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Age-based ultrasonographic criteria for diagnosis of autosomal dominant polycystic kidney disease in Persian cats

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Abstract

Objectives The aim of this study was to establish ultrasound criteria for the diagnosis of autosomal dominant polycystic kidney disease (ADPKD) in Persian cats.

Methods Eighty-two Persian cats were assessed using renal ultrasound and genotyped for the C→A transversion in exon 29 of *PKD1*. The animals were also submitted to hematological characterization, serum biochemistry analyses and urinalysis.

Results Age, sex and neutering status did not differ between ADPKD (n = 12) and non-ADPKD (n = 70) cats. After integrated molecular genetics/ultrasonographic analysis, the presence of at least one renal cyst was sufficient to establish a diagnosis of ADPKD in animals up to 15 months of age. Two or more cysts were required for diagnosis in cats aged 16–32 months, and at least three cysts warranted diagnosis of ADPKD in animals aged 33–49 months. Finally, four or more cysts led to diagnosis in cats aged 50–66 months. Although cats with ADPKD exhibited higher serum calcium levels than non-affected cats, hematological, urinalysis and other biochemical parameters did not differ between the two groups.

Conclusions and relevance Integrated analyses of imaging and molecular genetics data enabled, for the first time, the establishment of age-based ultrasonographic criteria for the diagnosis of ADPKD in Persian cats. The development of imaging criteria is particularly relevant and useful in the clinical setting given the current limitations to access and the cost of molecular genetics-based diagnostic tests.

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Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common life-threatening inherited disease in humans, affecting 1 in 500–1000 of the general population.[1] It is characterized by focal development and progressive enlargement of renal cysts, typically leading to gradual increase in kidney size and, after decades of relatively preserved renal function, a steady decline in glomerular filtration rate.[2] ADPKD is responsible for 4.4–10.0% of end-stage kidney disease cases worldwide, representing a major burden on public health.[3]

ADPKD is caused by a mutation in the polycystic kidney disease (PKD) genes, *PKD1* or *PKD2*. Given the complex, large transcript size and extensive distribution of *PKD1* and *PKD2* germline mutations in ADPKD

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families, access to direct gene testing and its high costs significantly limit the use of this tool to establish the diagnosis of disease in conventional clinical practice.[4] Ultrasonography (US), however, is widely available, and is a safe, inexpensive, and effective imaging method to detect and characterize renal cysts. In this context, Ravine et al[5] conducted an integrated linkage-ultrasonographic analysis in *PKD1*-linked families, establishing an imaging criterion for pre-symptomatic ADPKD diagnosis in at-risk family members. More recently, Pei et al performed a genotype-ultrasound analysis including *PKD1*- and *PKD2*-linked families.[6] This study enabled the establishment of ultrasound diagnostic criteria applicable to asymptomatic at-risk members of ADPKD families of unknown genotype.

The prevalence of PKD in Persian cats is extremely high, reaching 25.9–50% of tested cats in the UK, France, Italy, Slovenia and Taiwan.[7–16] The clinical manifestations and course of this disease in Persian and Persian-related cats closely resemble the features of the human illness.[17,18] Interestingly, all known cases of ADPKD in cats have been linked to a single germline mutation in the feline ortholog of the human *PKD1* gene, also named *PKD1*. This variant consists of a single nucleotide transversion (C→A) located at position 3284 of exon 29, which introduces a premature stop codon into the corresponding mRNA transcript.[19]

While to date the single mutation makes molecular testing an adequate tool for diagnosis of ADPKD in Persian and Persian-related cats, its limited access and high cost, especially in developing countries, restricts its application in cats. US, however, has been demonstrated to be the most cost-effective imaging modality for large-scale renal phenotypical evaluation of potentially affected cats.[9,10,18] Notably, a previous study involving affected Persian cats reported a US sensitivity and specificity for renal cyst detection of 91% and 100%, respectively, at 36 weeks of life compared with kidney histopathology.[18]

Cysts can be identified as anechoic structures within the renal parenchyma by US.[20,21] The criterion of at least one anechoic cyst in at least one kidney has been previously used to diagnose PKD in 13-week–10-year-old cats,[9] and in Persian cats >10 months of age.[10] However, these criteria did not take into account the genetic molecular basis of the disease, which was unknown at the time of the studies. Moreover, the development of renal cysts secondary to other clinical circumstances, such as obstruction due to nephrolithiasis, lymphoma and chronic kidney disease with interstitial nephritis, is well recognized, especially in old cats.[10,22]

Previous reports have illustrated how problematic it is to use the one-cyst criterion to diagnose ADPKD in Persian cats. In fact, Kappe et al described three Persian cats with a renal cyst and a negative genetic test.[22] Bonnazzi et al reported four cats with at least one renal cyst in only one kidney that were negative according to PCR-restriction fragment length polymorphism (PCR-RFLP) and sequencing for feline ADPKD,[14] and one cat negative at ultrasound that was positive in the genetic test. Finally, in a study by Lee et al,[16] three cats that were diagnosed with PKD using US did not harbor the mutation within exon 29.

The establishment of ultrasound criteria for ADPKD diagnosis based on appropriate age ranges in Persian cats is highly desirable for large-scale application in clinical practice. The present study, comprising an integrated molecular genetics–ultrasonographic analysis, achieved this goal. The development of such a criterion is therefore anticipated to have a significant impact on conventional feline clinical evaluation.

Materials and methods

Study population

Eighty-two Persian cats belonging to 33 distinct pedigrees were included in the current study. Persian cats of both sexes and >3 months of age were selected from catteries of accredited breeders. All cats were privately owned and the owners were thoroughly informed of the research aims and protocols. The study was approved by the institution's ethics committee (protocol 1812010514). This study was performed at the Department of Clinical Medicine and Department of Pathology, School of Veterinary Medicine and Animal Science, University of São Paulo, São Paulo, Brazil.

The animals were submitted to routine physical examination (including thyroid gland palpation), abdominal US, genotype analyses, complete blood count (CBC), serum biochemistry profile (including total thyroxine concentration) and urinalysis.

Genotype analyses

The presence or absence of the C→A transversion at position 3284 of exon 29 in the feline *PKD1* gene was investigated in all cats included in this study using a PCR-RFLP assay. Blood samples were collected by venipuncture, enabling genomic DNA extraction from whole blood. This procedure was performed using a commercially available kit (Illustra Blood Genomic Prep Mini Spin Kit; GE Healthcare) according to the manufacturer's instructions.

A 559 base pair (bp) PCR fragment containing exon 29 was amplified using the same pair of primers previously reported by Lyons et al:[19] *PKD1*F1 (5′–CAGGTAGAC GGGATAGACGA–3′) and *PKD1*R1 (5′–TTCTTCCTGGT CAACGACTG–3′). The reaction included genomic DNA template, 2.5 mM of each dNTP, 50 mM $MgCl_2$, 10 µM *PKD1*F1, 10 µM *PKD1*R1, PCR buffer and 5 U/µl Taq DNA Polymerase (Thermo Fisher Scientific), and was run on a thermal cycler (Eppendorf). The PCR conditions included an initial 3 mins of denaturation at 94°C;

35 amplification cycles, including 1 min denaturation at 94°C, 1 min primer annealing at 58°C and 1 min extension at 72°C; and a final 1 min extension at 72°C. The amplified product was digested with 10 U of *MLY1* (New England Biolabs) and analyzed by electrophoresis on 2% agarose gels and subsequent documentation using the Chemidoc system (BioRad). Product digestion resulting in 316 bp and 243 bp fragments indicated the presence of the C→A transversion, whereas observation of only the non-digested 559 bp fragment revealed the absence of this genetic variant. Positive and negative controls were used in all reactions.

All positive results were confirmed by direct automated Sanger sequencing of the respective amplified PCR products. Cats with this pathogenic mutation received definitive diagnosis of ADPKD, whereas cats without such a variant had the diagnosis of ADPKD excluded.

Ultrasonographic analysis

The sonographic assessments were performed by a veterinary specialist in US using a multifrequency (7.5–12 MHz) linear transducer (Logiq 7; GE Healthcare), with harmonic and Doppler image features (color, spectral and amplitude). The resolution was sufficient to detect 0.3 cm (diameter) renal cysts. The cats were not sedated for examination. Hair clipping was not performed or, when necessary, was reduced to a small window on each side of the abdomen. The kidneys, liver and pancreas were scanned for evidence of anechoic cysts within the parenchyma. Renal length was measured according to the longitudinal axis. Cysts were quantified and measured.

Laboratory evaluation

A CBC was obtained within 1 h of blood collection using a hematology analyzer (BC-2800Vet; Shenzhen Mindray Bio-Medical Electronics). Evaluated parameters included red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean cell hemoglobin, concentration, platelet count, and white blood cell count and differential. Air-dried Wright–Giemsa-stained blood films were performed immediately after blood collection. Differential white blood cell counts were performed manually by counting 100 nucleated cells per smear.

Selected biochemical analytes in the serum were quantified using an automated spectrophotometer clinical wet chemistry analyzer (Liasys; Analyzer Medical System). The analytes included serum urea nitrogen, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), albumin, total protein (TP), phosphate and calcium. Sodium, potassium and chloride were measured using an ion-specific electrolyte analyzer (AVL-OMNI4-Roche), whereas thyroxin concentration was quantified using radioimmunoassay (Abbot Laboratories). The analyzers were calibrated before each sample run, the runs followed the manufacturers' instructions, and the measurements were appropriately performed using control samples. Grossly hemolyzed or lipemic samples were discarded.

Laboratory urine evaluation

All urine samples were collected via cystocentesis and sample volumes ranged from 3.5–12 ml. Urinalysis consisted of a urinary dipstick test, determination of urine-specific gravity (USG) with a manual refractometer, measurement of urinary pH and urinary protein-to-creatinine ratio (UPC) using an analyzer (Liasys), and sediment examination. The sediment was prepared as described previously,[23,24] and evaluated using microscopy within 1 h of urine collection.

Statistical analysis

Continuous variables were tested for normality using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The values were expressed as mean and SD for parametric data, and as median and quartiles for non-parametric data. Categorical data were presented as absolute (n) values and percentages (%), and were tested using Pearson's χ^2 test and Fisher's exact test, if applicable. Non-parametric data were compared using the Mann–Whitney U-test for two independent samples. Parametric data were compared using the Student's *t*-test for two independent samples. Discrimination of variables was calculated using receiver–operator characteristic (ROC) curve analysis with area under the curve and asymptotic significance. Some continuous variables were categorized via ROC curve analysis.[25] The cut-off points were calculated using the value associated with the best sensitivity and specificity. Correlations were calculated using the Spearman rank test and predicted associations by linear regression. Values with an $r^2 \geqslant 0.7$ were determined to have good performance or linearity. An alpha risk $\leqslant 5\%$ for committing type I error and beta risk $\leqslant 20\%$ for type II error were accepted.[26,27] Analyses were performed using SPSS version 19.0 (IBM) and GraphPad Prism 5 (GraphPad).

Results

Feline population and determination of molecular diagnostic status

Twelve of 82 (14.6%) Persian cats were heterozygous for the C→A mutation in exon 29 of *PKD1*. The presence of this allele established a definitive diagnosis of ADPKD in such cats. The remaining 70 (85.4%) animals were homozygous for the wild-type *PKD1* allele. Because no other genetic variant in *PKD1* or other genes have been linked to ADPKD in cats to date, the absence of the C→A transversion in *PKD1* was considered to be sufficient to exclude the diagnosis of ADPKD in such cats. The ADPKD group consisted of seven intact females, two

neutered females, two intact males and one neutered male. Among the non-ADPKD cats, 43 were intact females, two were neutered males and 25 were intact males. The age range was 11–162 months in the ADPKD group and 5–168 months among non-ADPKD animals. The ADPKD and non-ADPKD groups did not differ with regard to sex ($P = 0.531$) and age (87.4 ± 52.6 vs 75.8 ± 35.8 months, respectively; $P = 0.345$). Of a total of 33 pedigrees, 11 pedigrees including 12 cats had at least one affected animal, whereas in 22 pedigrees comprising 70 cats, no animal was diagnosed with ADPKD.

Ultrasonographic characterization and imaging criteria for diagnosis of ADPKD

All 12 genetically confirmed ADPKD cats exhibited renal cysts (Table 1). The lowest number of cysts was detected in the affected group – two in each kidney – observed in an 11-month-old animal (Table 2). The diameter of the cystic lesions ranged from 0.3–1.9 cm. The renal longitudinal length was higher in ADPKD than in non-ADPKD cats (3.848 ± 0.528 cm vs 3.542 ± 0.496 cm; $P < 0.01$) (Table 1). Affected kidneys also exhibited increased cortical echogenicity, irregular contour and corticomedullary blurring (75% vs 38.57% [$P < 0.01$]; 50% vs 3.57% [$P < 0.001$]; 62.5% vs 6.43% [$P < 0.001$], respectively) (Table 1). Additionally, 33.3% (n = 4/12) of ADPKD animals had cysts in the liver (Table 2). No pancreatic cysts were observed.

The area under the ROC curve was 0.636 for age (95% confidence interval [CI] 43.7–83.5%; $P < 0.05$) and 1 for the number of cysts (95% CI 100–100%; $P < 0.05$). The cut-off value for the number of cysts to discriminate between ADPKD and non-ADPKD animals associated with the highest sensitivity and specificity was three cysts in one or both kidneys (Figure 1a). Sensitivity and specificity were calculated using the positive differential rates, yielding values of 64.3% and 81.3%, respectively. The best cut-off for age, in turn, was 66 months (100% sensitivity and 100% specificity).

Linear regression analysis was performed only for cats with ADPKD because the non-ADPKD animals did not have cysts and therefore did not constitute a measurable group for this statistical method. The lower limit value of the 95% CI was the minimum number of cysts in ADPKD animals over the animal's life (in months). A separate analysis of the animals' age groups was not performed for the calculation of minimum number of cysts, but rather a trend line that accounted for all evaluated animals was used. The most central region of the curve was used to select the age at which the cut-off should be made.

Linear regression analysis revealed a strong linear correlation ($R^2 = 0.74$; $P < 0.0001$) between age and the number of kidney cysts. The trend curve was used to extrapolate the number of cysts vs age according to the CI, enabling the establishment of specific thresholds for the diagnosis of ADPKD in Persian cats (Figure 1b). This

Table 1 Descriptive statistics for ultrasonography variables in the right kidney (RK) and left kidney (LK) from autosomal dominant polycystic kidney disease (ADPKD) and control (non-ADPKD) Persian cats

	ADPKD	Control	*P* value
	n = 24 (RK + LK)	n = 140 (RK + LK)	
Number of cysts per kidney (n)			
Mean ± SD	6.20 ± 2.17	0	
Median	6	0	
Range	2–10	0	
Renal longitudinal length (cm)			**<0.01***
Mean ± SD	3.848 ± 0.528	3.542 ± 0.496	
Median	0.279	0.246	
Range	2.53–4.61	1.75–4.95	
Renal echogenicity, n (%)			**<0.01**†
Normal	6 (25)	86 (61.4)	
Increased	18 (75)	54 (38.6)	
Renal contour, n (%)			**<0.0001**†
Regular	12 (50)	135 (96.4)	
Irregular	12 (50)	5 (3.6)	
Corticomedullary junction, n (%)			**<0.000001**†
Preserved	9 (37.5)	131 (93.6)	
Blurring	15 (62.5)	9 (6.4)	

Bold denotes significance
*Student's *t*-test for equal variances
†Fisher's exact test

Table 2 Descriptive data for sex, age and renal and hepatic cysts from Persian cats with autosomal dominant polycystic kidney disease (ADPKD)

Animal	Sex	Age (months)	Number of renal cysts			Presence of hepatic cysts
			RK	LK	Total	
1	Female	18	10	10	20	No
2	Male	18	5	5	10	No
3	Female	120	6	4	10	No
4	Female	60	5	5	10	No
5	Male	72	3	2	5	No
6	Female	138	6	6	12	Yes
7	Female	123	7	8	15	Yes
8	Female	162	10	6	16	Yes
9	Female	99	4	6	10	No
10	Female	11	2	2	4	No
11	Male	144	10	10	20	No
12	Female	84	7	10	17	Yes

RK = right kidney; LK = left kidney

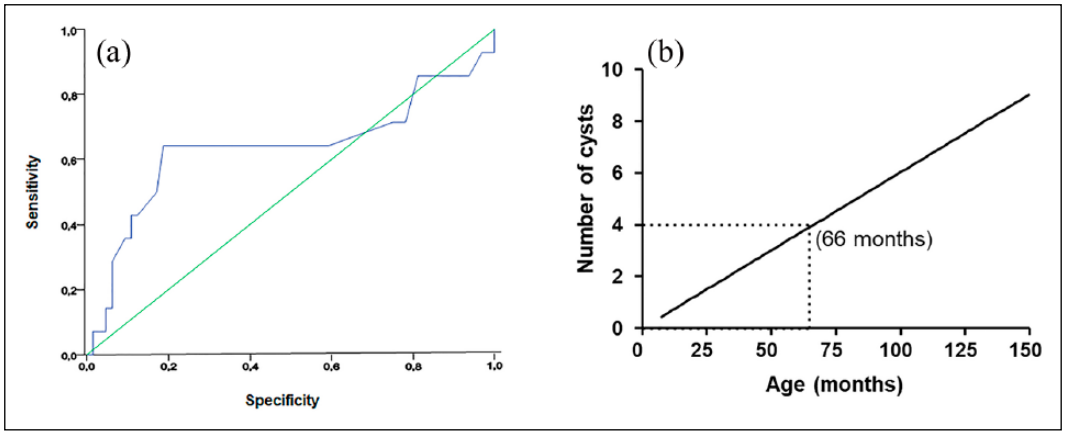

Figure 1 (a) Receiver–operating characteristic (ROC) curves for age and number of cysts detected in Persian cats with autosomal dominant polycystic kidney disease (ADPKD). The area under the ROC curve for age was 0.636 (95% confidence interval [CI] 0.437–0.835; $P < 0.05$) and 1 for number of cysts (95% CI 100–100%; $P < 0.05$) for ADPKD diagnosis. (b) Linear regression curve trend line for age and number of cysts detected in Persian cats with ADPKD

analysis confirmed the data yielded by the ROC curve assessment, supporting a cut-off point of four cysts in animals at 66 months of age.

Based on the integrated analysis, it was possible to determine statistically supported ultrasonographic criteria for the diagnosis of ADPKD in Persian cats. The risk was determined based on the probability of a positive diagnosis as a function of the expected number of cysts for a given age. The presence of at least one renal cyst was sufficient to establish the diagnosis of ADPKD in animals aged 15 months or younger; two or more cysts in one or both kidneys are required for this diagnosis in cats aged 16–32 months; at least three cysts in one or both kidneys warrant the diagnosis of ADPKD in animals aged 33–49 months; and four or more cysts in one or both kidneys resulted in the diagnosis in cats aged 50–66 months (Table 3). The absence of renal cysts in all 70 non-affected cats at all evaluated ages, including ADPKD-containing and no ADPKD-containing pedigrees, associated with the high prevalence of ADPKD in Persian cats, enables the use of the proposed diagnostic criteria in all Persian cats, not restricting its application to animals that belong to ADPKD-linked pedigrees.

Blood and urine analyses

None of the blood-based samples was discarded as a result of gross hemolysis or significant hyperlipidemia. No differences in hematological parameters were observed between ADPKD and non-ADPKD cats (see Table 1 in the supplementary material). ADPKD animals, however, exhibited higher serum calcium levels than non-affected cats (10.56 and 9.46 mg/dl, respectively;

Table 3 Diagnostic criteria for age-dependent kidney ultrasound in Persian cats with autosomal dominant polycystic kidney disease

Age (months)	Diagnostic criteria for kidney ultrasound
⩽15	⩾1 cysts
16–32	⩾2 cysts
33–49	⩾3 cysts
50–66	⩾4 cysts

Table 4 Descriptive statistics for serum biochemical parameters in autosomal dominant polycystic kidney disease (ADPKD) and control (non-ADPKD) Persian cats

Analyte	Group	Mean ± SE	Median	Range	n	*P* value
BUN (mg/dl)	ADPKD	23.04 ± 6.78	21.54	13.41–35.00	12	0.212*
	Control	20.87 ± 4.40	20.05	14.58–37.76	70	
Creatinine (mg/dl)	ADPKD	1.283 ± 0.350	1.24	0.78–2.10	12	0.269*
	Control	1.084 ± 0.199	1.05	0.70–1.61	70	
ALT (U/dl)	ADPKD	45.76 ± 21.18	34.7	24.6–83.8	10	0.160*
	Control	64.22 ± 47.74	46.25	19.2–241.8	70	
AST (U/dl)	ADPKD	15.83 ± 4.615	15.25	9.3–24.77	10	0.667*
	Control	15.03 ± 6.303	14.25	7.1–43.0	70	
ALP (U/dl)	ADPKD	18.70 ± 9.32	16.65	9.2–35.82	10	0.133*
	Control	27.66 ± 21.21	22.75	6.0–139.9	68	
GGT (U/dl)	ADPKD	0.667 ± 1.385	0.667	0.0–4.2	10	1.00*
	Control	0.33 ± 0.64	0.329	0.0–2.9	70	
TP (g/dl)	ADPKD	7.289 ± 0.40	7.285	6.65–8.00	12	0.615*
	Control	7.05 ± 0.70	7.160	5.23–9.05	70	
ALB (g/dl)	ADPKD	3.063 ± 0.16	3.03	2.80–3.38	10	0.969†
	Control	3.06 ± 0.30	3.12	2.13–3.60	70	
Calcium (mg/dl)	ADPKD	10.55 ± 1.43	10.56	8.88–12.71	09	**0.021***
	Control	9.45 ± 1.00	9.46	7.45–13.28	69	
Phosphorus (mg/dl)	ADPKD	4.63 ± 0.868	4.76	2.65–5.62	09	0.133*
	Control	5.34 ± 1.09	5.21	3.80–8.88	69	
Sodium (mEq/l)	ADPKD	152.92 ± 2.16	152.2	150.8–156.0	09	0.484*
	Control	154.64 ± 4.89	153.1	147.3–172.3	67	
Potassium (mEq/l)	ADPKD	4.60 ± 0.408	4.61	3.90–5.21	09	0.557*
	Control	4.74 ± 0.46	4.70	3.72–6.02	67	
Chloride (mEq/l)	ADPKD	117.82 ± 1.68	117.30	115.5–120.4	09	0.910*
	Control	118.36 ± 3.89	117.40	112.3–129.2	67	
Total T4 (µg/dl)	ADPKD	1.561 ± 0.185	1.64	1.24–1.74	07	0.725*
	Control	1.684 ± 0.496	1.58	0.89–2.50	39	

*Mann–Whitney U-test
†Student's *t*-test for different variances
Bold indicates $P < 0.05$
BUN = blood urea nitrogen; ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; GGT = gamma-glutamyl transferase; TP = total protein; ALB = albumin; calcium = total serum calcium; phosphorus = serum inorganic phosphorus; sodium = serum sodium; potassium = serum potassium; chloride = serum chloride; T4 = thyroxine

$P < 0.05$) (Table 4). Serum creatinine concentration, as well as the proportion of cats with serum creatinine exceeding the reference intervals, did not significantly differ between the disease and non-disease groups. The levels of ALT, AST, GGT, ALP, TP, phosphate, sodium, potassium, chloride, and thyroxine also did not differ between the two groups (Table 4).

Urine samples were not available for one ADPKD and 29 non-ADPKD cats owing to empty bladder or pregnancy. In addition, the amount of collected urine was insufficient for all analyses in three ADPKD and one control cat (<4 ml). The determination of USG, UPC and the proportion of cats with borderline proteinuria (UPC 0.2–0.4 [4/11 ADPKD cats, 11/41 non-ADPKD cats])[28] or

overt proteinuria (UPC >0.4 [1/11 ADPKD cats, 10/41 non-ADPKD cats]) did not differ significantly between affected and non-affected animals. Urinary dipstick testing, however, did not reveal significant differences in hemoglobin, acetone, protein, urobilinogen, glucose, bilirubin and nitrites between the ADPKD and non-ADPKD animals. The amount and type of urinary crystals and the proportion of cats with casts also did not differ between the two groups (Table 2 in the supplementary material).

Discussion

ADPKD is the most common inherited renal disease of domestic cats but primarily affects Persian cats and Persian-derived exotic breeds such as Himalayans, British Shorthairs and Scottish Folds.[19] The disease is characterized by the slow development of multiple renal and, occasionally, hepatic and pancreatic cysts, leading to end-stage renal disease late in life (>7 years of age).[17] Owing to the high morbidity/mortality of the disease, an early diagnosis of mutation-carrying animals is essential for a sustainable and healthy breeding program. Moreover, in symptomatic animals, complementary tests are necessary for the evaluation and clinical follow-up of the animal.

The only current molecular diagnosis with adequate accuracy is a PCR-RFLP,[19] or a real time-PCR assay.[12] However, because these molecular-genetic tests are available in so few international clinical veterinary centers, which limits access, and are relatively expensive, such tests are seldom performed in the clinical setting in developing countries. Consequently, US continues to be used routinely for disease investigation because it is an inexpensive and widely available modality in veterinary clinics.[14] However, the risk for false-positives compromises the quality of this test for the diagnosis of the disease. In this scenario, we propose here an ultrasonographic, age-based inclusion criterion supported by the certainty of genetic-molecular diagnosis for ADPKD in Persian cats, similar to the strategy used for the development of US diagnostic criteria for ADPKD in humans.[5,6]

Despite the occurrence of some cases of Persian cats with renal cystic disease not associated with the C→A transversion in exon 29 of *PKD1*, for which the diagnosis of ADPKD could not be excluded,[14,16,22,29] this is the only pathogenic mutation to be linked to ADPKD in cats to date.[19,30] The C→A transversion introduces a stop codon in the *PKD1* transcript, leading to the truncation of polycystin-1, the protein product encoded by this gene.[19] In this context, other pathogenic mutations in *PKD1* or other genes, such as *PKD2*, cannot be completely discarded as potential causes of feline ADPKD; however, heterozygosity for the C→A variant should be considered the cause of this disease in the absolute majority of – if not all – ADPKD-affected cats.[19] Based on these data, Persian cats without the C→A mutation can be virtually excluded to be affected by the disease.

Renal US remains not only the most practical non-invasive diagnostic method used in feline clinical practice, but also enables the assessment of severity and monitoring disease progression in affected cats.[15] Our study showed several non-cystic structural alterations in the kidneys of most cats diagnosed with ADPKD, from early adulthood onward. Such abnormalities included higher renal longitudinal length, irregular contour, increased cortical echogenicity and corticomedullary blurring. Increased cortical or cortical and medullary echogenicity can be a normal finding in cats, but is also one of the most common signs of chronic or acute kidney disease in this species.[31] In this study, therefore, this finding was not considered to be a reliable biomarker of renal injury; nevertheless, its prevalence was higher in ADPKD vs non-ADPKD animals. Renal cysts were not found in the genetically unaffected Persian cats, revealing that ultrasound specificity for cyst detection in our study was extremely high.

This finding is consistent with previous data for at-risk PKD cats.[14,16] In addition, Gerwing et al examined the kidneys of 1527 non-Persian cats and found renal cysts in only 0.5%,[32] indicating a very low incidence of both PKD and acquired renal cysts in such cats.

The mean number of cysts in ADPKD cats was 7.0 ± 2.6, which were distributed in one or both kidneys. Ultrasonographic evaluation of severely affected cats is usually simple given the large number of renal cysts. However, small cysts in limited numbers can be more difficult to identify with certainty, especially when they are located in the medulla. This can occur as a result of low contrast between the anechoic cystic fluid and medullary echogenicity.[15] Ultrasound sensitivity for ADPKD diagnosis is expected to increase with age and to be associated with high specificity.[9–11,18] A previous study comparing ultrasound results obtained at 3 and 12 months of age reported a diagnostic sensitivity of 100%; however, only a small number of cats were evaluated, the diagnosis was not anchored in genetic testing, and animal assessment was not performed.[33] An additional study, appropriately controlled by genetic testing, reported US sensitivity and specificity for ADPKD diagnosis of 96.2% and 91%, respectively, in Persian and Persian-related cats >10 months of age.[14] No analyses and criteria based on age and number of cysts, however, were proposed.

The age-specific positive and negative predictive values obtained in our analysis are only approximations of the post-test predictive values. However, the trends enable the generation of age-specific diagnostic criteria for ADPKD in Persian cats. The presence of at least one renal cyst in cats ⩽15 months of age is sufficient to establish the diagnosis; among those aged 16–32 months, ⩾2 uni- or bilateral cysts are needed to make this

diagnosis; for animals aged 33–49 months, at least three cysts in one or both kidneys are required for diagnosis; and in cats 50–66 months of age, ⩾4 uni- or bilateral cysts are sufficient for the diagnosis of ADPKD. It must be noted that the absence of renal cysts in all non-ADPKD analyzed animals, the inclusion of all evaluated ages and pedigrees with and without cases of this disease, and the high prevalence of this disorder in Persian cats, do not limit the proposed diagnostic criteria to at-risk cats, but instead extends such criteria to the evaluation of all Persian cats. It is also important to note that these diagnostic criteria were developed for, and therefore should be applied to, ultrasound analysis performed at a renal cyst resolution of 0.3 cm.

Our data do not underplay the relevance of other imaging information for the potential diagnosis of ADPKD, including the presence of hepatic or pancreatic cysts, and at very young ages and renal enlargement.[18] However, our study revealed that most ADPKD-affected cats develop multiple bilateral renal cysts, indicating that equivocal results are exceptions rather than common events. This information is particularly important because our data were obtained from affected animals with serum creatinine levels not significantly different from control cats and therefore in ADPKD animals that, in large part, had not reached advanced stages of chronic kidney disease.

We currently have no robust explanations, however, for the increased serum calcium levels observed in ADPKD animals. The lack of other significant differences in hematological, serum biochemical and urine parameters between the ADPKD and non-ADPKD groups were consistent with previously reported data, given that most of the evaluated affected cats had not reached advanced phases of chronic kidney disease.[17,18]

Conclusions

Our study established ultrasonographic criteria for the diagnosis of ADPKD in Persian cats, supporting and guiding the use of this imaging technique for this purpose. The development of accurate age-based US criteria for this diagnosis is particularly relevant and useful in the clinical setting, given the current limitations to access and the cost of molecular genetics-based diagnostic tests.

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Supplementary material The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.
Supplementary Table 1: Descriptive statistics for hematological parameters and urinary protein-to-creatinine ratio in autosomal dominant polycystic kidney disease (ADPKD) and control (non-ADPKD) Persian cats.
Supplementary Table 2: Descriptive statistics for urinary parameters in autosomal dominant polycystic kidney disease (ADPKD) and control (non-ADPKD) Persian cats.

Conflict of interest The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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