Exclusion of the candidate genes ACE and Bcl-2 for six families with nephronophthisis not linked to the NPH1 locus

Heymut Omran1, Karlsten Häfner1, Martin Vollmer1, Johannes Pigulla1, Giskard Wagner1, Gianluca Caridi2 and Friedhelm Hildebrandt1

1University Children’s Hospital Freiburg, Freiburg, Germany and 2Division of Pediatric Nephrology, G. Gaslini Children’s Hospital, Genova, Italy

Abstract

Background. Nephronophthisis (NPH) is an autosomal recessively transmitted kidney disease, characterized by cyst formation at the cortico-medullary junction, and a sclerosing tubulointerstitial nephropathy. Juvenile nephronophthisis (NPH1) is the most common genetic cause of renal failure in children and maps to chromosome 2q12-q13. The responsible gene NPHP1 has been identified and encodes for nephrocystin. Not all families with NPH demonstrate linkage to that locus.

Methods. We studied six families with NPH without linkage to the NPH1 locus. In order to attempt identification of a new causative gene, the candidate genes ACE (angiotensin converting enzyme) and Bcl-2 (B cell leukaemia/lymphoma 2 gene) originating from mouse models, were examined. For the six families highly polymorphic microsatellites covering the whole candidate gene regions were haplotyped and linkage analysis was performed.

Results. Haplotype analyses of all families examined were incompatible with linkage of the disease status to ACE or Bcl-2. Linkage analysis excluded both candidate gene regions with a LOD-score of \(< -2\).

Conclusions. This study excluded the candidate genes ACE and Bcl-2 for NPH. Additional linkage studies need to be performed in order to identify further genes responsible for nephronophthisis.

Key words: ACE; Bcl-2; chronic renal failure; linkage analysis; nephronophthisis

Introduction

Nephronophthisis (NPH), a hereditary kidney disease, is the major genetic cause for chronic renal failure in children [1]. Fanconi et al. introduced the term familial juvenile nephronophthisis to describe a disease characterized by autosomal recessive inheritance pattern, defect in urinary concentrating capacity, severe anemia and progressive renal failure leading to death before puberty [2]. Juvenile nephronophthisis presents with renal symptoms such as polyuria, polydipsia, failure to thrive, pallor, unexplained fever and decreased urine concentrating ability. End-stage renal disease is reached on average at the age of 13 years [3]. Renal pathology is characterized by irregularly thickened tubular basement membranes, tubular atrophy and dilatation, sclerosing tubulointerstitial nephropathy, and cysts at the cortico-medullary junction [4]. By linkage analysis a gene for juvenile nephronophthisis (NPH1) was mapped to chromosome 2 [5], and subsequently a disease causing gene (NPHP1) was identified [6]. About 80% of affected individuals exhibit large homozygous deletions involving NPHP1 [7].

Previously, we showed genetic heterogeneity in juvenile NPH and excluded for most of the studied families with NPH the NPH1 locus by haplotype analysis and deletion testing [3]. In order to attempt identification of a new causative gene, candidate genes originating from mouse models were examined. In mouse models of targeted gene disruption, deficiency of both gene doses of ACE (angiotensin converting enzyme) [8–10] and Bcl-2 (B cell leukaemia/lymphoma 2 gene) [11] phenotypes strongly resembling that of human nephronophthisis have been described. Both genes have been proposed as candidate genes for human NPH [9,11]. We therefore examined the respective loci by linkage analysis in six families with NPH.

Subjects and methods

Clinical details

Only families with multiple affected individuals were included in the study. None of the NPH-families studied had evidence of extrarenal manifestations such as retinitis pigmentosa,
liver fibrosis or cerebellar dysfunction. Four families (F10, F32, F86, F92) had two affected offspring and two families (F30, F93) had three affected offspring. Pedigrees of all families apart from F93 had been reported previously [3]. Parents were not consanguineous and had no history of kidney disease. Most of the affected individuals became symptomatic when they reached end-stage renal disease. End-stage renal disease ranged from 9 to 22 years (median age 18.5 years). Preceding symptoms consisted of polyuria and polydipsia. At least in one affected individual of every family a renal biopsy was performed. Histology showed in all biopsies a sclerosing tubulointerstitial nephropathy, tubular dilatation and atrophy and tubular basement membrane changes such as folding, splitting, thickening and attenuation, and was therefore fully consistent with NPH. In families F10, F30, F32, F86 and F92 exclusion of the NPH1 locus was reported previously [3]. Haplotype analysis using the same marker set excluded linkage to the NPH1 locus also for F93 (data are not shown).

**PCR analysis and haplotype studies**

Genomic DNA was isolated by standard methods [12] directly from blood samples or after EBV transformation of peripheral blood lymphocytes [13]. PCR primers were synthesized with an Applied Biosystems Model 392 DNA/RNA synthesizer. PCR was performed in a volume of 10 ml containing 0–18 pmol of primers, 0.2 mM each dATP, dGTP, and dTTP, 2.5 mM dCTP, 0.1 ml C/miI [aza-3P]dCTP, 10 mM Tris–HCl (pH 7.3), 50 mM KCl, 0.001% gelatine (w/v), and 0.3 U of Thermus aquaticus DNA polymerase (Perkin-Elmer Cetus). Amplification was carried out with denaturation at 94°C for 30 s, annealing at 55–58°C for 90 s, and extension at 72°C for 40 s. PCR products were separated by electrophoresis in 8% 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 ACE and Bcl-2 regions were examined [14,15]. LOD score analysis of linkage between the presence of a potential disease causing gene and each of the marker genes was performed using the computer program MLINK of the LINKAGE package version 5.01 [16]. On the basis of pedigree data, an autosomal recessive mode of inheritance was assumed for the linkage analysis. For the calculation 100% penetrance and a gene frequency 0.00010 was used. Only affected individuals were used for LOD score calculation. Haplotypes were produced using the CYRILLIC program (version 2.0). LOD scores were analysed using the program LODVIEW [17].

**Results**

Linkage analysis in the six families with NPH yielded maximum two point LOD score results < −2 covering the whole critical region of ACE flanked by markers D17S807 and D17S789 (Fig. 1A). Linkage analysis in the examined six families yielded maximum two point LOD score results < −2 covering the whole critical region of Bcl-2 flanked by markers D18S1147 and D18S555 (Fig. 1B). These results exclude linkage of the disease status of these families with NPH to ACE and Bcl-2. Haplotype analyses of all families were not compatible with linkage to the examined candidate gene loci (data are not shown).

**Discussion**

Little is known about disease mechanisms of nephronophthisis (NPH). Even with the identification of NPH1 and its gene product nephrocytacin, which contains a SH3 domain and is mutated in NPH1 [6], only hypotheses can be generated about putative pathogenetic mechanisms of nephronophthisis. This study used a candidate gene approach to identify further genes responsible for nephronophthisis. We analysed the candidate genes ACE and Bcl-2 for the following reasons: (i) mutant mice deficient for both gene doses of ACE showed decreased urinary concentrating capacity, low blood pressure and progressive renal failure, and exhibited histological findings highly reminiscent of human NPH and consisting of hypoplasia of the renal papilla, medullary cysts, tubular atrophy and dilatation, and interstitial inflammation [8,10]. Apart from hypoplasia of the renal papilla these findings are characteristic for the nephronophthisis-medullary cystic disease complex Carpenter et al. assumed that diseases of this complex could be caused by ACE deficiency [9]. Therefore the ACE gene appears as strong candidate gene for nephronophthisis. (ii) Bcl-2 deficient mice demonstrated hypopigmented hair, lymphoid apoptosis, growth retardation and progressive renal failure. On patholgy polycystic kidneys with dilatation of distal tubules and collecting ducts, and hyperproliferation of the interstitium and epithelium were noted [11]. In addition to this very similar renal pathology, human nephronophthisis shares with Bcl-2 deficient mice the association with hypopigmented hair [18]. However, other findings in Bcl-2 deficient mice such as severe apoptosis of the spleen and thymus are not associated with NPH. Cell–cell and cell–matrix interactions influence tissue architecture by modulating cell proliferation, migration, differentiation, and apoptosis. The demonstration of an altered distribution of beta-catenin and actin, in kidneys from Bcl-2 deficient mice, may indicate improper cell–cell interactions, which might interfere with renal maturation and contribute to renal cyst formation. A similar disease process is assumed in NPH1, because nephrocytacin encodes a src-homology (SH3)-domain [19], and src-homology domains are known to be implicated in highly specific protein–protein interactions as well as in cell adhesion processes.

In this study we excluded the candidate genes ACE and Bcl-2 for the six examined families with NPH not linked to the NPH1 locus by linkage analysis. In none of the families haplotype analysis was compatible with linkage, thus ruling out a primary defect of ACE and Bcl-2 as responsible for NPH in the examined families. Future identification of other genes responsible for nephronophthisis might help to elucidate disease mechanisms of tubulointerstitial fibrosis and renal cyst.
Fig. 1. Two point LOD score results of highly polymorphic microsatellites covering the candidate gene regions of ACE (A) and Bcl-2 (B). The x-axes represent the respective candidate gene regions. Examined microsatellites are positioned according to corresponding intermarker distances. The funnel-shaped curves centred around most of the examined microsatellites represent the sum of LOD scores in relation to the distance to the examined marker. Flanking markers are underlined. A LOD score < −2 (dotted line) is significant for exclusion of a potential disease locus.

development. Therefore additional linkage studies including total genome scans need to be performed.

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