Seven novel mutations of the PKD2 gene in families with autosomal dominant polycystic kidney disease

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Background. Autosomal dominant polycystic kidney disease (ADPKD) is genetically heterogeneous, with at least three chromosomal loci accounting for the disease. Mutations in the PKD2 gene on the long arm of chromosome 4 are expected to be responsible for approximately 15% of cases of ADPKD.

Methods. We report a systematic screening for mutations covering the 15 exons of the PKD2 gene in eight unrelated families with ADPKD type 2, using the heteroduplex technique.

Results. Seven novel mutations were identified and characterized that, together with the previously described changes, amount to a detection rate of 85% in the population studied. The newly described mutations are two nonsense mutations, a 1 bp deletion, a 1 bp insertion, a mutation that involves both a substitution and a deletion (2511AG→C), a complex mutation in exon 6 consisting of a simultaneous 7 bp inversion and a 4 bp deletion, and the last one is a G→C transversion at the PKD1 locus in cystic epithelia that may be a missense mutation. Most of these mutations are expected to lead to the formation of shorter truncated proteins lacking the carboxyl terminus of PKD2. We have also characterized a frequent polymorphism, Arg-Pro, at codon 28 in this gene. The clinical features of these PKD2 patients are similar to the previously described, with the mean age of end-stage renal disease being 75.5 years (SE ± 3.8 years).

Conclusions. Our results confirm that many different mutations are likely to be responsible for the disease and that most pathogenic defects probably are point or small changes in the coding region of the gene.

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common Mendelian disorders in humans and the most frequent genetic cause of renal failure in adults [1]. ADPKD is a genetically heterogeneous condition, with at least three genes involved: PKD1, which is located in 16p13.3 and which accounts for the majority of ADPKD cases [2–4]; PKD2, which is located in 4q21-q22 [5, 6]; and the much rarer PKD3, which is not yet mapped [7, 8]. The PKD1 and PKD2 genes have been recently cloned and characterized [9–13].

Mutation screening in the PKD1 gene has been difficult because of its size (cDNA of approximately 14 kb) and complexity (approximately 75% of the gene is duplicated). Most of the mutations reported in PKD1 produce premature translational termination resulting in truncated proteins [14–16]. These truncating mutations suggest that ADPKD is caused by the lack of normal protein. Furthermore, recent evidence of loss of heterozygosity at the PKD1 locus in cystic epithelia has been reported [17, 18], suggesting that cystogenesis in ADPKD results from the inactivation of the normal copy of the gene by a second somatic mutation.

The PKD2 gene consists of 15 exons with an open reading frame of 2904 bp and a 3’UTR of 2086 bp [13, 19]. Polycystin-2, the PKD2 gene product, is predicted to be a 968-amino acid integral membrane protein with six membrane-spanning domains and intracellular amino termini and carboxyl termini. There is a 25 to 30% identity and a 45 to 50% similarity between PKD2 and either a 450-amino acid portion of polycystin-1 or a 270 residue region of voltage-gated calcium channel a1E (VACCa1E). The cytoplasmic COOH terminal region of polycystin-2 contains a 29-amino acid segment similar to an EF-hand domain. Recent evidence suggests that the COOH terminus of PKD2 interacts with the coiled-coil domain of PKD1 protein [20, 21]. As the protein product of the PKD2 gene has homology to a family of voltage-gated Ca2+ channels in addition to PKD1, a physiological role in regulating transmembrane Ca2+ fluxes is suggested [13].

Key words: PKD2, cysts, ADPKD, gene mutations, heteroduplex.
Table 1. Mutations and polymorphism in the PKD2 gene

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Location</th>
<th>Nucleotide change</th>
<th>Effect on coding sequence</th>
<th>Reference</th>
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<td></td>
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<tr>
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</tr>
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<td>Ins of C at 534</td>
<td>Frameshifting after 180</td>
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<td>Frameshifting after 222</td>
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<tr>
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<tr>
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<td>Splice mutation</td>
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<td>A→G at 710 – 2</td>
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<td>EX4</td>
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<td>Ala→Pro at 356</td>
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<td>EX5</td>
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<td>Trp→Gly at 414</td>
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Polymorphism | Location | Heterozygosity | Nucleotide change | Effect on coding sequence | Reference |
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<td>0.49</td>
<td>G→C at 83</td>
<td>Arg→Pro at 28</td>
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* Mutation found in two families
* Mutations identified in our sample

Currently, several mutations have been described in the PKD2 gene, and most of them are expected to produce truncated proteins [13, 22–24] (Table 1). Here we report the identification of seven new mutations in the PKD2 gene in unrelated Spanish and British families.

**METHODS**

**Family description**

Autosomal dominant polycystic kidney disease diagnosis was made according to standard criteria [25]. Linkage studies were performed with PKD1- and PKD2-linked markers to confirm PKD2 gene segregation in all of these families [4, 26, 27]. Eight families were screened for mutations in the PKD2 gene. Seven of them were from Spain (5 from Catalonia and 2 from the center of Spain), and one was from Great Britain. A brief clinical summary of those in which the mutation was found is shown in Table 2.

To evaluate the renal survival, the age of family members whose cause of death was uremia was considered, as well as age of end-stage renal disease (ESRD) for those who died after entering renal replacement therapy.

**Mutation screening**

DNA was prepared from peripheral blood following standard procedures. The 15 coding exons of the PKD2 gene and the associated splice donor and accepted sites
were amplified by polymerase chain reaction (PCR) using a set of 17 primer pairs described previously [19]. Exon 1 is amplified in three overlapping PCR products. Heteroduplex analysis was performed using Hydrolink Mutation Detection Enhancement (MDE™) gel solution (FMC Bioproducts, Rockland, ME, USA) with the addition of 15% (wt/vol) urea, as recommended by the manufacturer. Briefly, 20 μl of the PCR product were denatured by heating at 95°C for five minutes, were cooled at 37°C, and were then loaded. Gels were typically 25 cm long and 1.5 mm thick and were run 10 V/cm, stained with ethidium bromide, and photographed under ultraviolet light.

Automated DNA sequencing and restriction analysis

Automated sequencing of PCR products was performed on an ABI310 DNA sequencer (Perkin Elmer, Norwalk, CT, USA) using the cycle-sequencing method. Sequencing was carried out in both directions with the exon-specific primers. When a deletion or insertion was suspected, the PCR product was cloned in pMosBlue plasmid vector (Amersham, Arlington Heights, IL, USA) to confirm the mutation by sequencing individual bacterial clones. Restriction analysis was performed according to the manufacturer’s instruction.

Mutation designation

The mutations described in this study were designated following the recommendations of Beaudet and Tsui [28]. Nucleotides and codons were numbered according to the sequence published by Mochizuki et al [13].

Survival analysis

The survival time to the onset of ESRD was calculated through the method of product limit [29].

RESULTS

Mutation screening was performed by heteroduplex analysis of PCR products obtained from genomic DNA and automated DNA sequencing. The whole PKD2 coding region and all intron/exon boundaries were investigated in eight probands from unrelated ADPKD families in which linkage of the disease to the PKD2 locus has been demonstrated. Mutations were found in seven of the eight families and are reported in Table 1. Segregation of the mutation with the disease in each family was tested by either heteroduplex analysis or restriction site change.

Nonsense mutations

Two nonsense mutations were identified in our sample. In family C21, a 1249C→T transition that substitutes an arginine for a stop codon at codon 417 (R417X) was identified in exon 5. This mutation creates a novel DdeI restriction site in the sequence.

The mutation in family C85 consists of a 1753C→T transition in exon 8 that introduces a stop in codon 585 (Q585X). This mutation creates a NheI restriction site.

Mutations causing frameshift

In family P326, the disease was shown to be caused by the insertion of one C in a stretch of 6 Cs (nucleotides 198 to 203). This mutation (198 ins C) is expected to produce a translation frameshift after codon 68 that introduces 22 novel amino acids before premature termination.

The mutation in family 6019 consists of a 1753C→T transition in exon 8 that introduces a stop in codon 585 (Q585X). This mutation creates a NheI restriction site.

In family C113, the sequence of exon 13 revealed a mutation involving both substitution and deletion that affected the dinucleotide AG at position 2511-2 (2511 AG→C). This mutation caused a translation frameshift that introduced six novel amino acids before premature termination. This mutation produced the loss of a MboII restriction site.

In family 6138, the sequence of exon 6 revealed a complex mutation comprising both a substitution of a 7 bp (nucleotides from 1367 to 1373) and a 4 bp deletion (nucleotides 1377 to 1380). The substituted 7 bp stretch was, in fact, an inversion because the sequence matched that in the complementary original strand. Closer inspection of this region showed two pairs of short complementary sequences. The repeats of the first one, of 6 bp, are flanking both the inverted and the deleted sequence, and
the second pair, of 3 bp, flanks the inverted sequence (Fig. 1). The 5' copies of both pairs are together, whereas the 3' copies are separated by the deleted sequence. It is expected that an intracatenary alignment of this pairs followed by recombination would simultaneously produce the inversion and the deletion. The predicted effect of this mutation is a frameshift from amino acid 456 with a premature termination TGA codon after five residues. This mutation produces the loss of a DraI restriction site.

**Missense mutation**

The only change observed in family C76 was a 1066G→C substitution in exon 4 converting Ala356 to Pro (A356P). This transversion creates a StuI restriction site. To try to determine whether this amino acid change is a disease-causing mutation, another 15 unrelated PKD2 patients and 100 normal individuals were tested with StuI, and no similar change was observed.

**Polymorphism in exon 1**

Heteroduplex analysis of exon 1 showed a frequent polymorphism in several PKD2 patients. It consists of a 83G→C transversion converting Arg28 to Pro (R28P). This change creates a BanII restriction site. This polymorphism was assayed on 15 PKD2 patients and 25 normal individuals, and a heterozygosity of 0.49 was detected. There was no difference in the frequency of any allele between the affected or normal samples.

**Survival analysis**

The survival analysis, estimated through the method of Kaplan and Meier, showed a mean survival to ESRD of 75.5 years (± 3.8 years; data not shown).

**DISCUSSION**

Mutations in the PKD2 gene are expected to be responsible for approximately 15% of the cases of polycystic kidney disease [3, 4]. The PKD2 disease tends to run a milder course than the more common PKD1 disease, with longer patient survival and slower progression toward end-stage renal failure [4, 30–34]. The age at diagnosis is also higher with fewer renal cysts present at that time than in PKD1. It is unclear if extrarenal complications of the disease in PKD2 are less severe than in PKD1 [4], but liver cysts seem to occur to the same extent as in PKD1, and a PKD2 family with intracranial aneurysms has been reported [35]. The clinical features of the patients reported here closely resemble those reported in previous studies [4, 33].

In the search for PKD2-causing defects in Spanish and English families, we have identified seven novel mutations in exons 1, 4, 5, 6, 8, 11, and 13 of the PKD2 gene. They are two nonsense mutations, a 1 bp deletion, a 1 bp insertion, a change AG→C, and a complex mutation involving both an inversion and a deletion in exon 6. This complex mutation involves exonic sequences that are flanked by short inverted repeats that might allow the rearrangement to occur by intracatenary recombination. The clinical course of the disease in this family is that of typical PKD2. Two deceased members exhibited characteristic clinical signs and symptoms as a result of intracranial aneurysms. The expected functional consequence of this mutation is a frameshift translational change with a premature termination of protein synthesis. The shortened product would lack the five final transmembrane domains, as well as the carboxyl terminus. Therefore, no evident relationship could be established between this mutation and the presence of intracranial aneurysms in this family.

The seventh mutation is a missense mutation that remains to be confirmed as pathogenic. After an extensive study of the proband of this family (C76) by both heteroduplex and single strand conformational polymorphism (SSCP) analyses, we only found a change in exon 4, where a G is replaced by a C at position 1066. This alteration cosegregates with the disease in the family and is not present in 100 control individuals. The mutation results in the substitution of a alanine for a proline at codon 356 of the protein. An alanine at this position is conserved in the murine polycystin-2 [36]. According
to the predicted model of the PKD2 gene product, alanine-356 is located in the first extracellular loop. It is interesting that the other missense mutation described so far in this gene (W414G) [23] is also located in this loop. It is possible that this extracellular loop could interact with other molecules or be important for the folding of the protein in the membrane. The introduction of a proline at this position may disrupt the secondary structure of the molecule, resulting in a hampered protein function. The proband of this family entered ESRD at 53 years, an age that can be considered early for this disease. It should be noted that in this study, it has not been determined whether any of the seven mutations are actually stable post-transcriptionally or post-translationally.

No mutation was found in one family with classic PKD2 and strong familiar evidence of linkage to this locus. All coding regions have been studied by heteroduplex and SSCA (not shown) analyses in this family, and the results suggest that a mutation could lie in the promoter region, or elsewhere in an intron, or could be due to a large rearrangement.

In summary, the germinal disease-causing mutation has been defined in 14 of 16 families of our sample (discussed in this article; Table 1) [24]. This high mutation detection rate suggests that most pathogenic defects are probably point or small changes in the coding region of the PKD2 gene. This fact makes the direct diagnosis of this type of ADPKD feasible and is especially useful for presymptomatic diagnosis as sonography scan may be ambiguous in young carriers.

Our findings also indicate that many different mutations, none present in more than one family, are responsible for the disease. However, three cases of recurrence have been described [23]. Thus, allelic heterogeneity appears to be a feature of PKD2. It should be noted that a mutation in family C113 (2511AG→C) occurs in the same region as that in family 6030 (2509G→T) previously described [24]. Similarly, the mutations in families 6019 (2152delA) and 6129 (2151delG) [24] occur close to a polyadenosine tract (nt 2152 to 2159) of exon 11 [37]. This could be considered a warm spot for changes because other frameshift mutations have been recently reported in this stretch [37, 38].

As these mutations occur throughout the gene and most of them are predicted to give rise to truncated proteins, the possibility that they are inactivating seems more likely. These mutations suggest that ADPKD type 2 is caused by a lack of normal protein (that is, haploinsufficiency), although the possibility of a second mutation in the normal copy of the gene, as suggested by a recent study in an animal model [39], cannot be excluded by our results. The truncation of polycystin-2, with the removal of different regions of its carboxy terminal portion, might disrupt the stability of the anchoring of the molecule to the cell membrane or its binding to other components, or the transduction of extracellular signals. Three different structures have been identified in the cytoplasmic end of this protein by computational analysis and/or preliminary in vitro studies. They are an EF-hand domain that could have Ca²⁺ binding activity and are encoded by exon 12 of the PKD2 gene [13,19], a region responsible for binding of the coiled-coil domain of PKD1, which resides in a fragment spanning the last 97 amino acids of PKD2, and an undefined domain that seems to be implicated in homodimeric interaction with the PKD2 protein itself [20, 21]. The most carboxy terminal mutations described so far are in exon 13: 2509G→T [24] and 2511AG→C (discussed in this article).

Even though the number of mutations detected in PKD2 patients is increasing, it is not possible at this stage to correlate genotype with phenotype. It is imperative to perform multicentric studies in order to establish if there is some kind of correlation between the mutations and the clinical features of the patients.

ACKNOWLEDGMENTS

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REFERENCES


Torra et al.: Mutations in the PKD2 gene