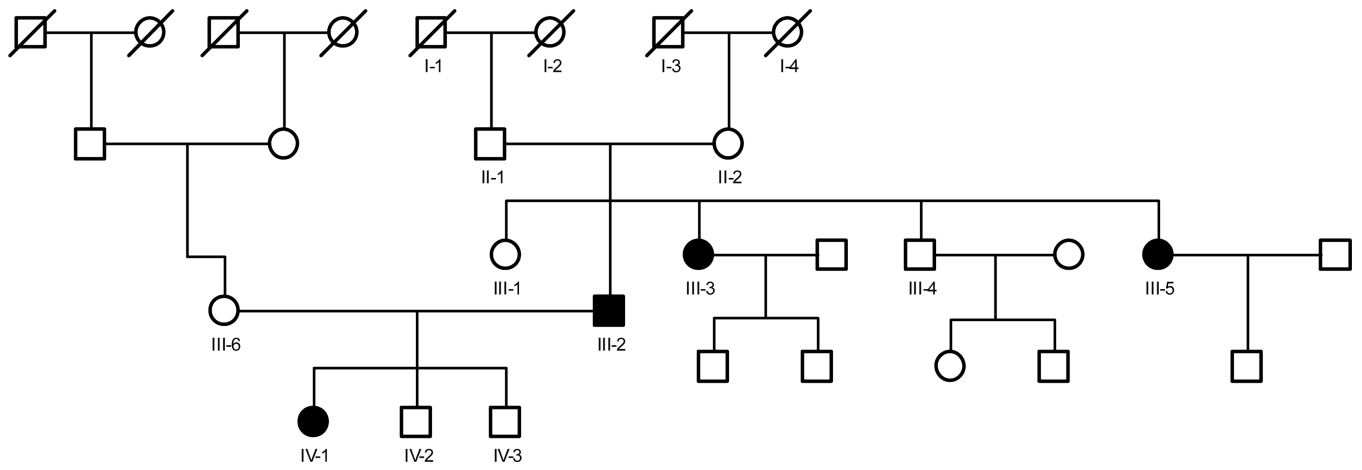
Figure 1.
Pedigree of the family F852. Solid symbols, affected individuals; circles, females; squares, males.
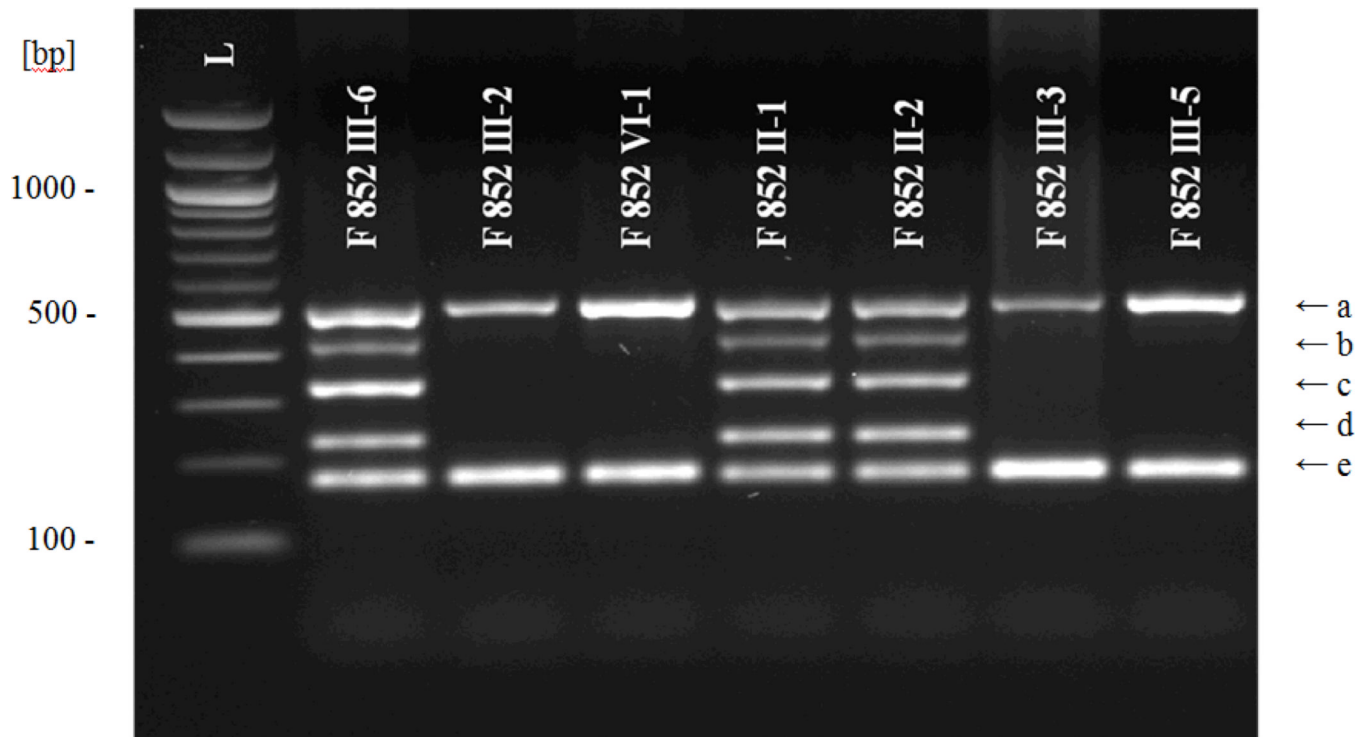
Figure 2.
Screening for a homozygous deletions in the *NPHP1* gene in family F852 using a multiplex PCR approach amplifying five different markers. Two control markers (a, e) and three *NPHP1* exon markers [d (exon 7, 236 bp), c (exon 5, 339 bp), b (exon 20, 436 bp)] were amplified in a single multiplex PCR. Note that patients III-2, III-3, III-5, and IV-1 lack the bands for the respective *NPHP1* exons, indicating a homozygous *NPHP1* deletion and confirming the diagnosis NPHP type 1. L, 100-bp ladder DNA size marker (New England Biolabs, Beverly, MA).
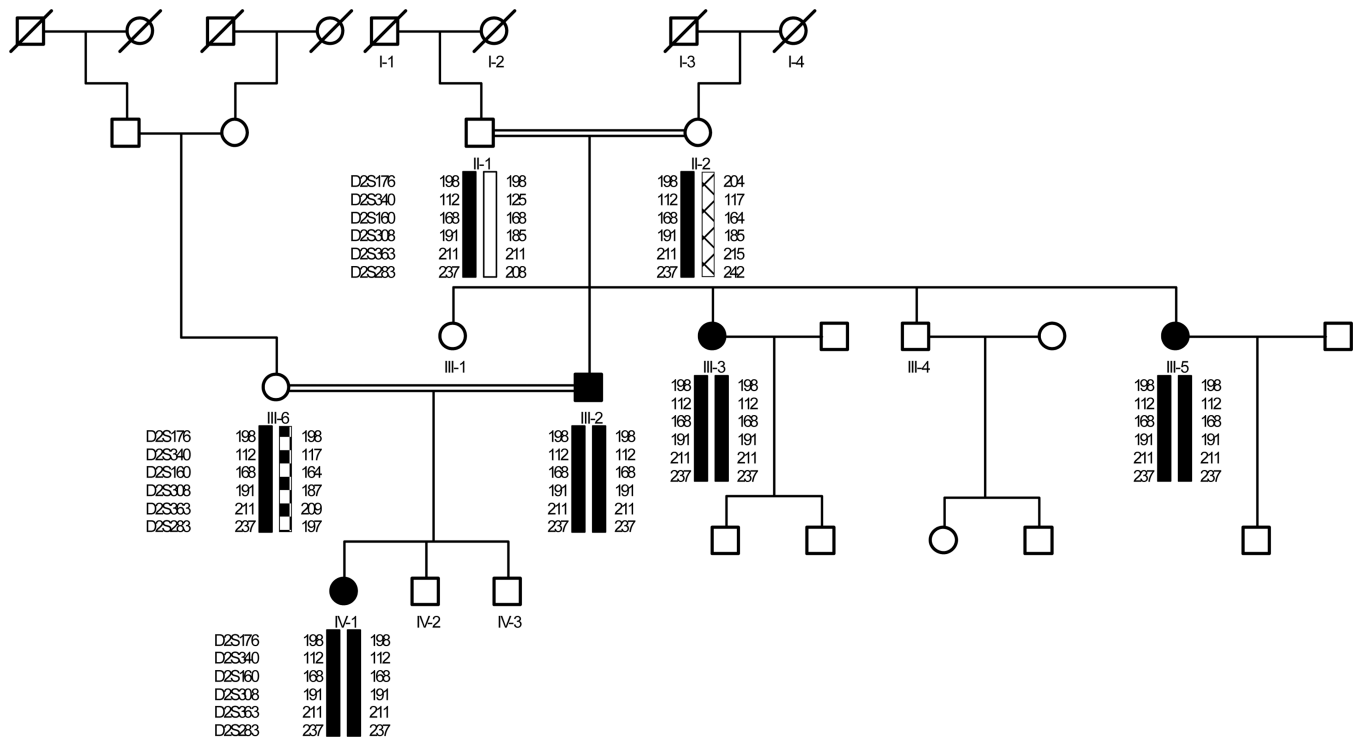
Figure 3.
Haplotypes on chromosome 2q12.2 to 2q14.2 of family F852. Six microsatellite markers, from cen to q-ter (*top to bottom*), are shown to the left of the pedigrees. Symbols are as in figure 1.