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A Loss-of-Function Model for Cystogenesis in Human Autosomal Dominant Polycystic Kidney Disease Type 2

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Summary

Autosomal dominant polycystic kidney disease (ADPKD) is genetically heterogeneous, with at least three chromosomal loci (*PKD1*, *PKD2*, and *PKD3*) that account for the disease. Mutations in the *PKD2* gene, on the long arm of chromosome 4, are expected to be responsible for ~15% of cases of ADPKD. Although ADPKD is a systemic disease, it shows a focal expression, because <1% of nephrons become cystic. A feasible explanation for the focal nature of events in *PKD1*, proposed on the basis of the two-hit theory, suggests that cystogenesis results from the inactivation of the normal copy of the *PKD1* gene by a second somatic mutation. The aim of this study is to demonstrate that somatic mutations are present in renal cysts from a *PKD2* kidney. We have studied 30 renal cysts from a patient with *PKD2* in which the germline mutation was shown to be a deletion that encompassed most of the disease gene. Loss-of-heterozygosity (LOH) studies showed loss of the wild-type allele in 10% of cysts. Screening of six exons of the gene by SSCP detected eight different somatic mutations, all of them expected to produce truncated proteins. Overall, ⩾37% of the cysts studied presented somatic mutations. No LOH for the *PKD1* gene or locus *D3S1478* were observed in those cysts, which demonstrates that somatic alterations are specific. We have identified second-hit mutations in human *PKD2* cysts, which suggests that this mechanism could be a crucial event in the development of cystogenesis in human ADPKD-type 2.

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD; MIM 173900) is one of the most common Mendelian disorders in humans and the most frequent genetic cause of renal failure in adults. The main feature of the disease is the bilateral progressive cystic dilation of the renal tubules, which may lead to end-stage renal disease (ESRD). Hepatic cysts, cerebral aneurysms, and cardiac valve abnormalities may also be found (Gabow 1993). Although ADPKD is a systemic disease, it shows a focal expression, because <1% of nephrons become cystic (Baert 1978). ADPKD is a genetically heterogeneous condition, with at least three genes involved: (1) *PKD1*, located in 16p13.3, which accounts for most ADPKD cases (Reeders et al. 1985; Peters and Sandkuijl 1992; European Polycystic Kidney Disease Consortium 1994; Torra et al. 1996); (2) *PKD2*, located in 4q21–q22 (Kimberling et al. 1993; Peters et al. 1993); and (3) the much rarer PKD3, not yet mapped (Daoust et al. 1995; de Almeida et al. 1995). *PKD1* and *PKD2* genes have been recently cloned and characterized (European Polycystic Kidney Disease Consortium 1994; Burn et al. 1995; Hughes et al. 1995; International PKD Consortium 1995; Mochizuki et al. 1996).

The *PKD2* gene consists of 15 exons with an open reading frame of 2,904 bp (Mochizuki et al. 1996; Hayashi et al. 1997). 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 and carboxyl termini (Mochizuki et al. 1996). At present, several germline mutations have been described in the *PKD2* gene, including nonsense, splice-site, frame-shift, and two putative missense mutations (Mochizuki et al. 1996; Veldhuisen et al. 1997; Viribay et al. 1997; Xenophontos et al. 1997; Pei et al. 1998; Torra et al., in press). Most of the *PKD2* mutations are expected to produce truncated proteins, since they also occur for mutations in the *PKD1* gene. Recent studies have demonstrated that polycystin-2 interacts with polycystin-1 through its cytoplasmic carboxyl end (Qian et al. 1997; Tsiokas et al. 1997); thus, truncating mutations

would interfere with this interaction. These truncating mutations, in both *PKD1* and *PKD2,* suggest that ADPKD is caused by inadequate levels of polycystin (i.e., haploinsufficiency). The process of cystogenesis could also be explained by a model of dominant/negative function, in which a mutated form of polycystin would inactivate the normal polycystin produced by the normal allele. On the basis of loss-of-heterozygosity (LOH) studies, it has been proposed that the process of cystogenesis in *PKD1* results from focal somatic second hits on the wild-type allele (Qian et al. 1996; Brasier and Henske 1997). However, the strong immunoreactivity for polycystin-1 observed in the majority of *PKD1* cysts has challenged this hypothesis (Geng et al. 1996; Ward et al. 1996; Ibraghimov-Beskrovnaya et al. 1997; Ong et al., 1999). Alternatively, it has been suggested that, as polycystin-1 interacts with polycystin-2, somatic mutations in *PKD2* could cooperate with the germline mutation in *PKD1* (Qian et al. 1996). Wu et al. (1998) showed results that support a two-hit hypothesis in mice. Recent studies on a mouse model for *PKD2* found that mice that carry a null allele and an allele that can undergo genomic rearrangements that lead to a null allele develop renal cysts at a higher frequency than those that are heterozygous for any of these alleles (Wu et al. 1998). More recently, Koptides et al. (1999) showed evidence of somatic point mutations for human *PKD2* cysts, but did not find evidence of LOH. The purpose of the present study was to demonstrate a loss-of-function mechanism for *PKD2* in humans through the study of LOH and point somatic mutations within the *PKD2* gene.

Material and Methods

Patients and Specimens

The cysts studied were obtained from a kidney that belonged to a patient with ADPKD who underwent an orthotopic transplantation at age 69 years. His kidney was removed during the transplantation procedure. This patient belongs to a large family we had previously studied that had clear evidence of linkage to *PKD2* (LOD score 4.0) (Torra et al. 1996). This family was the only one of our families with *PKD2* in which no mutation had been detected by heteroduplex or SSCP analyses (Torra et al., in press). The clinical features of the persons in this family were those typical of persons with *PKD2* with late-onset disease and with ESRD at a mean age of 72 years. The kidney was studied immediately after removal, which took place in the Hospital Clínic of Barcelona. The study was approved by the hospital's ethical committee.

Preparation of Cystic Epithelial Cells and DNA Preamplification

To avoid contamination from neighboring cells, we followed the procedure of Qian et al. (1996) and introduced some modifications. Throughout the procedure, the kidney was placed on ice in a tray (fig. 1). To obtain as few contaminating cells as possible, the surface of the cyst was first rinsed with PBS. We then drained the cyst content with a needle and a syringe. The needle was left inserted in the cyst during the washing steps. The cavity of the cyst was rinsed four to eight times with Ca^{2+} and Mg^{2+}-free PBS, until the liquid that was obtained appeared to be clear. At this point, PBS/2 mM EDTA was introduced into the cysts and was energetically pulled in and out of the cyst several times to facilitate the detachment of the epithelial cells from the basement membrane. Only the cysts that remained intact were considered for the study. The cells that were obtained from the cyst cavity were suspended in PBS/2 mM EDTA and centrifuged at 14,000 rpm for 5 min, and the supernatant was discarded. The pellet was resuspended with 600 μl of urea solution (8 M urea, 0.3 M NaCl, 10 mM EDTA, 10 mM TRIS, 2% SDS) and 12 μl of proteinase K solution (10 mg/ml), incubated overnight at 37°C, and treated with a phenol/chloroform extraction. Next, 0.5 μl glycogen and 2.5 V absolute ethanol were added, and the pellet was incubated for 1 h at −80°C. After incubation, it was centrifuged for 20 min at 14,000 rpm and the supernatant was discarded. Eventually, the pellet was dried and resuspended in 45 μl 1 × TE pH 7.5.

To increase the amount of DNA for subsequent studies, the material obtained from cystic cells was preamplified. The reaction included 1–2 μl DNA, 6 μl 10 × buffer (Perkin-Elmer), 4mM DTT, 2.5 mM $MgCl_2$, 10 mM of each dNTPs, 5 U of *Taq* polymerase (Perkin-Elmer), 400 μM of each random primer (15-mer oligonucleotides in which any one of the four possible bases could be present at each position), and distilled water

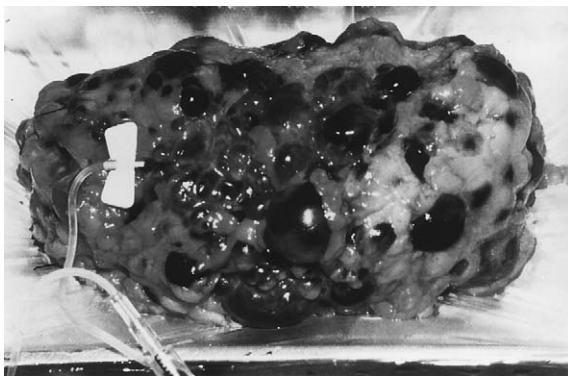

Figure 1 *PKD2* kidney with a needle inserted into a cyst.

to 60 μl. PCR amplification was performed as follows: 5 min at 94°C; 50 cycles: 1 min at 94°C, 2 min at 37°C, and 4 min at 55°C.

LOH Analysis

Each DNA sample was typed by PCR by using microsatellites *D4S1542* and *D4S1563* for *PKD2* locus and by using *D16S663, D16S291,* and *KG8* [*PKD1-3′UTR*] for *PKD1*. The distance of these flanking markers to the corresponding gene was <1 cM. Marker *D3S1478* was used as a control. The patient was informative for all the microsatellites studied. One microliter of preamplified DNA was used for each PCR reaction. We used 5% DMSO and 1.5 mM (*D16S663, KG8* [*PKD1-3′UTR*], *D4S1563, D4S1542,* and *D3S1478*) or 1 mM (*D16S291*) $MgCl_2$. The PCR conditions were as described elsewhere (Torra et al. 1996; Brasier et al. 1997). The PCR products were run on an acrylamide gel and were silver stained (Bassam et al. 1991).

Search for PKD2 Mutations, DNA Sequencing, and Restriction Enzyme Analysis

We analyzed exons 2, 4–6, 11, and 12 of *PKD2*. The primers used for exon amplification and PCR conditions have been described elsewhere (Hayashi et al. 1997; Viribay et al. 1997). PCR products were analyzed by SSCP. Three microliters of denatured PCR product were combined with loading buffer, were loaded into GeneGel Excel 12.5 acrylamide gels (Amersham Pharmacia Biotech), and were run according to the manufacturer's instructions. The different migrations were silver stained (Bassam et al. 1991). DNA samples with abnormal migrations were sequenced. PCR products were purified by using the QIAquick PCR purification kit (Qiagen) and were automatically sequenced by means of the Dye Terminator Cycle Sequencing Ready Reaction (Perkin Elmer Cetus) and an automatic sequencer (ABI310). To exclude the possibility that the mutations were the result of the preamplification step, we analyzed nonpreamplified DNA from two cysts that harbor mutations that disrupted an endonuclease recognition site (see table 1) and confirmed the presence of the corresponding mutations. We also preamplified and twice sequenced the other mutations that do not abolish a restriction site, which confirmed the presence of the nucleotide changes.

DNA Dosage Assessment

Five micrograms of genomic DNA from affected and normal individuals were digested with 50 U of *Eco*RI (Boehringer-Mannheim) and run on a 0.8% agarose gel at 40 V overnight. Southern blotting onto a nylon filter was done according to standard protocols. DNA probes were prepared by PCR amplification of genomic DNA, followed by extraction of the corresponding band from low–melting-point agarose gels. For *PKD2,* two probes were obtained by amplification of part of exon 1 of the gene with primers F42 and IR19d (Hayashi et al. 1997) or by amplification of a fragment containing exons 11 and 12 with primers IF10 and IR3. A control DNA probe was obtained by amplification of exon 4 of the *CFTR* gene (Zielenski et al. 1991). DNA probes were labeled with [^{32}P]-dCTP to high specific activity by the random hexamer primer method. Hybridizations, simultaneously containing one of the *PKD2* probes and the control probe, were done at 65°C. The sizes of the target *Eco*RI fragments were 5.2 and 5.4 kb for exon 1 and exons 11–13 of the *PKD2* gene, respectively (Hayashi et al. 1997), and 4.7 kb for exon 4 of *CFTR* (Zielenski et al. 1991). The band densities of patients and normal individuals were analyzed by densitometry.

Table 1

Somatic Mutations in the *PKD2* Gene in a Polycystic Kidney of a Patient with Autosomal Dominant *PKD2*

Cyst	LOH for Microsatellites	Mutation	Exon or Intron
C1		IVS4+1G→A	Intron 4
C3		934insG	Exon 4
C5		990delC[a]	Exon 4
C6	D4S1542, D4S1563	PKD2 deletion	Exons 1–15
C8		2236insA	Exon 11
C10	D4S1542, D4S1563	PKD2 deletion	Exons 1–15
C17	D4S1563	PKD2 deletion	?
C23		Y429X[b]	Exon 5
C25		2198del13	Exon 11
C29		1217delT	Exon 5
C30		2207delT	Exon 11

[a]Mutation 990delC abolishes a *Bam*HI restriction site.
[b]Mutation Y429X abolishes an *Rsa*I restriction site.

Results

Germline Mutation

The germline mutation causing the disease in this family had not been identified after repeated screenings of exonic sequences and exon-intron boundaries by SSCP or by heteroduplex analyses in previous studies (Torra et al., in press). However, analysis with a frequent polymorphism recently identified within exon 1 of *PKD2* (Arg28Pro, Torra et al., in press) detected an apparent non-Mendelian segregation of alleles for this polymorphism in the family (fig. 2*A*). Thus, although most affected individuals were apparently homozygous for one allele, two affected siblings (II2 and II3) were apparently homozygous for the other allele (fig. 2*B*). Densitometric analyses of genomic *Eco*RI DNA digests with probes that could detect exon 1 or exons 11–13 of *PKD2* detected half intensity of *PKD2* in the affected subjects (fig. 2*C*). This result supports a deletion that encom-

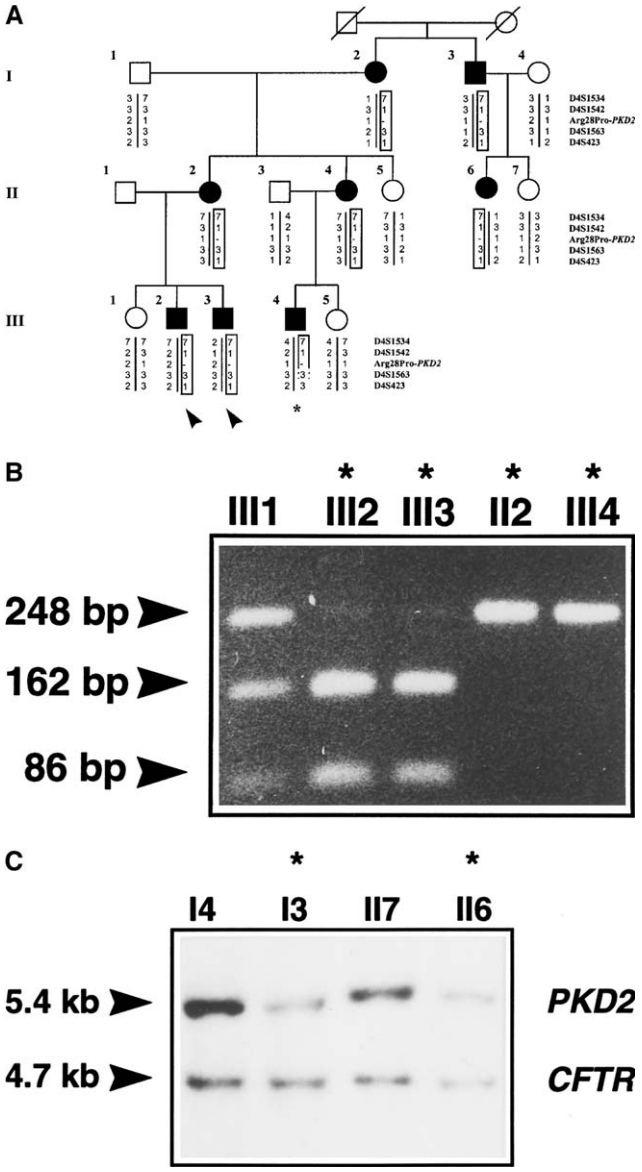

Figure 2 Genomic deletion of the *PKD2* gene in a family with ADPKD-type 2. *A,* Pedigree of the family. Haplotypes with *PKD2* intragenic and flanking markers. Hemizygosity for Arg28Pro is shown (1: Arg, 2: Pro, -:del). Individuals with abnormal segregation of the polymorphism are indicated with an arrow. Asterisk indicates recombination among *PKD2* flanking markers. *B, Ban*II digestion of DNA region containing the Arg28Pro polymorphism. The polymorphism creates a new *Ban*II restriction site, originating two fragments of 162 and 86 bp instead of the original 248-bp fragment. Asterisks indicate affected individuals. *C, Eco*RI digestion Southern blot analysis of subjects of the *PKD2* family with a probe for exons 11–13 of *PKD2*. Control hybridization was done with a probe corresponding to exon 4 of *CFTR*. Densitometric analysis indicated that the *PKD2* subjects (*) had approximately a half dosage of the *PKD2* gene.

passes at least exons 1–13 of the gene as the germline mutation in this family. The analysis with microsatellite markers close to the disease gene (distance <1 cM) failed to detect LOH, which indicates that the deletion is probably not very large (fig. 2*A*).

PKD2 Somatic Mutations

Large deletions.—First we performed an LOH study with two microsatellite markers (*D4S1542* and *D4S1563*) flanking *PKD2* in 30 cysts from a kidney of individual I3 (fig. 2*A*). We found that three cysts had lost alleles for at least one of these markers (table 1). As expected, in all cases the allele lost corresponded to the wild-type *PKD2* gene, as determined by segregation analysis. LOH was not always absolute, probably because of contamination from noncystic cells (fig. 3). When the *PKD2* cysts were analyzed for three microsatellite markers linked to or within the *PKD1* gene or with the *D3S1478* marker, we failed to detect any evidence of LOH.

Point mutations.—Because no evidence of LOH for the *PKD2* region was found in 27 cysts, we concentrated on screening for subtle changes in this gene, and in the first instance we focused on six exons. After SSCP analysis, eight cases with an abnormal migration pattern were observed, which on sequencing corresponded to

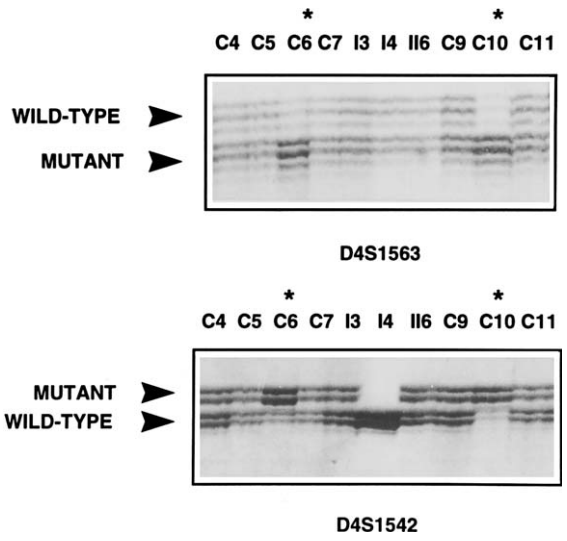

Figure 3 LOH of alleles at microsatellites *D4S1563* and *D4S1542* linked to the *PKD2* gene in cysts from a patient (I3) with ADPKD-type 2. Lanes C4–C11 correspond to cysts from patient I3. LOH from cysts C6 and C10 is indicated with an asterisk. In both cases the lost allele corresponds to the wild-type allele. Lymphocyte DNA from subjects II6 (affected daughter) and I4 (spouse) is also shown. Patient numbers correspond to those shown in figure 2. LOH was determined by visual comparison of signals obtained from amplification of cysts and control DNA. All cysts showing LOH were typed three times, for confirmation, for both microsatellites.

eight different mutations (table 1 andfig. 4). All of them should result in a truncated *PKD2* protein, six being frame-shift mutations due to the insertion or deletion of one or several nucleotides—one creating a premature stop codon and one affecting the splicing of intron 4. In most cases, sequencing revealed only the somatic-mutated allele, because the germline mutation was a deletion; however, since a small amount of noncystic cells are frequently present, in some sequences we could detect traces of sequence that corresponded to the wild-type allele (fig. 4*B*).

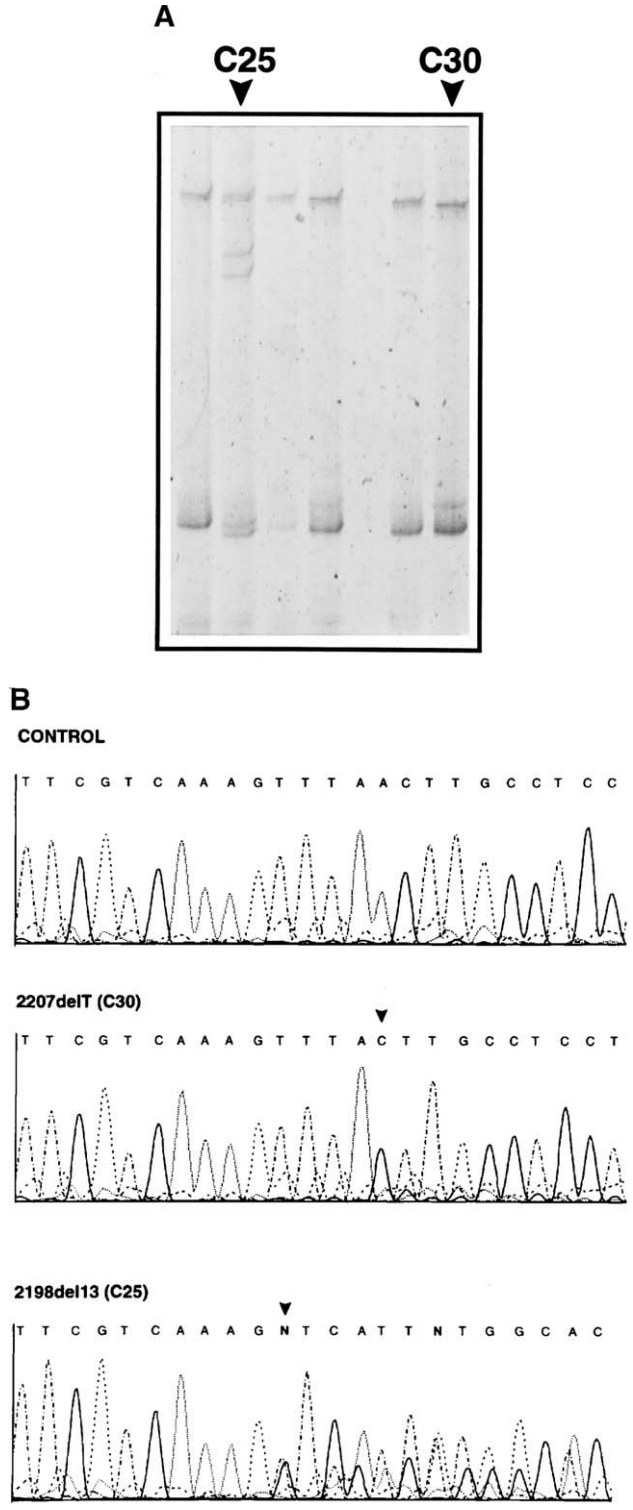

Figure 4 Two exon 11 *PKD2* point mutations in cysts of a patient with ADPKD-type 2. *A*, SSCP analysis of exon 11 of the *PKD2* gene of cysts C30 and C25. *B*, Sequencing of exon 11 from control and cysts C25 and C30 detected two frame-shift mutations (2207delT in C30 and 2198del13 in C25). Sequences correspond to the reverse strands. Nucleotide positions where mutations occur are marked with an arrow. Sequencing of C30 detected only the mutant allele as a result of the germline deletion at the site of the mutation. Sequencing of C25 detected both the mutated and wild-type alleles, which resulted from contamination with noncystic cells.

Discussion

Mutations in the *PKD2* gene are expected to be responsible for ~15% of cases of polycystic kidney disease (Peters and Sandkuijl 1992; Torra et al. 1996). 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 (Parfrey et al. 1990; Gabow et al. 1992; Ravine et al. 1992; Wright et al. 1993; Torra et al. 1996). The search for mutations within the *PKD2* gene has proven to be a much more efficient process than the search for the *PKD1* gene, because the *PKD2* gene is smaller and is not partially reiterated, as is the *PKD1* gene. All the germline mutations reported so far in *PKD2* are small nucleotide changes, most of which lead to truncated proteins. The identification of the apparent severe mutation that we report in this paper, which gives rise to a regular *PKD2* phenotype, confirms our previous suspicion about the lack of genotype/phenotype correlation in *PKD2* (Torra et al., in press). The mean age of ESRD for this family is 72 years, thus being completely concordant with the mean age of ESRD for *PKD2* (Hateboer et al. 1999).

Clear evidence that somatic second-hit mutations could be involved in the process of cystogenesis in *PKD1* disease has been obtained by different authors on the basis of LOH studies in both renal and hepatic cysts (Qian et al. 1996; Brasier and Henske 1997; Watnick et al. 1998). When the phenotypic similarity between types 1 and 2 of the disease is taken into account, the possibility arises that this mechanism could also play a role in *PKD2*. Evidence in favor of this hypothesis has been presented in a study with a mouse model for *PKD2* (Wu et al. 1998) and, more recently, in human *PKD2* cysts (Koptides et al. 1999). To confirm the existence of this phenomenon in humans, we studied 30 renal cysts from a patient carrying a germline deletion in the *PKD2* gene.

We obtained evidence of LOH in at least three cysts and demonstrated that the wild-type allele was deleted. Since the rate of LOH detected was too low (10%) to fully explain the two-hit theory, we decided to search for point mutations within the *PKD2* gene that could account for a second hit. Because about 70% of the germline mutations in *PKD2* have been found in exons 2, 4–6, 11, and 12, SSCP analysis was done for these exons. In this search, we found eight new somatic mutations within the *PKD2* gene. The results presented here demonstrate, for the first time, the occurrence of somatic mutations within the *PKD2* gene and suggest that *PKD2* in humans occurs through a molecular recessive mechanism. For somatic alterations in renal cysts, we have found LOH in 3 of 30 cysts and eight point mutations in the remaining 27 cysts. All these mutations are probably inactivating, because they are expected to produce truncated proteins. Although the analysis did not cover the whole *PKD2* gene, the mutational events detected account for 37% of *PKD2* cysts in the current study. The remaining cysts may have mutations in other regions of the *PKD2* gene, or perhaps LOH is masked by contamination from noncystic cells.

Renal epithelial cells have been demonstrated to be a frequent target tissue for somatic mutation. Martin et al. (1996) demonstrated that somatic mutation in renal epithelial cells constitutes a frequent event. Renal epithelial cells may be the target of somatic mutations for several reasons: (1) they are metabolically active and consume large amounts of oxygen; (2) they perform most of the secretory and resorptive work to obtain the renal filtrate; (3) they are mitotically competent, especially when an injury to the kidney occurs; and (4) they are the cells from which a primary renal tumor and adenocarcinoma arise. An important question, then, is whether the observed LOH in this *PKD2* kidney is specific for the *PKD2* gene. To prove whether LOH was specific for the *PKD2* gene, we tested one microsatellite from a region in chromosome 3 (*D3S1478*), which is frequently deleted in human tumors (Brasier and Henske 1997), but we did not find any LOH for this marker in the studied cysts. Another possibility is that somatic mutations in the *PKD1* gene were also involved in *PKD2*–cyst formation, but we did not observe any LOH for *PKD1*-linked markers in *PKD2* cysts. Conversely, we failed to detect any LOH for *PKD2* markers in 60 cysts from patients with *PKD1* (data not shown). These data suggest that the observed somatic alterations were specific to the *PKD2* gene.

Although somatic second hits in *PKD1* cysts have been demonstrated (Qian et al. 1996; Brasier and Henske 1997), the strong immunoreactivity for polycystin-1 observed in most *PKD1* cysts has challenged current thinking about its role in cystogenesis (Geng et al. 1996; Ward et al. 1996; Ibraghimov-Beskrovnaya et al. 1997). As in the case for *PKD1,* Ong et al. (1999), by working with samples from the *PKD2* kidney presented here, have found staining for polycystin-2 in ~85% of cysts, although 25% of them were partial positives. The rest of the samples were not immunoreactive, which suggests the presence of a second *PKD2* somatic mutation. Perhaps the only way these findings can be reconciled with the two-hit hypothesis would be if the somatic mutations were predominantly missense changes that maintain the epitope that the antibody is directed against. However, the mutations detected here cause either a loss of the gene product or truncated proteins that lack the carboxyl end. Moreover, the germline mutation in this case is a large deletion, which probably fails to produce a target for the antibody. Unfortunately, our results do not offer a satisfactory explanation to the immunoreactivity for polycystin-2 observed in those cysts. Alternatively, it can be speculated that somatic mutations are not necessary for cyst formation but arise later and confer an advantage on the mutant cell such that it repopulates the growing cyst. Further studies will be necessary to provide an explanation to this paradox.

Our data reveal that a molecular recessive mechanism is implicated in the process of cystogenesis for *PKD2* disease. Polycystin-2 seems to be a member of a superfamily of proteins that are involved in the regulation of renal epithelial cell growth. Thus, the loss of both copies of an *ADPKD* gene disrupts this cascade and causes hyperplasia of a given cell, which results in the formation of a cyst. This fact has important implications for the understanding of the pathophysiology of the disease and the development of new strategies to prevent or retard the progressive formation and enlargement of cysts. It remains to be answered whether or not other noncystic features of ADPKD (types 1 and 2), such as intracranial aneurysms, also arise from a second-hit mechanism.

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Electronic-Database Information

The accession number and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for polycystic kidneys, polycystic kidney disease type 2 [MIM 173900])

References

Baert L (1978) Hereditary polycystic kidney disease (adult form): a microdissection study of two cases at an early stage of the disease. Kidney Int 13:519–525

Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196:80–83

Brasier JL, Henske EP (1997) Loss of the polycystic kidney disease (*PKD1*) region of chromosome 16p13 in renal cyst cells supports a loss-of-function model for cyst pathogenesis. J Clin Invest 99:194–199

Burn TC , Connors TD, Dackowski WR, Petry LR, Van Raay TJ, Millholland JM, Venet M, et al (1995) Analysis of the genomic sequence for the autosomal dominant polycystic kidney disease (PKD1) gene predicts the presence of a leucine-rich repeat. Hum Mol Genet 4:575–582

Daoust MC, Reynolds DM, Bichet DG, Somlo S (1995) Evidence for a third genetic locus for autosomal dominant polycystic kidney disease. Genomics 25:733–736

De Almeida S, de Almeida E, Peters DJM, Pinto JR, Tavora I, Lavinha J, Breuning MH, et al (1995) Autosomal dominant polycystic kidney disease: evidence for the existence of a third locus in a Portuguese family. Hum Genet 96:83–88

European Polycystic Kidney Disease Consortium, The (1994) The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. Cell 77:881–894

Gabow PA (1993) Autosomal dominant polycystic kidney disease. N Engl J Med 329:332–342

Gabow PA, Johnson AM, Kaehny WD, Kimberling WJ, Lezotte DC, Duley IT, Jones RH (1992) Factors affecting the progression of renal disease in autosomal dominant polycystic kidney disease. Kidney Int 41:1311–1319

Geng L, Segal Y, Peissel B, Deng N, Pei Y, Carone F, Rennke HG, et al (1996) Identification and localization of polycystin, the *PKD1* gene product. J Clin Invest 98:2674–2682

Hateboer N, Van Dijk MA, Bogdanova N, Coto E, Saggar-Malik A, San Millán JL, Torra R, et al (1999) Comparison of phenotypes of polycystic kidney disease types 1 and 2. Lancet 353:103–107

Hayashi T, Mochizuki T, Reynolds DM, Wu GQ, Cai Y, Somlo S (1997) Characterization of the exon structure of the polycystic kidney disease 2 gene (PKD2). Genomics 44:131–136

Hughes J, Ward CJ, Peral B, Aspinwall R, Clark K, San Millán JL, Gamble V, et al (1995) The polycystic kidney disease 1 (*PKD1*) gene encodes a novel protein with multiple cell recognition domains. Nat Genet 10:151–160

Ibraghimov-Beskrovnaya O, Dackowski WR, Foggensteiner L, Coleman N, Thiru S, Petry LR (1997) Polycystin: in vitro synthesis, in vivo tissue expression, and subcellular localization identifies a large membrane-associated protein. Proc Natl Acad Sci USA 94:6397–6402

International PKD Consortium (1995) Polycystic kidney disease: the complete structure of the *PKD1* gene and its protein. Cell 81:289–298

Kimberling WJ, Kumar S, Gabow PA, Kenyon JB, Connolly CJ, Somlo S (1993) Autosomal dominant polycystic kidney disease: localization of the second gene to chromosome 4q13–q23. Genomics 18:467–472

Koptides M, Hadjimichael C, Koupepidou P, Pierides A, Constantinou Deltas C (1999) Germinal and somatic mutations in the *PKD2* gene of renal cysts in autosomal dominant polycystic kidney disease. Hum Mol Genet 8:509–513

Martin GM, Ogburn CE, Colgin LM, Gown AM, Edland SD, Monnat RJ (1996) Somatic mutations are frequent and increase with age in human kidney epithelial cells. Hum Mol Genet 5:215–221

Mochizuki T, Wu G, Hayashi T, Xenophontos SL, Veldhuisen B, Saris JJ (1996) *PKD2*, a gene for polycystic kidney disease that encodes an integral membrane protein. Science 272: 1339–1342

Ong ACM, Ward CJ, Butler RJ, Biddolph S, Bowker C, Torra R, Pei Y, et al (1999) Coordinate expression of the ADPKD proteins, polycystin-2 and polycystin-1, in normal and cystic tissue. Am J Pathol 154:1721–1729

Parfrey PS, Bear JC, Morgan J, Cramer BC, McManamon PJ, Gault MH, Churchill DN, et al (1990) The diagnosis and prognosis of autosomal dominant polycystic kidney disease. N Engl J Med 323:1085–1090

Pei Y, He N, Wang K, Kasenda M, Paterson AD, Chan G, Liang Y, et al (1998) A spectrum of mutations in the polycystic kidney disease-2 (*PKD2*) gene from eight Canadian kindreds. J Am Soc Nephrol 9:1853–1860

Peters DJM, Sandkuijl LA (1992) Genetic heterogeneity of polycystic kidney disease in Europe. In: Breuning MH, Devoto M, Romeo G (eds) Contributions to nephrology: polycystic kidney disease. Karger, Basel, pp 128–139

Peters DJM, Spruit L, Saris JJ, Ravine D, Sandkuijl LA, Fossdal R, Boersma J, et al (1993) Chromosome 4 localization of a second gene for autosomal dominant polycystic kidney disease. Nat Genet 5:359–362

Qian F, Germino FJ, Cai Y, Zhang X, Somlo S, Germino GG (1997) PKD1 interacts with PKD2 through a probable coiled-coil domain. Nat Genet 16:179–183

Qian F, Watnick TJ, Onuchic LF, Germino GG (1996) The molecular basis of cyst formation in human autosomal dominant polycystic kidney disease type 1. Cell 87:979–987

Ravine D, Walker RG, Gibson RN, Forrest SM, Richards RI, Friend K, Sheffield LJ, et al (1992) Phenotype and genotype heterogeneity in autosomal dominant polycystic kidney disease. Lancet 340:1330–1333

Reeders ST, Breuning MH, Davies KE, Nicholls RD, Jarman AP, Higgs RD, Pearson PL, et al (1985) A highly polymorphic DNA marker linked to adult polycystic kidney disease on chromosome 16. Nature 317:542–544

Torra R, Badenas C, Darnell A, Nicolau C, Volpini V, Revert L, Estivill X (1996) Linkage, clinical features and prognosis of ADPKD types 1 and 2. J Am Soc Nephrol 7:2142–2151

Torra R, Viribay M, Tellería D, Badenas C, Watson M, Harris PC, Darnell A, et al. Seven novel mutations of the *PKD2* gene in families with autosomal dominant polycystic kidney disease. Kidney Int (in press)

Tsiokas L, Kim E, Arnould T, Sukhatme UP, Walz G (1997) Homo- and heterodimeric interactions between the gene products of *PKD1* and *PKD2*. Proc Natl Acad Sci USA 94: 6965–6970

Veldhuisen B, Saris JJ, de Haij S, Hayashi T, Reynolds DM, Mochizuki T, Elles R, et al (1997) A spectrum of mutations

in the second gene for autosomal dominant polycystic kidney disease (*PKD2*). Am J Hum Genet 61:547–555

Viribay M, Hayashi T, Tellería D, Mochizuki T, Reynolds DM, Alonso R, Lens XM, et al (1997) Novel stop and frameshifting mutations in the autosomal dominant polycystic kidney disease 2 (*PKD2*) gene. Hum Genet 101:229–234

Ward CJ, Turley H, Ong AC, Comley M, Biddolph S, Chetty R, Ratcliffe PJ, et al (1996) Polycystin, the polycystic kidney disease 1 protein, is expressed by epithelial cells in fetal, adult, and polycystic kidney. Proc Natl Acad Sci USA 93: 1524–1528

Watnick TJ, Torres VE, Gandolph MA, Qian F, Onuchic LF, Klinger KW, Landes G, et al (1998) Somatic mutation in individual liver cysts supports a two-hit model of cystogenesis in autosomal dominant polycystic kidney disease. Mol Cell 2:247–251

Wright GD, Hughes AE, Larkin KA, Doherty CC, Nevin NC (1993) Autosomal dominant polycystic disease with minimal clinical expression unlinked to the *PKD1* locus. Nephrol Dial Transplant 8:491–494

Wu G, D'Agati V, Cai Y, Markowitz G, Park JH, Reynolds DM, Maeda Y, et al (1998) Somatic inactivation of *PKD2* results in polycystic kidney disease. Cell 93:177–188

Xenophontos S, Constantinides R, Hayashi T, Mochizuki T, Somlo S, Pierides A, Constantinou-Deltas C (1997) A translation frameshift mutation induced by a cytosine insertion in the polycystic kidney disease 2 gene (*PDK2*). Hum Mol Genet 6:949–952

Zielenski J, Rozmahel R, Bozon D, Kerem BS, Grzelczak Z, Riordan JR, Rommens J, et al (1991) Genomic DNA sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Genomics 10:214–28