

Intrafamilial Variability and Clinical Heterogeneity in Two Siblings with NPHP4 loss of Function Mutations

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Abstract

Joubert syndrome-related disorders (JSRDs) are a group of clinically and genetically pleiotropic conditions that share a midbrain-hindbrain malformation, the pathognomonic molar tooth sign (MTS) visible on brain imaging, with variable involvement of other organs and systems mainly the eyes and the kidneys. Nevertheless, the definition of JSRDs remained problematical due to the extreme phenotypic heterogeneity, often with intrafamilial variability, and the significant clinical overlap among distinct forms. Here we describe two siblings with nephronophthisis (NPHP), the elder is best categorized as JSRD. Nevertheless, his younger sibling lacked the characteristic molar tooth sign; hence best categorized as NPHP-related ciliopathy. Both siblings had NPHP as the common renal phenotype, yet with variable neurological and ocular involvement. Genetic linkage and mutation analysis revealed a novel homozygous, potential loss of function mutation (c.2618dupA, p.H873G) in the gene NPHP4 in both siblings. This finding extends the phenotypic spectrum associated with NPHP4 mutations, with discernible clinical heterogeneity and intrafamilial variability.

Keywords: Nephronophthisis; Mutations; Joubert syndrome related disorders; NPHP4

Introduction

Nephronophthisis (NPHP) is an autosomal recessive renal cystic disease and a leading cause of chronic renal impairment with eventual end-stage renal disease in the first three decades of life [1]. NPHP is characterized clinically by polyuria/polydipsia, growth failure, anemia out of proportion to the degree of renal failure and insidious onset of chronic renal failure [2].

Renal ultrasound scan shows moderately small kidneys with poor cortico-medullary differentiation and cysts at the cortico-medullary junction which appear late in the course of the disease [3]. To date 18 NPHP genes have been identified in patients with nephronophthisis and associated ciliopathies and their encoded proteins are located at the primary cilia-centrosomes complex giving the unifying theory of ciliopathies [4].

Extra-renal manifestations of NPHP include mainly retinal degeneration (Senior-Lokens syndrome) [5,6], cerebellar vermis hypoplasia (Joubert syndrome related disorders) [7] and ocular motor apraxia [8] in addition to many other described features within the spectrum of nephronophthisis associated ciliopathies [9,10].

NPHP4 gene was first mapped to chromosome 1p36 in 2002 extending over 130kb and consisting of 30 exons encoding 1,426 amino acids [10,11]. The nephrocystin-4 protein (nephroretinin) encoded by the NPHP4 gene localizes to the cilia transition zone as well as to the cortical actin cytoskeleton of renal epithelial cells [12].

NPHP4 is thought to function in conjunction with NPHP1 at the transition zone to regulate entry and exit of ciliary cargos [13].

NPHP4 has also been shown to interact with two other ciliopathy proteins, RPGRIP and RPGRIP1L, which are mutated in Leber congenital amaurosis and Joubert syndrome, respectively [14].

Mutations in NPHP4 have been described before in patients with isolated nephronophthisis, in association with oculomotor apraxia or retinal degeneration [15], and recently for the first time with Joubert syndrome related disorder [16].

We here describe two siblings with a variable clinical phenotype of NPHP related ciliopathies caused by NPHP4 loss of function mutations.

Case Report

Case 1

A ten year old male patient was referred to our center (Cairo University Center of Pediatric Nephrology and Transplantation) from a district hospital where he presented with fever, respiratory distress and pallor. Routine laboratory investigations revealed anemia (Hb 5 g/dL) and deteriorating renal function (BUN 65 mg/dL, Cr 8.8 mg/dL).

His past history was relevant for polyuria, polydipsia and nocturnal enuresis dating since the age of 4 years. He is the first offspring of a consanguineous marriage. His perinatal history was uneventful, but yet the mother noticed that her child had a visual problem with

difficulty fixing on objects together coupled with abnormal eye movements.

Patient had dysmorphic facies (prominent forehead, broad nasal bridge and hypertelorism) (Figure 1). On presentation to our center, he looked pale and had respiratory distress, tachycardia, and hypertension with as blood pressure of 140/90 (above the 95th centile for age, gender and height). His weight was on the 10th centile and his height was below the 3rd centile for his age and gender. He had an ataxic gait and incoordination of fine movements.

His serum BUN was 65 mg/dL, creatinine 8.8 mg/dL, estimated GFR 7 ml/min/1.73 m², Na 131 mEq/L, K 5 mEq/L, hemoglobin 5 g/dL and venous blood gas analysis showed metabolic acidosis with pH 7.32 and HCO₃ 14 mEq/L. Urine analysis showed no sediments and a specific gravity of 1015.

Abdominal ultrasound scan showed normal sized hyper echogenic kidneys with poor cortico-medullary differentiation and multiple variable sized cysts at the cortico-medullary junction (Figure 1). His IQ was 80% of the same aged child and his bone age was delayed (only 84 months at a chronological age of 120 months).

Upon ophthalmological evaluation, he had oculomotor apraxia with normal funduscopy. Nevertheless, his electroretinogram (ERG) demonstrated subnormal response with significant evidence of cone dysfunction.

Brain MRI showed the pathognomonic molar tooth sign (MTS) with elongated but non-thickened superior cerebellar peduncles and cerebellar vermis hypoplasia (Figure 2). Being in ESRD, the patient was therefore started on regular hemodialysis program.

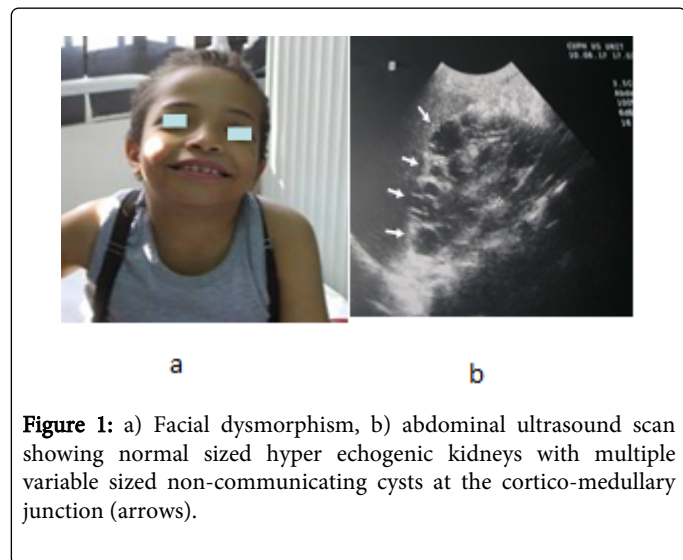


Figure 1: a) Facial dysmorphism, b) abdominal ultrasound scan showing normal sized hyper echogenic kidneys with multiple variable sized non-communicating cysts at the cortico-medullary junction (arrows).

Case 2

Screening the siblings of case (1) showed that his 7 year old brother was also complaining of polyuria, polydipsia and secondary enuresis. The brother (case 2) was the first-born of preterm triplets via a cesarean section and his birth weight was 2 kg. His initial and discharge physical examinations as a newborn were normal and uneventful with no history of admission to NICU unlike the other two new borns who had to be admitted to NICU (respiratory distress and very low birth weight) for mechanical ventilation and were discharged after a month.

His weight and height were 10th centile for age and gender. Laboratory investigations showed BUN 20 mg/dL, creatinine 1.3 mg/dL, estimated GFR 45 ml/min/1.73 m², Na 140 mEq/L, K 4.2 mEq/L, hemoglobin 9 g/dL and his venous blood gas analysis showed evidence of metabolic acidosis with pH 7.31 and HCO₃ 16.4 mEq/L. His bone age was also delayed (only 54 months at a chronological age of 84 months).

The abdominal ultrasound scan showed normal sized hyper echogenic kidneys with fair cortico-medullary differentiation, but no cysts could be detected. Absence of cysts was further confirmed by thin slice abdominal computed tomography (CT) scan. He had no ataxic symptoms but his IQ was 83% of the same aged child. Brain MRI showed no evidence of MTS. Fundoscopy was inconspicuous yet his ERG revealed a subnormal response indicative of retinal dystrophy. Unlike his brother, he showed no evidence of oculomotor apraxia.

Ultrasound guided renal biopsy showed the characteristic triad of thickened tubular basement membrane, tubular atrophy and dilatation with mild interstitial infiltration and fibrosis suggestive of NPHP.

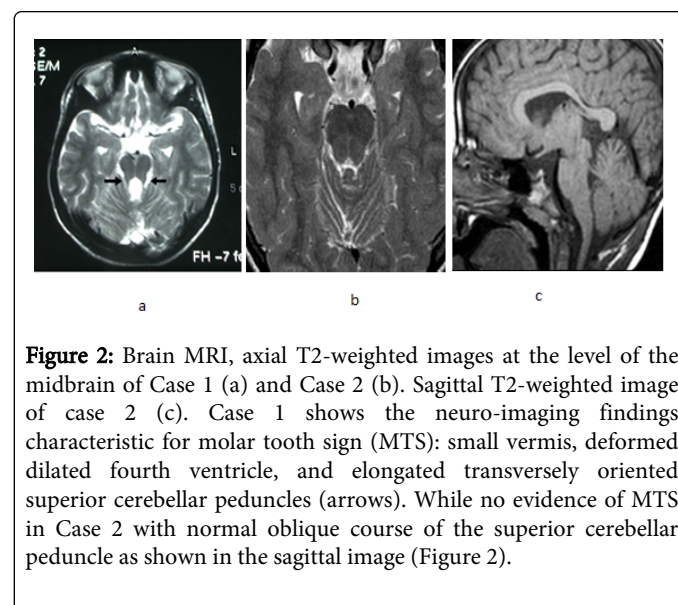


Figure 2: Brain MRI, axial T2-weighted images at the level of the midbrain of Case 1 (a) and Case 2 (b). Sagittal T2-weighted image of case 2 (c). Case 1 shows the neuro-imaging findings characteristic for molar tooth sign (MTS): small vermis, deformed dilated fourth ventricle, and elongated transversely oriented superior cerebellar peduncles (arrows). While no evidence of MTS in Case 2 with normal oblique course of the superior cerebellar peduncle as shown in the sagittal image (Figure 2).

Genetic analysis

In both sibling cases, we performed genome wide linkage and homozygosity mapping using 1 million single nucleotide polymorphism (SNP) microarrays from Affymetrix. We identified extensive homozygosity on chromosome 1, 7, and 8 indicating inheritance from a common ancestor. The homozygous region on chromosome 1 included the NPHP4 gene. Sanger sequencing of all 30 NPHP4 exons revealed a homozygous one base pair insertion (c. 2618dupA) leading to a frameshift (pH is 873Glnfs*14) and most likely loss of function at the amino acid level (Figure 3).

Results and Discussion

The phenotypic complexity of JSRDs mirrors their genetic heterogeneity. To date, mutations in 21 different genes have been associated with Joubert syndrome including AHI1, NPHP1, CEP290, TMEM67/MKS3, ARL13B, CC2D2A, RPGRIP1L and more recently INPP5E which have been detected in JSRD patients with variable phenotypes. Adding more complexity, mutations in many JSRD genes

have been found also to cause other ciliopathies, including isolated NPHP (NPHP1), Senior-Looken syndrome (NPHP1 and CEP290), Leber congenital amaurosis (CEP290) and Meckel Gruber syndrome (TMEM67/MKS3, CEP290 and RPGRIP1L) [17,18].

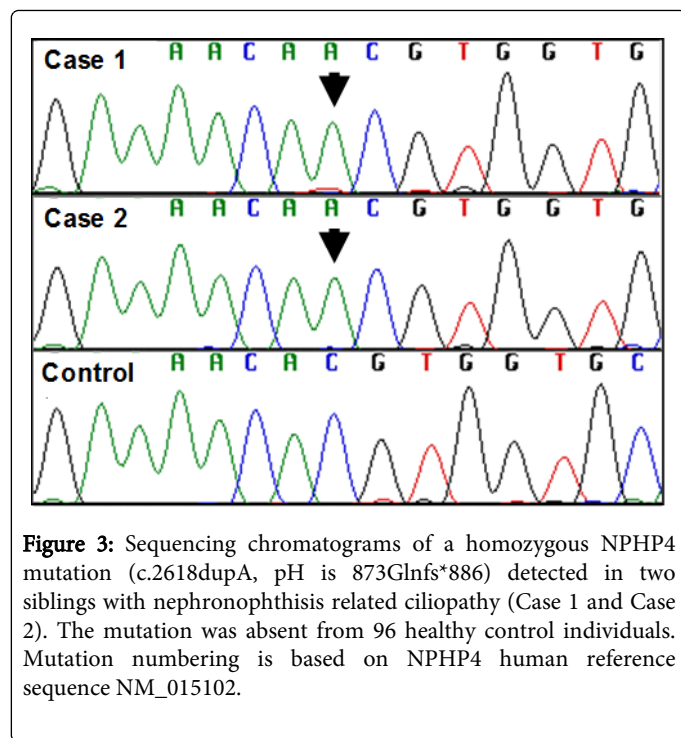


Figure 3: Sequencing chromatograms of a homozygous NPHP4 mutation (c.2618dupA, pH is 873Glnfs*886) detected in two siblings with nephronophthisis related ciliopathy (Case 1 and Case 2). The mutation was absent from 96 healthy control individuals. Mutation numbering is based on NPHP4 human reference sequence NM_015102.

JSRD results from ciliary dysfunction which also includes Meckel syndrome (MKS [MIM 249000]), Bardet-Biedl syndrome (BBS [MIM 209900]), nephronophthisis (MIM 256100), and Leber congenital amaurosis (LCA [MIM 204000]). These disorders, termed ciliopathies, [19] share both phenotypic features (retinal dystrophy, polydactyl, cystic renal disease, and hepatic fibrosis) and molecular causes [20,21]. These features significantly overlap with other disorders with cerebello-oculo-renal involvement, most notably NPHP; the significance of this relationship is strengthened by the identification of deletions of NPHP1, a gene commonly mutated in NPHP in a subset of JS patients [22,23]. The data suggest a tantalizing connection between intraflagellar transport in cilia and brain development [24].

Here we report on two siblings with variable clinical phenotypes of ciliopathy of JSRD where genetic linkage and mutation analysis identified the pathogenic mutation in the NPHP4 gene. Phenotypically the siblings were best categorized as cerebro-oculo-renal syndrome (CORS) using the Joubert syndrome and related cerebellar disorders classification system tested in Egyptian families [25].

Interestingly both siblings shared the progressive renal disease, albeit the different chronic kidney disease (CKD) stages and absence of cysts in the younger sibling (case 2). This can be explained by the fact that cysts might appear later in the course of NPHP. Regarding the eye phenotype, both siblings had retinal dystrophy as evidenced by subnormal ERG response though more advanced in the elder sibling with solid evidence of cone dysfunction and oculomotor apraxia that couldn't be elicited in his younger brother. Most notably, the index case (case 1) had significant ataxic symptoms and signs that started early in life but no ataxic manifestations could be detected in the younger sibling to date. The brain MRI of the index case demonstrated

mild MTS which could not be detected in his younger sibling. Also the elder sibling had facial dysmorphism that, again, was lacking in his younger brother. This highlights the clinical and phenotypic intrafamilial variability within this family with a homozygous truncating NPHP4 mutation.

The NPHP4 gene located on chromosome 1p36 encodes a 1,426 amino acid protein called nephrocystin-4/nephroretinin. Nephrocystin-4 interacts with nephrocystin-1 and is probably involved in the same intracellular signaling pathway [26]. Mutations in NPHP1 have been identified in patients with Joubert syndrome [21]. Of note, nephrocystin-4 localizes to the connecting cilium of photoreceptor cells and interacts with RPGRIP1 [RP guanosine riphosphatase (GTPase) regulator interacting protein 1], a component of cone and rod photoreceptors that is mutated in patients with Leber amaurosis [27].

To date, 93 different mutations have been described for the NPHP4 gene, 46 of which are predicted to be truncating the encoded protein. These mutations have been exclusively identified in patients with nephronophthisis, with or without retinal involvement. The identification of a truncating mutation in 2 siblings with neurological signs, presented in the current study, expands the spectrum of phenotypes associated with NPHP4. For some of the NPHP genes it has been shown that mutations within the very same gene can cause a wide range of different phenotypes spanning from very severe dysplastic to mild degenerative phenotypes. This multiple allelism has been described for the NPHP genes NPHP1, NPHP3, NPHP6, and NPHP8. The question, whether the presence of this homozygous truncating mutation, identified here, is necessary and sufficient to cause the variable neurological symptoms in both siblings cannot be finally answered. Modifying alleles in other genes might as well be implicated in the overall phenotypic disease outcome. Such epistatic effects have been described recently in patients who carry deleterious homozygous NPHP1 deletions in combination with heterozygous NPHP6 or AHI1 mutations [28,29].

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