Evidence for further genetic heterogeneity in nephronophthisis

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Abstract

Background. A new type of nephronophthisis (NPH) has been recently identified in a large Venezuelan kindred: adolescent nephronophthisis (NPH3) causes end-stage renal disease (ESRD) at a median age of 19 years. The responsible gene (NPHP3) maps to 3q21-q22. NPH3 shares with juvenile nephronophthisis (NPH1) the same disease manifestations such as polyuria, polydipsia, and secondary enuresis. Histopathological findings consist of tubular basement membrane changes, cysts at the corticomedullary junction, and a chronic sclerosing tubulointerstitial nephropathy. The only difference is a younger age at ESRD in NPH1 (median age of 13 years) when compared with NPH3.

Methods. In order to evaluate whether there might be a fourth locus of isolated nephronophthisis, we studied eight NPH families without extrarenal disease manifestations and without linkage to the NPH1 locus (NPHP1) on chromosome 2q12-q13. ESRD was reached at ages ranging from 7 to 33 years. Individuals were haplotyped with microsatellites covering the genetic locus of NPHP3. Infantile NPH (NPH2) was excluded in all families by the clinical history and histological findings.

Results. In four of the examined families haplotype analysis was compatible with linkage to the NPHP3 locus. In one of these families identity by descent was observed. In contrast, in another four families linkage was excluded for NPHP3.

Conclusion. Four NPH-families were neither linked to NPHP1 nor to NPHP3, indicating further genetic heterogeneity within the group of nephronophthisis. The finding of further genetic heterogeneity in NPH has important implications for genetic counselling.

Keywords: nephronophthisis; NPHP1; NPHP3; chronic renal failure; linkage analysis

Introduction

Nephronophthisis (NPH), an autosomal recessive inherited tubulointerstitial nephropathy, is the major genetic cause for chronic renal failure in children [1]. We recently described in a large Venezuelan kindred a novel type of NPH, adolescent nephronophthisis (NPH3). Using a homozogosity mapping strategy we localized the responsible gene (NPHP3) within a 2.4 cM interval on human chromosome 3q21-q22 [2], which is syntenic to the murine renal cystic disease locus pcy on mouse chromosome 9 [3]. Adolescent nephronophthisis shares with juvenile nephronophthisis (NPH1) the same clinical symptoms and renal pathology findings. These findings consist of symptoms such as polyuria, polydipsia, and secondary enuresis, together with severe anaemia and progressive renal failure. As a result, many patients first come to medical attention when they have already reached ESRD.

Renal pathology is characterized by irregularly thickened tubular basement membranes, atrophy and dilatation of tubules, interstitial cell infiltration, and cysts at the corticomedullary junction. The only difference between both variants of NPH is a younger age at ESRD in juvenile nephronophthisis (median age at ESRD 13 years, range 7–25 years) when compared with adolescent nephronophthisis (median age at ESRD 19 years, range 11–46 years). However, both variants overlap within a wide age range where ESRD can occur. Thus both entities can only be distinguished by molecular genetic studies.

The gene locus for NPH1 was localized to 2q12-q13. The responsible gene NPHP1 has been identified [4–6]. In about 70–80% of patients with juvenile nephronophthisis, large homozygous deletions involving this gene are found [7,8]. An additional gene locus for infantile nephronophthisis (NPHP2) was localized to 9q22-q31 [9]. Clinical and morphological findings of infantile nephronophthisis (NPH2) clearly differ from juvenile and adolescent nephronophthisis. NPH2 has an early disease onset and causes end-stage renal disease (ESRD) within the first 3 years of life [9–11]. The patients present with enlarged and echogenic
kidneys with lack of corticomedullary differentiation on sonography. They develop anaemia, hyperkalaemic metabolic acidosis, and hypertension [9]. Renal morphology in NPH2 consists of a diffuse sclerosing tubulointerstitial nephritis and microcystic dilatation of proximal tubules and Bowman’s space. In contrast to other forms of NPH predominantly microcysts are observed in the cortex, and medullary cysts and thickened tubular basement membranes are absent [11].

In this study, we examined eight families with nephronophthisis without extrarenal involvement and absence of linkage to NPHP1. Onset of ESRD ranged from 7 to 33 years of age. Infantile NPH was excluded by clinical history and histopathological findings in these families. All families were haplotyped for highly polymorphic markers covering the NPHP3 locus to test whether adolescent nephronophthisis may contribute to the disease in the families examined. Four of the eight NPH-families showed a haplotype analysis compatible with linkage to NPHP3. Thus we found evidence of further genetic heterogeneity in nephronophthisis, because in the other four families linkage to NPHP3 was excluded.

Subjects and methods

Clinical details

Only families with isolated nephronophthisis and no extrarenal disease manifestation were included in the study. In families (F26, F30, F32, F443, F444, F623, and F624) clinical details and exclusion of the NPHP1 locus [12–14], and in F430 clinical details have been reported previously [15]. For F430 we excluded the NPHP1 locus by haplotype analysis using polymorphic microsatellites covering the critical region of the NPHP1 locus (Figure 1) [12,16]. Diagnosis of nephronophthisis was based on pedigree data (multiple affected siblings and absence of renal disease in the parents), chronic renal failure and typical findings on renal pathology. At least in one affected individual of every family a renal biopsy was performed. Histology showed in all biopsies tubular basement membrane changes such as folding, splitting, thickening and attenuation, tubular dilatation and atrophy, and a sclerosing tubulointerstitial nephropathy, and was therefore fully consistent with NPH1 or NPHP3. Only in F623 was a family history for consanguinity present.

Polymerase chain reaction (PCR) analysis and haplotype studies

Blood samples were obtained following informed consent. Genomic DNA was isolated by standard methods directly from blood samples [17] or after Epstein–Barr virus (EBV) transformation of peripheral blood lymphocytes [18]. PCR was performed in a volume of 10 μl containing 6–18 pmol of primers, 0.2 mmol/l each dATP, dGTP, and dTTP, 2.5 mmol/l dCTP, 0.1 mmol/l [γ-32P]ATP, 10 mmol Tris–HCl (pH 7.3), 50 mmol KCl, 0.001% gelatin (w/v), and 0.3 U of Thermus aquaticus DNA polymerase (Gibco BRL, Karlsruhe, Germany). Amplification was carried out with denaturation at 94°C for 30 s, annealing at 52–64°C for 90 s, and extension at 72°C for 40 s. PCR products were separated by electrophoresis in 7% denaturing polyacrylamide sequencing gels. The gels were blotted onto Whatman paper and dried. Autoradiography was performed for 2–16 h. Microsatellite results were interpreted independently by two investigators. Highly polymorphic microsatellite markers that localize to the NPHP3 locus were studied [2]. Haplotype analysis was performed using the program Cyrillic 2.0 (Cherwell Scientific, Oxford, UK).

Results

Haplotype analysis for the NPHP3 locus

Four of the eight families with NPH examined (F26, F32, F430, F624) showed haplotype analyses compatible with linkage to the NPHP3 locus (Figure 2A). In F624 we identified a homozygous haplotype for markers D3S1292, D3S1273, D3S1290, D3S3713, and D3S1238 in both affected siblings, compatible with homozygosity by descent, indicating consanguinity among the parents. We did not observe any haplotype

Fig. 1. Exclusion of the NPHP1 locus for NPH family F430 by haplotype analysis. Haplotype analysis of four consecutive microsatellite markers of the NPHP1 locus on chromosome 2q12-q13 for NPH family F430. Flanking markers are D2S1893 (centromeric) and D2S1888 (telomeric). Solid symbols denote affected individuals; open symbols denote unaffected individuals. Maker order is from top to bottom: D2S1890, D2S1893, D2S1888, and D2S160. Haplotypes in the affected individual and one healthy sibling are the same, thus excluding linkage of the disease status to the NPHP1 locus.

Fig. 2. Haplotype analysis of five consecutive microsatellite markers of the NPHP3 locus on chromosome 3q21-q22 in the families with NPH examined. Flanking markers are D3S1292 (centromeric) and D3S1238 (telomeric). Solid symbols denote affected individuals; open symbols denote unaffected individuals. Maker order is from top to bottom: D3S1292, D3S1273, D3S1290, D3S3713 and D3S1238. Panel A shows the results of families with haplotype analyses that were compatible with linkage to the NPHP3 locus (F26, F32, F430, and F624). All affected siblings share the same haplotype and have a haplotype that is different to that of healthy siblings. Note that both affected individuals of F624 are homozygous for the same haplotype, indicating homozygosity by descent. Panel B shows the haplotypes of families without linkage to the NPHP3 region (F30, F443, F444, F622, and F623). Affected siblings do not share the same haplotype, thus excluding linkage of the disease status to the NPHP3 locus.
sharing between the families with NPH examined. Four of the examined families had haplotype analyses that exclude linkage to the \textit{NPHP3} locus (Figure 2B). Since there was no linkage to \textit{NPHP1} either, we conclude that there must exist at least one additional locus for isolated NPH.
The median age at ESRD is 12 years (range 7–22 years) in the families in whom haplotypes were compatible with linkage to the \( NP\text{HP}3 \) locus, whereas it is 22 years (range 11–33 years) in the NPH families without linkage to \( NP\text{HP}3 \) and \( NP\text{HP}1 \).

**Discussion**

In this study we performed haplotype analyses in eight families with NPH and absence of extrarenal disease manifestations with highly polymorphic markers covering the \( NP\text{HP}3 \) locus [2]. Four NPH families showed a haplotype analysis compatible with linkage to the \( NP\text{HP}3 \) locus. We observed no haplotype sharing between the families examined that might indicate any relationship between the examined families. However, in F624 we identified a homozygous haplotype in both affected siblings compatible with homozygosity by descent. Both parents originate from the same remote area. There was no family history for consanguinity in that family, indicating a very remote relationship between both parents. However, haplotype analyses of this particular family and the other three NPH families are not sufficient to prove that adolescent nephropathy may be responsible for NPH in a subset of the examined families, because each individual family is too small to obtain a significant LOD score for linkage. Interestingly in the families with haplotype analyses that were compatible with linkage to \( NP\text{HP}3 \) the age range at which ESRD occurred was compatible with the range found in juvenile NPH, and unlike the age range found in the original Venezuelan kindred, in which linkage to \( NP\text{HP}3 \) was first discovered.

The other four families with NPH did not show evidence of linkage to any examined NPH locus, demonstrating further genetic heterogeneity within the group of nephropathies (Figure 2B). From this result, we conclude that at least one other NPH locus remains to be identified. The finding of further genetic heterogeneity in NPH is important implications for both genetic counselling and the isolation of the \( NP\text{HP}3 \) gene. The existence of genetic heterogeneity in this disorder confounds the clinical use of linkage analysis in NPH families without mutations of the \( NP\text{HP}1 \) gene, because families are often too small to allow definitive determination of linkage to \( NP\text{HP}1 \) or \( NP\text{HP}3 \). Additional linkage studies including total genome scans need to be performed to identify other NPH loci.

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**References**


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