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Downregulation of Ke 6, a Novel Gene Encoded within the Major Histocompatibility Complex, in Murine Polycystic Kidney Disease

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Polycystic kidney disease (PKD) is characterized by progressive enlargement of the kidneys due to numerous expanding cysts ultimately leading to renal failure. We have identified a gene, Ke 6, located within the H-2K/t^{w5} region on mouse chromosome 17, which is downregulated in two distinct murine models of heritable PKD. Ke 6 is a member of the short-chain alcohol dehydrogenase family and possesses remarkable amino acid sequence conservation with several bacterial proteins with oxidoreductase function. The Ke 6 gene gives rise to two transcripts—a 1-kb Ke 6a mRNA which is abundant in kidney and liver tissue and a 1.4-kb Ke 6b mRNA which is found at a moderate level in spleen tissue. We report here the complete nucleotide sequence of Ke 6a cDNA and the expression of the Ke 6 gene in murine models of PKD. The Ke 6 gene may be intimately involved in the manifestation of these cystic kidney diseases.

There are two forms of heritable polycystic kidney disease (PKD) in humans—autosomal dominant (ADPKD) and autosomal recessive (ARPKD). ADPKD is the major inherited cause of kidney failure in adults and occurs at a frequency of 1 in 1,000 (13, 15). The human ADPKD has been linked to two different loci (22), one of which has been mapped to chromosome 16 (32). ARPKD occurs with an incidence as high as 1 in 6,000 live births (15) and is a more aggressive form of the disease, resulting in death shortly after birth. Recently, several mouse models of PKD which are useful for investigating the pathogenesis of the disease have been described. In mice with congenital polycystic kidney (*cpk*) disease, inheritance, progression, and histopathology closely resemble human ARPKD (12), and animals die within 20 to 25 days of life. In mice with progressive polycystic kidney (*pcy*) disease (39) and in recently described juvenile cystic kidney (*jck*) mice (4), the disease progresses more slowly and affected mice usually survive for 6 to 8 months (4, 39). In this respect, the *pcy* and *jck* murine models resemble human ADPKD, even though the pattern of inheritance of the disease is strictly autosomal recessive (4, 38). The *cpk* and *pcy* genes have been mapped to chromosomes 12 (8) and 9 (38), respectively, and although the *jck* gene has not yet been mapped, it is not allelic to either *cpk* or *pcy* (4). Several other disorders in humans (for example, Von Hipple-Lindau syndrome and tuberous sclerosis) and diseases in rats, cats, and minks in which renal cysts develop have been reported (31). PKD has also been induced in laboratory mice by disrupting a gene, *Tg737*, on mouse chromosome 14 by transgenic insertion (26). It is thus evident that mutations at multiple loci can give rise to renal cystic diseases (31). We have identified a gene, Ke 6, within the H-2K/t^{w5} region of mouse chromosome 17 whose pattern of aberrant expression in two different murine models of PKD appears to imply its intimate involvement in the manifestation of disease. In this paper, we report the complete nucleotide sequence of Ke 6 cDNA and the pattern of Ke 6 gene expression in normal and cystic kidneys.

MATERIALS AND METHODS

Animals. *cpk* heterozygous mice of the congeneic strain DBA/2J were obtained from Jackson Laboratories. Homozygous *cpk* mice were detected by the distension of the abdomen by day 14 of age. Cystic mice and normal littermates were sacrificed at 14 and 18 days of age, and tissues were obtained for RNA extraction. Homozygous *jck* mice with a C57BL/6J background at different stages of the disease (11 and 25 weeks) were provided by David Beier (Brigham and Women's Hospital). Wild-type mice of C57BL/6J (6 to 9 weeks of age) and DBA/2J (3 to 4 weeks of age) backgrounds were also sacrificed to obtain tissue for RNA extraction.

Poly(A)$^+$ RNA extraction. Poly(A)$^+$ RNA was extracted from tissues directly by the method described previously (34). Briefly, this method involves homogenizing tissue in 100 mM NaCl–1 mM EDTA–2.0% sodium dodecyl sulfate (SDS)–20 mM Tris (pH 7.4) in the presence of 500 μg of proteinase K per ml. After incubation at 45°C for 0.5 h, oligo(dT) cellulose was added to the homogenate after the salt concentration was increased to 500 mM. This mixture was rocked at room temperature for 45 min to bind the poly(A)$^+$ RNA to the oligo(dT) cellulose. The oligo(dT) cellulose was washed three times in 400 mM NaCl–1 mM EDTA–0.2% SDS–10 mM Tris, pH 7.4, and packed into a Econo column (Bio-Rad). The poly(A)$^+$ RNA was eluted with 10 mM Tris (pH 7.4)–1 mM EDTA buffer.

Northern (RNA) blot analysis. Poly(A)$^+$ RNA was separated on an agarose-formaldehyde gel (1% agarose) and then capillary blotted onto a nylon membrane MagnaGraph (MSI). The RNA was UV cross-linked to the membrane with a Stratalinker (Stratagene). Membranes were prehybridized and hybridized in Church's buffer containing 1% bovine

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serum albumin, 7% SDS, 1 mM EDTA, and 0.5 M sodium phosphate, pH 7.0, at 65°C for 16 to 18 h. DNA probes were labeled by a random-primed labeling method (10), cleaned by spin column, and added to the hybridization fluid at 10^6 cpm/ml. Membranes were washed at 50°C in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS, washed in 0.1× SSC–0.1% SDS for approximately 1 h, and then exposed to X-ray film with intensifying screens at −70°C. All of the Northern blots were probed with mouse β-actin cDNA, protein tyrosine phosphatase (PTP) cDNA, and/or L-ferritin cDNA to check for equal loading. Blots hybridized with PTP were washed in 2× SSC–0.1% SDS at 50°C. In addition, RNA gels were stained with ethidium bromide and photographed to check for equal ethidium bromide staining intensity across lanes. Quantitation was performed by densitometric scanning of autoradiograms with a Microtek MSF-300GS image scanner, and the autoradiograms were analyzed with Image 1.31n software.

cDNA cloning. A total of 10^6 PFU of a mouse kidney cDNA library in λgt 10 (Clonetech) was screened with a 0.7-kb *Sma*I fragment obtained from cosmid 32 of the t^{w5} haplotype. Three positive clones were selected and cloned into pBluescript II SK+ phagemid vector (Stratagene). An insert from one of these clones, p 11.6, was then used to screen 10^6 PFU of a mouse kidney cDNA library constructed in λgt 22A vector with a Bethesda Research Laboratories superscript cDNA synthesis kit. Four full-length positives clones were cloned into pBluescript II SK+ phagemid vector for further analysis. Replicas of the plaques were made with MSI magnagraph membranes by standard methods. Prehybridization, hybridization, and washes were done as described for Northern blots. Positive bacteriophage were plaque purified and the cDNA insert was excised, isolated by gel electrophoresis, and purified by using Geneclean (Bio 101) for cloning into the Bluescript vector.

DNA sequencing. cDNA sequences were determined by the dideoxy chain-termination method (33) by using the Sequenase version 2.0 kit (United States Biochemical Corp.) and α-^{35}S-dATP as specified by the manufacturers. Regions of ambiguous DNA sequence were further analyzed by the use of dITP in the sequencing reactions. The reaction products were resolved on denaturing polyacrylamide gels which were fixed, dried, and autoradiographed. All sequence information was obtained by several sequencing reaction runs and by sequencing overlapping subclones. A gap in sequence information toward the 3′ end of the cDNA was determined by synthesizing a 17-mer custom primer (Oligos, Inc.) and used in the sequencing reaction. The sequence alignment, identity, and similarity scores were determined with the aid of the BLASTP program (2) at the National Center for Biotechnology Information.

Reticulocyte lysate translation of in vitro-synthesized Ke 6 RNA. Clone p13.3 containing the full-length cDNA of Ke 6a and ferritin H cDNA were linearized and transcribed in vitro with T7 and T3 polymerase (Stratagene) according to the manufacturer's specifications in the presence of 1 mM ribonucleoside triphosphates. The synthetic RNA was separated from unincorporated nucleotides by using a spin column and translated in a rabbit reticulocyte lysate system (Promega) as directed in the presence of L-[^{35}S]methionine. β-Lactamase RNA and ferritin H RNA were also used as templates for in vitro translation as controls. Labeled products were separated by SDS-polyacrylamide gel electrophoresis (PAGE) and visualized by autoradiography.

RESULTS

Identification of the location of the Ke 6 gene within cosmid 32. Previously, in a search for expressed genes in the t^{w5} haplotype, 170 kb of DNA containing the entire H-2K region within chromosome 17 had been cloned in a series of overlapping cosmids (1). Any gene identified in this region is a potential candidate for the tcl-w5 gene, which is an early-acting embryonic lethal mutation demonstrated to be recombinationally inseparable from the H-2K gene (3). Five expressed genes, Ke 1 through Ke 5, within this region have been identified (1). Characterization of the Ke 4 gene led to detection of a transcript expressed at a very high level in the kidney (37). To precisely locate this gene, which we identify as Ke 6, we sequentially probed strips of Northern blots containing poly(A)$^+$ RNA from the kidney and spleen with smaller pieces of the 4.9-kb *Bam*HI fragment. Six internal *Sma*I sites within the 4.9-kb *Bam*HI fragment yield subfragments of sizes 1.7, 0.25, 0.7, 0.065, 1.9, 0.140, and 0.130 kb (Fig. 1A). Because the 5′ portion of the 1.7-kb fragment contains the 3′ end of the previously isolated Ke 4 gene (37), it hybridized to the Ke 4 mRNA as expected (Fig. 1B). A 1.0-kb Ke 6 transcript that is abundant in the kidney was detectable when the 0.7 and 1.9 *Sma*I fragments were used as hybridization probes. In addition, a transcript of about 1.4 kb in size was detected at moderate levels in spleen cells with the 0.7- and 1.9-kb genomic *Sma*I probes. We postulate that the longer transcript is formed by alternative splicing of the Ke 6 gene's primary transcript because we observe only one band in genomic Southern blots of mouse DNA treated with several restriction enzymes that do not cut within the gene (not shown). Because there is only a single gene, the longer spleen transcript must therefore arise from the same transcriptional unit by an alternate splicing event. The 0.25-kb *Sma*I fragment faintly hybridizes to the 1.0-kb kidney transcript but is able to clearly detect the 1.4-kb transcript in spleen RNA, presumably because it contains more of the exon sequence specific for the longer mRNA. A smear of hybridization signal noticeable with this probe is most likely due to the presence of repeated sequences. We have identified the 1.0-kb transcript as Ke 6a and the 1.4-kb transcript as Ke 6b.

Isolation and determination of the primary structure of Ke 6 cDNA. To clone Ke 6 cDNA, a mouse kidney cDNA library was screened with a 0.7-kb genomic *Sma*I fragment (Fig. 1), and three Ke 6a clones (11.6, 12.3, 20.1.1) with insert sizes between 0.6 and 0.7 kb were isolated. To isolate a full-length cDNA, a second kidney cDNA library with a larger average insert size was constructed and screened with clone 11.6, and four 1-kb Ke 6a clones (4.2, 4.3, 13.1, 13.3) were isolated. Clones 11.6, 4.3, and 13.1 were fully sequenced, and clones 20.1.1, 4.2, and 13.3 were partially sequenced. Nucleotide sequence determination has revealed an open reading frame of 780 nucleotides which predicts a protein of 259 amino acids with a molecular mass of 26.6 kDa (Fig. 2). The nucleotide sequence surrounding the initiation codon perfectly matches the consensus initiation sequence (23). Reticulocyte lysate translation of in vitro-transcribed Ke 6a mRNA yields a protein with a molecular mass of 31.5 kDa on SDS-PAGE (Fig. 3). The slight difference between apparent and expected molecular mass has been seen elsewhere (19). Ke 6a is highly homologous to several proteins in bacteria which are either known or presumed to have oxidoreductase or dehydrogenase function (Fig. 4); this similarity is striking, considering the evolutionary distance between mammals and bacteria. The two regions of Ke 6a

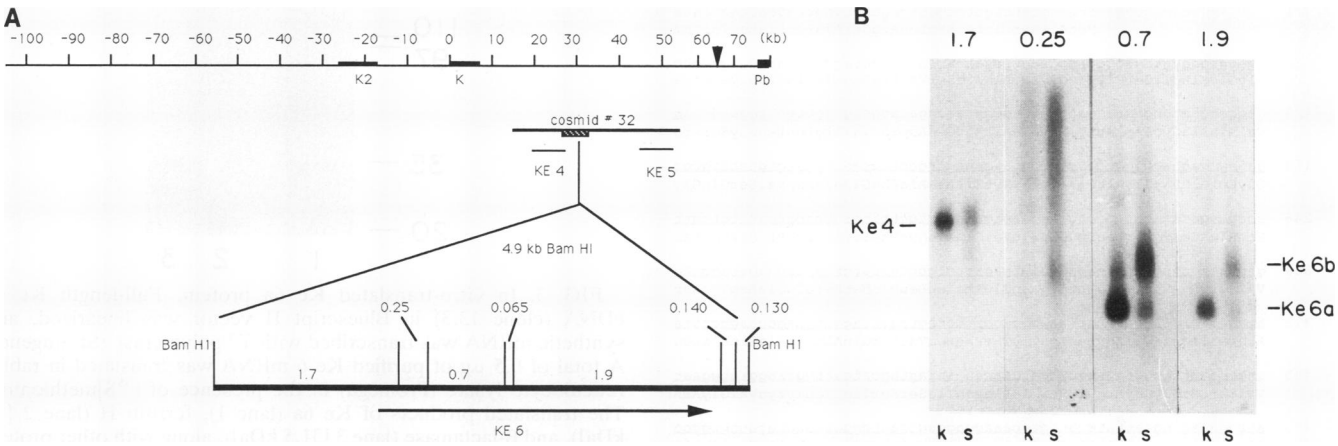

FIG. 1. Location of the Ke 6 gene within the H-2K region of the t^{w5} haplotype chromosome 17 (A) and localization of Ke 6 gene transcripts within cosmid 32 (B). (A) The locations of the H-2K and K2 class I genes and of the class II gene Pb are shown as solid boxes. Because the MHC is inverted in t haplotypes (35), the centromere is on the right side and the telomere is on the left. The arrow at about +65 kb indicates the centromeric limit of the region where the tcl-w5 gene should reside. The relative positions of previously identified genes Ke 4 and Ke 5 (1) are indicated. The crosshatched box within cosmid 32 indicates the 4.9-kb *Bam*HI (Bam HI) fragment where the Ke 6 gene is located. This region is enlarged below to indicate the locations of the *Sma*I restriction fragments, some of which were used as probes to detect the transcripts of the Ke 6 gene as shown in panel B. The direction of transcription and approximate position of Ke 6 gene are indicated by the arrow. (B) Northern blot analysis of poly(A)$^+$ RNA from kidney and spleen tissue probed with the *Sma*I genomic fragments shown in panel A indicated that there are two Ke 6 transcripts—a 1-kb mRNA, Ke 6a, and a 1.4-kb mRNA, Ke 6b. Northern blot analysis was performed with 4 μg of poly(A)$^+$ RNA from mouse kidney (k) and mouse spleen (s) tissue. Poly(A)$^+$ RNA was separated on a 1% agarose–2.2 M formaldehyde gel and blotted onto nylon membranes (0.22 μm; Magnagraph, MSI). [α-^{32}P]dCTP-labeled (10) genomic restriction fragments depicted in panel A and noted above the lanes were used as probes for hybridization at 65°C in Church's buffer for 16 to 20 h, and membranes were washed in 0.2× SSC–0.1% SDS at 50°C. Ke 6a, Ke 6b, and Ke 4 mRNAs are indicated.

protein with the greatest homology with compared proteins (Fig. 4) lie between amino acids 11 to 37 and 97 to 259. In the latter carboxy-terminal region of 163 residues, Ke 6a protein shares 42% identity and 74% similarity with *Escherichia coli* 3-oxoacyl-[acyl-carrier protein] reductase (30), 44% identity and 70% similarity with *Rhizobium meliloti* nodulation protein G (11), 44% identity and 66% similarity with *Alcaligenes eutrophus* acetoacetyl-coenzyme A reductase (29), 36% identity and 61% similarity with *Bacillus megaterium* glucose dehydrogenase (25), 33% identity and 58% similarity with *Flavobacterium* sp. *N*-acyl-D-mannosamine dehydrogenase (41), and 35% identity and 63% similarity with *Eubacterium* sp. 7-alpha-hydroxysteroid dehydrogenase (14). Ke 6a protein contains a sequence motif typical of members of the short-chain alcohol dehydrogenase family (Fig. 2 and 4). Interestingly, all of the Ke 6a homologous proteins are of similar sizes (240 to 260 residues), and most have been found to be members of a superfamily of pyridine nucleotide-linked sugar-alcohol-polyol dehydrogenases (5). We postulate that Ke 6a protein has oxidoreductase or dehydrogenase activity.

Gene expression of Ke 6 in normal and polycystic kidneys. The steady-state level of Ke 6a mRNA is high in kidney and liver tissue and low in all other tissues examined (Fig. 5). The longer Ke 6b mRNA is found at moderate abundance in the spleen. The Ke 6b transcript is detected by the Ke 6a cDNA (Fig. 5) and by the genomic *Sma*I fragments (0.7 and 1.9 kb, Fig. 1B), presumably because the two transcripts have largely similar sequences at the 3′ end. Because Ke 6 is a novel mammalian gene with its highest level of expression in kidney and in liver, both tissues which are affected to the greatest degree in PKD (15, 24), we investigated the expression of the Ke 6 gene in different rodent models of PKD by using Northern blot analysis. The *cpk* homozygous mice are affected with a rapidly progressive form of PKD that is fatal by 20 to 25 days of life and closely resembles the human ARPKD (12). Interestingly, we found a 5- to 10-fold-lower level of Ke 6a mRNA in kidney and liver tissue of 14- to 18-day-old DBA/2J *cpk/cpk* mice than in DBA/2J +/+ mice (Fig. 6A). The extent of Ke 6 repression in the liver is similar to that in the kidney, even though the liver tissue of *cpk/cpk* mice appears morphologically normal upon gross examination and is also determined not to contain cysts by histological investigations (12). Moreover, there is also a reduction in the level of Ke 6a mRNA in other tissues, such as brain (Fig. 6A) and heart (not shown), in *cpk/cpk* mice, which implies that the downregulation of the Ke 6 gene in the kidney is not just a reflection of the loss of functional tissue. In order to determine that the decrease in Ke 6 gene expression in the tissues of the *cpk/cpk* mice is a specific alteration, the same Northern blot membrane was sequentially probed with protein tyrosine phosphatase, actin, and ferritin cDNAs. The lower panels of Fig. 6A demonstrate the unaltered level of expression of these genes in the tissues of *cpk/cpk* mice. The hybridization signals of these mRNAs also serve as an internal control to indicate that the decrease in Ke 6 hybridization signal is not due to any loading error of RNA samples during Northern blot analysis. The level of PTP mRNA is uniformly equal in kidney, liver, and brain tissue (16); however, the varying hybridization intensities of actin and ferritin signals reflect their normal basal level of gene expression (9, 28) in those tissues.

In a different murine model of PKD, the *jck* homozygous mice, the animals develop cystic kidneys considerably later in life and the progression of the disease is slower. Cystic changes in the kidney slowly enlarge with increasing age, whereas no histological abnormalities are found in any other organs (4). We found a progressive decrease in the level of Ke 6a mRNA in the kidneys of the C57BL/6 *jck* homozygous mice which correlates with the advancement of the disease (Fig. 6B). The reduction in the level of Ke 6a mRNA is more

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1          cacagccgccatggcgtctcagctccggctccgctctgcgctggccctggtcaca
                    MetAlaSerGlnLeuArgLeuArgSerAlaLeuAlaLeuValThr

56  ggtggtgcgggtagcggcatcggccgtgcgatcagcgtgcgcctagcggcagagggcgccgcc
    GlyGlyAlaGlySerGlyIleGlyArgAlaIleSerValArgLeuAlaAlaGluGlyAlaAla
                  §                    Δ
120 gtggccgcctgcgacctggacggggccgcggcacaggacacggtgcggctgctgggaagcccg
    ValAlaAlaCysAspLeuAspGlyAlaAlaAlaGlnAspThrValArgLeuLeuGlySerPro
                                       Δ
183 gggagcgaggacggggcgccgcgcggcaagcacgctgccttccaagcggatgtgtctcagggc
    GlySerGluAspGlyAlaProArgGlyLysHisAlaAlaPheGlnAlaAspValSerGlnGly

246 cccgcagccagacgcctgctggaggaagtgcaggcctgcttttctcgcccgccatctgtcgtt
    ProAlaAlaArgArgLeuLeuGluGluValGlnAlaCysPheSerArgProProSerValVal

309 gtgtcctgtgcgggcatcacacgcgatgagtttctgctccacatgtcagaagaagactgggac
    ValSerCysAlaGlyIleThrArgAspGluPheLeuLeuHisMetSerGluGluAspTrpAsp
                    ·                                ·
372 agagtcatagctgtcaacctcaagggcaccttcctagtcactcaggctgcagcccaggcttta
    ArgValIleAlaValAsnLeuLysGlyThrPheLeuValThrGlnAlaAlaAlaGlnAlaLeu

435 gtgtccagtggcggtcgtggctccatcatcaacattagtagcatcattggaaaggtggggaat
    ValSerSerGlyGlyArgGlySerIleIleAsnIleSerSerIleIleGlyLysValGlyAsn
                                    ¥
495 atcggacaaacgaatttatgcgtcgtccaaagcaggagtgattgggctcacccagactgcggcc
    IleGlyGlnThrAsnTyrAlaSerSerLysAlaGlyValIleGlyLeuThrGlnThrAlaAla
                  *******Δ****************************
558 cgggagcttggacgacatggaatccgatgtaactcggtcctcccagggttcattgcaacgccc
    ArgGluLeuGlyArgHisGlyIleArgCysAsnSerValLeuProGlyPheIleAlaThrPro

621 atgacccagaaaatgccagagaaagtgaaggacaaggtaactgcaatgattccgttgggacac
    MetThrGlnLysMetProGluLysValLysAspLysValThrAlaMetIleProLeuGlyHis
       Δ
683 atgggggaccctgaggatgtggcagatgtggttgcattcttggcatctgaagacagtggatac
    MetGlyAspProGluAspValAlaAspValValAlaPheLeuAlaSerGluAspSerGlyTyr

746 atcacaggggcctccgtggaagtcagtggaggtcttttcatgtaactgcctgatggaagccgg
    IleThrGlyAlaSerValGluValSerGlyGlyLeuPheMet###

809 actctgctcactcccccaccactgcctggccctcctgctgatgaggacgctaagttcccaggc

872 tacaaaagagatggcagtgtgtggctcaggcatgctgaatatggaaggcaggggtgcttgtga

935 ccctaataaattccaaatcctcttccctgcc(a)n
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FIG. 2. The primary structure of Ke 6a cDNA and encoded protein. The sequence around the initiation codon perfectly matches Kozak's consensus sequence (23). The region of alignment with the 0.7-kb *Sma*I genomic sequence is underlined. The termination codon is marked with #, and the poly(A)$^+$ addition signal is shown in boldface letters. Putative N-linked glycosylation sites (¥), the glycosaminoglycan attachment site (§), protein kinase C phosphorylation sites (Δ), and casein kinase II phosphorylation sites (·) are shown. The region which fits the consensus sequence pattern found within one of the best conserved regions for the family of short-chain alcohol dehydrogenases is marked with asterisks.

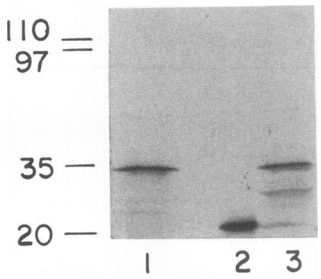

FIG. 3. In vitro-translated Ke 6a protein. Full-length Ke 6a cDNA (clone 13.3) in Bluescript II vector was linearized, and synthetic mRNA was transcribed with T7 polymerase (Stratagene). A total of 0.5 μg of purified Ke 6 mRNA was translated in rabbit reticulocyte lysate (Promega) in the presence of [^{35}S]methionine. The translated products of Ke 6a (lane 1), ferritin H (lane 2 [22 kDa]), and β-lactamase (lane 3 [31.5 kDa]), along with other protein molecular weight standards whose positions are marked, were run on SDS-PAGE (10% polyacrylamide) gels. The estimated molecular mass of Ke 6a is about 31.5 kDa.

pronounced in a 25-week-old affected animal (5- to 10-fold) than in an 11-week-old affected animal (3-fold) compared with C57BL +/+ mice. Intriguingly, however, in this PKD model, there is no downregulation of Ke 6a mRNA in either liver, brain, or heart tissue. Once again, the lower panels of Fig. 6B indicate that the inhibition in Ke 6 gene expression is a specific alteration in the kidneys of *jck/jck* mice because no change in the level of expression of PTP and actin genes between normal and affected kidneys is observed.

We have also examined the pattern of Ke 6 gene expression in the kidneys of Han:SPRD-*cy* rats (20), which carry an autosomal incomplete dominant trait of PKD. The progression of the disease in homozygote *cy* rats is very similar to that in the *cpk/cpk* mice in that animals develop grossly cystic kidneys by 2 weeks and they do not survive beyond the 3rd week of life (7). Heterozygote *cy* rats develop cystic kidneys later on in life, and males usually die by 6 months even though females may live longer. Interestingly, although the female *cy* rats develop cystic kidneys at the same age as males, they do not become as severely azotemic as their male counterparts (20). There is no significant change in Ke 6 gene expression at various stages of the disease in heterozygotes (2, 3, 8, and 24 weeks of age) and homozygotes (2 and 3 weeks of age) in both male and female rats (Fig. 7). It is important to emphasize that there is no decrease in Ke 6 mRNA levels in the kidneys of young homozygotes (2 and 3 weeks of age) and older heterozygotes (8 and 24 weeks of age) in spite of them developing overtly cystic kidneys. The decrease in the Ke 6 hybridization signal seen in the 3-week-old female homozygote in this blot is due to degradation of the RNA, which was confirmed by ethidium bromide staining, and the level of actin and PTP mRNA (not shown) and is therefore not a real change. It is important to note that the *cy* rat is different from the *cpk* and *jck* murine models of PKD in that the rats develop several other disorders such as secondary hyperparathyroidism, metastatic calcification, and ulcerative enteritis, abnormalities not usually seen in murine PKD (20). However, this observation indicates that the inhibition of Ke 6 gene expression is not merely a phenomenon that occurs as a consequence of the development of cysts in kidneys.

DISCUSSION

By using the positional cloning technique, genes whose chromosomal location is relevant can be identified even if the nature of the gene product is unknown. This region of mouse chromosome 17, which is occupied by the H-2 complex and the t complex, represents such a location in which it is of great interest to characterize novel genes. Several diseases are associated with the major histocompatibility complex (MHC), some of which are autoimmune in nature (40). It has been suggested that non-class I and class II genes in linkage disequilibrium with antigen-presenting genes may be responsible for these diseases themselves (17). Identification of novel genes in the MHC is therefore of particular importance. The mouse H-2 complex and the human MHC have been extensively characterized, with the result that substantial portions of this region have been cloned by chromosome walking. The search for expressed genes in this region has led to the discovery of several new genes which encode proteins of diverse function, such as the tumor necrosis factor genes (27) and such genes as Ring4, Ring10, Ring11, and Ring12 (21) which play a critical role in immune function. It is possible that the Ring2 gene is the human homolog of the mouse Ke 6 gene merely because of its relative position in the MHC. Several newly identified genes (Ring1 through Ring4) in the class II region of the

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[1]      MASQLRLRSALALVTGAGSGIGRAISVRLAAEGAAVAACDLDGAAAQDTVRLLGSPGSEDGAPRGKHAAFQADVSQGPAARRLLEE  86
[2]          MnfeGkIALVTGASrGIGRAIAetLAArGGkViGtatsenGAQaisdyLGAnGkglmlnvTdpASiESVLekirAeFgeVD.  82
[3]         mfeLtGrkALVTGASGAIGgAIArvLhAQGAiVGlhgtQieklEtlateLGdrvklfpAnlANRdevkAlgqRAeAdlegVD.  83
[4]            MtqrIAyVTGGmGGIGtAIcqRLAkDGfrVvAgcgpnSPrREkwleqqkalGfDfiasegNvADwdstktAfdkvKsevg  80
[5]        mytdLKdkVvVVTGGSKGLGRAMAVRfGqEqSkVvvnyrsneeealeVKkeieeAGgQAiivrgdvTkEeDVvnlveTavkefg  84
[6] ttagvsrrpgRLaGkaAIVTGAAGGIGRAtveaylrEGASVvAmDLaprlAatryeepGAipiacdladraaidaamadAvArlGgldI..  90
[7]         mklVQdkITIITGGtrGIGfAaAklfieNGAkVSifgetqeevDtALaqLkelyPEEevlgfapdltsrDavmAAvGtQaqky  83

[1] VQACFSRPPSVVVSCAGITRDEFLLHMSEEDWDRVIAVNLKGTFLVTQAAAQALVSSGG.....RGSIINISSIIGKVGNIGQTNYASSKA 172
[2] ..........ILVNnAGITRDNlLMRMkDEEWNdIIetNLsSvFrLSkAvmRAMMkkr......HGrIItIGSVVGtMGNgGQANYAAAKA 156
[3] ..........ILVNnAGITKDglfLHMADpDWDiVLeVNLtAmFrLTReiTQqMIrrr......nGrIINVTSVaGaIGNpGQTNYcASKA 157
[4] evd.......VLINnAGITRDvvfrkMTraDWDaVIdtNLtSlFnVTkqviDGMadrG......WGrIVNISSVnGqkGqfGQTNYSTAKA 158
[5] sld.......VMINnAGVenpvpsheLSlENWNQVIdtNLtGAFLgSReAikyfVeNdi.....KGNVINMSSVhemIpwplfvHYAASKG 163
[6] ..........LVaGgAlkggtgnfLdLSDaDWDRyVdVNMtGTFLtcRAGARmaVAAGAgkdgrsArIItIGSVnSfMAEpeaAaYvAAKG 170
[7] gRld......VMINnAGITmNsvfsRVSEEDfknIMdINVNGvFngAwSAyQcMkdAk......QGvIINtASVtGiyGSLSgigYPTSKA 163

[1] GVIGLTQTAARELGRHGIRCNSVLPGFIATPMTQK..MPEKVKDKVTAMIPLGHMGDPEDVADVVAFLASEDSGYITGASVEVSGGLFM  259
[2] GLIGfSkSlAREVAsRGItvNvVaPGFIeTdMTRa..LSDdqRagIlAqVPaGRLGgAQEIANaVAFLASDEAAYITGeTLhVNGGMYMv  244
[3] GMIGfSkSlAQEIAtRnItvNcVaPGFIeSAMTDK..LnhDqKEKImvaIPIhRMGtgtEVAsaVAYLASDhAAYVTGqTIhVNGGMaMi  245
[4] GLhGfTmAlAQEVAtkGVtvNTVsPGYIATdMvka..IrQdVlDKIvAtIPVkRLGlPEEIAsIcAwLSSEESGFsTGAdfsLNGGLhMg  246
[5] GMklMTETlAlEyApkGIRvNNIgPGaIdTPInaekfadpeqRadVeSMIPMGyIGkPEEIAsVaAFLASsQASYVTGiTLfadGGMtky  253
[6] GVamLTRAmAvDLARHGIlvNmIaPGpVdvtgnntgysepRLaEqVldeVALGRpGlPEEVAtaavFLAeDgSSFITGSTItIdGGLsam  260
[7] GVIGLTHglGREIiRknIRvvGVaPGvVdTdMTkg..LPpeIlEdylktLPMkRMlkPEEIANVylFLASDlASgITATTIsVdGAyrp  249
```

FIG. 4. Alignment of the amino acid sequences of the six proteins in bacteria with highest levels of homology to Ke 6. Proteins are numbered as follows: 1, Ke 6a protein; 2, *E. coli* 3-oxoacyl-[acyl-carrier protein] reductase (30); 3, *R. meliloti* nodulation protein G (11); 4, *A. eutrophus* acetoacetyl-coenzyme A reductase (29); 5, *B. megaterium* glucose dehydrogenase (25); 6, *Flavobacterium* sp. *N*-acyl-D-mannosamine dehydrogenase (41); and 7, *Eubacterium* sp. 7-alpha-hydroxysteroid dehydrogenase (14). The sequence alignment, identity, and similarity scores were determined with the aid of the BLASTP program (2) at the National Center for Biotechnology Information. Identical and conservative substitutions are indicated by capital letters, and identical residues are shaded. The alcohol dehydrogenase family consensus motif is overlined.

human MHC were determined to be colinear in arrangement to their putative mouse homologs (18). The sequence of the Ring2 gene is not available to enable direct homology comparison to determine this conclusively.

The t complex, which extends for 15 to 20 centimorgans of the proximal part of mouse chromosome 17, has also been well studied genetically (6, 36). Mouse t haplotypes are variant forms of this region of chromosome 17 which are found in wild mouse populations (36). Each t haplotype contains at least one recessive lethal or semilethal mutation. Several of these lethal genes map close to or within the H-2 complex (35). Specifically, tcl-w5, an early-acting embryonic lethal mutation in which development fails beyond the egg cylinder stage, was shown to be recombinationally inseparable from the H-2K gene (3). In a search for the tcl-w5 gene, Abe et al. (1) searched a span of 170 kb containing the entire H-2K region from the t^{w5} haplotype and identified five expressed genes—Ke 1 through Ke 5. The genomic location of Ke 6 makes it an additional candidate for the tcl-w5 gene.

The repression of the Ke 6 gene is observed in two different mouse models of PKD. Ke 6 gene expression in homozygous *cpk* mice is downregulated in the liver to the same extent as in the kidney, which indicates that Ke 6 gene repression is not merely due to loss of functional tissue but perhaps is being aberrantly regulated by the mutant *cpk* gene. The downregulation of the Ke 6 gene is more pronounced in 25-week-old *jck/jck* mice in the terminal stage of the disease than in 11-week-old affected mice. The observations that Ke 6 gene expression is not altered in *cy* rats and that in *jck* mice it is downregulated only in the kidney argue strongly against uremia or some other physiologic aspect of renal dysfunction as causes for repression of the Ke 6 gene, because both of these animals develop severe azotemia. We therefore postulate that the Ke 6 gene may play a role either directly or indirectly in the development of PKD in *cpk* and *jck* mice. Moreover, the Ke 6a protein is very likely required for normal kidney function and/or structure. The *cpk* and *jck* gene products are probably required for the basal regulation of the Ke 6 gene, and in this respect, the Ke 6 gene may be closer in the cascade of events leading to the development of PKD than the mutant *cpk* or *jck* genes. It is possible that the *cpk* gene is ubiquitously expressed, and therefore the downregulation of the Ke 6 gene is seen in all tissues examined. On the other hand, the *jck* gene may be kidney specific, and

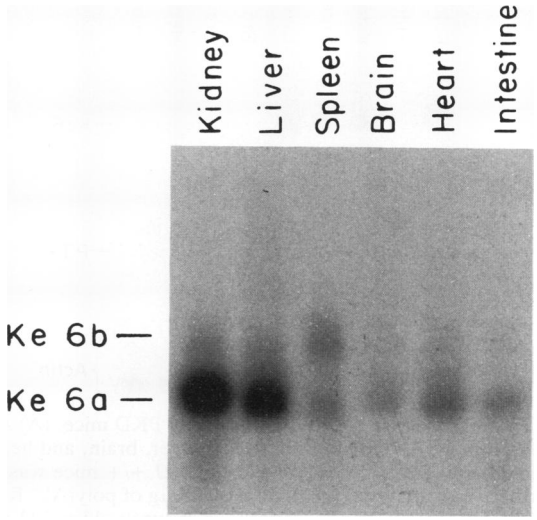

FIG. 5. Normal expression pattern of the Ke 6 gene. Northern blot analysis was performed with 3 μg of poly(A)$^+$ RNA from mouse kidney, liver, spleen, brain, heart, and intestine. RNA was separated on a 1% agarose–2.2 M formaldehyde gel and blotted onto nylon membranes. [α-^{32}P]dCTP-labeled insert from Ke 6a clone 13.1 was used as a probe for hybridization at 65°C in Church's buffer for 16 to 20 h, and the membrane was washed in 0.1× SSC–0.1% SDS at 50°C and exposed to X-ray film.

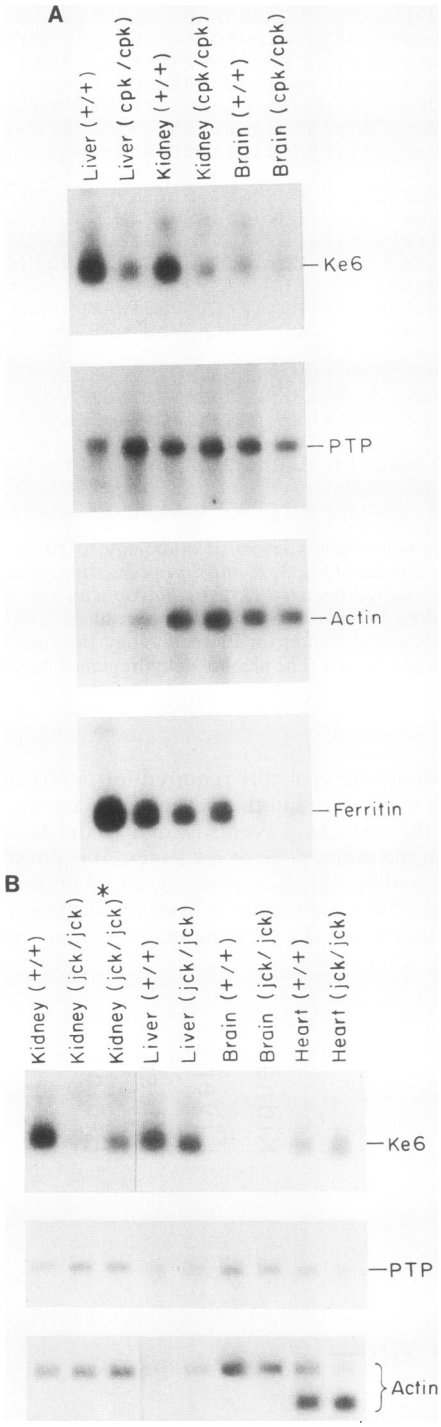

FIG. 6. Expression of the Ke 6 gene in PKD mice. (A) A total of 3.5 μg of poly(A)$^+$ RNA from kidney, liver, brain, and heart tissue of 18-day-old DBA/2J *cpk/cpk* and DBA/2J +/+ mice was used for Northern blot analysis. (B) A total of 3.5 μg of poly(A)$^+$ RNA from kidney, liver, brain, and heart tissue of 25-week-old and 11-week-old (*) C57BL/6 *jck/jck* and C57BL/6 +/+ mice was used for Northern blot analysis. There was no gross morphologic difference in any organs besides the kidney in either *cpk* or *jck* mice. Northern blot analysis was performed as described in Fig. 5. Equal loading of RNA was confirmed by ethidium bromide staining intensity and by hybridizing each membrane sequentially with [α-^{32}P]dCTP-labeled protein tyrosine phosphatase and β-actin cDNAs. The membrane in panel A was also probed with L-ferritin cDNA.

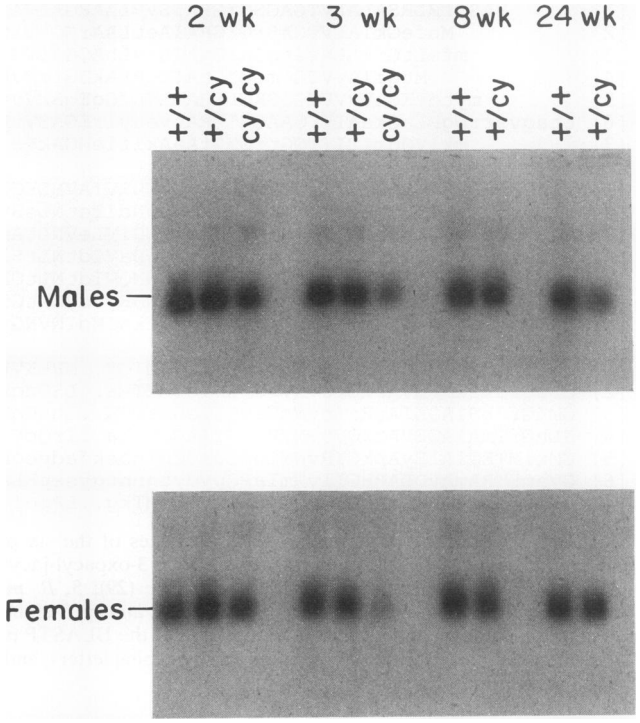

FIG. 7. Expression of the Ke 6 gene in the kidneys of Han:SPRD-*cy* rats. A total of 5 μg of total RNA from the kidneys of 2-, 3-, 8-, and 24-week-old +/+, +/*cy*, and *cy/cy* (2 and 3 weeks of age only) male and female rats was used for Northern blot analysis. [α-^{32}P]dCTP-labeled insert from Ke 6a cDNA clone 13.1 was used as a probe for hybridization at 65°C in Church's buffer for 16 to 20 h. The membrane was washed in 2× SSC–0.1% SDS at 50°C.

thus we see repression of Ke 6 expression only in the kidneys of *jck/jck* mice. The etiology and the basic biochemical defect(s) of PKDs are unknown. Ke 6 could serve as a useful molecular tool to study the pathogenesis of PKD, and investigating the function of the Ke 6 protein could lead to greater understanding of the molecular basis of this group of heritable renal disorders. Because the syntenic relationship of genes has been maintained during mammalian evolution, identification of the Ke 6 gene and other mouse mutations, e.g., *cpk*, *pcy*, and *Tg737*, whose chromosomal locations have been mapped, may be useful in directing linkage analysis studies of heritable renal cystic diseases in humans in view of the limited availability of kindreds for genetic study.

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