Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 4289–4293, May 1991
Genetics

CpG island in the region of an autosomal dominant polycystic kidney disease locus defines the 5′ end of a gene encoding a putative proton channel

(*PKD1* locus/positional cloning/cDNA/DNA sequence homology/ion channel)

GERALD A. J. GILLESPIE*, STEFAN SOMLO, GREGORY G. GERMINO, DEBRA WEINSTAT-SASLOW, AND STEPHEN T. REEDERS

Departments of Internal Medicine and Human Genetics, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510

Communicated by Edward A. Adelberg, January 31, 1991

ABSTRACT **In an attempt to isolate candidate genes for autosomal dominant polycystic kidney disease, a number of CpG-rich islands have been identified from a region defined genetically as the site of disease mutations. Genomic fragments adjacent to one of these islands were used to isolate cDNAs from both HeLa cells and cultured cystic epithelium that encode a 155-amino acid peptide having four putative transmembrane domains. The corresponding transcript was found in all tissues tested but was most abundant in brain and kidney. Potential control response elements were identified in the genomic region 5′ to the initiation codon. The deduced amino acid sequence has 93% similarity to the 16-kDa proteolipid component that is believed to be part of the proton channel of the vacuolar H^+-ATPase. Possible roles for a mutated proton channel in the pathogenesis of cystic disease were considered. However, sequencing of cDNAs corresponding to both alleles of an affected individual revealed no differences in the deduced amino acid sequence. Moreover, transcript size and abundance were not altered in cystic kidney.**

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by progressive dilatation of renal tubules leading to the formation of renal cysts and irreversible renal failure. Cysts are not confined to the kidney but are also seen in the liver and, occasionally, in the pancreas and spleen. The pathogenesis of ADPKD is not understood.

As a first step toward a better understanding of the molecular basis of ADPKD, a "positional cloning" approach has been adopted. Genetic linkage was established between an ADPKD locus that is the site of ≈96% of disease mutations, *PKD1*, and the α-globin complex on chromosome 16 (1). Extensive linkage studies using a variety of probes (2–4) have led to a detailed genetic map of the region and have refined the initial assignment of *PKD1* to 16p13.3.

In order to identify the *PKD1* gene and its mutations, we have begun to isolate genomic DNA from the *PKD1* region (ref. 5; G.G.G., unpublished work) using, as anchor points, two genetic markers (26.6PROX and GGG1) known to flank the PKD1 locus (refs. 2–5 and S.S., unpublished work). These markers are separated by <750 kilobases (kb) and define the genetic and physical intervals within which *PKD1* lies (G.G.G., unpublished work). To expedite our search for genes within this area, we have concentrated on CpG islands as gene markers; CpG islands usually coincide with the 5′ (or less frequently 3′) end of genes (6). The *PKD1* region is known to be CpG-rich and contains many CpG islands and, therefore, many genes. Three such islands were previously identified by a combination of cosmid walking and directional chromosome jumping (5). Here we report that one of these islands marks the position of a gene that we have subsequently isolated from HeLa and cultured cystic kidney epithelial cell cDNA libraries.

The nucleotide sequences† obtained from the HeLa and cyst cDNAs were identical except for a single base and revealed 77% identity with the gene encoding the 16-kDa proteolipid subunit of bovine chromaffin granule H^+-ATPase, which is believed to be the proton channel of the vacuolar H^+-ATPase (7). A similar, if not identical, H^+-ATPase has been implicated in H^+ secretion and urinary acidification in the intercalated cells of the distal nephron (8, 9). Moreover, a 16-kDa proteolipid having a highly homologous amino acid sequence has also been isolated from gap junction preparations of bovine brain (10), from yeast (11), from mediatophore preparations of *Torpedo* electric organ (12), and from *Drosophila* (13).

MATERIALS AND METHODS

Probes. JA14 is an ≈6.0-kb *Eco*RI fragment subcloned from a genomic cosmid, NK14, and spans a previously described CpG island in the *PKD1* region (5) (Fig. 1). Two *Eag* I fragments from JA14 were used as hybridization probes in cDNA library screening (Fig. 1). Radioactive hybridization probes were prepared using [α-^{32}P]dCTP as described (14).

Cell Lines. The DE-02 cell line was established and characterized by J. Grantham (University of Kansas Medical Center) as follows. Domes from several large cysts were removed, rinsed, treated with collagenase, and placed into T flasks for primary culture in Dulbecco's modified Eagle's medium/Ham's nutrient mixture F-12, 1:1 (GIBCO, BRL), supplemented with 5% fetal bovine serum, insulin, thyroxine, and selenium. After primary cultures reached confluence they were passaged and either expanded or stored frozen in culture medium containing 10% (vol/vol) dimethyl sulfoxide at −90°C. Cells were analyzed by flow cytometry using an antibody to cytokeratin and were 91% cytokeratin positive. After growth on plastic dishes they had a morphological appearance typical of epithelial cells obtained from polycystic kidneys and normal human kidney cortex.

RNA Extraction. Total cellular RNA was prepared from DE-02 cells by the method of Chomczynski and Sacchi (15). Polyadenylylated RNA was isolated by oligo(dT)-cellulose chromatography (16).

cDNA Library Construction. An oligo(dT)-primed cDNA library was constructed from DE-02 poly(A)-enriched RNA by standard procedures (17), except that *Eco*RI/*Not* I adap-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: ADPKD, autosomal dominant polycystic kidney disease.

*To whom reprint requests should be addressed.

†The sequence reported in this paper has been deposited in the GenBank data base (accession no. M62762).

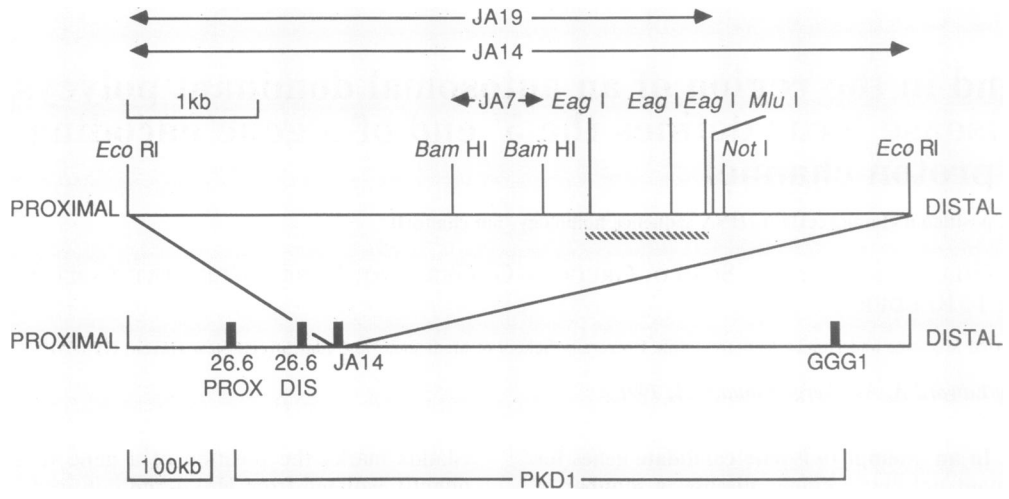

FIG. 1. Restriction map of the 6.0-kb *Eco*RI fragment JA14, which spans the CpG island marking the position of the AJ1 transcript. The hatched bar underlines the *Eag* I fragments used as probes. The relationship of the *Mlu* I site to the transcript is shown in Fig. 2. JA7 is a 0.7-kb *Bam*HI fragment previously used in physical mapping studies (5). The position of this CpG island within the *PKD1* region is indicated below the restriction map. 26.6PROX and GGG1 are the closest known flanking markers for the *PKD1* gene (refs. 4 and 5; S.S., unpublished work).

tors were used instead of *Eco*RI linkers. Size-selected (>0.7 kb) cDNA was ligated to *Eco*RI-digested λgt10 (18), packaged *in vitro*, and plated on *Escherichia coli* C600HfI (18). This library was screened directly prior to amplification. A HeLa cell cDNA library, similarly constructed, was a gift of J. Germino (Yale University School of Medicine). A. Swaroop (University of Michigan) kindly provided transformed lymphocyte and teratocarcinoma cDNA libraries. Fetal and adult kidney cDNA libraries (Clontech) were also screened.

Library Screening. Six hundred thousand plaques from each of the above cDNA libraries were screened. Plaques were transferred to nylon membranes by standard methods (17). Hybridization was carried out according to Church and Gilbert (19). Posthybridization washes of the HeLa, teratocarcinoma, lymphoblast, and adult and fetal kidney cDNA libraries following screening with fragments adjacent to the CpG island were carried out in 1× standard saline/citrate (SSC)/0.1% SDS at 20°C. Subsequent screening of the DE-02 cDNA library with a HeLa cDNA probe was followed by a final stringent wash (0.1× SSC/0.1% SDS at 65°C).

Northern Analysis. Total cellular RNA was denatured with formamide and formaldehyde and electrophoresed in 1.3% agarose/Mops/formaldehyde gels (17). Following electrophoresis, RNA was transferred in 20× SSC to nylon membranes. Hybridization to labeled probes (14) was carried out in Church–Gilbert buffer (19). Stringent washes were performed as described in figure legends.

Subcloning of Recombinant Bacteriophage Inserts. Bacteriophage DNA was digested with *Eco*RI and fractionated in 1% agarose/TAE gels (TAE is 0.04 M Tris acetate, pH 8.0/1 mM EDTA). cDNA inserts were excised from the gel, purified using Prep-A-Gene (Bio-Rad) according to the manufacturer's instructions, and subcloned into *Eco*RI-digested plasmid pUC18 (20) (for restriction analysis and double stranded DNA sequencing) or *Eco*RI-digested phage M13 (21) (for single-stranded DNA sequencing).

DNA Sequencing. Single- and double-stranded sequencing was performed according to standard procedures incorporating the dideoxy method (17, 21).

RESULTS

Isolation of cDNA Clones. Five cDNA libraries, representing renal and nonrenal tissues, were screened with genomic fragments adjacent to a previously described (5) CpG island within the *PKD1* region (Fig. 1). A single positive clone (λAJ1) was isolated from the HeLa library. We postulated that both allelic *PKD1* transcripts would be present in a cDNA library prepared from cyst epithelium of a *PKD1* patient. Therefore, AJ1 was used to screen a sixth cDNA library, which was prepared from mRNA isolated from a polycystic kidney epithelial cell line, DE-02, that had been established from explanted cysts obtained from the kidney of an ADPKD patient. Approximately 20–40 positives were obtained per 50,000 plaques. Phage DNA was isolated from 6 plaques after purification. Single *Eco*RI fragments (0.6–1.2 kb) were found in all but 1.

DNA Sequence Analysis of cDNA Clones. The HeLa cDNA (AJ1) was sequenced. The deduced amino acid sequence predicted a peptide of 155 amino acids containing four hydropathic regions (amino acids Ala^{11}–Ala^{31}, Met^{53}–Leu^{80}, Phe^{90}–Gly^{114}, Phe^{128}–Ala^{138}) suggestive of four transmembrane domains. We reasoned that demonstration of homology with previously isolated and characterized sequences might suggest a function for transcripts isolated from the *PKD1* region of chromosome 16 and allow the latter to be assessed as candidate genes for the *PKD1* locus. Therefore, the DNA sequence of AJ1 was used to search the GenBank data base (22) (January 1990). AJ1 exhibited 77% identity with a previously isolated gene corresponding to the 16-kDa proteolipid H^+-conducting channel of bovine chromaffin granule H^+-ATPase (8). The DNA sequences of six clones from the DE-02 library (AJ2, -3, -4, -5, -8, and -11) were determined and found to be identical with that of AJ1 with the exception of a single base substitution, a conservative T-to-C transition at the third position of the 10th codon in AJ3. The single base substitution seen in AJ3 does not affect the deduced amino acid sequence (see Fig. 2) and presumably reflects allelic variation at this locus. Since the sequences of the HeLa and cyst cDNAs are the same, except for the conservative difference noted, the gene is referred to as AJ1 below.

The deduced amino acid sequence of AJ1 is compared with that of the bovine homolog in Fig. 2. The human and bovine proteins show ≈93% similarity (including conservative substitutions) at the amino acid level, with amino acid differences at positions 4, 6, 51, 83, and 88 (Fig. 2). In addition, a region in which there are numerous differences in amino acid sequence can be seen between amino acid residues 107 and 120. However, in this region the deduced amino acid sequence of AJ1 is identical to that found in *Torpedo*, yeast, and

```
      MluI
-230  ACGCGTCTCCCCCACGGTGCGAAGTGGGTACGGCTCGCAGGGGGGGGCCAGGTCATGT  -171
-170  GACGCGGCCGCGCCGCATTTTGTTCTGCGGTGCTGGTATTTAGAGCGCACGCTGACGGC  -111
-110  CGGATCGCCTTCGCCGCCGCCGCCGCAAACCTTCGTGCCCGCCCGTCCTCGCCCCCG   -51
 -50  CCTCCGCCACCGCCTCGGCCCCGCAGAGCTTGCCCCCTCCCCACCCGCAGACATGTCCGAG    9
                                    Human              M  S  E
                                    Bovine
                                    Yeast                 T
  10  TCCAAGAGCGGCCCCCGAGTATGCTTCGTTTTTCGCCGTCATGGGCGCCTCGGCCGCCATG   69
      S  K  S  G  P  E  Y  A  S  F  F  A  V  M  G  A  S  A  A  M
      A     N
      L  C  -  -     V        P        G  A  I  G  C  A  S     I
  70  GTCTTCAGCGCCCTGGGCGCTGCCTATGGCACAGCCAAGAGCGGTACCGGCATTGCGGCC   129
      V  F  S  A  L  G  A  A  Y  G  T  A  K  S  G  T  G  I  A  A

      I     T  S                                    V        C
 130  ATGTCTGTCATGCGGCCGGAGCAGATCATGAAGTCCATCATCCCAGTGGTCATGGCTGGC   189
      M  S  V  M  R  P  E  Q  I  M  K  S  I  I  P  V  V  M  A  G
                        M
      T  C     L        D  L  L  F     N     V        I
 190  ATCATCGCCATCTACGGCCTGGTGGTGGCAGTCCTCATCGCCAACTCCCTGAATGACGAC   249
      I  I  A  I  Y  G  L  V  V  A  V  L  I  A  N  S  L  N  D  D
                                                            G
                                 S        V  C  Y        G  Q  K
 250  ATCAGCCTCTACAAGAGCTTCCTCCAGCTGGGCGCCGGCCTGAGCGTGGGCCTGAGCGGC   309
      I  S  L  Y  K  S  F  L  Q  L  G  A  G  L  S  V  G  L  S  G
               R
      Q  A        T  G     I
 310  CTGGCAGCCGGCTTTGCCATCGGCATCGTGGGGGACGCTGGCGTGCGGGGCACCGCCCAG   369
      L  A  A  G  F  A  I  G  I  V  G  D  A  G  V  R  G  T  A  Q
               R  S  P  S  A  L  L     T  Q     R  A  C
                                                      S  S
 370  CAGCCCCGACTATTCGTGGGCATGATCCTGATTCTCATCTTCGCCGAGGTGCTCGGCCTC   429
      Q  P  R  L  F  V  G  M  I  L  I  L  I  F  A  E  V  L  G  L


 430  TACGGTCTCATCGTCGCCCTCATCCTCTCCACAAAGTAGACCCTCTCCGAGCCCACCAGC   489
      Y  G  L  I  V  A  L  I  L  S  T  K

                              L     N  S  R  A  T  Q  D  V  V  C
 490  CACAGAATATTATGTAAAGACCACCCCTCCTCATTCCAGAACGAACAGCCTGACACATAC   549
 550  GCAGCGCCGCCCCGCCCCCAGTAGTTGGTCTTGTACATGCGCAGTATCCTAGTGCCCATCG   609
 610  TCTGTTTCCCCGCCTTGCCCCCGCCCGCCCCCGTGCCGTGGACATCTGGGCCCACTCATCG   669
 670  CCCCTCCAGGCCCCCCGGCGCCCCACCCCCTAAAGTGCTCTAGTATGCGGATGATTTAGAA   729
 730  TTGTCATTTCTCTTTACTGGATGTTTATTATTAAAGATCTCGCCTGTTCCTGCGTCTGCG   789
 790  GAGCCGCCCTTGTCTCCCAGCTATCTATAACCTTAGCTTGTGTGTCGCCTTGTGGGTTCC   849
 850  TGTTGCTGAGACTTTTCCTGGATGGAGCCGCCCTCACCGCGCCCGTGGCCCTGCGCGGAG   909
 910  CTGTGTCCAATAAAGTTCTTGCT                                       932
```

FIG. 2. Nucleotide sequence of AJ1 (cDNA) and 130 base pairs of 5′ genomic sequence. The deduced amino acid sequence of AJ1 is compared with the peptide sequences of the bovine and yeast 16-kDa proteolipid component of the vacuolar H^+-ATPase. Nucleotides are numbered 5′ to 3′, beginning with the initiator methionine codon. The 5′ untranslated region extends at least as far as nucleotide −99, since AJ1 contains sequence up to this point. The transcription initiation point has not been mapped. Sequence from the *Mlu* I site at −230 (part of the CpG island shown in Fig. 1) to −100 was derived from the genomic clone JA19. Potential binding sites for transcription factor Sp1 are underlined and the polyadenylylation signal is depicted in bold type.

Drosophila, raising the possibility that the deduced bovine sequence is incorrect.

Genomic Analysis. Hybridization of AJ1 to Southern-blotted genomic digests, even when washed at high stringency (0.1× SSC, 65°C), revealed several hybridizing fragments. Comparison of the sizes of these bands with those predicted from hybridization of AJ1 to a set of overlapping cosmids from the *PKD1* region (5) indicated that some of the hybridizing fragments were from other (homologous) loci. For example, in *Taq* I genomic DNA digests (Fig. 3), AJ1 hybridized strongly to two fragments (one of which is allelic with a third fragment as described below). Somatic-cell hybrid analysis using several chromosome 16-only human–rodent lines (4) showed that only the 1.4/1.7-kb allelic fragments derive from chromosome 16 (data not shown). The chromosome 16 origin of these fragments was supported by the observation that the alleles segregate with 26.6PROX and GGG1, two genetic markers that are known to flank *PKD1* (refs. 2–5; S.S., unpublished work). A somatic-cell hybrid panel was used to show that two of the non-chromosome 16 hybridizing fragments map to chromosomes 3 and 6. At lower stringency many more cross-hybridizing fragments were observed.

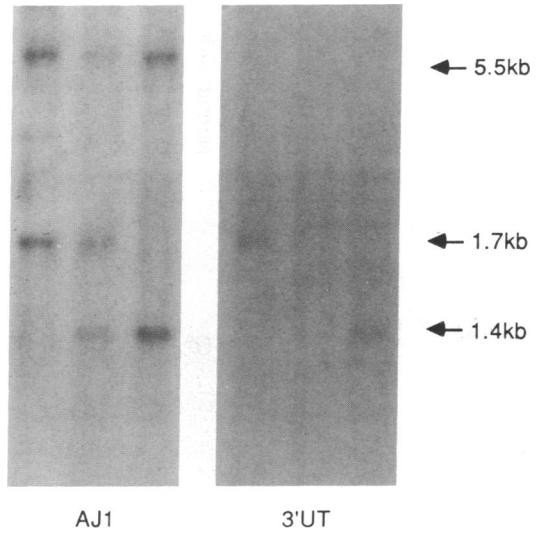

FIG. 3. *Taq* I-digested genomic DNA from three unrelated individuals was hybridized with AJ1 (*Left*) and with a 3′ untranslated-region (3′UT) probe from AJ1 (*Right*). Individuals 1 and 3 are homozygous for the 1.7-kb and 1.4-kb *Taq* I alleles, respectively, whereas individual 2 is heterozygous. The 5.5-kb fragment hybridizes with AJ1 but not with the 3′UT probe. Final stringent washing was in 0.1× SSC/0.1% SDS at 65°C for 20 min.

In view of the fact that there are several AJ1-hybridizing loci in the genome, we set out to confirm that AJ1 is indeed encoded by a gene in the *PKD1* region. Two sets of experiments were carried out. First, the sequence of a genomic cosmid clone from chromosome 16 was compared with AJ1. Second, a fragment from the 3′ untranslated region of AJ1 that detects a single locus was prepared and studied.

Genomic DNA Sequence. In the course of cloning the *PKD1* region, a number of genomic cosmids and subclones were generated. One of these, JA19, a 4.3-kb *Eco*RI–*Mlu* I fragment (Fig. 1) corresponds to part of the CpG island that hybridizes to the AJ1 transcript (5). DNA sequence was obtained from the *Mlu* I end of JA19. One hundred percent sequence identity to the 5′ untranslated region and first 40 bases of the coding region of AJ1 was observed, the homologous region commencing 124 bases from the *Mlu* I site. Additional sequence obtained using a primer corresponding to nucleotides −85 to −63 of AJ1 again revealed 100% sequence identity with JA19 up to the point where intervening sequence is encountered in the genomic subclone (at nucleotide 79 of the cDNA). Sequence analysis of JA19 using primers homologous to the 3′ untranslated region and various parts of the coding region of AJ1 also demonstrated perfect identity between JA19 and AJ1. These data strongly suggest that AJ1 is encoded by the JA19 genomic fragment of chromosome 16 and not by a homologous locus and also indicate that AJ1 is transcribed in a distal–proximal direction with respect to the chromosome.

A 0.27-kb *Apa* I–*Eco*RI fragment from the 3′ untranslated part of AJ1 was shown to hybridize specifically to the *PKD1*-linked polymorphic *Taq* I fragments mentioned above and not to any of the other homologous loci (Fig. 3). These data confirm that AJ1 is encoded by a gene in the *PKD1* region of chromosome 16.

Northern Blot Analysis. The pattern of expression of AJ1 was investigated using Northern blots prepared with total cellular RNA from a number of tissues (Fig. 4). Expression of AJ1 appears to be ubiquitous, with the highest levels of mRNA detected in brain and kidney. No difference in hybridization pattern was observed when RNA preparations from normal and polycystic kidneys were compared or when epithelial cell line DE-02 RNA was compared with normal

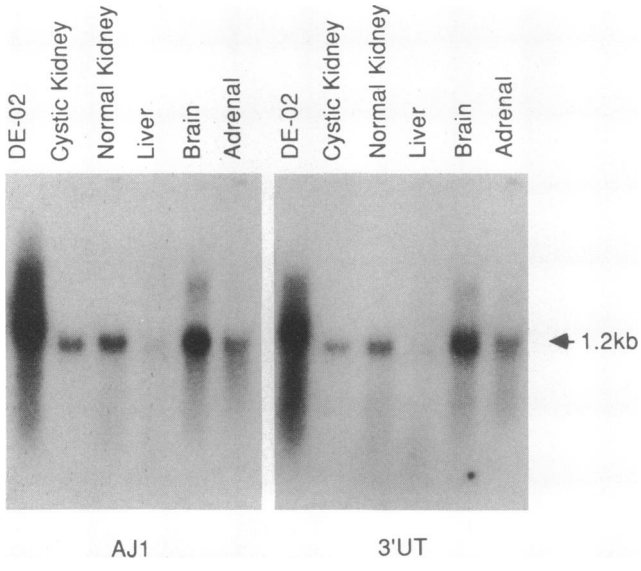

FIG. 4. Northern blot of human total RNA samples from human tissues hybridized with the entire cDNA, AJ1 (*Left*), and 3′ untranslated region (3′UT) alone (*Right*). A 1.2-kb transcript was detected with both probes in all tissues. Final stringent washing was in 0.1× SSC/0.1% SDS at 65°C for 10 min.

kidney RNA. Identical results were obtained using the chromosome 16 locus-specific probe derived from the 3′ untranslated region of AJ1 (Fig. 4), indicating that the transcripts detected by AJ1 originated from the *PKD1* region.

DISCUSSION

Identification and use of CpG island probes in the isolation of expressed sequences is well documented (23, 24). We previously described three CpG islands in the region of *PKD1* (5). One of these has been used in this study to isolate a human cDNA clone, AJ1, with sequence similarity to the proteolipid subunit that, as part of a multimer, forms the proton channel of the bovine chromaffin granule H^+-ATPase (7). Homologous genes and their proteins have also been isolated from yeast (11), bovine brain gap junctions (10), *Torpedo* electric organ (12), and *Drosophila* (13). The H^+-ATPase in question belongs to the vacuolar class of proton pump (25) that is found associated with intracellular vesicles. A similar H^+-ATPase has been found in renal tubular epithelial cells (8) and, in particular, in the intercalated cells of rat kidney cortical collecting duct, where it functions in urinary acidification (9).

The differences in sequence between the bovine and human sequence suggested that AJ1 did not encode the counterpart of the bovine H^+-ATPase 16-kDa proteolipid subunit but represented a related gene. However, the major (nonconservative) differences between the bovine and human sequences are concentrated in a region between amino acids 107 and 120. In this region there is complete identity between the human, *Drosophila*, *Torpedo*, and yeast sequences, leading us to conclude that AJ1 indeed represents the human counterpart of the 16-kDa proton-channel component. Even so, genomic hybridization studies in the human show that there are at least two other homologs of AJ1. It remains to be determined whether these are functional sequences or pseudogenes. The sequence similarity of the 16-kDa proteolipid component of the vacuolar H^+-ATPase, the acetylcholine-transporting *Torpedo* mediatophore, and a fragment of the sequence of a gap junction component raises the possibility that this transmembrane peptide has several distinct cellular roles.

It has been suggested (26) that the vacuolar H^+-ATPase maintains a significant pH gradient across the vacuolar membrane. The existence of a low-pH compartment within polarized epithelial cells appears to be required for appropriate targeting of secreted proteins to the basolateral cell surface (27). This has been concluded from studies in which laminin and heparan sulfate proteoglycan (both basement membrane components) were shown to be misdirected to both the apical and basolateral membranes of MDCK cells in the presence of 10 mM NH_4Cl. Presumably NH_4Cl acts to perturb the normal targeting of secretory proteins to the basolateral surface by raising the pH of intracellular compartments. Of note in this regard are reports of tubular basement membrane changes associated with cystic kidney disease. In particular, defective biosynthesis, secretion, and/or degradation of heparan sulfate proteoglycans as judged by loss of ruthenium red-staining material has been observed (28) and postulated to lead to defective assembly and structure of the basement membrane. These defects in basement membrane could be the result of incorrect targeting of basement membrane components in cystic disease. However, membrane-associated proteins, whose correct targeting is not thought to depend on vacuolar pH, are also misdirected in ADPKD. For example, Na^+/K^+-ATPase is expressed on the apical surface of the epithelial cells in early ADPKD (29). Thus, the polarization abnormalities in ADPKD do not appear to be specific for targeting mechanisms dependent on vacuolar acidification.

To assess further the candidacy of AJ1, we searched for mutations in the coding sequence of AJ1 that could be responsible for abnormal protein function. The DNA sequence of allelic cDNAs isolated from a primary culture of cyst epithelium obtained from an ADPKD patient revealed only a single base difference between the two alleles. This change does not affect the deduced amino acid sequence. Thus, the coding region of the gene for the 16-kDa proton channel of the H^+-ATPase is not mutated in *PKD1*. In addition, Northern blot analysis suggests that there are no qualitative or quantitative differences in expression of this gene between normal and ADPKD kidneys, or between normal kidney and a cyst-derived renal epithelial cell line. Therefore, involvement of a regulatory abnormality or RNA processing defect in the proton-channel gene seems unlikely to be responsible for ADPKD.

DNA sequencing of the chromosome 16 genomic locus encoding AJ1 revealed a C+G-rich region 5′ to the coding region, in which a number of motifs resembling Sp1 binding sites (30) reside (see Fig. 2). No other regulatory motifs (e.g., TATA or CAAT) could be discerned. Other cases in which upstream C+G-rich regions are found in the absence of TATA and CAAT boxes have been documented (31, 32), and it has been suggested that such findings are typical characteristics of "housekeeping" genes (33).

We are grateful to Dr. C. Slayman for her critical review of the manuscript. We thank Dr. J. Grantham (University of Kansas Medical Center) for cyst explant cultures. G.A.J.G. is supported by National Institutes of Health Grant DK40703. S.S. is a National Kidney Foundation/Polycystic Kidney Disease Research Foundation fellow. G.G.G. is a Physician-Scientist Award recipient, Grant DK01423. S.T.R. is an assistant investigator with the Howard Hughes Medical Institute.

1. Reeders, S. T., Breuning, M. H., Davies, K. E., Nicholls, D. R., Jarman, A. J., Higgs, D. R., Pearson, P. L. & Weatherall, D. J. (1985) *Nature (London)* **317**, 542–544.
2. Breuning, M. H., Snijdewint, F. G. M., Brunner, H., Verwest, A., IJdo, J. W., Saris, J. J., Dauwerse, J. G., Blonden, L., Keith, T., Callen, D. F., Hyland, V. J., Ziao, G. H., Scherer, G., Higgs, D. R., Harris, P., Bachner, L., Reeders, S. T.,

Germino, G., Pearson, P. L. & van Ommen, G. J. B. (1990) *J. Med. Genet.* **27,** 603–613.
3. Reeders, S. T., Keith, T., Green, P., Germino, G. G., Barton, N. J., Lehmann, O. J., Brown, V. A., Phipps, P., Morgan, J., Bear, J. C. & Parfrey, P. (1988) *Genomics* **3,** 150–155.
4. Germino, G. G., Barton, N. J., Lamb, J., Higgs, D. R., Harris, P., Scherer, G., Nakamura, Y. & Reeders, S. T. (1990) *Am. J. Hum. Genet.* **46,** 925–933.
5. Gillespie, G. A. J., Germino, G. G., Somlo, S., Weinstat-Saslow, D., Breuning, M. H. & Reeders, S. T. (1990) *Nucleic Acids Res.* **18,** 7071–7075.
6. Lindsay, S. & Bird, A. P. (1987) *Nature (London)* **327,** 336–338.
7. Mandel, M., Moriyama, Y., Hulmes, J. D., Pan, Y.-C. E., Nelson, H. & Nelson, N. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 5521–5524.
8. Hirsch, S., Strauss, A., Masood, K., Lee, S., Sukhatme, V. & Gluck, S. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3004–3008.
9. Alper, S. L., Natale, J., Gluck, S., Lodish, H. F. & Brown, D. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5429–5433.
10. Dermietzel, R., Völker, M., Hwang, T.-K., Berzborn, R. J. & Meyer, H. E. (1989) *FEBS Lett.* **253,** 1–5.
11. Nelson, H. & Nelson, N. (1989) *FEBS Lett.* **247,** 147–153.
12. Birman, S., Meunier, F.-M., Lesbats, B., LeCaer, J.-P., Rossier, J. & Israël, M. (1990) *FEBS Lett.* **261,** 303–306.
13. Meagher, L., McLean, P. & Finbow, M. E. (1990) *Nucleic Acids Res.* **18,** 6712.
14. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
15. Chomczynski, P. & Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
16. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
17. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K., eds. (1987) *Current Protocols in Molecular Biology* (Wiley, New York), Vol. 1.
18. Huynh, T. V., Young, R. A. & Davis, R. W. (1984) *DNA Cloning Techniques: A Practical Approach*, ed. Glover, D. (IRL, Oxford, U.K.), pp. 49–78.
19. Church, G. M. & Gilbert, W. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1991–1995.
20. Yanisch-Perron, C., Vieirra, J. & Messing, J. (1988) *Gene* **33,** 103–119.
21. Sanger, F., Coulson, A. R., Hong, G. F., Hill, D. F. & Peterson, G. Z. (1982) *Proc. Natl. Acad. Sci. USA* **162,** 729–733.
22. Bilofsky, H. S. & Burks, C. (1988) *Nucleic Acids Res.* **16,** 1861–1864.
23. Sargent, C. A., Dunham, I. & Campbell, R. D. (1989) *EMBO J.* **8,** 2305–2312.
24. Spies, T., Bresnahan, M. & Strominger, J. L. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8955–8958.
25. Forgac, M. (1989) *Physiol. Rev.* **69,** 765–796.
26. Mellman, I., Fuchs, R. & Helenius, A. (1986) *Annu. Rev. Biochem.* **55,** 663–700.
27. Caplan, M. J., Stow, J. L., Newmann, A. P., Madri, J., Anderson, H. C., Farquar, M. G., Palade, G. E. & Jamieson, J. D. (1987) *Nature (London)* **329,** 632–635.
28. Carone, F. A., Hollenberg, P. F., Nakamura, S., Punyarit, P., Glogowski, W. & Flouret, G. (1989) *Kidney Int.* **36,** 1034–1040.
29. Wilson, P. D., Sherwood, A. C., Palla, K., Du, J., Watson, R. & Norman, J. T. (1991) *Am. J. Physiol.* **260,** F420–F430.
30. Mitchell, P. & Tjian, R. (1989) *Science* **245,** 371–378.
31. Dynan, W. S. & Tjian, R. (1983) *Cell* **35,** 79–87.
32. Dynan, W. S. (1986) *Trends Genet.* **2,** 196–197.
33. Shull, M. M., Pugh, D. G. & Lingrel, J. G. (1990) *Genomics* **6,** 451–460.