Stop codon at arginine 586 is the prevalent nephronophthisis type 1 mutation in Italy

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Introduction

Nephronophthisis (NPHP) is an autosomal recessive disease with prevalent renal manifestations, characterized by occasional cysts in medulla and severe tubulo-interstitial fibrosis, evolving to end-stage renal failure [1]. It represents the most frequent cause of uremia in children, with major clinical, physiological and social consequences including high costs for substitutive approaches and renal transplant. NPHP is a clinical and genetic heterogeneous disease with at least five genes (NPHP1–5) identified and variable extra-renal manifestations [2–6]. Retinal dysfunction constituting Senior Loken syndrome (SLS1–5) is the most common association [1]. Other organ defects identify specific subsets such as liver fibrosis in NPHP3 and situs inversus in NPHP2. NPHP1 [OMIM #256100] represents the most frequent variant. On a molecular basis, the large majority of patients with NPHP1 present a large homozygous deletion [7] at 2q13 [NPHP1-del] that includes nephrocystin, the NPHP1 gene [2,8]. Patients with NPHP1 gene mutations are rare. A revision of the literature reveals only one case with compound heterozygous mutation, while less uncommon is the combination of [NPHP1-del] with a point mutation of NPHP1 ‘in trans’ [2,8–13], which has been reported in 11 cases (Table 1). Hildebrandt et al. [12] calculated the probability of compound heterozygous NPHP1 mutation in 1 out of 10⁹. Based on these findings, molecular analysis of NPHP1 is indicated only in patients with heterozygous [NPHP1-del] [12].

We report here the final results of the screening for NPHP1 mutations in patients with clinical NPHP, for whom the presence of homozygous [NPHP1-del] had been previously excluded. Four cases with NPHP1 mutations were found, one with compound heterozygous mutation and three with an association with [NPHP1-del] that challenges the concept of rarity and suggests that mutation analysis be done in patients with clinical signs of NPHP.

Case

We considered a cohort of 122 children who were followed at different nephrology units in Italy for a clinical suspicion of NPHP based on selected clinical and pathological criteria. They included a history of polyuria and polydipsia with urinary concentration defect (failure to increase fresh urine osmolality over 400 mOsm after Desmopressin stimulation), and ultrasound images with small hyper-echoic kidneys without cortico-medullary differentiation. The presence of extra-renal manifestations were evaluated with appropriate clinical approaches depending on the organ of interest (i.e. fundus oculi, electroretinogram for retinal defects, liver biochemistry and ultrasound for liver fibrosis). All patients were from Italy, with a predominance of patients from southern Italy (60%). Our focus was on NPHP1, and all patients were first tested for the presence of homozygous [NPHP1-del] at 2q13 [14] independently from the clinical background (i.e. presence or absence of extra-renal manifestations). Fifty-six presented homozygous [NPHP1-del], while the remaining 62 patients were checked for mutations involving nephrocystin (NPHP1). In the four cases presenting mutations, molecular analysis was extended to the other family members (father, mother and siblings when available).
Results

Four children out of 66 of our study cohort presented an NPHP1 mutation. In three unrelated cases, coming from different parts of Italy, there was the same nucleotide change [c. 1756C>T] resulting in a premature stop codon at Arginine 586 [p. R586X]. This change was associated with heterozygous [NPHP1-del] in two and with compound heterozygous [c. 1122+2dupT] in one (Table 1). Prediction analysis of this duplication, using the Neural Network Splice site program (NNSplice), showed low probability for maintenance of splicing effect on this obligatory donor splice site [15]. The fourth child presented another stop mutation that was [c. 1122+2dupT] [1122+2dupT] p. [Arg586X]+[NPHP1-del]p

Table 2. Clinical data of Italian patients with NPHP1 mutations. Patient G510, G1500, G800 carrying the same [p. R586X] mutation

<table>
<thead>
<tr>
<th>Code No</th>
<th>Born (d/m/y)</th>
<th>Age onset</th>
<th>ESRD</th>
<th>HD/TX</th>
<th>Clinical data</th>
<th>Nucleotide change</th>
</tr>
</thead>
<tbody>
<tr>
<td>G510</td>
<td>09/08/91</td>
<td>8 years</td>
<td>N/N</td>
<td>Low</td>
<td>Small/Hyperechoic</td>
<td>c. [1557T&gt;G]m+[NPHP1-del]m</td>
</tr>
<tr>
<td>G1500</td>
<td>30/08/87</td>
<td>12 years</td>
<td>Creat. 3 mg%</td>
<td>Low</td>
<td>Small/Hyperechoic</td>
<td>c. [1576C&gt;T]m+[1122+2dupT]m</td>
</tr>
<tr>
<td>G800</td>
<td>19/09/85</td>
<td>5 years</td>
<td>Y/N</td>
<td>Low</td>
<td>Small/Hyperechoic</td>
<td>c. [1576C&gt;T]m+[1122+2dupT]m</td>
</tr>
</tbody>
</table>

Creat., serum creatinine; Ur. Osm., urine osmolality; Y, yes; N, no; pmaternal origin; mmaternal origin.

Discussion

There are a few main results of our screening study that deserve consideration. The first is that the frequency of NPHP1 mutation in Italy is not as rare as previously reported and affects four patients out of 60 with clinical and molecular diagnosis of NPHP1. One of these presented an NPHP1 compound heterozygous mutation that is a very rare occurrence, being so far described in only one patient [11]. A parallel aspect is that we found the same mutation [p. R586X] in three patients, which represents, therefore, a prevalent mutation in our country. The possibility of a common ancestor in our patients cannot be ruled out. Unfortunately, the unique polymorphic markers inside the deletion region are not informative to define a haplotype. The unique information we obtained was about the association of [p. R586X] mutation with the G allele of Single Nucleotide Polymorphism (SNP) Rs11675767 at exon 7, which is the only coding SNP of NPHP1 gene and is present in 60%
of Caucasians (NCBI, dbSNP). Our cohort should be considered representative of the Italian population of NPHP since our laboratory serves as referral for NPHP1 diagnosis for several nephrology services. Assuming the former group of 56 patients with homozygous [NPHP1-del] plus the four with NPHP1 mutations described here as representative of the Italian frequency, we can conclude that the percentage of cases with NPHP1 mutation (4 out of 60) is 6% of the entire population of NPHP1 patients, which is not negligible on statistical basis.

In conclusion, mutations of NPHP1 can be found in 6% of all proven NPHP1 patients, which seems not negligible for diagnostic purposes. A premature stop codon mutation at Arginine 586 was found in Italy. Routine NPHP1 mutation analysis should be included in diagnostic flow chart of NPHP.

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Conflict of interest statement. None declared.

References


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