Identification of a gene for nephronophthisis

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Nephronophthisis Type 1 (NPH1)

Juvenile nephronophthisis (NPH) is an autosomal recessive cystic kidney disease [1,2], which leads to end-stage renal failure at an average age of 13 years following the initial symptoms of polyuria, polydipsia, renal salt loss, and secondary enuresis [3,4]. NPH constitutes the most common genetic cause of chronic renal failure in the first two decades of life. The histologic characteristics of NPH consist of thickening and disintegration of the tubular basement membrane, together with interstitial lymphohistiocytic infiltrations. At the bases of the tubular epithelial cells a marked increase of microfilaments is seen. Following the development of tubular atrophy and cysts the picture finally merges into a chronic tubulointerstitial fibrosis [5]. Extrarenal manifestations of NPH are well known. One example is the association of NPH with retinitis pigmentosa in the so-called Senior-Løken syndrome [6,7]. Since no specific protein defect was known to be responsible for the histologic changes of NPH, we followed the strategy of positional cloning to isolate a gene responsible for NPH.

By linkage analysis a gene locus for NPH, termed NPH1, had been localized to chromosome 2q12-q13 by Antignac et al. [8]. Subsequently, this localization was confirmed [9], and it was shown that there is at least one additional gene locus present in the human genome [10]. The NPH1 genetic region was cloned as a means to identifying the responsible gene [11–13], and the presence of large homozygous deletions of the NPH1 critical region was demonstrated in 80% of inbred or multiplex NPH1 families [14,15].

The NPH1 gene is a novel gene

In order to identify the gene responsible for NPH1 we set out to clone the NPH1 critical region in three extensive PAC contigs containing 66 novel PAC clones and a partial transcriptional map of 27 expressed sequences [16–18]. Three of these PAC clones (107o20, 62p20, 146c2) were found to be at least partially deleted in patients with NPH1. The ends of these PAC clones were sequenced, and two of them, when compared with electronic sequence data bases identified two partial cDNAs (zc07a11 and yy63e10). A DNA probe was derived from zc07a11 and additional cDNAs were isolated to yield a composite cDNA of a novel gene termed NPHP1 [19]. The NPHP1 gene was characterized and consists of 20 exons, as was recently confirmed [20]. Almost the entire gene was found to be deleted on both chromosomes in 16 of 22 families with NPH1, with one family exhibiting a somewhat smaller deletion. In the affected individuals of three additional NPH1 families we demonstrated the presence of a deletion on the paternal chromosome together with a maternal point mutation on the other chromosome, which most likely conveys loss of function. From these results we conclude, that in NPHP1 we have identified the gene responsible for NPH1.

Northern blot analysis revealed a transcript of 4.5 kb, with strong expression in skeletal muscle and weak expression in kidney, pancreas and heart. Sequence data base comparison of the full-length cDNA sequence showed that in NPHP1 we identified a novel gene, which is unrelated to known gene families. However, a short partial sequence of the deduced amino acid sequence of the gene product nephrocystin showed strong similarity to src-homology 3 (SH3) domains. There was strong similarity to the c-ckr proto-oncogenes from mouse and man as well as to other SH3-containing genes of Drosophila, the nematode Caenorhabditis elegans and the yeast Schizosaccharomyces pombe. Secondary structure prediction yielded a putative amphipathic helix at the amino terminus of nephrocystin, which in way of a coiled-coil domain might play a role in protein–protein interaction. Such interactions have been described for the two gene products of autosomal-dominant polycystic kidney disease (ADPKD1 and ADPKD2) [21,22].

A functional hypothesis for nephrocystin

The pathogenesis of NPH is still obscure. There are many functional changes in NPH that point to the
convergence of a pathological mechanism at the level of tubular function, like the early occurrence of decreased urinary concentration ability and the histologic changes that emphasize a central role of altered tubular structure in the pathogenesis [23]. Rahilly and Fleming have favoured the hypothesis of a primary tubular basement membrane (TBM) defect in NPH by demonstration of abnormal expression of the α5 integrin fibronectin receptor in the tubular basement membrane of patients with NPH [24]. This lead to the hypothesis that renal tubular cells in NPH express α5 integrin fibronectin receptor as a compensatory mechanism for defective function of the α6 integrin molecule. This, in turn, might lead to destruction of the TBM, which is a typical histologic finding early in the course of NPH.

From this first information on putative functional domains of nephrocystin we derive a testable pathogenetic hypothesis on its function: since SH3 domains constitute binding domains of adaptor proteins like crk in focal adhesions, and since there is high sequence conservation between nephrocystin and crk, nephrocystin by analogy might play a role in cell-matrix interactions through focal adhesions (Figure 1). Focal adhesions function in signal transduction from the extracellular matrix to the nucleus. One of the signaling pathways is following the route over integrin molecules, focal adhesion kinase, the proto-oncogene crk, a guanine nucleotide exchange factor and Ras to the nucleus (Figure 1). The hypothesis, that NPHP1 might play a role in focal adhesion signalling, is underlined by the fact that the best animal model for NPH so far is represented by a mouse model of targeted gene disruption for tensin [25], which is yet another component of focal adhesion signalling.

Conclusion

In summary, we have identified a novel gene, termed NPHP1, which is mutated in nephronophthisis type 1. This will provide a start point to study the function of its gene product nephrocystin in tubular cell-matrix interaction and its pathogenetic role for renal cyst development, and tubulointerstitial fibrosis. Through the demonstration of homozygous deletions in NPH1 patients and through the identification of the NPHP1 gene, molecular genetic diagnosis has become available for NPH1.

Acknowledgements. F. H. was generously supported by a grant from the Deutsche Forschungsgemeinschaft (Hi 381/3-3) and the Zentrum Klinische Forschung I, Freiburg (A1).

References