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Mutation Detection in the Repeated Part of the PKD1 Gene

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Summary

The principle cause of one of the most prevalent genetic disorders, autosomal dominant polycystic kidney disease, involves mutations in the PKD1 gene. However, since its identification in 1994, only 27 mutations have been published. Detection of mutations has been complicated because the greater part of the gene lies within a genomic region that is reiterated several times at another locus on chromosome 16. Amplification of DNA fragments in the repeated part of the PKD1 gene will lead to coamplification of highly homologous fragments derived from this other locus. These additional fragments severely hamper point-mutation detection. None of the point mutations published to date are located in the repeated part of the PKD1 gene. However, we have reduced the problems posed by the strong homology, by using the protein-truncation test, and we have identified eight novel mutations, seven of which are located in the repeated part of the PKD1 gene.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most frequently inherited disorders, with an estimated incidence of 1/1,000 in the Caucasian population (Dalgaard 1957). Mutations in the PKD1 gene account for ~85% of all ADPKD cases (Peters and Sandkuijl 1992). The gene was identified in 1994 (The European Polycystic Kidney Disease Consortium 1994). One year later the genomic sequence of the entire gene and its 14-kb transcript were elucidated (The American PKD1 Consortium 1995; Hughes et al. 1995; The International Polycystic Kidney Disease Consortium 1995).

However, few mutations have been found in patients with ADPKD. To date, 23 small mutations have been published (The European Polycystic Kidney Disease Consortium 1994; Peral et al. 1995, 1996*a*, 1996*b*; Turco et al. 1995; Neophytou et al. 1996; Rosetti et al. 1996), in addition to three mutations detectable on Southern blots (The European Polycystic Kidney Disease Consortium 1994; Roelfsema et al. 1996*a*) and one de novo translocation (The European Polycystic Kidney Disease Consortium 1994). The main problem involved here is the very strong homology between the greater part of the PKD1 gene and another locus, proximal on chromosome 16p (Germino et al. 1992; The European Polycystic Kidney Disease Consortium 1994). Only the 3′ end of the PKD1 gene is unique (fig. 1). Of the 23 small mutations published to date, 17 are within the unique region.

A large-scale screening for point mutations at this unique part of the PKD1 gene revealed a detection rate of 10%–15% (Peral et al. 1996*b*). This suggests that most mutations will be located in the repeat area. The homology poses an enormous problem, because all standard techniques used in point-mutation detection make use of PCR (Myers et al. 1987; Cotton et al. 1988; Orita et al. 1989; White et al. 1992). Amplification in the repeated area will, unfortunately, result in coamplification of homologous sequences derived from the proximal locus. It is extremely difficult to detect a mutated allele among this large number of wild type–like alleles. Moreover, the fragments derived from the homologous proximal locus do not have exactly the same sequence (The American PKD1 Consortium 1995) and could therefore result in false positives.

We chose the protein-truncation test (PTT) (Roest et al. 1993; Roelfsema et al. 1996*b*) as a solution to these problems instead of using technically demanding methods such as either cloning of genomic DNA from patients or long-range PCR with PKD1-specific primers. The PTT detects translation-terminating mutations on the basis of coupled transcription/translation reactions of PCR products. The artificially produced proteins represent small parts of polycystin-1, the PKD1 gene product. After size

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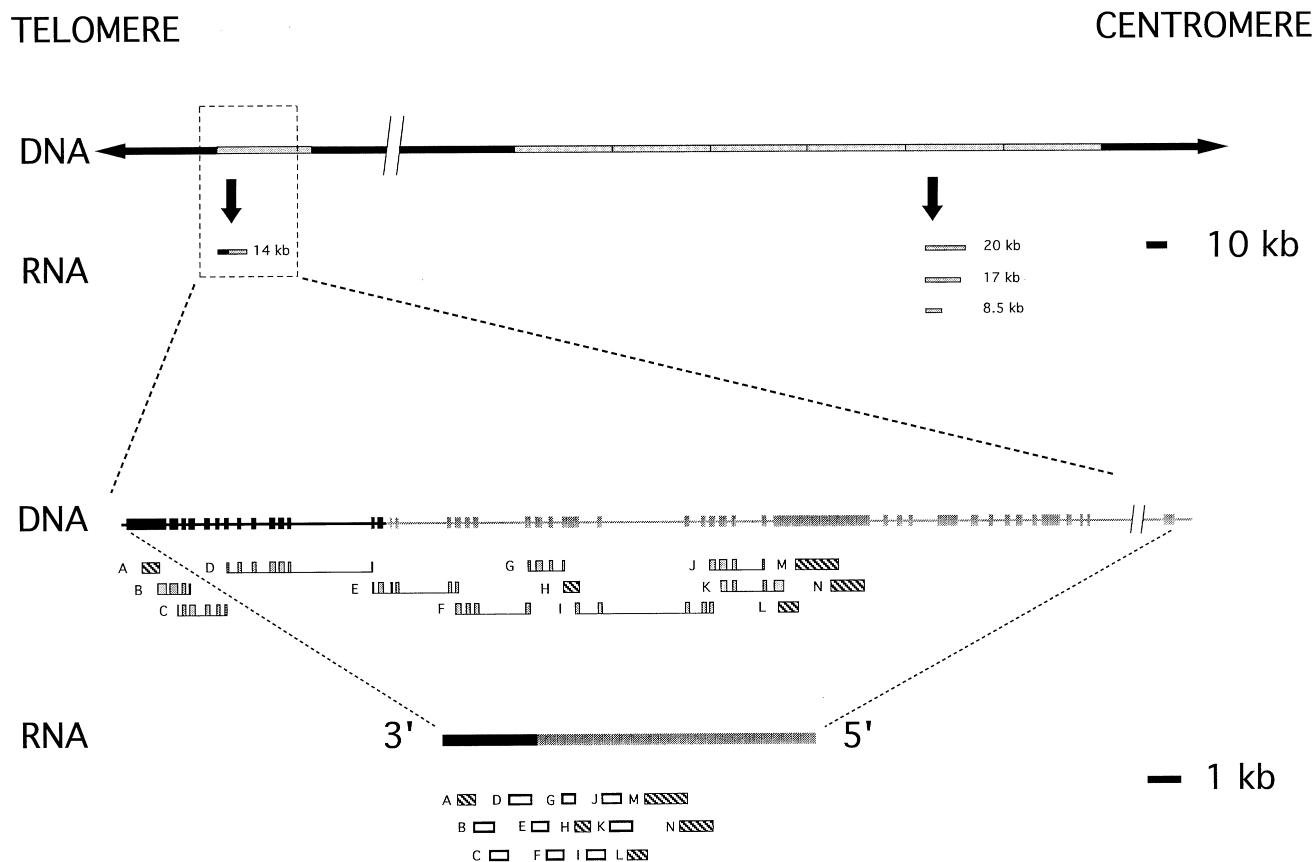

Figure 1 Model of repeated structure of PKD1 gene on chromosome 16p. The top line represents parts of chromosome 16p: the PKD1 gene and a region with strong homology to the gene. A large part of the PKD1 gene, encompassing ~75% of its coding sequence, lies in a region that shares very strong homology with a region, reiterated several times, at a proximal locus; six iterations are shown, but the precise number is unknown. This proximal locus encodes three transcripts, of 20 kb, 17 kb, and 8.5 kb. There is little sequence divergence between the PKD1 gene and the homologous locus: the homology is estimated to reach 97% (The American PKD1 Consortium 1995; The International Polycystic Kidney Disease Consortium 1995). The homologous parts are gray shaded, and the unique parts are blackened. Depicted in detail is the PKD1 gene, with its 46 exons and the ~14-kb transcript. The PCR fragments used in this study are indicated on the DNA and RNA level. Fragments that could be amplified directly from genomic DNA are indicated as diagonally hatched boxes.

determination by SDS-PAGE, truncated proteins that correspond to premature stops in the PCR product can then be detected. The PTT detects aberrant proteins instead of aberrant DNA fragments and is a very sensitive method.

Recently, the first mutations in the repeated part of the PKD1 gene were found by the PTT or by a nonistopic RNase-cleavage assay in combination with long-range PCR and an anchored PKD1-specific primer (Peral et al. 1997). We report mutations detected in the repeated part by only the PTT. The gene comprises either 12,906 bases of coding sequence (Hughes et al. 1995) or 12,909 bases of coding sequence (The International Polycystic Kidney Disease Consortium 1995). In our screening we covered 9,412 contiguous bases from the 3′ end onward. In total, eight mutations were found, seven of which are located in the repeated region.

Subjects and Methods

Family Selection and Isolation of DNA and RNA

In this study we analyzed RNA from a group of 20 individuals and DNA from 135 individuals of Dutch origin, all of whom were diagnosed as having ADPKD. When linkage analysis could be performed and the mutation was found not to be linked to the PKD1 locus, the family was not included in this study. Of the 135 individuals, 36 are single patients.

DNA was isolated from peripheral blood by use of standard procedures (Breuning et al. 1990). RNA was extracted from peripheral blood as well. Total RNA was isolated with RNAzol, according to the manufacturer's instructions (Cinna; Biotecx Laboratories).

Amplification of DNA and RNA

RNA was reverse transcribed by use of Superscript reverse transcriptase (Life Technologies). The cDNA synthesis was either randomly primed or primed with the KG8BP1A oligonucleotide, which anneals to a specific sequence in the 3′ UTR of the PKD1 transcript (Peral et al. 1994). In each reaction cDNA was used, synthesized from ~75 ng of total RNA.

Total genomic DNA was used for PCR on DNA. Concentrations were 200–300 ng in each reaction.

The PCR was performed in 50-ml reactions with *Taq* DNA Polymerase (Life Technologies). PCR buffer contained 50 mM KCl, 10 mM Tris-HCl pH 8.9, 100 mg of BSA, 0.01% gelatin, 400 pmol of each dNTP, and 10% glycerol. In all reactions 10 pmol of each primer was used. All fragments could be amplified with 1.5 mM $MgCl_2$, except for fragment G, for which 2.0 mM was used. The PCR was performed on a Hybaid Omnigene thermocycler, in 40 cycles. The forward primer contains a T7 phage promoter sequence and eukaryotic translation-initiation sequence (Kozak 1984), needed for the in vitro transcription/translation (Roest et al. 1993). The primers used to generate the fragments for the in vitro transcription/translation are listed in table 1. Amplification on RNA necessitated a booster PCR. The first PCR was performed with forward primers that were located 2–10 bp toward the 5′ end and that did not have a tail with the T7 promoter and Kozak consensus sequence.

PTT and Sequence Analysis

The PTT analyzes PCR fragments representing part of the open reading frame. The fragments, preferably >500 bp, are translated into small proteins. Therefore, amplification with genomic DNA as target can only be done with large exons, limiting our screening to exons 15 and 23. The remaining exons were amplified from RNA, by reverse transcriptase–PCR (RT-PCR).

The PTT was performed according to the method of Roest et al. (1993). All PCR products were analyzed on 2.5% agarose gels. In each in vitro transcription/translation reaction, 3.5 ml of PCR product was used. In addition to 6.25 ml of lysate, T7 polymerase, leucine-depleted amino acids, and buffer were added, all from the TNT Coupled Reticulocyte Lysate kit (Promega). In order to radioactively label the proteins, tritium-labeled leucine (Amersham) was added. The reactions were performed for 60 min at 30°C. Size determination of the reaction products was performed on a 14% SDS-polyacrylamide gel. Typically, 28 samples were loaded onto one gel. The gel was dehydrated by a wash in dimethyl sulfoxide (DMSO), followed by an incubation in DMSO/2,5-diphenyloxazole and a final wash in water. The gel was then dried on a vacuum slab-gel dryer and subsequently was autoradiographed by use of Kodak X-Omat film. Truncated proteins can be seen as strong bands with an intensity that nearly equals and sometimes even exceeds the intensity of the wild-type band. Additional fainter bands can also be seen on film and are probably caused by second translation-initiation sites, present in all samples, and mutations during PCR. In order to distinguish truncated proteins caused by true mutations, ⩾10 samples were loaded onto one gel. In addition, all positive individuals were analyzed again, in duplicate.

The PTT results in weak signals when PCR fragments with a short open reading frame are used. Fragment A in our study has a short open reading frame and was therefore used only for the individuals who were analyzed with RT-PCR as well, even though fragment A could be amplified on genomic DNA. After an exposure time of 2 wk, results were obtained for this group of patients.

PCR fragments were cloned with the pGEM-T vector (Promega) or with pBluescript. The clones were gridded in a square. The clones of each row and column were pooled. These pools, containing five to seven clones, were analyzed by the PTT. The positive rows and columns indicate the clones with the mutated allele. Subsequent sequence analysis was performed either with the automated-laser fluorescent-DNA sequencer (ALF) or manually with either the Amplicycle kit (Perkin Elmer) or the Sequenase kit (USB), according to the manufacturer's protocol. When it was possible, the sequence data were confirmed by restriction analysis. The mutant sequence of PK167 is based on two clones; the mutant sequences of PK56 and PK71 are each based on one.

Results

We screened exons 16–46 by RT-PCR, using 11 overlapping primer pairs, called "A"–"K," which together cover 6,336 bases (fig. 1). Each fragment is ⩽1 kb. In our experience, it is difficult to amplify larger fragments. Not only is the efficiency reduced, but extra products are generated as a result of faulty annealing of primers.

Amplification of genomic DNA is not hampered by these problems. A booster PCR is not needed, and much larger PCR products can be generated. Exon 15 is ideal for this purpose. We covered most of exon 15 by using three PCR fragments, called "L," "M," and "N," which are 0.8 kb, 1.7 kb, and 1.3 kb, respectively (fig. 1).

To test the possibility of genomic DNA–based screening, we first performed the PTT in combination with genomic PCR on patient PK153-1, amplifying exon 23. We had already found a truncation with fragment H, corresponding to exon 23, by using RT-PCR on RNA from this patient. Despite the fact that a genomic PCR results in a relatively greater proportion of homologous sequences, the truncated protein could be detected as clearly as it could be by use of RT-PCR. We screened

Table 1

Primers Used in Present Study

Fragment	Primer		
	Forward	Forward with Tail[a]	Reverse
A	TGACCGACTCAACCAGGCCACA	CTCAACCAGGCCACAGAGGACG	CGCAGGCCCCTCAGCCCTAGT
B	CGCCGCTTCACTAGCTTCGAC	GACCAGGTGGCGCACGTGAGCT	GGCCTTGCAGGCTGTGCAGCTG
C	GGCCCCAGGGTCCACACGTGC	CACACGTGCTCGGCCGCAGG	ACGCTCCAGAGGGAGTCCAC
D	CAGGTCCTTGCCGAGGGGGTC	GTCAGCAGCCCAGCCCCTACCC	GGACTCTCCCAGCCAACGTCG
E	TGGCTCTCCATATGGGACCGG	AGCCGTTTCACTCGCATCCAG	CGTTTCCATGTGGGTGTCTTGGG
F	TCTTCGTGCCCCCAAGCCATGTC	CGCTTTGTGTTTCCTGAGCCGA	AGTCGCCAACAGCCCCGTACC
G	GGTCCAGCCCCAGGCCTCCG	GTCGGTGCTGTGGTCACCCTG	ACTGGTCCAGCTTGTGCAGGATG
H		CTCATCCACCTGGCCAGCTCGG	TAGTGGCCGTCCAGCAGCGT
I	GCTGTCGCCAGGGCCACTGC	CGCTGTCGCCAGGGCCACTGC	CCAGCGTCAGGGGCTCCTCGT
J	GGCTGGCGGCTGGCGTGGAGTA	CTGGCGGCTGGCGTGGAGTACA	GGGCTCTGGGAGGGTGATGGC
K	AGTACCGCTGGGAGGTGTATCG	TACCGCTGGGAGGTGTATCGC	ACCTTGGTGGTGAGGGCGTG
L		AACTTCACAGCCCGCGTGCAG	TGATGGGCACCAGGCGCTCG
M		GATCTGGGGGACGGTGGGTGG	GAGCGGTTGGTGAAGCAGG
N		ACGTGGGACTTCGGGGACGG	AGGGATGGGCGTGCAGCGGT

[a] The tail consists of a T7 phage promoter and a eukaryotic translation-initiation site (sequence not shown).

genomic DNA from other individuals, but only PK153-1 has a mutation in exon 23.

Screening of exon 15 was performed with the same ease that characterized the screening of exon 23, except for fragment N, which showed a slightly complicated banding pattern on autoradiographs. Sequence divergence in some part of the region homologous to fragment N results in a premature stop. This leads to a truncated protein in all individuals. Performing the PTT on DNA from the somatic-cell hybrid HY145.19, which contains only the PKD1 locus and not the homologous proximal locus (Ceccherini et al. 1989), proved that the translation stop originates from the homologous locus. This truncated protein causes a complex pattern (fig. 2, right-hand gel, lanes C, PK71, and PK44). Nevertheless, mutations can be detected: two truncated proteins were clearly visible after screening of this fragment.

Screening the RNA of 24 affected individuals revealed two mutations (table 2 and fig. 2). One of these is the aforementioned mutation in exon 23; the other is a base substitution found both with fragment B and with fragment C, in exon 44. The screening of genomic DNA was performed on 135 affected individuals, and six additional mutations in exon 15 were detected (table 2 and fig. 2). One mutation was found in two individuals from separate families living in the same town, who therefore are likely to be related.

After the initial detection of a truncated protein in an individual, other family members were analyzed as well. However, additional family members were not available for all individuals with positive PTT results. Family 5, probably connected to family 133, is a large pedigree with $\geqslant 75$ additional family members. Family 167 is also reasonably large, with 12 members, 7 of whom are affected. Family 71 has five members: the two parents, one of whom is affected, and three children, one of whom is affected. Family 55 consists of two affected brothers. Cosegregation of truncated proteins with the disease was always seen in the additional affected members and never in unaffected individuals. However, the possibility remains that the mutation causing the truncation is present at the homologous proximal locus rather than in the PKD1 gene. Therefore, we performed haplotype analysis on families 5, 71, 133, and 167, with markers HBAP1 (Fougerousse et al. 1992), KG8 (Snarey et al. 1994), CW2 (Peral et al. 1994), D16S423 (Weissenbach et al. 1992), D16S509 (Gyapay et al. 1994), D16S406 (Weissenbach et al. 1992), and D16S3103 (Dib et al. 1996). These markers range from the PKD1 locus toward the homologous proximal locus. HBAP1 is distal to the PKD1 gene, and KG8 is located in the 3′ UTR of the PKD1 transcript. The other markers are proximal from the PKD1 gene, marker D16S3103 being close to the homologous proximal locus. The analysis revealed recombination events between the homologous proximal locus and the PKD1 gene in selected individuals from families 5 and 167 (fig. 3). These recombination events prove that their truncated proteins are derived from the PKD1 locus.

Premature stops in the repeated part of the gene can be detected by the PTT, without difficulty. However, sequence analysis of the fragments cannot be done by direct sequencing. Direct sequencing is hampered by the abundance of homologous fragments (fig. 4). Therefore, PCR fragments were cloned. For each fragment, 30–49 clones were divided into pools in such a way that each clone was present in two separate pools. The cloned fragments were analyzed with the PTT. This procedure enabled us to identify those clones that resulted in a truncated protein with the same size as that of the truncated protein from the patient. These clones were sequenced either manually or with an automated se-

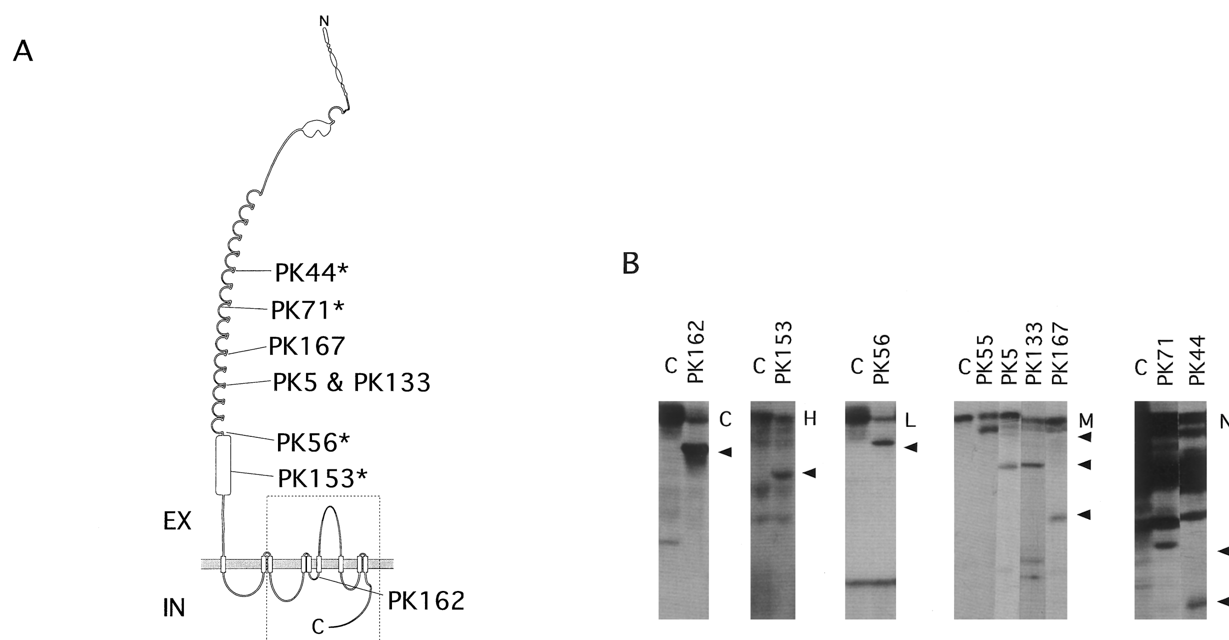

Figure 2 *A,* Model of polycystin-1, the PKD1 gene product, showing positions of mutations presented in this study. The patients indicated by an asterisk (*) have mutations that could not be confirmed by haplotype analysis. Their mutations could also derive from the homologous locus. The protein model was constructed on the basis of predictions by Hughes et al. (1995) and Moy et al. (1996). The part of polycystin-1 surrounded by a dotted-line box is encoded by the unique 3′ end of the transcript, and the remainder is encoded by the duplicated part. *B,* Panel of selected fragments from different SDS-PAGE gels. Truncated proteins, indicated by arrowheads, are recognizable in lanes containing samples from patients. The wild-type proteins vary in size but, for clarity, are shown at the same height. C = control. Additional, faint bands can be seen on the gels; these are probably caused either by second translation-initiation sites or by mutations during the PCR.

quencer. The PTT detects only translation-terminating mutations. Therefore, all mutations that we found are either base substitutions leading to a stop codon or frameshifts. The sequence of four mutations revealed small deletions leading to frameshifts. The precise location of the mutation in family 71, a deletion of C and A in a sequence (ACACACA) at positions 4036–4042, cannot be determined. Base substitutions were found in three individuals. The sequence analysis of the mutation in PK55 is in progress (table 2).

The mutant sequence was compared with the wild-type sequence, to determine the loss or creation of a restriction site. PCR fragments derived from genomic DNA from the patients were digested if a site was lost or gained. However, restriction enzymes that cut DNA very frequently are not practical for this purpose, because the aberrant restriction fragments will be overshadowed by the many other fragments that these enzymes produce. The restriction sites used to confirm mutations are listed in table 2.

Discussion

We successfully applied the PTT to detect translation-terminating mutations in the repeated area of the PKD1

Table 2

Mutations in the PKD1 Gene

Family/families	Mutation	Position[a]	PCR Fragment(s)	Exon	Restriction Site(s)[b]	Family Member Tested
162	G→A	12036	B, C	44	*Sau*3AI creation/*Ava*II loss	4
153	C→T	8692	H	23	*Ava*II loss	1
56	Deletion of 7 bp	6574–6580	L	15		1
5, 133	G→A	5622	M	15	*Hinf*I creation	34
55	Unknown		M	15		2
167	Deletion of A	5014	M	15		8
71	Deletion of 2 bp	4036/4042[c]	N	15		5
44	Deletion of T	3866	N	15	*Bgl*II creation	4

[a] According to the open reading frame presented by The International Polycystic Kidney Disease Consortium (1995).
[b] Only restriction sites tested on PCR fragments derived from genomic DNA are listed.
[c] Position cannot be determined precisely.

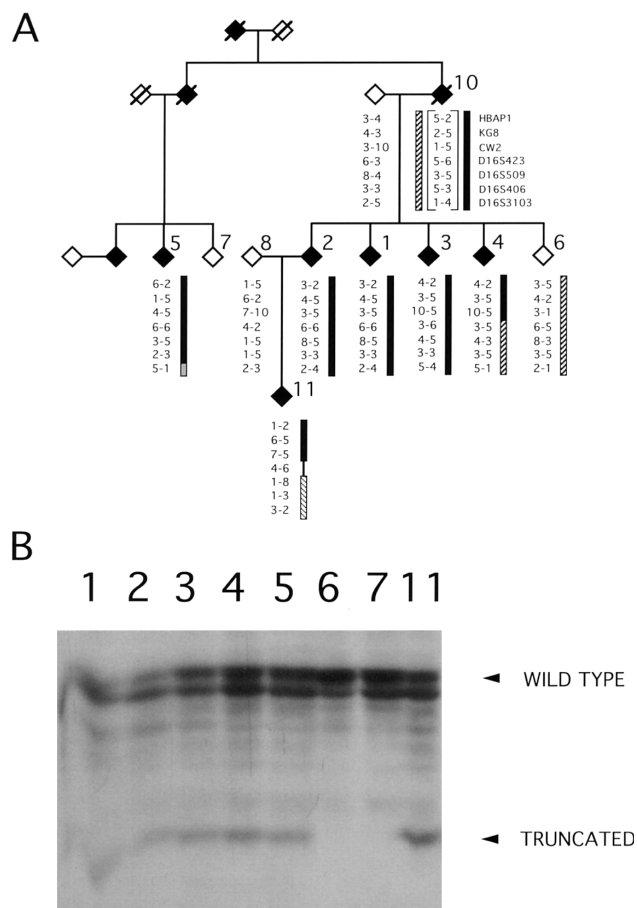

Figure 3 *A,* Pedigree of family 167, with haplotypes constructed of markers distal to the PKD1 gene, toward the proximal homologous locus. The haplotype cosegregating with ADPKD is blackened. The haplotypes of individuals 4 and 11 are the result of a recombination event between the PKD1 gene and the homologous proximal locus. The precise breakpoint of the recombination in individual 11 is not clear, because his parent is not informative for marker D16S423. A third recombination event must have occurred in this family as well, because the haplotype of individual 5 is recombined compared with those in the other branch of the family. *B,* SDS-PAGE gel with the in vitro translation products from family 167. All affected individuals show truncated proteins.

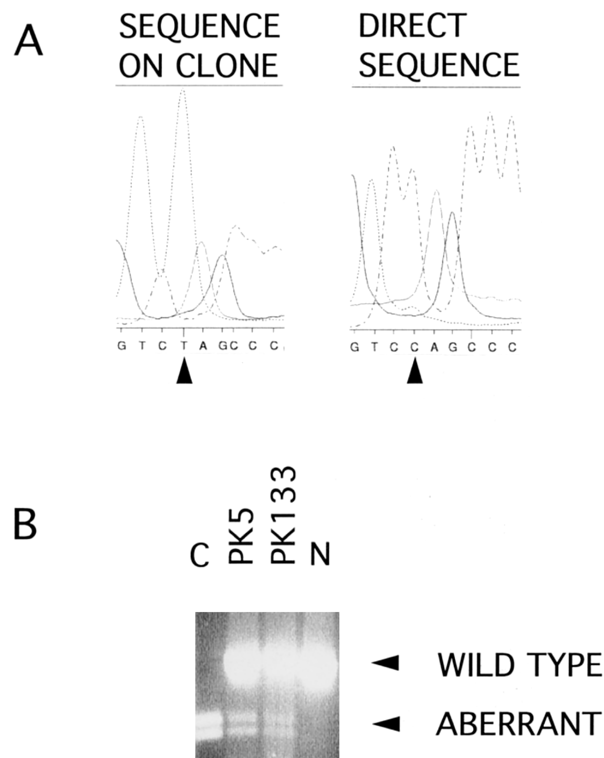

Figure 4 *A,* Comparison between direct sequence of PCR fragment H of patient 153-1 and clone of PCR fragment carrying the mutation in this patient. A very small peak indicating a thymine can be seen in the direct sequence, but it is almost completely overshadowed by the cytosine peak. *B, Hinf*I digest of a PCR product on an agarose gel. The mutation in families 5 and 133 creates a *Hinf*I site that produces two very weak bands, compared with the enormous amount of DNA from the wild type–like sequence. The PCR was performed on genomic DNA from two affected individuals, one each from families 5 and 133. N = healthy individual serving as a negative control; and C = PCR product derived from a clone carrying this particular mutation, used as a positive control.

gene. Mutation detection in the repeated part of the PKD1 gene is probably impossible with most standard methods, without cloning, long-range PCR, or PKD1-specific primers. Methods such as denaturing gradient-gel electrophoresis (Myers et al. 1987), SSCP analysis (Orita et al. 1989), chemical cleavage of mismatch (Cotton et al. 1988), and heteroduplex analysis (White et al. 1992) rely on detection of aberrantly migrating DNA fragments. The DNA fragments from mutations are likely to be overshadowed by the enormous abundance of the homologous sequences. The procedure presented in the present paper does not require PKD1-specific primers or long-range PCR (Barnes 1994). However, to characterize the mutations, cloning and subsequent screening of PCR fragments are needed.

The PTT is limited to detection of translation-terminating mutations. Therefore, missense mutations, causing amino acid substitutions, cannot be detected. In the case of PKD1 we have used this fact, which otherwise would be a limitation, to our advantage. The PTT misses the sequence variants derived from the homologous locus. A procedure for detection of missense mutations will probably be technically demanding. Moreover, the screening can be hampered by polymorphisms. Therefore, we chose to screen affected individuals for premature stops, as the first step.

The proportion of mutations identified in our screening is not very high. To a certain extent this is due to genetic heterogeneity, since we could not establish linkage with the PKD1 locus in all of the families that we

studied. However, genetic heterogeneity can account only for a relatively small number of cases; therefore, there must be other explanations as well. Missense mutations will not be found, and, if ADPKD is caused by a high frequency of missense mutations, this will account for the low proportion. However, to date, only one possible missense mutation at the unique part has been published (Peral et al. 1996*b*). Alternatively, mutations may cluster in a PKD1 region that we either did not screen extensively or did not screen at all. A similar effect could be caused by a high frequency of only a few mutations in our patients. These mutations would then remain to be identified. Furthermore, there can be various other, less obvious explanations. For example, inversions may occur between the PKD1 gene and its homologous region, similar to the mutation found in one-half of the individuals with hemophilia A (Naylor et al. 1993). However, FISH using two cosmids flanking the PKD1 gene in $\geqslant 10$ ADPKD patients has not revealed any evidence for inversions (J. G. Dauwerse, personal communication).

ADPKD is characterized by the formation of multiple cysts in the kidneys, cysts that grow during the course of a lifetime (Dalgaard 1957). Cysts can occur in other organs, particularly in the liver (Milutinovic et al. 1980; Gabow et al. 1984) but also in the pancreas and spleen (Milutinovic et al. 1989). Furthermore, patients have a higher risk for intracranial aneurysms (Chapman et al. 1992). Cardiac valvular abnormalities (Leier et al. 1984; Hossack et al. 1988) and colonic diverticula have been reported as well (Scheff et al. 1980). A striking aspect of ADPKD is that a minority, 1%–2%, of all nephrons are involved in cyst formation (Scheff et al. 1980). Nevertheless, all affected individuals will develop cysts in the kidney, but the extrarenal manifestations are subject to much inter- and intrafamilial variation. Although the PKD1 and PKD2 genes have both been identified (The European Polycystic Kidney Disease Consortium 1994; Mochizuki et al. 1996), it is not clear how the symptoms are caused. A second-hit model has been proposed to explain both the variability in phenotype and the fact that most nephrons remain apparently normal (Reeders 1992). Other models explaining the dominant nature of the disease include either a dominant-negative effect from the mutated allele or the lack of one functional copy of the gene (i.e., haploinsufficiency). Most premature translation stops that we have found predict small proteins, with a complete removal of the predicted integral membrane and cytoplasmic part of polycystin-1. Whether such small proteins have a biological role remains to be established. Not all mutations found lead to small proteins. The mutation in family 162 is a stop codon in exon 44, leaving the greater part of polycystin-1 intact. Others studies also have shown mutations in exon 44 (Turco et al. 1995; Neophytou et al. 1996; Rosetti et al. 1996). Peral et al. (1996*b*) have detected the mutation closest to the 3′ end of the transcript. This mutation causes a stop in exon 46, removing only 76 amino acids, which clearly indicates that this part has an important function. Mutations leaving the greater part of polycystin-1 intact can still lead to severe polycystic kidney disease, as has been shown by a case of infantile onset of the disease caused by a translation stop in exon 41 (Peral et al. 1996*a*).

Recently, evidence for somatic mutations in the PKD1 gene of epithelial cells of cysts has been presented (Qian et al. 1996). These mutations, occurring in the normal copy of the PKD1 gene, could be a second hit, leading to cyst formation. The mutations were deletions in eight cysts, found by detection of loss of heterozygosity of two intragenic polymorphic markers 2.5 kb apart in the unique region of the PKD1 gene, and a 2-bp deletion in one cyst. These nine somatic mutations were found in a total of 46 cysts, which means that the other cysts are still to be accounted for. Perhaps mutations will be found in other parts of the gene.

The apparent stochastic nature of renal cyst formation and other symptoms of ADPKD could be explained by a second-hit model. However, several other characterizations of ADPKD cannot be explained. Despite the intrafamilial variation, there is a recurrence risk for early onset of ADPKD in some families (Zerres et al. 1993). Recurrence of early onset has been observed in family 5 (Eulderink and Hogewind 1978), in which the mutation has now been characterized. Another feature of ADPKD is the formation of massive hepatic cysts, which is predominantly a disorder in women (Gabow et al. 1990). These findings suggest that other factors besides a second hit are significant in cyst formation. Genetic modifiers may influence the cause of ADPKD. Possibly this is the case in deletions removing or disrupting both the TSC2 gene and the PKD1 gene, causing severe early-onset renal cyst formation (Brook-Carter et al. 1994).

Cyst formation is a complex process, and the elucidation of this process, which includes mutation analysis, will probably take some time. Mutation detection in the repeated part of the PKD1 gene is now possible, at least for translation-terminating mutations. A procedure for detection of all types of mutations will be the next important step.

Acknowledgments

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