Abstract. Autosomal dominant polycystic kidney disease (ADPKD) is a commonly inherited disorder in humans that causes the formation of fluid-filled renal cysts, often leading to renal failure. PKD1 mutations cause 85% of ADPKD. Feline PKD is autosomal dominant and has clinical presentations similar to humans. PKD affects ~38% of Persian cats worldwide, which is ~6% of cats, making it the most prominent inherited feline disease. Previous analyses have shown significant linkage between the PKD phenotype and microsatellite markers linked to the feline homolog for PKD1. In this report, the feline PKD1 gene was scanned for causative mutations and a C>A transversion was identified at c.10063 (human ref NM_000296) in exon 29, resulting in a stop mutation at position 3284, which suggests a loss of ~25% of the C-terminus of the protein. The same mutation has not been identified in humans, although similar regions of the protein are truncated. The C>A transversion has been identified in the heterozygous state in 48 affected cats examined, including 41 Persians, a Siamese, and several other breeds that have been known to outcross with Persians. In addition, the mutation is segregating concordantly in all available PKD families. No unaffected cats have been identified with the mutation. No homozygous cats have been identified, supporting the suggestion that the mutation is embryonic lethal. These data suggest that the stop mutation causes feline PKD, providing a test to identify cats that will develop PKD and demonstrating that the domestic cat is an ideal model for human PKD.

Companion animal species such as the domestic cat are effective models for several inherited diseases. Cats and humans show strong conservation of biology and anatomy and share >30 homologous hereditary diseases (1,2). One inherited disease for which the cat is an exceptional animal model is polycystic kidney disease.

Autosomal dominant polycystic kidney disease (ADPKD) is a commonly inherited disorder in humans, with a frequency in the general population of 1 in 1000 (3). Approximately 85% of ADPKD cases are caused by mutations in the PKD1 gene (4,5), located on human chromosome 16p13.3; the remaining 15% are caused by mutations in the PKD2 gene, located on human chromosome 4q21–23 (6,7). ADPKD is characterized by the formation of fluid-filled cysts in the kidneys, and the average age of onset is 40 yr, with ESRD occurring by age 60 in 50% of cases (8). This suggests that ~4% of ESRD patients are a result of PKD.

Feline polycystic kidney disease is an inherited disease in Persian and Persian-related cats. PKD in cats is characterized by renal as well as hepatic and pancreatic cysts (9) and has an autosomal dominant mode of inheritance (10,11). Approximately 38% of Persian cats in the United States (12) and worldwide (13–17) are positive for PKD. Purebred cats represent ~20% of the cat population in the United States, and Persian-type breeds constitute 80% of the cat fancy; hence, PKD is the most prominent inherited feline disease.

A linkage analysis for feline PKD was performed by genotyping 43 feline-derived microsatellites in seven extended feline pedigrees segregating for PKD (18). The results showed a significant linkage and no recombinants (\(Z = 5.83, \theta = 0\)) between feline PKD and the microsatellite marker FCA476 that is within 10 cR to the PKD1 gene on cat chromosome E3 (18).

A BAC clone that contains the feline PKD1 gene was identified and submitted for sequencing at The University of Oklahoma’s Advanced Center for Genome Technology. PCR amplification and sequence analysis identified a C>A transversion causing a stop codon (OPA) in exon 29 of the feline PKD1 gene. This mutation has been identified in the heterozygous state in all 48 affected cats examined to date, including all affected Persians, a Siamese, Ragdolls, domestic shorthairs, and several other breeds that have been known to outcross with Persians, such as Exotic Shorthair, Selkirk Rex, and Scottish Folds. The mutation is segregating consistently in the largest feline PKD pedigree and in several individuals from all other available pedigrees. The causative mutation has not been observed in 33 unaffected cats, and no homozygous affected cats have been identified, suggesting that the mutation is embryonic lethal and is consistent with previous
data (18). These data suggest that the stop mutation causes feline PKD and that a DNA test is now possible to identify cats that will develop PKD in the future. Along with the similar clinical presentation, these data support the use of the domestic cat as a model for human PKD. The cat has the same mode of inheritance, a mutation in the PKD1 gene; the affected cats have only the heterozygous state, and they can be used for long-term drug and potentially gene therapy trials.

Materials and Methods

All animal experimentation described in this article was conducted in accord with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Sample Identification

Samples from cats that represent families segregating with PKD were collected from five feline PKD ultrasound screening clinics that were held at the University of California, Davis, School of Veterinary Medicine between June 2000 and September 2002. Cats of 10 mo of age or older were determined to be affected or normal by the visualization of singular bilateral or multiple unilateral cysts using ultrasonography. The same two board-certified radiologists (D.A.B. and Dr. E. Herrgesell of the University of California, Davis, School of Veterinary Medicine) scanned all cats. Details regarding the disease criteria and the pedigrees developed from these clinics have been previously described (18). Forty-eight affected and 33 normal cats were analyzed. Representatives from each pedigree were genotyped for the PKD mutation, including 41 affected and 26 unaffected Persians. One PKD-affected cat and one normal cat from other breeds, including Siamese, Siberian, Exotic Shorthair, Domestic Shorthair, Selkirk Rexes, Scottish Folds, and Ragdolls, were identified by the authors (D.J.B.) and/or from the University of California, Davis, ultrasound screening clinics and were also analyzed.

BAC Clone Sequencing

The sequence of the feline BAC clone (GenBank accession no. AC145332.26) that contains the feline PKD1 homolog was obtained using standard BAC isolation, shotgun sequencing, and finishing strategies as described previously (19–23). Primers from exon 17 that had an 8-bp overlap in the 3’ portion of their sequence were constructed. Thus, when amplified, they produced a product of 40 bp that was used as a probe to isolate the BAC clone that contains the feline PKD1 gene. Commonly termed “overgo” primers, the sequences for these primers were PKD1F tcggcattgtgtccttggagtgtg and PKD1R tgt-gcgcttttaacaggcttactgg. Briefly, 50 μg of purified BAC DNA was randomly sheared and made blunt-ended. After kinase treatment and Terrific Broth (TB) medium supplemented with 100 μg of ampicillin, E. coli strain XL1BlueMRF’ (Stratagene, La Jolla, CA) were transformed by electroporation into Escherichia coli, strain XL1BlueMRF’ (Stratagene, La Jolla, CA). A random library of ~2500 colonies were picked from the transformation and grown in Terrific Broth (TB) medium supplemented with 100 μg of ampicillin for 14 h at 37°C with shaking at 250 rpm, and the sequencing templates were isolated by a cleared lysate-based protocol. Sequencing reactions were performed as described previously using TaqDNA polymerase with the Amersham ET Fluorescence-labeled terminators (19–22). The reactions were incubated for 60 cycles in a Perkin-Elmer Cetus DNA Thermocycler 9600, and after removal of unincorporated dye terminators by ethanol precipitation, the fluorescence-labeled nested fragment sets were resolved by electrophoresis on an ABI 3700 Capillary DNA Sequencer. The resulting sequence data were transferred to a Sun Workstation Cluster, where it was base-called and assembled using the Phred and Phrap programs (24,25). Overlapping sequences and contigs were analyzed using Consed (26).

Fluorescence in situ hybridization (FISH) of the PKD1 BAC clone was performed on mitotic spreads of feline chromosomes using standard methods. Labeling procedures and probe concentrations were the same as described previously (27). Cot-1 DNA was substituted in the hybridization solution with a twofold amount of cat genomic DNA.

PKD1 Sequence Analysis

Sequence of the PKD1-containing BAC clone (GenBank accession no. AC145332.26) was aligned to the human (GenBank accession no. AC009065.8), dog (GenBank accession no. AY102170.1), and mouse (GenBank accession no. AC132367.3) PKD1 sequences to identify potential intron/exon boundaries for the cat using the software PipMaker (28) (Figure 1). Primers were developed in intronic regions for amplification of complete exons using the software Primer3 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) (29) and NetPrimer (http://www.premierbiosoft.com/netprimer/netprlaunch/netprlaunch. html). Primers and the GenBank accession numbers for the exons analyzed are presented in Table 1. Primers (MWG Biotech, High Point, NC) were used to amplify PKD-negative control cat DNA. Each primer was tested in the cat as described previously (30) on a Stratagene 96-well temperature gradient Robocycler. The amplified products were separated on 1.8% agarose gels at 100 Vhr. Gels were visualized by UV exposure after ethidium bromide staining and photodocumented using the Alpha Imaging System (Alpha Innotech, San Leandro, CA). A positive optimization of the primers produced a single PCR product that was excised from the gel and purified using the Qiagen gel extraction column (Qiagen, Valencia, CA), or PCR products were purified directly using the Qiagen PCR clean up kit. Purified products were sequenced directly in both directions using the ABI Dye Terminator Sequencing chemistry v3.1 (Applied Biosystems, Foster City, CA). Sequencing reactions were separated on an ABI 377 DNA Analyzer, and the DNA contig sequence was assembled using the Sequencer Software package (Gene Codes, Ann Arbor, MI). Integrity of the sequence contig was confirmed by visual inspection and verified to be the correct gene by comparison with sequences in GenBank using BLAST (31).

Genotyping

DNA from 48 affected and 33 normal cats was isolated from white cells by standard phenol/chloroform techniques. EDTA anticoagulated blood was collected by venipuncture at the PKD clinics or sent by private clinicians. PKD exon products were amplified by PCR from genomic DNA of two normal and two affected cats using the optimized primers (Table 1). Individual exons were amplified independently in feline DNA samples using optimal PCR conditions on a Stratagene 96-well temperature gradient Robocycler. Approximately 12.5 ng of DNA was used per PCR reaction. Reaction conditions for each primer pair were as follows: ~1 pmol of each forward and reverse primer, 1.25 mM dNTP, 1.75 mM MgCl2, 1× PCR buffer II, and 0.375U of AmpliTaq (Applied Biosystems) polymerase in 10 μl reaction volumes. Cycling parameters included an initial 3 min denaturation at 94°C followed by 35 cycles of 1 min denaturation at 94°C, annealing for 1 min at 58°C, and a 72°C extension for 1 min. The cycling parameters were followed by a final extension at 72°C for 10 min. Products were generated, visualized, gel-extracted, purified, and sequenced as described above and analyzed for mutations associated with
PKD. Sequences generated from each exon were aligned (DNAS- tar, Madison, WI) with wild-type cat sequence to identify possible causative mutations for the observed phenotype. When polymorphisms were detected, sequence data were translated to determine whether the mutation resulted in an amino acid change.

RFLP Analysis

Once the mutation was identified, 46 affected and 31 normal cats were screened for the PKD mutation using RFLP typing on agarose gels. The amplification product for exon 29 is 559 bp. The identified mutation causes a restriction enzyme site alteration for MLY1, producing two fragments of 316 and 243 bp. Approximately 5 μl of amplification product was digested with 10 U of MLY1 (New England Biolabs, Beverly, MA) in a 10 μl reaction that contained 1× NE Buffer 4 at 37°C for 3 h followed by inactivation of the enzyme at 65°C for 10 min. The complete digestion reaction was analyzed on 1.8 to 2% agarose gels as described above.

Results

The sequenced BAC clone (GenBank accession no. AC145332.26) is in eight contigs that cover 167 kb. The region

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**Table 1.** PCR analysis of PKD1 in the domestic cat

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<th>Exon Size (bp)</th>
<th>Product Size (bp)</th>
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<th>Reverse Primer 5'-3'</th>
<th>GenBank Accession No.</th>
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PKD1. Schematic diagram of the feline PKD1 gene as suggested from the BAC clone sequence. Intron/exon boundaries are predicted by comparison with human, mouse, and dog sequence as determined with the program PipMaker (28). The y axis represents nucleotide identity of the cat to the human sequence, and the x axis represents nucleotide position in the gene. The exon number is represented above each exon symbol. □, simple repeat; ■, UTR; →, exon; ■, gene; , CpG/GpC ≥ 0.75; , CpG/GpC ≥ 0.60.
that contains the PKD1 gene is represented by two contigs that are separated in intron 4. The 5' region of exon 1 is not complete, with an estimated 278 bp not represented in the assembled sequence. A schematic of the feline PKD1 gene is presented in Figure 1, which predicts exon sizes in the genomic BAC clone and sequence identity to the annotated human mRNA sequence. Intron and exon sizes as well as estimates of sequence and protein identity to human, mouse, and dog are presented in Table 2.
presented in Table 2. Over all exons, the cat sequence is most similar to dog (83.66%; range, 75.0 to 94.4%), least similar to mouse (73.44%; range, 45.3 to 89.6%), and intermediate to humans (80.02%; range, 64.9 to 94.4%). Exon 10 has the lowest similarity with the cat as compared with each species. The similarity of cat sequence to that from any second species (mouse, human, or dog) varies between exons. One would expect the cat always to be most similar to the dog, a fellow carnivore, but this was not the case. The predicted mRNA and protein sequence is provided in a Supplemental figure. FISH of the PKD1 BAC clone does not suggest duplication of the feline PKD1 gene (Figure 2), and the feline gene is located on cat chromosome E3. Nine of 46 PKD1 exons were scanned for mutations by direct sequencing. A C>A transversion at c.10063 (human ref NM_000296) in exon 29 resulting in a C3284X protein change was identified, which is an OPA stop codon that should cause a loss of ~25% of the protein (Figure 3). The mutation causes a unique RFLP site in the amplification product of exon 29. An example of the RFLP typing is presented in Figure 4. A total of 48 affected and 33 unaffected cats were scanned for this stop mutation. Ten cats were confirmed by sequence analyses and all cats by RFLP. All 48 affected cats had the stop mutation, including the non-Persian cats. None of the 33 unaffected cats was identified with the mutation. Pedigree analysis of feline PKD family 5 (18) showed complete co-segregation of the stop mutation with the disease phenotype. None of the 48 affected cats was found to be homozygous for the mutation.

Four of the nine exons (exons 6, 14, 15, 23, 24, 29, 30, 37, 38) and several intron regions had nucleotide variants as identified between two sequenced Persian cats and the PKD1 sequence from the BAC clone (Table 1). One Persian was affected with PKD; thus, the sequence comparison represents three normal alleles. Each identified variant was homozygous in the two Persian cats, except for a mutation in exon 29. Ten variants were identified, but only four were in translated regions of the exons. None was identified at exon/intron splice sites. Two of the four variants produced silent mutations and were homozygous in the two Persian cats sequenced. One mutation, a C>T transition at position 127 of exon 38, caused an amino acid change, but both amino acids are hydrophobic and it is not anticipated that this substitution alters the protein conformation. One primer set amplified exons 21 and 22 and the intervening intron 21, and another set amplified exons 22 and 23 and the intervening intron 22 (Table 1). Both cat introns are shorter than the corresponding human intron (Table 2), and no sequence data from the BAC clone and/or amplified genomic DNA supported the presence of polypyrimidine tracts.

Discussion

Domestic cats are effective models for several inherited diseases and should be used to develop better drug and gene therapies for PKD. Feline PKD is an inherited disease in Persian and Persian-related cats. As with humans, PKD in cats is characterized by renal as well as hepatic and pancreatic cysts (9) and has an autosomal dominant mode of inheritance (10). Approximately 37% of Persian cats worldwide (14) are PKD affected. Only 20% of the cat population in the United States is represented by purebred cats, but of purebreds, Persians and Persian-derived breeds constitute ~80% of the cat fancy (32). This suggests that ~6% of the cat population in the United States has PKD, making it the most prominent inherited feline disease.

Our previous linkage analyses strongly implicated PKD1 as the causative gene for feline PKD (18). Each newly identified family for human PKD has generally been found to be a novel mutation in the PKD1 gene. Mutations are found throughout the gene, with no single mutation being highly prevalent in the population. Because no particular mutation or region of the PKD1 gene in humans is highly prevalent for mutations, identification of the feline PKD mutation could have entailed the complete sequencing of the feline homolog from an affected cat. Once the draft sequence of the feline BAC clone containing PKD1 was obtained, we began a systematic scan for

Figure 2. Localization of the feline PKD1 gene by fluorescence in situ hybridization to domestic cat chromosome E3q13. Presented is a mitotic spread of feline chromosomes from lymphocytes with the hybridization of the feline PKD1 BAC clone (a; arrows). Hybridization signals are observed as red dots on both E3 chromosomes. Each dot represents the signal of a single chromatid. (b) The inverted DAPI-banding pattern of the same metaphase. Feline chromosome E3 is the smallest metacentric cat chromosome and is easily distinguished by its morphology and DAPI-banding pattern.
mutations. A nucleotide transversion causing a stop codon was identified in exon 29 that suggests a truncation of the protein with a loss of 25% of its C-terminus. Thus, this feature is a very strong candidate for the causative mutation resulting in the PKD phenotype.

In addition, concordant segregation of the mutation with the disease in a large cat family segregating for PKD supports the stop mutation as causative for feline PKD. A different mutation still possibly could cause feline PKD but would have to be in strong linkage disequilibrium with this stop codon. In addition, the stop codon mutation is consistent with the microsatellite haplotype that shows complete linkage to the PKD phenotype (data not shown).

The feline *PKD1* gene is represented by two contigs, with the 5′ region of exon 1 and intron 4 not yet represented by sequence. A majority of feline exon and intron sizes are estimated from the BAC clone sequence. In humans, exon 1 is 424 bp; thus, ~141 amino acids cannot be compared with the cat. Intron 4 is 213 bp in humans, and this entire intron may not yet be represented in the cat sequence. Over all exons, the cat sequence is most similar to dog, followed by human, and least similar to mouse, which is consistent with the evolutionary relationship of the species. Exon 10 is the least similar for all species, as compared with the cat.

There are two distinctive features found in the human *PKD1* gene that distinguish it from the homolog in mice and dogs (33–36): (1) the presence of polypyrimidine tracts in intron 21 (~2.5 kb) and intron 22 (~600 bp) and (2) the presence of several replicates of a 13.5-kb region of the 5′ portion of the gene on human chromosome 16. Neither feature is present in the cat. The genomic amplifications of introns 21 and 22 from both normal and affected cats are consistent with the size determined by the feline BAC clone sequencing. Introns 21 and 22 both are smaller in cats than in humans. When human-derived primers were used to amplify a region that consisted of a majority of exon 21, exon 22, and the intervening intron 21, this region is ~950 bp in cats and could not be amplified from genomic human DNA, suggesting that the 2.5-kb tract could not be amplified by standard PCR. The polypyrimidine tract is
not represented in the human reference sequence for PKD1 but has been demonstrated elsewhere and confirmed by long-PCR techniques (34). Intron 22 is only 349 bp as compared with 602 bp in humans. Thus, the polypyrinidine features are not found in cats.

Likewise, three aspects of our data suggest that the 5' portion of the feline PKD1 gene homolog is not replicated as in humans. For the cats sequenced, heterozygous sequence was not identified within the exons and introns that correspond to the region duplicated in humans. Pseudogenes acquire mutations rapidly; thus, it would be anticipated that some variants would be detected. FISH of the PKD1 BAC clone does not suggest duplication and was located on feline chromosome E3, which is as predicted by genetic mapping (37) and chromosome painting (38) and is the region homologous to human chromosome 16. In addition, because this region is not replicated in both mice and dogs, it is more likely that this replication event is found in humans and/or primates and is not an ancestral event for mammals.

Only the Persian and Persian-derived cat breeds (Exotic Short-hairs and Himalayans) have been recognized to have a high frequency of PKD. Other breeds—Ragdolls, Scottish Folds, and Selkirk Rexes—have either purposely or accidentally bred to Persians. We hypothesize the PKD mutation in the other breeds to be identical by descent with the Persian mutation. All affected cats from the other breeds have the identical mutation as the Persians. Because a majority of human cases are de novo, more extensive sequence analyses are required to differentiate de novo, identical by state mutations from mutations that are identical by descent within various cat breeds. This disease is occurring within a closed breed, suggesting that identity by descent and disease homogeneity is expected. Persians, however, are one of the oldest and most popular breeds, having a large population that is dispersed throughout the world. All cases analyzed here represent cats from the United States; thus, more extensive surveys should be conducted to validate the causative mutation in different regions of the world.

Further investigation into the cause of PKD will be valuable for feline health as well as provide insights into human AD-PKD. As with humans, cats have a wide range of disease progression and severity; thus, other genetic and environmental factors could influence disease progression (39–42). Currently, the disease is highly prevalent in the cat population, making the identification of both severe, early-onset cases and mild, late-onset cases feasible. This could lead to the identification of genetic modifiers. As cats have similar clinical presentations, therapies that are under development for epidermal growth factor receptor (EGFR) could be tested for efficacy in the cat, before use in humans (43–47).

Persian and Persian-related cats should be screened for PKD by ultrasound before they are bred (47). Although breeders are advised not to breed two positive cats, they are often bred for several unrelated reasons: (1) clinical signs have not yet appeared; (2) many breeders are still unaware of the disease; (3) ultrasound is either unavailable or cost-prohibitive; (4) breeding decisions are made before adequate accuracy of diagnosis; and (5) the disease is highly prevalent; thus, many catteries could lose ~40% of their breeding population. A genetic test for feline PKD will provide breeders with an efficient and accurate means to selectively breed their cats and remove PKD from the population. Because PKD has been found in other cat breeds related to Persians, the incidence of PKD in these breeds should be evaluated.

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