Detection of two different nonsense mutations in exon 44 of the PKD1 gene in two unrelated Italian families with severe autosomal dominant polycystic kidney disease

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Abstract. Sixty-seven Italian patients with autosomal dominant polycystic kidney disease (ADPKD) were screened for mutations in the PKD1 gene. We used PCR, heteroduplex and single-strand conformation polymorphism DNA analysis, and automated DNA sequencing for exons 35, 36, 38, 44 and 45. We detected abnormal heteroduplexes in affected individuals from two unrelated families with clinically severe ADPKD phenotype. These changes were absent in other, unaffected members, as well as in the probands of the other families studied. DNA sequencing revealed in both cases different C to T transitions in exon 44, which created premature stop codons. Both mutations altered restriction sites, and the abnormal patterns were observed in all the affected family members. To our knowledge these are the first nonsense mutations described in the PKD1 gene.

Key words: ADPKD; mutation detection; nonsense mutations; PCR; PKD1 gene

Introduction

With an estimated incidence of ≥1 in 1000, ADPKD is one of the most common inherited diseases, and the most frequent genetic cause of renal failure in adults. It accounts for ~10% of cases of end-stage renal disease (ESRD). The disease is characterized by bilateral progressive cystic dilatation of the renal tubules, leading to ESRD in adult life. Hepatic cysts, cerebral aneurysms and cardiac valve abnormalities may also be found [1]. At least three different genes are involved: PKD1, the major locus, on chromosome 16p13.3; PKD2 on chromosome 4q13-23; and PKD3, still not localized [2-5]. The identification of PKD1 was reported in 1994, along with its complete sequence [6-8]. However, mutation detection has been hindered by the reiteration of over three-quarters of the PKD1 gene proximally on the same chromosome. Moreover, these repeated PKD1-like sequences are transcriptionally active [6]. The PKD1 product, polycystin, appears to belong to a new class of membrane-bound protein. The precise role(s) of the PKD1 protein is still unknown, even though the deduced peptide sequence has revealed that it has multifunctional domains, likely to be involved in cell–cell and/or cell–matrix interactions [9]. In order to better characterize the molecular defects responsible for ADPKD in affected Italian families, we set up a mutation screening strategy based on PCR, heteroduplex and single-strand conformation polymorphism (SSCP) analysis, and DNA sequencing. We detected novel nonsense mutations in affected individuals from two families with a clinically severe ADPKD phenotype. These are the first nonsense mutations described in the PKD1 gene.

Materials and methods

Description of the families

The two families (VR4001 and VR4137T) come from northern Italy, and have five and three examined affected members respectively. They have been described previously [10,11].

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Table 1. The PKD1 nonsense described in this study

<table>
<thead>
<tr>
<th>Family</th>
<th>Mutation</th>
<th>Exon</th>
<th>RE</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>VR4001</td>
<td>C→T12332&lt;sup&gt;a&lt;/sup&gt;</td>
<td>stop at 4041</td>
<td>-MspAlI</td>
<td>10</td>
</tr>
<tr>
<td>VR4137</td>
<td>C→T12269</td>
<td>stop at 4020</td>
<td>+DdeI</td>
<td>11</td>
</tr>
</tbody>
</table>

<sup>a</sup>Previously described as C3817T (Q1273X) [10], when only the partial cDNA sequence of PKD1 was published [6]. Nt = nucleotide; RE = restriction enzyme site created (+) or abolished (−).

DNA studies

DNA extraction, electrophoresis, PCR, primers and conditions were as described previously [10–14].

Heteroduplex analysis

Heteroduplex analysis was performed using hydrolink mutation detection enhancement (MDE) gels (AT Biochem, Malvern, PA), following the recommendations of the manufacturer, including the addition of 15% (wt/vol) urea. Briefly, 20 µl of the PCR product was denatured by heating at 95°C for 5 min. DNA samples were then allowed to cool down at 37°C for at least 2 h before loading. Gels were 40 cm long and 0.8 mm thick, were run at 10 V/cm, stained with ethidium bromide and photographed under UV light [15].

RNA extraction and reverse transcription (RT)-PCR

Total RNA was extracted from freshly prepared Ficoll-Paque (Pharmacia, Uppsala, Sweden) containing peripheral lymphocytes using the RNaseasy Total RNA kit (QIAGEN, Chatsworth, CA), following the instructions of the manufacturer. cDNA was synthesized from 2 µg of total RNA in a volume of 50 µl. RT-PCR was performed using either random hexamers or polyT.

Restriction analysis

Restriction analyses were performed according to the instructions of the manufacturer (New England Biolabs).

Automated DNA sequencing

The PCR products for sequencing were cloned in pCR-Script™ SK(+) plasmid vector (Stratagene), in the presence of Sr/I. Automated sequencing was performed on an ALF DNA Sequencer (Pharmacia LKB) using an Autoread Sequencing Kit (Pharmacia), following the instructions of the manufacturer.

Results

We looked for disease-causing mutations in the PKD1 gene in 67 ADPKD patients from 50 unrelated Italian families. Using PCR, heteroduplex analysis and SSCP for exons 35, 36, 38, 44 and 45, we detected novel aberrant bands in affected individuals from two families (VR4001 and VR4137T) which were absent in other unaffected members, in the probands of other families studied and in normal controls. Cloning and automated DNA sequencing revealed C to T transitions in the same exon 44, which created different premature stop codons (Q4041X and R4020X) (Table 1). Both mutations altered restriction sites for MspAlI and DdeI, and the abnormal restriction patterns were observed both on genomic DNA and on cDNA from all the affected family members. RT-PCR performed on lymphocyte mRNA showed that both the mutant and the normal transcript are represented. Thus the PKD1 message appeared stable.

Discussion

The search for PKD1 gene mutations in a large number of patients has thus far led to the identification of only a small number of defects, limited to single families, and including deletions, splicing mutations and nonsense mutations, indicating allelic heterogeneity [6,16]. In all cases aberrant transcripts were identified. Moreover, large deletions disrupting and inactivating the PKD1 and TCS2 genes have been described in six severely affected infantile PKD patients [17].

Sixty-seven Italian ADPKD patients from 50 unrelated families were screened in our lab for mutations in the 3’ unique region of the PKD1 gene, using heteroduplex DNA analysis. We have detected novel aberrant bands in affected individuals from two families segregating with the disease and associated with a severe ADPKD phenotype. Our findings show that the mutant PKD1 allele is efficiently transcribed, at least in lymphocytes, and that the loss of this portion of the PKD1 protein is sufficient to generate the disease. The mutations here described are expected to cause the loss of the last C-terminal portion of polycystin, including the whole intracytoplasmic tail terminus. This C-tail domain of the protein, ~230 amino acids long, is encoded by the unique 3’ end of the PKD1 gene, and is probably involved in the transduction of extracellular signals [9]. The PKD1 gene product, polycystin, has been shown to be a complex large multifunctional integral membrane glycoprotein, involved in cell–matrix or cell–cell interactions [7,9]. It might also work as a receptor for soluble factors, and/or be involved in controlling apoptosis [18]. In any case, its precise role is
still unknown, but polycystin would normally mediate interactions with the extracellular environment.

The severe phenotype associated with these mutations suggests that this part of the PKD1 gene is functionally critical. Moreover, we suggest that nonsense mutations in the PKD1 gene might be a common cause of disease.

Mutation C12269T occurs at a CpG site, consistent with the high mutation rate of this dinucleotide due to methylation-mediated deamination of 5-methylcytosine [19]. Moreover, exon 44 might also be a mutational hot spot, but further mutation screening along the entire length of the PKD1 gene will be necessary to confirm this hypothesis. To our knowledge, these are the first nonsense mutations described in the PKD1 gene.

Given the large size and the unusual rearrangements of the PKD1 gene, and the technology now available, we anticipate that detection of further mutations will not be a straightforward task. However, the complete sequence of the PKD1 gene is now known, which will allow mutation detection along the entire gene. Characterization of additional mutations, and correlations between genotype and phenotype should in turn lead to a better knowledge of the physiological role(s) of the PKD1 gene product and of the underlying molecular mechanism of disease.

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