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A Spectrum of Mutations in the Second Gene for Autosomal Dominant Polycystic Kidney Disease (PKD2)

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Summary

Recently the second gene for autosomal dominant polycystic kidney disease (ADPKD), located on chromosome 4q21-q22, has been cloned and characterized. The gene encodes an integral membrane protein, polycystin-2, that shows amino acid similarity to the PKD1 gene product and to the family of voltage-activated calcium (and sodium) channels. We have systematically screened the gene for mutations by single-strand conformation–polymorphism analysis in 35 families with the second type of ADPKD and have identified 20 mutations. So far, most mutations found seem to be unique and occur throughout the gene, without any evidence of clustering. In addition to small deletions, insertions, and substitutions leading to premature translation stops, one amino acid substitution and five possible splice-site mutations have been found. These findings suggest that the first step toward cyst formation in PKD2 patients is the loss of one functional copy of polycystin-2.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most frequently inherited disorders, with an incidence of 1/1,000 individuals (Dalgaard 1957). The disease is mainly characterized by the formation and enlargement of multiple cysts in both kidneys, which can lead to renal failure in adults. In addition, extrarenal manifestations can occur, including cysts in other organs (such as the liver, pancreas, and spleen) (Milutinovic et al. 1980; Gabow 1990), hypertension (Florijn et al. 1992), and intracranial aneurysms (Chapman et al. 1992; van Dijk et al. 1995).

ADPKD is a genetically heterogeneous disease that can be caused by an alteration in at least three different genes. In 85% of families, the disease is caused by a mutation in the PKD1 gene, located on chromosome 16p13.3 (Reeders et al. 1985; Peters and Sandkuijl 1992). The gene was identified in 1994, and the gene product, polycystin-1, is predicted to be a transmembrane protein involved in cell-cell or cell-matrix interaction (The European Polycystic Kidney Disease Consortium 1994; Hughes et al. 1995; The American PKD1 Consortium 1995; The International Polycystic Kidney Disease Consortium 1995). Recently the PKD2 gene, located on chromosome 4q21-q22 (Kimberling et al. 1993; Peters et al. 1993), has been cloned and characterized (Mochizuki et al. 1996). Mutations in this gene are responsible for the disease in ~15% of the families (authors' unpublished observation). The gene consists of 15 exons with an open reading frame of 2,904 bp and a 3′ UTR of 2,086 bp (Mochizuki et al. 1996). The PKD2 gene product, polycystin-2, is predicted to be an integral membrane protein with six transmembrane domains and internal amino and carboxy termini. Polycystin-2 shows ~50% similarity to 450 amino acids of polycystin-1 and its *Caenorhabditis elegans* homologue ZK945.9 and to 270 residues of a voltage-activated calcium channel (Mochizuki et al. 1996). Only a few families have been reported in whom the ADPKD-causing mutation has not been linked to either chromosome 4 or chromosome 16 (Bogdanova et al. 1995; Daoust et al. 1995; de Almeida et al. 1995). A possible third gene, however, has not been identified yet.

In addition to the genetic heterogeneity of ADPKD, phenotypic variability has been observed (Parfrey et al. 1990; Bear et al. 1992; Gabow et al. 1992; Ravine et al. 1992). Individuals with the second type of ADPKD tend to show a milder clinical phenotype, compared with individuals with a mutation in the PKD1 gene. At the

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time of diagnosis, usually at an older age, these patients have fewer cysts and are less likely to have hypertension. They show a slower progression toward renal failure and have a longer life span. Nevertheless, ADPKD is essentially similar in both groups of patients.

Now that the PKD2 gene is identified, we can search for mutations in individuals affected with the second type of ADPKD. We have analyzed 35 families, using SSCP analysis (SSCA). After screening ~80% of the gene, we found mutations in 20 families. In addition to disease-causing mutations, a polymorphism was detected in exon 4. Further functional analysis of these mutations will have to show how inactivation of one copy of the PKD2 gene leads to cyst formation.

Subjects, Material, and Methods

Family Selection and DNA isolation

A total of 35 families with the second type of ADPKD were selected for mutation screening in the PKD2 gene. For all families, clear evidence against linkage to chromosome 16 was obtained (Nørby and Schwartz 1990; Ravine et al. 1992; Fossdal et al. 1993; Peters et al. 1993; Bogdanova et al. 1995; Coto et al. 1995; Veldhuisen et al. 1996; authors' unpublished observations). Linkage to chromosome 4 was established by at least two—but, in most families, more—informative CA-repeat markers around the PKD2 gene. Most of the asymptomatic at-risk individuals participating in this study were examined by ultrasonography, according to the criteria described by Bear et al. (1984). Mutations were found in families collected in the Netherlands (PK5069, PK5197, PK5179, PK5144, PK5118, PK5120, and PK5183), Spain (PK6526, PK6533, PK6534, and PK6537), Iceland (PK9502), Denmark (PK1080), England (PK5414), Australia (PK5403 and PK5404), Bulgaria (PK7827, PK7852, and PK7808), and Belgium (PK5609).

Several of these families have been described elsewhere: PK5069 (Peters et al. 1993; van Dijk et al. 1995; Veldhuisen et al. 1996); PK6526, PK6533, PK6534, and PK6537 (families 13, 14, 15, and 16, respectively, in Coto et al. 1995); PK9502 (family F2 in Fossdal et al. 1993; Peters et al. 1993); PK1080 (Nørby and Schwartz 1990; Peters et al. 1993); PK5403 and PK5404 (pedigrees 2 and 5 respectively in Ravine et al. 1992); PK5403 (family 13868 in Peters et al. 1993); and PK7827 and PK7852 (Bogdanova et al. 1995).

DNA was isolated from either blood leukocytes or Epstein barr virus–transformed lymphocytes by use of standard procedures (Breuning et al. 1990). DNA from one affected individual of each family was selected for SSCA. RNA was isolated from a lymphocyte cell line of one affected individual of family PK9502 and from blood leukocytes of several control individuals.

SSCA

Intronic primers were selected to amplify all exons and adjacent splice junction sites of the PKD2 gene (for primer sequences, see Hayashi et al., in press). Because of the size of the coding sequence of exon 1 (595 bp), primers were selected to amplify three overlapping products, of 248, 202, and 335 bp. Table 1 shows the primer pairs and annealing temperatures used for amplification, as well as the sizes of the PCR products. SSCA was performed according to protocols, with modifications described by Orita et al. (1989). Between 30 and 100 ng genomic DNA of one affected individual per family was amplified in a total volume of 15 μl, by use of 15 pmol primers (synthesized by Pharmacia), 0.2 mM each of dGTP, dATP, and dTTP and 0.025 mM dCTP, 0.5 μCi α-^{32}P-dCTP, 0.06 units Supertrouper polymerase (HT Biotechnology), and two different types of PCR buffers (Supertaq buffer [0.1 M Tris-HCl pH 9.0, 0.5 M KCl, 0.1% gelatin, 1.5 mM $MgCl_2$, and 1% Triton X-100] and LR PCR buffer [0.5 M KCl, 0.1 M Tris-HCl pH 8.9, 2 mg BSA/ml, 0.1% gelatin, and 1–2 mM $MgCl_2$]). The amplification protocol used was denaturing for 2 min at 94°C, followed by 30 cycles of 1 min at 94°C, 2 min at annealing temperature (see table 1), and 1 min at 72°C and then a final extension for 9 min at 72°C. The PCR products were diluted 1:5 in SSCA loading buffer (47.5% formamide, 15 mM EDTA, 0.05% SDS, 0.05% xylene cyanole, and 0.05% bromphenol blue) and were denatured at 95°C. The products were separated on either 5% or 10% nondenaturing polyacrylamide gels (49:1, acrylamide:bis-acrylamide) or 8% gels (29:1) with or without 10% glycerol at either room temperature or 4°C. Most samples were analyzed at four different conditions. The optimal conditions are shown in table 2. Depending on the PCR product and the condition of the gel, electrophoresis at 4°C was performed at 50 W for 2.5–4 h, and electrophoresis at room temperature took place at 7–15 W for 15–20 h. Gels were exposed to Kodak XAR5 films. For samples with an aberrantly migrating product, SSCA was performed on all family members, to check segregation of the fragment with the disease. In the case of the missense mutation in exon 5 (family PK5403), 116 control chromosomes of unrelated individuals were screened by use of the same SSCA conditions, to investigate whether an aberrant pattern is caused by a polymorphism present in the population. The control chromosomes were derived from 42 individuals obtained from CEPH (Weissenbach et al. 1992), 26 PKD2 patients (chromosomes not in phase with the PKD2 mutation), and 3 unaffected relatives.

DNA Sequencing

Sequencing was performed manually or with the Automated Laser Fluorescent DNA sequencer (ALF; Phar-

Table 1

Primer Pairs Used for SSCA and RT-PCR

Exon	Primer Name[a]	Sequence (5′→3′)	Product Size (bp)	Annealing Temperature (°C)
SSCA:				
1	F23	AGGAGGTGGAAGGGGAAGAA	335	63
	IR19b	TTCTGGTTCGTGCATCTGCC		
2	IF5	AAATGATATCTTTTCTTTTCTTCA	188	52
	IR20	AACTTTCCCATTAGTGCAAG		
3	IF6	CCAAAATGTTTATCCACAGG	268	55
	IR5	AGGTACTTTCAAAGTTATTTCCA		
4	IF7	TGGTTATGCAACGATGCAGG	355	60
	IR7b	CCGAGTGCCAATGAGTCACA		
5	IF1c	GCCTCAAGTGTTCCACTGAT	362	58
	IR1	AGGTTTTTCTGGGTAACCCTAG		
6	IF2	TTTAATTGTTCTTATTTACATGCA	298	52
	IR8	TTGTAGAATAGAATAGGAAATTTGG		
8	IF9b	TTTTATTATACACAGTCACACCA	282	55
	IR10	CTACTCTGACTAAATTTTTCTTCTT		
12	IF11	GATGAATGTTATCTGTATCCTCTC	221	55
	IR3	TAGGTACCAAATCAAATCCG		
RT-PCR:				
1	F exon1c	GCGGAATGGTGGTGGAGATG		
	F23	AGGAGGTGGAAGGGGAAGAA		
2	R exon2	TTAGTGCTGCTTTCCTCCATGAGT		
3	R exon3	CGGGGGTGTCTAGGAAGAGCTG		
4	R exon4	TTCAGTCTGGTTGCTGGGCT		

[a] F = forward primer in exon; IF = forward primer in intron; R = reverse primer in exon; and IR = reverse primer in intron.

macia). Products for manual sequencing were obtained by PCR on genomic DNA, with SSCA primers (table 1). Forward and reverse cycle sequence reactions were performed by use of the Amplicycle Kit according to manufacturer's protocol (Perkin-Elmer), by use of the PCR amplification primers. Products for the ALF were obtained by amplification of genomic DNA by use of SSCA primers (table 1) with an M13 extension (CGACGTTGTAAAACGACGGCCAGT) at the 5′ end of the forward primers and with a biotin label at the 5′ end of the reverse primers. To sequence the mutations in families PK6533 and PK6534, the PCR products were cloned in a pGEM-T7 vector (Promega) and were sequenced with either biotinylated M13 master primer

Table 2

Optimal SSCA Conditions

Exon	Polyacrylamide Gel (%)	Glycerol (%)	Temperature[a] (°C)	Time (h)	Power (W)
1	10	10	RT	15	12
2	10	10	RT	17	12
3	10	10	RT	15	12
4	8	10	RT	15	15
	10	10	RT	15	15
5	8	10	RT	15	15
	10	10	RT	15	15
6	5	10	RT	15	12
8	5	0	4	3	50
	10	10	RT	20	15
12	5	0	4	3	50
	10	10	RT	22	12

[a] RT = room temperature.

Table 3

Mutations in PKD2 Gene

Family	Exon	Sequence Change[a]	Codon Change	Restriction Site and Product Sizes (bp)
PK5069	1	ins C 534–538	Frameshift 180→212X	*Xcm*I creation (336→234+102)
PK9502	2	del 16 bp (596–12)→599 or (596–13)→598	Splice mutation	
PK5609	2	sub G→A (709+1)	Splice mutation	
PK5197	3	sub A→G (710−2)	Splice mutation	*Hpa*II creation[b]
PK7827	4	sub C→T 916	R306X	*Eco*571 creation[b]
PK7852	4	sub C→T 916	R306X	*Eco*571 creation[b]
PK5179	4	ins C 972–973	Frameshift 325→340X	*Ava*I creation (356→248+148)
PK5414	5	del C 1193	Frameshift 398→451X	
PK6537	5	del AA 1194/1195	Frameshift 398→407X	
PK5403	5	sub T→G 1240	W414G	
PK6534	6	del T 1362–1363	Frameshift 455→460X	
PK5144	6	del T 1443–1445	Frameshift 482→513X	
PK6533	6	del T 1443–1445	Frameshift 482→513X	
PK5404	6	sub G→T 1480	E494X	
PK6526	8	sub C→G (1717-3)	Splice mutation	*Sac*I creation (282→59+223)
PK5118	8	del C 1781–1782	Frameshift 594→609X	*Cvi*RI creation[b]
PK5120	8	del C 1781–1782	Frameshift 594→609X	*Cvi*RI creation[b]
PK1080	12	sub A→G (2241-2)	Splice mutation	
PK5183	12	sub C→A 2286	Y762X	*Rsa*I loss (106+115→121)
PK7808	12	sub C→A 2286	Y762X	*Rsa*I loss (106+115→121)

[a] ins = insertion; del = deletion; and sub = substitution.
[b] Restriction enzyme creates a complex pattern on agarose gel.

and the M13 reverse primer or the biotinylated M13 reverse primer with the M13 master primer. The PCR products were purified by use of an Easyprep kit (Pharmacia). Single-stranded fragments of biotinylated PCR products were obtained by use of magnetic beads (Dynabeads). The sequence reaction was performed with a fluorescent universal or reverse primer of the autoread kit (Pharmacia). Each mutation was sequenced in at least two affected individuals of a family.

Restriction Analysis

Digestion of purified nonradioactive PCR products was performed in a total volume of 20 μl with 10 units of enzyme (table 3) in 0.5–2 × One-Phor-All buffer (10 × [100 mM Tris-acetate pH 7.5, 100 mM magnesium acetate, and 500 mM potassium acetate]; Pharmacia), and separation was on a 2.5% agarose gel. The creation of the *Cvi*RI site in exon 8 (families PK5118 and PK5120) could not be tested, because of a large number of *Cvi*RI sites in this sequence. The pattern, created by digestion of the exon 4 PCR product with *Eco*57I (families PK7827 and PK7852) and by digestion of the exon 3 PCR product with *Hpa*II (family PK5197), was too complex to allow confirmation of the mutations.

Results

We have screened 80% of the PKD2 gene (all exons except exon 1) for mutations in 35 PKD2 patients. For each exon, SSCA was performed at four different conditions. Only exon 1 has not been screened systematically, because the PCR of most samples was hampered by the GC richness of this exon. Nevertheless, one alteration was found in this exon (family PK5069). In 20 (57%) of the 35 families the mutation in the PKD2 gene was identified and subsequently sequenced. The results are listed in table 3. Most mutations found so far are unique and dispersed over the entire gene, although four mutations occurred in more than one family (fig. 1 and table 3). In families PK5118 and PK5120, which we expected to be related, a deletion of a C in exon 8 was found. However, two seemingly unrelated family pairs (in families PK5144 and PK6533 and in families PK5183 and PK7808) have the same mutation, in exon 6 and exon 12, respectively. These pairs of families do not originate from the same countries, and haplotype analysis did not reveal a common haplotype (data not shown). Also, in two Bulgarian families (PK7827 and PK7852), which carry the same C→T substitution in exon 4, no common haplotype was found.

Truncating Mutations

The majority of the mutations found were small deletions or insertions, resulting in a frameshift leading to a premature translation stop (table 3). In seven families (PK6537, PK5414, PK6534, PK6533, PK5144, PK5118, and PK5120) a deletion of a C, a T, or AA

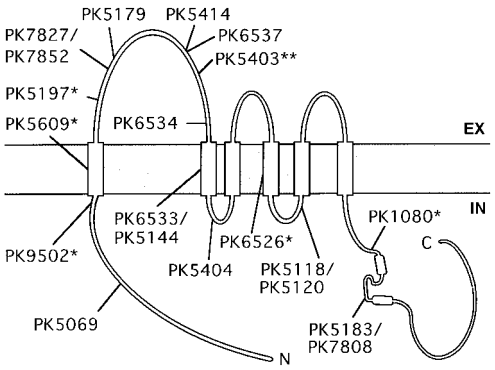

Figure 1 Model of polycystin-2, and localization of mutations. The predicted protein has six transmembrane domains (range 5–8) and intracellular amino and carboxy termini. The carboxy terminus contains an EF-hand domain (shown as two small rods). Most of the alterations lead to premature stops, although several splice mutations (*) and a missense mutation (**) are found. EX = extracellular; and IN = intracellular.

was found. In two families (PK5069 and PK5179) an insertion of a C caused the frameshift. A base-pair substitution leading to a nonsense mutation was found in five families (PK7827, PK7852, PK5404, PK5183, and PK7808). In all families, segregation of the mutation with the disease was confirmed by SSCA.

In several families the mutation causes the creation or disruption of a restriction site (table 3). Most of these mutations could be confirmed by digestion with restriction enzymes. In families PK7827 and PK7852 the nonsense mutations in exon 4 created an *Eco*57I site, 13 bp downstream from an already existing *Eco*57I site. Since *Eco*57I cuts 16 bp downstream from its recognition site, only one of the two sites is recognized by the enzyme. Restriction analysis did not show an aberrant pattern in affected individuals. The creation of a *Cvi*RI site in affected individuals of families PK5118 and PK5120 was not analyzed, because of a large number of *Cvi*RI sites present in the PCR product of exon 8.

Missense Mutation

In family PK5403 a missense variant was found, changing a tryptophan at codon 414 to a glycine (table 3). Segregation of the aberrant SSCA fragment with ADPKD was confirmed in this family (nine affected and seven unaffected individuals). Comparison of the sequence of polycystin-2 with that of polycystin-1 and its *Caenorhabditis elegans* homologue (Mochizuki et al. 1996) revealed that tryptophan is conserved between the three predicted proteins. SSCA of exon 5 on 116 control chromosomes did not show the aberrantly migrating product. No restriction site disappeared or was created by this mutation.

Splice-Site Mutations

Five mutations found are alterations of the splice sites (families PK9502, PK5609, PK5197, PK6526, and PK1080). The intronic positions of these mutations are shown between brackets in table 3. In families PK1080, PK5197, and PK6526 the intronic part of the well-conserved splice-acceptor site (yAG-gt) (Padgett et al. 1986) is changed by a base-pair substitution. In family PK5609 a base-pair substitution changes the second intronic base pair of the well-conserved splice-donor site (ag-GT) (Padgett et al. 1986). Segregation of the mutations with ADPKD in these families was confirmed by SSCA. Additionally, restriction analysis of family PK6526 (exon 8) by *Sac*I showed segregation of the mutation with the disease (data not shown). In family PK9502 a deletion of 16 bp (either 12 intronic bp and 4 exonic bp or 13 intronic bp and 3 exonic bp) was found at the boundary of intron 1 and exon 2, disrupting the splice-acceptor site (table 3). Reverse-transcriptase PCRs (RT-PCRs) with forward primers in exon 1 and with reverse primers in exons 2–4 (table 1) were performed on an affected individual of family PK9502, who carries the 16-bp deletion (individual not shown in fig. 2), four control individuals, and the cDNA clone cTM4, which contains part of the PKD2 gene (Mochizuki et al. 1996). Products with the expected sizes were detected in all samples; however, no aberrant spliced transcript was found in family PK9502 (data not shown). Segregation of the deletion with ADPKD in family PK9502 was shown in 19 affected and 15 unaffected individuals of this family, on a 10% polyacrylamide and 3% agarose gel (for analysis of part of the pedigree, see fig. 2). Affected individual PK9502-6 does not carry the deletion that was found in its affected family members. Absence of the mutation in this individual is in agreement with the haplotype analysis performed on this family (fig. 2). A normal copy of the gene was inherited because of a recombination event just proximal to the PKD2 gene. However, these findings are in conflict with the results obtained with ultrasonography. When the individual was age 36 years, three cysts in the right kidney and many small cysts in the left kidney were found.

Polymorphisms

In addition to disease-causing mutations, we identified a polymorphism in exon 4. SSCA analysis of 43 unrelated individuals obtained from CEPH (Weissenbach et al. 1992) revealed that 47% of the individuals are heterozygous for this polymorphism, 23% are homozygous for one allele, and 30% are homozygous for the other. Sequencing of the exon 4 PCR product of several homozygous and heterozygous individuals did not reveal a sequence difference in the coding part of the fragment, so the polymorphism is most likely located within one of the introns close to the boundary of exon 4.

Discussion

The pathways involved in cyst formation and other symptoms in ADPKD patients are largely unknown.

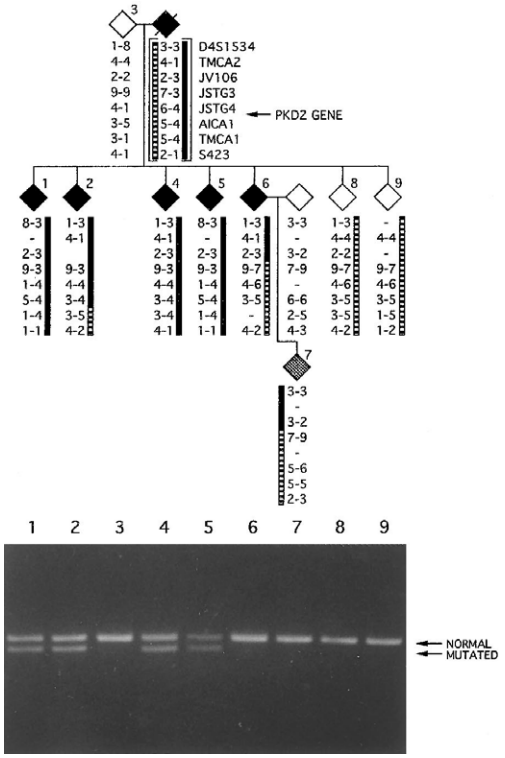

Figure 2 Segregation of a 16-bp deletion in a part of family PK9502, on a 3% agarose gel. The haplotypes are shown for chromosome 4 markers D4S1534, TMCA2, JV106, JSTG3, JSTG4, AICA1, TMCA1, and D4S423. Deduced haplotypes are shown between square brackets. The PKD2 gene is located between JSTG4 and AICA1. The 16-bp deletion segregates with haplotype 3-1-3-3-4-4-4-1 (*black bars*) in 19 family members (data shown for individuals 1, 2, 4, and 5). One affected individual (6), however, with a recombination event between JV106 and JSTG3, has not inherited the deletion from the affected parent. Also, the child (7), who has inherited the same recombined haplotype as the affected parent, does not carry the deletion. Cysts were detected in both kidneys of individual 6; however, no ultrasound data were available for individual 7. For reasons of confidentiality, individuals are indicated by diamonds. Affected individuals are represented by black symbols.

Mutations in at least two genes can cause essentially the same disease. In most of the families the PKD1 gene is mutated, and in ~15% an alteration in the PKD2 gene on chromosome 4q21-q22 is responsible for the disease. Both genes encode novel transmembrane proteins for which the functions are unknown. The PKD1 gene product, polycystin-1, has a predicted role in cell-cell or cell-matrix interactions (Hughes et al. 1995; The International Polycystic Kidney Disease Consortium 1995). The PKD2 gene product, polycystin-2, shows homology to polycystin-1, but the predicted protein model lacks the large extracellular N-terminal domain with associated motifs that has been described for polycystin-1 (Hughes et al. 1995; Mochizuki et al. 1996). In addition, polycystin-2 shows homology to the family of voltage-activated calcium (and sodium) channels and may, in association with either itself or other proteins, function as an ion channel (Mochizuki et al. 1996).

In addition to functional studies, the spectrum of mutations leading to ADPKD may provide insight into the role of both proteins. The mutation detection for PKD1 is severely hampered by the repetitive nature of the gene. Therefore, most mutations described thus far are truncating mutations located in the unique part of the gene (The European Polycystic Kidney Disease Consortium 1994; Peral et al. 1995, 1996*a,* 1996*b;* Turco et al. 1995; Neophytou et al. 1996; Roelfsema et al. 1996; Rosetti et al. 1996). However, recently a number of mutations have also been identified in the repeated part of the gene, resulting in relatively short proteins that lack the transmembrane and intracellular carboxy-terminal parts (J. H. Roelfsema, personal communications).

Mutation detection of the PKD2 gene can provide additional information about the mechanism involved in cyst formation. To unravel the molecular defect in patients with the second type of ADPKD, we performed SSCA on 35 families. After screening a large part of the open reading frame, we identified a mutation in 20 families. The undetected mutations in the remaining families are either located in the unscreened coding part of the gene (exon 1) or in intronic parts, or are not detected because of the limitations of SSCA. In addition, linkage to chromosome 4 could not be established with certainty in a few small families. Most mutations found in PKD2 patients are unique and dispersed over the entire gene, although four mutations occur also in a second family. The families from the Netherlands (PK5118 and PK5120) are most likely related. However, in the other family pairs no common haplotypes of markers flanking the PKD2 gene were found, suggesting three recurrent mutations (data not shown). We detected one polymorphism in the PKD2 gene, with a heterozygosity of .47. Since we did not see an alteration in the coding sequence of this product (exon 4), the polymorphism is probably located within one of the introns, close to the boundaries of this exon.

Fourteen (67%) of the mutations found are nonsense mutations and frameshifts resulting in premature translation stops. Also, the three alterations of the PKD2 gene originally described resulted in shorter proteins (Mochizuki et al. 1996). In family PK5069, where the mutated gene has a translation stop before the first predicted transmembrane domain, the translated protein of 212 amino acids is probably completely nonfunctional. In the other PKD2 families premature stops were found in exons 4–6, 8, and 12, suggesting that the domain(s) essential for a normal protein function are located in the carboxy-terminal part of the protein. For polycystin-2, an EF-hand domain has been described at the C-terminus, which can be involved in the binding of calcium (Mochizuki et al. 1996). All mutations are located

upstream of this domain, except for the exon 12 mutation in families PK5183 and PK7808, which is located within this domain.

In addition to truncating mutations, we also identified one missense mutation in exon 5 (family PK5403). The alteration in family PK5403, where a T is replaced by a G in the mutated gene, is found in eight affected individuals of this family and is not observed in seven unaffected individuals of this family. This alteration was absent in 116 control chromosomes. The mutation results in the substitution of a glycine for a tryptophan at codon 414 of the protein. A tryptophan at this position is conserved between polycystin-2, polycystin-1, and its *C. elegans* homologue. According to the predicted model of the PKD2 gene product (fig. 1), the substitution by a glycine is located in the first extracellular loop. Glycine, which can adopt a much wider range of conformations than any other amino acid, may have an important effect on the folding of this loop, resulting in a hampered protein function. Alternatively, the tryptophan-414 could be essential for interactions with other proteins.

In five families an alteration in the splice sites of exons 2, 3, 8, and 12 was found. In families PK6526, PK1080, and PK5197 the splice-acceptor site is changed by an intronic substitution, which cosegregates with the disease in the families (table 3). In family PK5609 the splice-donor site is changed by a base-pair substitution. Whether these splice mutations result in a premature stop or in the skipping of (part of) an exon needs to be established on transcript level. However, RNA of affected individuals is not available yet. In family PK9502 the splice-acceptor site is disrupted because of a deletion of 16 bp at the boundary of intron 1 and exon 2. RT-PCRs with forward primers in exon 1 and with reverse primers in exon 2–4 on cDNA of an affected and control individuals did not reveal an aberrant transcript, although we did amplify the normal-size transcript.

Splice-site mutations may result in several possible alterations of the transcript (reviewed in Krawczak et al. 1992; Nakai and Sakamoto 1994; Berget 1995; Maquat 1996). Either one or more exons are spliced out of the transcript, introns are retained in the transcript, or an alternative splice-acceptor site is used. Alternatively, the transcript could be unstable. So, deletion of one or more primer-adhesion sites, a large distance between the PCR primers, or absence of the transcript could explain the incapability to amplify the aberrant product. In the same family, one affected individual (PK9502-6) does not have the 16-bp deletion detected in other family members, which is in concordance with the haplotype analysis but not with the ultrasound data (fig. 2). Also, the child (PK9502-7), who has inherited the recombined chromosome, does not carry the deletion. However, no ultrasound data on this child are available. Thus, cystic kidneys in individual PK9502-6 are not caused by the 16-bp deletion, as is the case in the other affected family members, but are probably are caused by a de novo mutation in one of the ADPKD genes or are due to another cause.

Mutation analysis of the PKD2 gene by SSCA revealed an alteration in 57% of the families after screening of a large part of the gene. An equal amount of substitutions are found, compared with deletions and insertions. However, whether this ratio is a correct representation of PKD2 mutations in the population remains to be seen. Deletions and insertions are more likely to cause conformational changes in a PCR product, which can be detected by SSCA, than is the case for substitutions. Other mutation-detection techniques are needed to identify the alterations in the remaining families. Since mutation in the PKD1 and PKD2 gene result in an essentially similar phenotype, it is believed that the proteins are involved in the same pathway. An in vitro study suggested interaction between both transmembrane proteins, via coiled-coil domains at the carboxy termini of the proteins, although the coiled-coil domain predicted for polycystin-2 is not perfect (Qian et al. 1997). For both the PKD1 and PKD2 gene, truncating mutations have been found, resulting in loss of a large part of the protein. These truncating mutations suggest that ADPKD is caused by a lack of normal functioning protein (i.e., haploinsufficiency), although the possibility of a second somatic mutation in the normal copy of the gene, as suggested for PKD1, cannot be excluded by our results (Reeders 1992; Qian et al. 1996). We have shown that SSCA is an effective method to elucidate the primary defect in PKD2 patients; however, functional studies have to show how mutations in the ADPKD genes lead to the inactivation of an unknown pathway.

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