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RESEARCH ARTICLE

REVISED PKD1 gene mutation and ultrasonographic characterization in cats with renal cysts [version 3; peer review: 1 approved, 2 approved with reservations]

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Abstract

Background

Polycystic kidney disease (PKD) has a complex phenotype partly explained by genetic variants related to this disease. Ultrasonography is a promising approach for defining clinical signs. This study aimed to assess kidney characteristics in cats with Polycystin-1 (PKD1) gene mutations and wild-type cats. Kidney characteristics were identified by ultrasonography.

Methods

A total of 108 cats of variable breeds aged an average of 37.01±3.50 months were included. Blood examination and biochemical tests were evaluated. For cystic formation, renal ultrasound was performed. The PKD1 gene mutation was identified *via* polymerase chain reaction (PCR) and DNA sequencing. Matrix correlation and effectiveness of ultrasound for PKD1 mutation detection were determined.

Results

The results showed that 19.44% of cats had PKD1 mutations, a high

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prevalence in Persian and Persian-related breed cats. Our results demonstrated the characteristics of kidneys in wild-type cats and cats with gene mutations. Based on ultrasonography results, there was an association between cats with gene mutations and cyst formation. The findings indicated that ultrasound did not detect cysts in cats aged 4-36 months, supporting the evidence that PKD1 gene mutations may not be present. This study found high sensitivity and renal specificity ultrasound for PKD1 heterozygous mutation. Moreover, cystic formation *via* renal ultrasound showed an increased risk for PKD1 mutation 2,623 times compared to normal kidneys.

Conclusions

Ultrasonographic examination, coupled with genetic investigations, may help to clarify the phenotypic variability of PKD1. The phenotypic profile of PKD1 will guide therapeutic outcomes and reduce the prevalence of PKD morbidity and mortality in cats.

Keywords
Gene, kidney disease, mutation, polycystic, ultrasonography

This article is included in the Genomics and Genetics gateway.

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REVISED **Amendments from Version 2**

We have revised and discussed the point that an older domestic shorthair with unilateral cyst but no evidence of the relevant PKD1 mutation was found in one cat in this study. This cat was diagnosed with CKD. As a result, cysts could be the result of chronic kidney disease. Chronic kidney disease can lead to the development of renal cell carcinoma via oxidative stress from a uremic milieu or an underlying cystic disease. However, in this present study, we are still unable to determine whether the cat's cyst was caused by a novel PKD mutation or underlying cause. Moreover, we have revised the mistakes in Figure 3, Figure 4 and Table 2.

Any further responses from the reviewers can be found at the end of the article

Introduction

A previous study reported that the genetic condition of the *PKD1* gene affects Persian and Persian-related cats.[1] Renal, hepatic, and pancreatic cysts are the common symptoms of feline PKD, inherited in an autosomal dominant manner.[2] This disease is frequently detected in the Persian breed. It is one of this breed's most common feline genetic diseases, other than diabetes and feline lower urinary tract disease.[3] This disease not only impacts the Persian breed but other breeds, including the Exotic Shorthair, British Shorthair, American Shorthair, Himalayan, Scottish fold, Ragdoll, Chartreaux, and Maine Coon breeds, may also be affected by this disease.[4–6] The polycystin-1 (*PKD1*) gene mutation was indicated for 85% of human autosomal dominant polycystic kidney disease (ADPKD). The mutation of the *PKD1* gene in cats carried heterozygous CA transversion at c.10063 in exon 29, resulting in impaired renal function.[1,7]

Polycystin-1 upregulation was reported in the renal tissue from a mouse model with a *PKD1* mutation. The increased expression of the mammalian target of rapamycin (mTOR) affected autophagy and apoptosis signaling in cells with a *PKD1* gene mutation.[1,7–9] In addition, many studies have shown the mechanisms by which cyclic adenosine monophosphate (cAMP) levels influence PKD.[10–13] Oral administration of antioxidant agents such as DHA-enriched fish oils showed potential renoprotective effects in cats with chronic kidney disease related to *PKD* gene mutation.[14]

Ultrasonography is a useful diagnostic tool for evaluating the renal structure and renal parenchyma that cannot be evaluated through radiography.[15,16] This technique is a useful diagnostic tool for assessing and revealing renal parenchyma alteration, as that occurs when the disease progresses.[17] This technique is a practical and accurate approach to diagnosing PKD. Cats with PKD have multiple hypoechoic to anechoic cysts that are round or oval in shape and clearly distinguished from the renal parenchyma.[18] In a previous study, ultrasound had a sensitivity of 75% when performed at 16 weeks of age and 91% when performed at 36 weeks of age.[19]

This study aims to investigate the association between clinical presentations and the ultrasonography of the kidneys in wild-type cats and cats with gene mutations to provide the guide treatment and aid prevalence estimates of PKD in cats.

Methods

Animals

The Ethics Committee of Kasetsart University, Bangkok, Thailand, approved this research on September 12, 2022 (ACKU-65-VET-077). In addition, all cat owners in this study signed an informed consent agreement.[39] A total of 108 cats, including 18 Persian cats, 49 Persian-related breed cats, 22 domestic shorthair cats, and 19 Bengal cats, were enrolled in this study at the Veterinary Teaching Hospital, Faculty of Veterinary Medicine, Kasetsart University, from September 2022 to April 2023, as shown in Figure 1. The cat underwent a complete physical examination to evaluate the general condition. All procedures were refined as much as possible to minimize suffering to alleviate the harm to the cats in this study with a high standard that is internationally accepted ('Best Practice Guidelines') of veterinary clinical care for individual cats and following the ethical approvals. This study adhered to the ARRIVE guidelines.[40]

A complete blood count and serum biochemistry profile were performed for each cat. Kidney characterization was assessed by ultrasonography analysis in all cats using only gentle restraint and no sedation. In our ultrasonographic examination, all cats were clipped the hair at the abdominal area and the ultrasonographic gel was utilized in all cats. The non-invasive two-dimensional (2D)-mode ultrasonography was used to define the kidney structure and size. The ultrasonography images have demonstrated that multiple cysts are a common finding in *PKD1* heterozygous mutation cats, as shown in Figure 2.

Polymerase chain reaction and DNA sequencing

DNA amplification with polymerase chain reaction (PCR) was performed to detect *PKD1* gene mutation. PCR protocol obtained the specific forward and reversed primers from Lee *et al.*[18] The PCR primers were PKD-forward

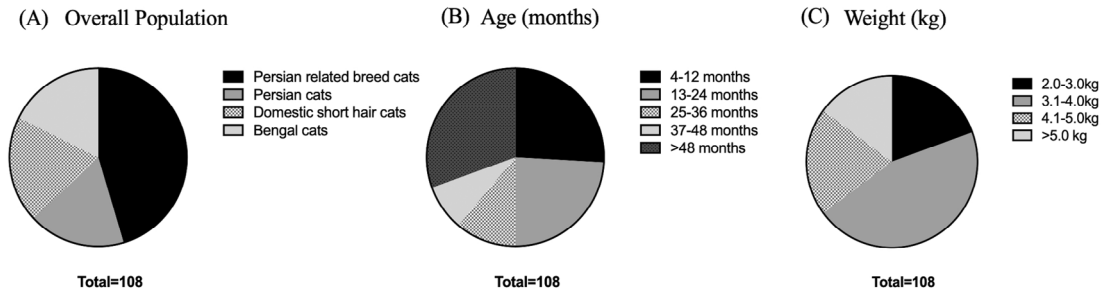


Figure 1. Overall population in this study. (A) Various breeds of enrolled cats. (B) Age in the different groups, including 4-12 months, 13-24 months, 25-36 months, 37-48 months, and more than 48 months. (C) Weight comprising 2.0-3.0, 3.1-4.0, 4.1-5.0, and more than 5.0 kg.

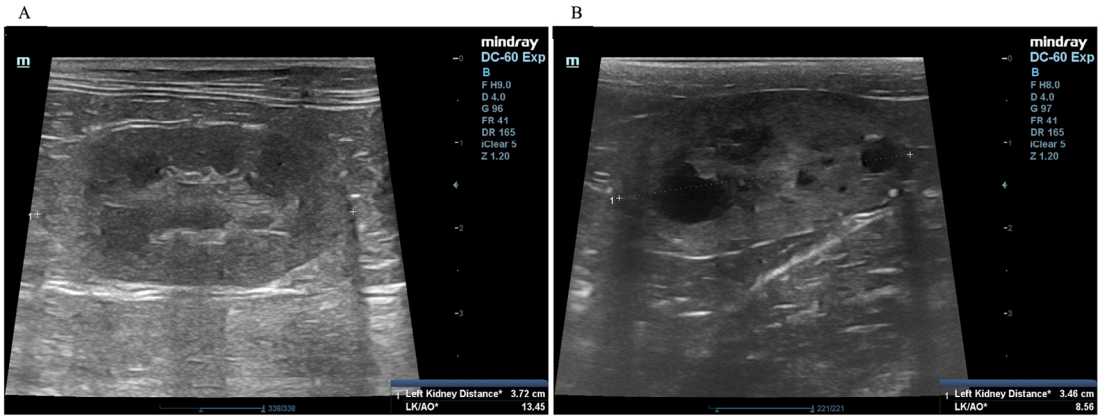


Figure 2. Ultrasound image of kidneys with cysts. (A) Normal kidney appearance without cystic formation. (B) Multiple cyst formation in renal parenchyma. In (A) and (B), the ultrasonography image was cropped for the proper size.

5′-CAGGTAGAC GGGATAGACGA-3′ and PKD-reverse 5′-TTCTTCCTGGTCAACGACTG-3′. In brief, DNA was extracted from the blood using FavorPrep Blood Genomic DNA Extraction Mini Kit (Farvogen, Taiwan). Then, 2 µl of extracted DNA from blood (Farvogen, Taiwan) was mixed with 2.5 µl of 10X Taq buffer, 0.2 µl of dNTPs (25 mM each dNTP) (Thermo Fisher Scientific, USA), 1.5 µl of $MgCl_2$ (25 mM), 1 µl of each custom forward and reverse primer from the manufacturer (Integrated DNA Technologies, USA) (10 µM each primer), 1 unit of Taq DNA polymerase (Thermo Fisher Scientific, USA), and 16.7 µl of nuclease-free water. The final volume of the PCR reaction was 25 µl per reaction. The PCR condition was as follows: heat-activation at 94°C for 3 minutes, 35 cycles of denaturation for 1 minute, annealing at 58°C for 1 minute, and extension at 72°C for 1 minute. Finally, the final extension was performed at 72°C for 10 minutes *via* Biometra Thermocycler T-Gradient ThermoBlock (Thermo Fisher Scientific, USA). The PCR product was detected with 1.5% agarose gel electrophoresis. The PCR product was visualized under gel documentation (Bio-rad, USA) at 559 bp. The PCR product was stored at -20°C until sequencing. The Barcode-tagged (BT) sequencing method was performed to detect the PCR product's nucleotide. According to BT sequencing, the PCR product was analyzed by Celemics, Inc. (Seoul, Korea). Single nucleotide polymorphism of the *PKD1* gene was detected using the BioEdit version 7.2 (RRID:SCR_007361) program and A plasmid Editor (ApE) version 2.0.60 (RRID:SCR_014266) program.

Ultrasonography

The procedure for ultrasound examination of the kidneys followed that of a previous report.[4,20] Briefly, ultrasonography was performed using a Mindray real-time ultrasound machine (model DC-7, Shenzhen Mindray Bio-medical Electronics, Nanshan, Shenzhen, China) with a linear transducer (frequency 7.5-12.0 MHz). The kidneys were examined in the longitudinal and transverse planes. Results were recorded for each cat. The three separated regions, the renal cortex, the renal medulla, and the renal sinus, were identified. The renal sinus is the most hyperechoic component of the kidney and is surrounded by the renal pelvis and vascular branches. In the renal pelvis, ultrasonography images can be assessed using the renal crest as a landmark. The renal size can be measured by measuring from the long axis view. The renal lengths of the right and left kidneys were measured to identify the renal structure, as shown in Figure 2.

Statistical analysis

Descriptive statistics were determined as mean ± standard error of the mean (SEM). An evaluation of the effectiveness of diagnostic tests was performed. The datasets were analyzed using a paired Student's- t-test. An odds ratio (OD) was performed to measure the association between cysts in renal parenchyma and *PKD1* mutation. A correlation matrix was used to represent the correlation coefficients for different variables. Pearson's correlation coefficient was used to determine the correlation between variables and to analyze the multiple linear regression models that contained several independent variables (GraphPad Prism (RRID: SCR_002798) Software version 9.0, USA). A *P*-value of 0.05 or less was indicated for statistical significance.

Results

Characteristics, ultrasonographic variables, and serum biochemical findings

The number of cats in the *PKD1* mutation group was 21 (19.44 %) from an overall population of 108 cats. The cats in the *PKD1* mutation group were older than those in the wild-type group (50.89 ± 9.19 *vs.* 33.93 ± 3.59 months, $P = 0.051$). However, there were no significant differences in weight between cats in the wild-type group and the PKD1 mutation group. General characteristics and ultrasonographic parameters for the cats are reported in Table 1 and Figure 3A-C.[37,38] The size of the left kidney was significantly larger in the *PKD1* mutation group than in the wild-type group. However, there was no statistically significant difference between right and left kidney size in cats affected with *PKD1* mutation ($P = 0.9980$). Blood profiles and serum biochemistry results are reported in Table 2 and Figure 3D-F. The levels of other

Table 1. Characteristics and ultrasonographic variables of cats.

Parameters (Mean ± SEM)	PKD1		*P* value
	Wild-type group	HET mutant group	
Age in months	33.93 ± 3.59	50.89 ± 9.19	$P = 0.051$
Weight (kg)	4.36 ± 0.46	3.34 ± 0.22	$P = 0.283$
Male (number [%])	72.4	61.9	-
Right kidney size (cm)	3.64 ± 0.39	4.44 ± 0.22	$P = 0.082$
Left kidney size (cm)	3.54 ± 0.37*	4.58 ± 0.28*	$P = 0.036$
Cysts number	0	12.61 ± 2.09	-
Bilateral Kidney Cysts (%)	0	100	-

PKD1 = Polycystin-1, HET = heterozygous mutation.
Values are presented as the mean ± SEM.
*$P < 0.05$.

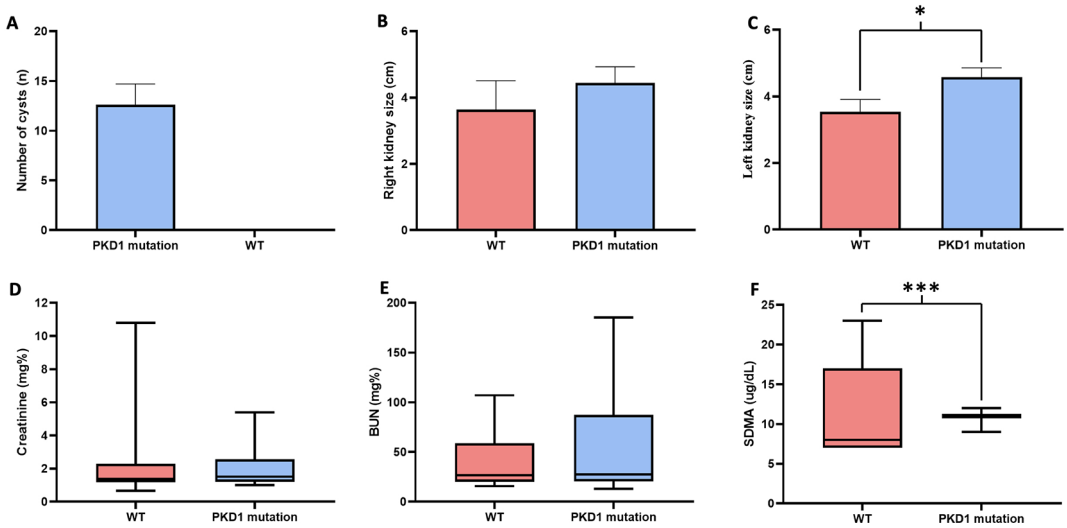


Figure 3. The results of renal ultrasonographic parameters, including (A) Number of cysts, (B) Right kidney size, (C) Left kidney size, and renal biochemistry, including (D) Creatinine levels (E) BUN levels, and (F) SDMA in cats with and without *PKD1* gene mutation.

Table 2. Serum biochemistry, red blood cell count, and hematocrit in all cats and groups.

Parameters (Mean ± SEM)	Overall population	Wild-type group	PKD1 mutant group	Reference value
BUN (mg%)	42.81 ± 3.54	38.18 ± 4.05	50.35 ± 11.39	15-34
Creatinine (mg%)	2.21 ± 0.19	1.95 ± 0.21	2.00 ± 0.33	<2.0
BUN/Creatinine ratio	19.4	19.6	25.2	7-37
Hematocrit (%)	35.87 ± 0.63	37.30 ± 3.95	33.01 ± 1.35	30-45
PP (g/dL)	7.64 ± 0.08	7.54 ± 0.80	8.0 ± 0.19	5-7.5
SDMA (ug/dL)	12.33 ± 0.74	9.36 ± 0.999.36***	20.5 ± 2.06***	<14

PKD1 = Polycystin-1, BUN = blood urea nitrogen, PP = plasma protein, SDMA = symmetric dimethylarginine.
***$P < 0.001$.

biochemical profiles, such as symmetric dimethyl arginine creatinine (SDMA), significantly differed between groups. However, the blood profile parameters of red blood cell count, hematocrit, and plasma protein were not different between the groups. Additionally, the results of average creatinine levels in overall cats were elevated. Nonetheless, our results demonstrated that increasing creatinine exceeding 2.0 mg% may slightly increase the possibility for renal cyst formation by 1.41 times (95% CI, 0.3827 to 5.1764, $P = 0.6070$). As indicated in Table 2, the levels of BUN were mildly elevated than the normal reference value. However, Pearson's correlation results revealed that there was no correlation between age and BUN levels in WT (r = 0.1436 $P = 0.62$) and *PKD1* mutation group (r = 0.3234 $P = 0.099$).

Sequencing results and ultrasonography correlation analysis of PKD1 in cats

The association between ultrasonography findings and the genotypes of *PKD1* mutations was analyzed for 108 cats. *PKD1* heterozygous mutations were identified in 21 cats, and the results showed significant differences between C/C and C/A genotypes for the homozygous wild-type and heterozygous mutation (Figure 4). In the present study, gene mutation

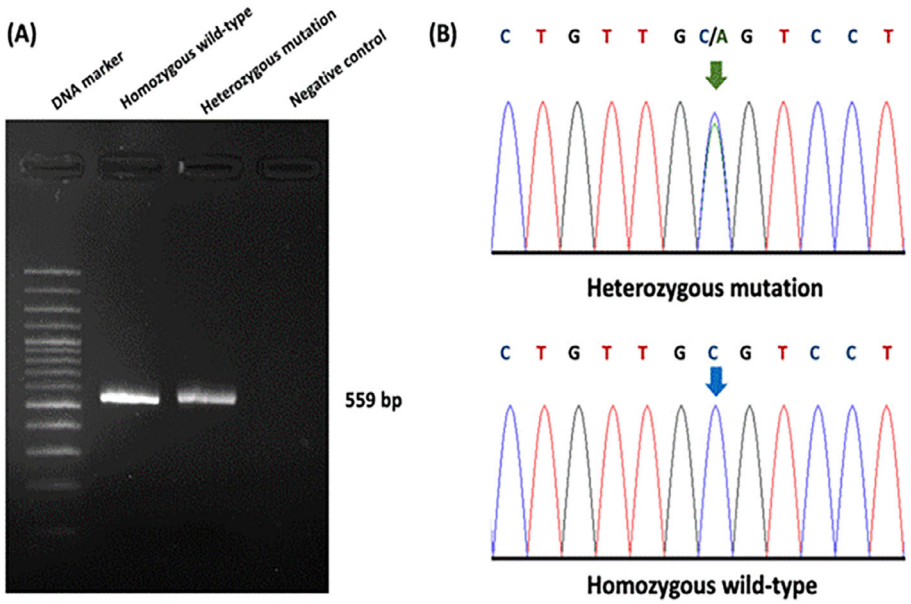


Figure 4. Results of Polycystin-1 (PKD1) polymorphism from genotyping by sequencing. (A) Gel electrophoresis represented 559 bp of target DNA of the PKD1 gene. Lane 1 represents DNA marker (M), while Lane 2 and Lane 3 show DNA products from cats with homozygous wild-type and heterozygous mutation, respectively. Lane 4 indicates negative control. (B) The barcode-tagged (BT) sequencing result of *PKD1* was shown in two groups, including homozygous wildtype (C/C) and heterozygous mutation (C/A). The green arrow pointed to two peaks of cytosine and adenine, while the blue arrow represented one peak of cytosine. In (A), the gel electrophoresis image was adjusted to the darkness of the background and cropped for the proper size. In (B), the chromatogram results were cropped from BT sequencing in the Bioedit program and the arrow cursors (green and blue) were added, pointing to the mutation area for clarity and easy understanding. The text of the chromatogram nucleotide above the sequencing picture was edited in another font with bold letters.

Table 3. Various breeds and the number of cats with and without *PKD1* gene mutation associated with renal cyst formation.

Breed	*PKD1* mutation cats with cysts (n=21)	Wild-type cats (n=86)	Wild-type cats with cyst (n=1)
Persian cats (n=18)	5	13	-
Persian-related cat (n=49)	12	37	-
Domestic short hair cats (n=22)	4	17	1
Bengal cats (n=19)	-	19	-

Table 4. Results of the effectiveness of ultrasonography for detecting PKD1 heterozygous mutation.

Statistical test	Value	95% Confidence Interval
Sensitivity	100.00%	84.54% to 100.00%
Specificity	98.91%	94.10% to 99.94%
Positive Predictive Value (PPV)	95.45%	78.20% to 99.77%
Negative Predictive Value (NPV)	100.00%	95.95% to 100.00%
Odds ratio (OD)	2623.00	103.2465 to 66637.8792

PKD1 = Polycystin-1.

cats were associated with cyst formation in cats. The ultrasonographic analysis showed renal cysts in 19 cats, and all of these cats harbored *PKD1* heterozygous mutations. However, there was a 51-month-old domestic shorthair cat without the *PKD1* gene mutation developing the cysts in renal parenchyma. Table 3 demonstrates the number of cats in various breeds with *PKD1* gene mutation and renal cyst development.

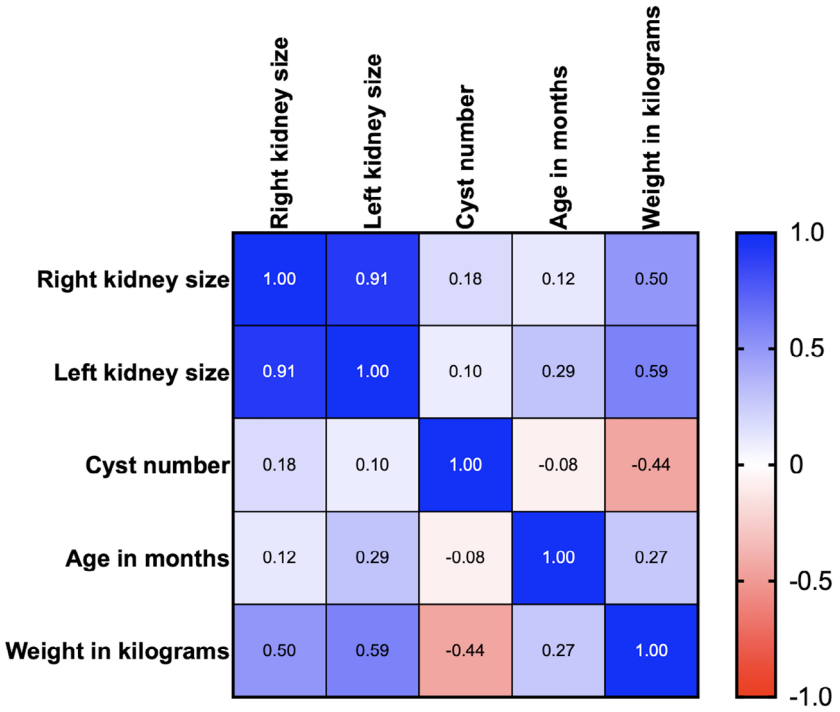


Figure 5. Correlation between Polycystin-1 (PKD1), ultrasonography profiles, and clinical data (age and body weight).

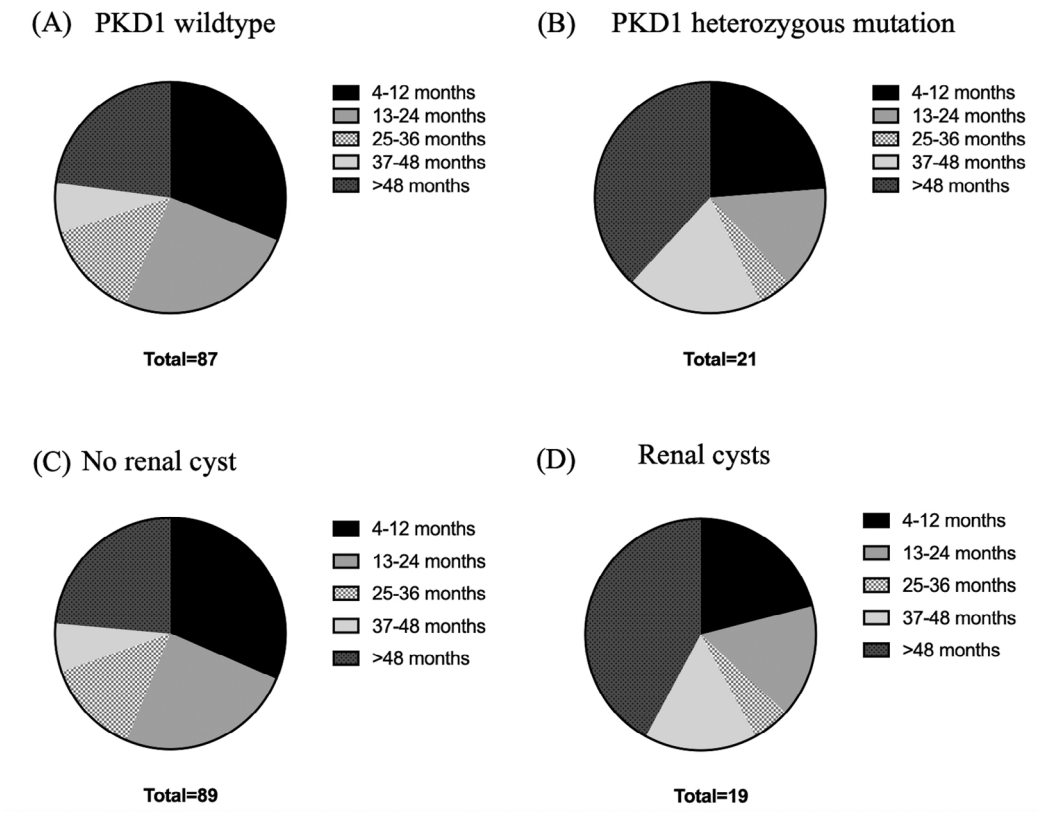


Figure 6. Relationships between ages, weight, and Polycystin-1 (PKD1) gene. (A) PKD1 wild-type, (B) PKD1 heterozygous mutation, (C) no renal cyst and (D) renal cysts.

Effectiveness of ultrasonography for detecting *PKD1* gene mutation

Regarding renal ultrasonography, the effectiveness of this method was evaluated for detecting the variation of *PKD1* gene polymorphism (Table 4). The sensitivity and specificity of renal ultrasound accounted for 100.00% (95% CI, 84.54 to 100.00) and 98.91% (95% CI, 94.10 to 99.94), respectively. In addition, positive predictive value (PPV) and negative predictive value (NPV) were indicated at 95.45% (95% CI, 78.20 to 99.77) and 100.00% (95% CI, 95.95 to 100.00), respectively. On top of that, according to the OD, presenting renal cysts from renal ultrasonography increased the possibility for *PKD1* heterozygous mutation 2,623.00 times (95% CI, 103.2465 to 66637.8792, $P < 0.0001$).

Relationships between renal cysts and *PKD1* genotypes in cats

Age, body weight, right kidney size, left kidney size, and the number of cysts were analyzed using correlation matrix analysis (Figure 5). The results showed that age and weight were negatively related to the number of cysts. However, the weight was related to kidney size. There is likely no gene mutation if the cyst is not found at less than 36 months of age. Cysts are usually found in small cats weighing between 2 and 4 kg (Figure 6). Therefore, cats with increasing SDMA, an age exceeding 36 months, body weight between 2-4 kg, and renal cyst formation from ultrasonographic examination are a high possibility of *PKD1* gene mutation with PKD disorder.

Discussion

This study's differential ultrasonographic profiles between cats with *PKD1* mutations and wild-type cats may provide complementary tools for detecting and evaluating polycystic kidney disease. The findings in our study are consistent with previously reported findings that the formation of the cysts is closely related to *PKD1* gene mutations with high sensitivity and specificity.[18] As well as that, reports from Italy and Taiwan revealed that the sensitivity of ultrasonography was approximately 78.6 to 92.6%, and the specificity was 91 to 100%.[18,20] However, a previous study reported that pure-breed Maine Coon cats developed cystic formation in renal parenchyma without *PKD1* gene mutation.[21] Therefore, the gold-standard technique of *PKD1* gene mutation, PCR, and DNA sequencing is still recommended for confirming the genotype and further breeding selection consideration.

This study found that the rate of *PKD1* gene mutation was high in Persian and Persian-related breed cats including Scottish Folds cats, Previous reports have suggested that *PKD1* mutation was found at very low rates in Persians and other breeds which is contrary to our results. The high number of *PKD1* mutations that had been reported may be due to the Persian and Persian-related breed cats being a popular cat breed in eastern countries and Thailand.

Of the total 108 cats examined by ultrasound, no cyst was found in 87 cats of the wild-type group. 19 cats were confirmed to have renal cysts, and in every cat with bilateral cysts, a *PKD1* mutation was present. However, one cat (51-month-old) in our study had unilateral cysts with no evidence of PKD1 mutation. This cat was diagnosed with CKD. As a result, cysts could be the result of chronic kidney disease. Chronic kidney disease can lead to the development of renal cell carcinoma via oxidative stress from a uremic milieu or an underlying cystic disease. However, in this present study, we are still unable to determine whether the cat's cyst was caused by a novel PKD mutation or underlying cause.

In a previous study, ultrasound had a sensitivity of 75% when performed at 16 weeks of age and 91% when performed at 36 weeks of age.[19]

Previous studies have indicated age-related cyst numbers for an ultrasound to diagnose *PKD1* mutations. Nevertheless, our study indicated a negative correlation between the age and the number of cysts in recruited cats. Moreover, the cats in this study have a negative correlation between cyst number and body weight. This could potentially result from the manifestation of PKD symptoms in cats with *PKD1* mutation. According to the elevated BUN in our study, the enrolled cats in this study are represented with either renal failure or non-renal failure. The high levels of BUN and increasing age were not related in contrast with the prior study from humans at that age and BUN indicated a positive correlation.[22]

The cysts detected are sufficient for a positive diagnosis in young cats up to 12 months of age. In addition, these criteria will help to determine a positive result for *PKD1* mutations if multiple bilateral cysts are detected (>10 per kidney) or if cysts are not detected in older cats. The results from this study are similar to a previous study that reported a positive correlation between the detection of renal cysts and age, as cyst detection was increased in older animals.[23]

The apoptosis pathway is associated with inflammation in PKD in humans and mouse models.[24–27] Bcl-2 and Erb-b2 may be related to the pathway of PKD in cats with sarcomeric protein mutations. Inhibition of the apoptosis pathway might be associated with renal fibrosis and cyst expansion in cats with PKD.[28,29]

Caspases are another essential target in the apoptosis mechanism.[30,31] Caspases are involved in preserving kidney function, suggesting caspases as a potential new target for the treatment of PKD.[26]

PKD1 mutation is caused by disrupting the mechanism that controls the tubular diameter. The cyst characteristics were reported to be involved in cAMP signal transduction. The vasopressin that acts on the V2 receptor is the most potent catalyst for cAMP formation. Vasopressin 2 receptors are located in the collecting ducts, connecting ducts, and thickened limbs of Henle, which is the area of cyst formation. Tolvaptan has been used in treating PKD because the anti-vasopressin 2 receptor plays an important role in cyst growth.[32–34] Other medications, such as metformin, which targets AMPK and cAMP signaling, have shown great promise in reducing cyst formation and cellular proliferation.[35,36] However, reports linking these novel therapeutic drugs with PKD in cats still require further confirmation. Future clinical trials of potential signaling pathways are crucial in PKD diagnosis and treatment in feline patients.

Conclusions

The frequency of *PKD1* mutations was high in Persian and Persian-related breed cats. Such modifications were associated with the polycystic kidney phenotype. Ultrasonographic examination of cats will be a helpful tool for the routine identification of carriers of the mutated gene for polycystic kidney disease in mature cats (≥ 9 months). The results from this study may provide important information on clinical presentation and a gene associated with feline PKD. Genetic insights from genes may enable a more precise diagnosis of the type of renal cyst, which allows treatment or prevention strategies. Furthermore, knowing a genetic susceptibility to kidney cysts early may help to more accurately predict those most at risk of chronic kidney disease in the future.

Ethical approval

The work described in this manuscript involved using non-experimental (owned) animals. Established internationally recognized high standards (‘best practice’) of veterinary clinical care for the individual patient always followed ethical approval, and written informed consent for publication of the participants was obtained from the owners.

Data availability

Underlying data

Figshare: Raw data PKD1 in cats. https://doi.org/10.6084/m9.figshare.22957526.v1.[37]

Extended data

Figshare: Figures. https://doi.org/10.6084/m9.figshare.22957715.v1.[38]

Figshare: Figure 3. https://doi.org/10.6084/m9.figshare.25230506.v1.[39]

Figshare: consent form. https://doi.org/10.6084/m9.figshare.22958243.v1.[40]

Reporting guidelines

Repository: ARRIVE checklist for 'PKD1 gene mutation and ultrasonographic characterizations in cats with renal cysts'. https://doi.org/10.6084/m9.figshare.23123414.v1.[41]

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

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Version 3

Reviewer Report 05 August 2024

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Jin He
[1] Zhejiang University, Hangzhou, Zhejiang, China
[2] College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China

The manuscript by Jaturanratsamee et al. presents a comprehensive study investigating the correlation between polycystic kidney disease (PKD) and the PKD1 gene mutation in cats. Utilizing a cohort of 108 cats across various breeds, the authors employ a multimodal approach, including blood examination, biochemical tests, renal ultrasound, and genetic analysis via polymerase chain reaction (PCR) and DNA sequencing. The primary objective is to assess the prevalence of PKD1 gene mutations and to characterize the associated renal phenotypes using ultrasonography, with the ultimate goal of informing treatment strategies and prevalence estimates for feline PKD.

The study's design is robust, incorporating a broad sample of cat breeds and a systematic methodology for data collection and analysis. The use of both imaging and genetic techniques provides a dual perspective on the disease's presentation, offering valuable insights into the phenotypic expression of the PKD1 mutation in cats. The manuscript also discusses the implications of these findings for clinical practice, emphasizing the potential of ultrasonography as a diagnostic tool for identifying PKD1 gene carriers.

However, as with any research, there are aspects of the study that warrant further consideration and clarification. The following comments will address specific areas where additional information, refinement, or alternative approaches may enhance the study's validity, reproducibility, and overall contribution to the field of veterinary genetics and nephrology.

1) Similar to Reviewer 1, I am curious about the selection of cats involved in the study. Are they typically normal-conditioned cats or sick ones? If they belong to the latter category, then the calculated ratios might be overestimated.
2) The authors stated in the statistics section that paired t-tests were utilized in the study. However, I am unsure how a paired test could be applied to analyze Table 1. As a biologist with extensive experience in statistics teaching, I would argue that only the comparison between the left kidney size and the right kidney size could be analyzed using this method. Besides,

normalization of kidney size with their respective body weights may be a better way of presenting the data.
3) The authors mentioned the use of multiple linear models; however, I was unable to locate them in the manuscript.
4) In Table 2, I am puzzled by how the overall creatinine level can be calculated as 2.21 when the constituent parts of the population are recorded as 1.95 and 2.00.
5) The authors should include a discussion on SDMA in comparison to creatinine, which could benefit biologists with limited clinical backgrounds, since creatinine levels are commonly utilized in research.
6) I am curious about the calculation of the odds ratio given that there are no false-negative cases. Additionally, the specificity presented differs from my own calculations.
7) The PCR genotyping method may only cover a portion of the feline PKD1 gene, implying that the 'wild-type' (WT) cats might also harbor PKD1 mutations. This concern was raised by other reviewers, but it seems to me that the authors have not adequately addressed it.
8) Is there a gold standard or diagnostic criterion in veterinary medicine for ADPKD, similar to the Ravine criterion used in human studies? Cyst formation could also be a result of other diseases.
9) Considering the above comments, the authors should carefully select appropriate terminology to describe and discuss their findings. Their conclusions may only be valid within the context of the specific population studied, potential undetected PKD1 mutations, and the diagnostic methods employed.

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Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
No

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Animal models of ADPKD. Statistical genetics and genomics.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 2

Reviewer Report 05 February 2024

https://doi.org/10.5256/f1000research.157775.r222872

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Stephen C Parnell
University of Kansas Medical Center, Kansas, USA

In the manuscript "PKD1 gene mutation and ultrasonographic characterization in cats with renal cyst" authors Jaturanratsamee and others use ultrasonography to investigate the association between clinical presentation of polycystic kidney disease (PKD) in a population of non-experimental house cats including animals with or without feline PKD1 gene mutation. The stated purpose of the study is to guide treatment options and to help estimate the prevalence of PKD in cats.

Animals enrolled in the study were examined by routine medical exam, ultrasonography, serum biochemistry, and targeted PCR analysis of the feline PKD1 gene. Relationships between genotype and phenotype are presented.

The stated conclusions for the study are that the frequency of PKD1 mutations are high in Persian and Persian-related breeds, that PKD1 mutation correlates with the PKD phenotype, and that ultrasonography can be a helpful tool to identify carriers of the mutated feline PKD1 gene.
The study is well-conducted for the most part, but I have a few questions and minor corrections that the authors should address.

The items listed below should be addressed in a revised version.

1. This portion of the manuscript is not clear to me: *Of the total 108 cats examined by ultrasound, no cyst was found in 87 cats of the wild-type group. However, 19 cats were confirmed to have renal cysts, and in every cat with cysts found, a PKD1 mutation was present.*
   An older domestic shorthair with cysts but no evidence of the relevant PKD1 mutation was reported elsewhere in the manuscript and should be addressed again at this point.
   Is it possible that the 51-month specimen represents a novel PKD1 mutation? Were the cysts in this animal unilateral or bilateral?

2. Have additional PKD1 mutations been identified within cats, or is the c.10063 CA transversion the only mutation identified to date? If so then this is interesting given the very

large number of unique PKD1 mutations in the ADPKD patient population, but raises a more fundamental question. The authors conclude that "ultrasonographic examination of cats will be a helpful tool for the routine identification of carriers of the mutated gene for polycystic kidney disease in cats." The manuscript reports sensitivity of only 75% at 16 week of age - isn't this close to sexual maturity? If there is a single founder mutation for PKD in cats then wouldn't a PCR-based assay (which could be performed well before sexual maturity) be much more useful for diagnosis than ultransonography? The authors should discuss this, and also note the age of sexual maturity in cats.

3. The cats with mutant PKD1 are on average almost 17-months older than the wild-type cats. This seems non-random. How were study animals selected, and has there been any consideration of whether there could be selection biases that affect interpretation of the data?

The items listed below should be corrected or amended in the final version.

1. In Figure 3 B/C are both labelled as Right Kidney. One should be the data for the left kidney.

2. In Figure 4 the chemical name is listed as "alanine" but it should be "adenine". Please indicate the resulting protein mutation caused by this transversion.

3. In Table 2 please indicate the percentage of cystic cats in which the cysts are unilateral vs bilateral.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 15 Feb 2024

Soontaree Petchdee

We have provided a point-by-point reply to each point raised by the reviewers as a separate file and indicated any changes or corrections within the text in blue on the resubmitted manuscript. Please see below for the list of changes.

Comments to the Author;

Reviewer 2:

This portion of the manuscript is not clear to me: Of the total 108 cats examined by ultrasound, no cyst was found in 87 cats of the wild-type group. However, 19 cats were confirmed to have renal cysts, and in every cat with cysts found, a PKD1 mutation was present. An older domestic shorthair with cysts but no evidence of the relevant PKD1 mutation was reported elsewhere in the manuscript and should be addressed again at this point. Is it possible that the 51-month specimen represents a novel PKD1 mutation? Were the cysts in this animal unilateral or bilateral?

Answer to the reviewer: We apologize for the unclear on this point. We have edited this point in the revised manuscript in the discussion section.

The 51-month-old cat may represent a PKD1 mutation, and it usually correlates with a bilateral cyst. The cysts detected are sufficient for a positive diagnosis in young cats up to 12 months of age. In addition, these criteria will help to determine a positive result for *PKD1* mutations if multiple bilateral cysts are detected (>10 per kidney) or if cysts are not detected in older cats. The results from this study are similar to a previous study that reported a positive correlation between the detection of renal cysts and age, as cyst detection was increased in older animals.

Have additional PKD1 mutations been identified within cats, or is the c.10063 CA transversion the only mutation identified to date? If so then this is interesting given the very large number of unique PKD1 mutations in the ADPKD patient population, but raises a more fundamental question. The authors conclude that "ultrasonographic examination of cats will be a helpful tool for the routine identification of carriers of the mutated gene for polycystic kidney disease in cats." The manuscript reports a sensitivity of only 75% at 16 weeks of age - isn't this close to sexual maturity? If there is a single founder mutation for PKD in cats then wouldn't a PCR-based assay (which could be performed well before sexual maturity) be much more useful for diagnosis than ultrasonography? The authors should discuss this and also note the age of sexual maturity in cats.

Answer to the reviewer: We apologize for the unclear on this point. We have edited this point in the revised manuscript in the discussion and conclusion section.

Similar to our study, in the previous study, ultrasound provided more sensitivity when diagnosing cats of maturity age, which had a sensitivity of 91% when performed at 36 weeks of age. In our study, we suggested that ultrasonographic examination of cats will be a helpful tool for the routine identification of carriers of the mutated gene for polycystic kidney disease in mature cats.

A previous study reported sexual maturation in the domestic cat based on histological examination of seminiferous tubules, cats were classified as juvenile (<9 months; only spermatogonia and Sertoli cells present), pubertal (≥9 months to <1 year; complete spermatogenesis), and adult (>2 years; spermiogenesis complete and cauda epididymis filled with spermatozoa).

(Ref.) Schön, J., Neumann, S., Wildt, D. E., Pukazhenthi, B. S., & Jewgenow, K. (2009). Localization of oestrogen receptors in the epididymis during sexual maturation of the domestic cat. *Reproduction in domestic animals = Zuchthygiene*, *44 Suppl 2*, 294–301. https://doi.org/10.1111/j.1439-0531.2009.01391.x

The cats with mutant PKD1 are on average almost 17 months older than the wild-type cats. This seems non-random. How were study animals selected, and has there been any consideration of whether there could be selection biases that affect the interpretation of the data?

Answer to the reviewer: We appreciate the helpful comment and apologize for the unclear on this point. The animals in this present study were truly randomized. We were random and blind in all the processes. The person who performed the ultrasounds, the person who recorded the genetic parameters, and the person who analyzed the data were different persons.

In Figure 3 B/C are both labelled as Right Kidney. One should be the data for the left kidney.

Answer to the reviewer: We apologize for this point. We have edited this point in the revised manuscript

In Figure 4 the chemical name is listed as "alanine" but it should be "adenine". Please indicate the resulting protein mutation caused by this transversion.

Answer to the reviewer: We apologize for this point. We have edited this point in the revised manuscript

In Table 2 please indicate the percentage of cystic cats in which the cysts are unilateral vs bilateral.

Answer to the reviewer: We apologize for this point. We have edited this point in the revised manuscript

Best regards

Soontaree Petchdee

Corresponding author

Competing Interests: No competing interests were disclosed.

Reviewer Report 03 November 2023

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Leslie A. Lyons

Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA

Authors have made appropriate corrections.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genetics PKD feline diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 16 October 2023

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Leslie A. Lyons
Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA

The manuscript by Jaturanratsamee et al., entitled "**PKD1 gene mutation and ultrasonographic characterization in cats with renal cysts"** describes the evaluation of 108 cats of variable breeds. Blood examination and biochemical tests were evaluated. For cyst detection, renal ultrasound was performed. The PKD1 gene mutation was identified *via* polymerase chain reaction (PCR) and DNA sequencing. 19.44% (21 of 108) of cats had PKD1 mutations, a high prevalence in Persian and Persian-related breed cats.

The authors present a dataset including 108 cats in which 21 had the variant for PKD1. The dataset is important however the authors need to do some different presentations that would be of more significant value to the veterinary and PKD research communities. Hence. “partly” for the interpretation of data. Partly for data presentation as all US images are not available.

The authors should note 87 cats had no cysts at all. What is the frequency of singular cysts in humans, I think this supports cats rarely have singular cysts, or at least less commonly than humans.

18 Persian cats, 49 Persian-related breed cats, 22 domestic shorthair cats, and 19 Bengal cats – need table with age at US for each cat – the figures are less useful. Produce a summary table that summarizes groups, per breed, the cats without the PKD1 variant and then provides each breed cat that had PKD individually. Which cat had PKD1 variant but no cysts – what was the cat’s age? Mention this in the text.

Scottish Folds are popular in eastern countries, however, the rate of PKD is high – contrast to other, recent population data suggesting PKD1 is a very low rate in Persians and other breeds.

BUN high for both populations – age correlation is important – please comment.

PKD is one of the most common hereditary disease – actually simple Mendelian diseases in cats. HCM may be more common but has a complex inheritance.

Was the hair clipped for the US evaluations or alcohol or gel applied for US?

Overall creatinine for overall population was 2.21 _ 0.19 – odd not intermediate – correct?

Which was most predictive of PKD – renal failure, number of cysts, age, BUN, SDMA? Any cats in renal failure?

Was left to right kidney size significantly different in PKD1 cats?

Figure 4 would be better with biochemistries and cysts, kidney size, in affected cats versus normal. More figures like this, less pie charts.

The results showed that age and weight were negatively related to the number of cysts? I think this is misstated in results.

"The feline Polycystin-1 (PKD1) gene was examined for the point of 85% of causal mutations" – not sure what this means.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genetics PKD feline diseases

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 18 Oct 2023

Soontaree Petchdee

Reviewer1:
The authors present a dataset including 108 cats in which 21 had the variant for PKD1. The dataset is important however the authors need to do some different presentations that would be of more significant value to the veterinary and PKD research communities. Hence. "partly" for the interpretation of data. Partly for data presentation as all US images are not available.
The authors should note 87 cats had no cysts at all. What is the frequency of singular cysts in humans, I think this supports cats rarely have singular cysts, or at least less commonly than humans.
Answer to the reviewer: We apologize for this point. We have edited this point in the revised manuscript in the discussion section.
Of the total 108 cats examined by ultrasound, no cyst was found in 87 cats of the wild-type group. However,19 cats were confirmed to have renal cysts, and in every cat with cysts found, a *PKD1* mutation was presented.

18 Persian cats, 49 Persian-related breed cats, 22 domestic shorthair cats, and 19 Bengal cats – need a table with age at US for each cat – the figures are less useful. Produce a summary table that summarizes groups, per breed, the cats without the PKD1 variant and then provide each breed cat that had PKD individually.
Which cat had PKD1 variant but no cysts – what was the cat's age? Mention this in the text.
Answer to the reviewer: We appreciate the helpful comment. We have edited this point in the revised manuscript in Table 3 and mentioned more information in the results part. We

apologize for this point.
However, there was a 51-month-old domestic shorthair cat without the *PKD1* gene mutation developing the cysts in renal parenchyma. Table 3 demonstrates the number of cats in various breeds with *PKD1* gene mutation and renal cyst development.

Scottish Folds are popular in eastern countries, however, the rate of PKD is high – contrast to other, recent population data suggesting PKD1 is a very low rate in Persians and other breeds.
Answer to the reviewer: We appreciate the helpful comment. We have edited this point in the revised manuscript in the discussion section.
This study found that the rate of *PKD1* gene mutation was high in Persian and Persian-related breed cats including Scottish Folds cats, Previous reports has been suggested that *PKD1* mutation was found at very low rates in Persians and other breeds which is contrary to our results. The high number of *PKD1* mutations that had been reported may be due to the Persian and Persian-related breed cats being a popular cat breed in eastern countries and Thailand.

BUN is high for both populations – age correlation is important – please comment.
Answer to the reviewer: We apologize for this point. We have edited this point in the revised manuscript in the results and discussion part.
As indicated in Table 2, the levels of BUN were mildly elevated than the normal reference value. However, Pearson's correlation results revealed that there was no correlation between age and BUN levels in WT (r=0.1436 *P* = 0.62) and *PKD1* mutation group (r=0.3234 *P* = 0.099).

PKD is one of the most common hereditary diseases – actually simple Mendelian diseases in cats. HCM may be more common but has a complex inheritance.
Answer to the reviewer: We apologize for this point. We have edited this point in the revised manuscript in the introduction part.
Feline polycystic kidney disease (PKD) is an inherited disorder that develops cysts in the renal parenchyma.

Was the hair clipped for the US evaluations or alcohol or gel applied for US?
Answer to the reviewer: We apologize for this point. We have edited this point in the revised manuscript in the materials and methods part.
In our ultrasonographic examination, all cats were clipped the hair in the abdominal area and the ultrasonographic gel was used in all cats.

Overall creatinine for overall population was 2.21 _ 0.19 – odd not intermediate – correct?
Answer to the reviewer: We apologize for this point. We have edited this point in the revised manuscript in the results part.
Additionally, the results of average creatinine levels in overall cats were elevated. Nonetheless, our results demonstrated that increasing creatinine exceeding 2.0 mg% may slightly increase the possibility for renal cyst formation by 1.41 times (95% CI, 0.3827 to 5.1764, *P* = 0.6070).

Which was most predictive of PKD – renal failure, number of cysts, age, BUN, SDMA? Any cats in renal failure?
Answer to the reviewer: We apologize for this point. We have edited this point in the revised manuscript in the results part. The enrolled cats in this study are represented with either renal failure or non-renal failure.
Therefore, cats with increasing SDMA, an age exceeding 36 months, body weight between 2-4 kg, and renal cyst formation from ultrasonographic examination are a high possibility of PKD1 gene mutation with PKD disorder.
According to the elevated BUN in our study, the enrolled cats in this study are represented with either renal failure or non-renal failure. The high levels of BUN and increasing age were not related in contrast with the prior study from humans at that age and BUN indicated a positive correlation.

Was left to right kidney size significantly different in PKD1 cats?
Answer to the reviewer: We apologize for this point. We have edited this point in the revised manuscript in the results part.
However, there was no statistically significant difference between right and left kidney size in cats affected with PKD1 mutation (*P*=0.9980).

Figure 4 would be better with biochemistries and cysts, kidney size, in affected cats versus normal. More figures like this, less pie charts.
Answer to the reviewer: We apologize for this point. We have edited this point in the revised manuscript and added Figure 4.

The results showed that age and weight were negatively related to the number of cysts? I think this is misstated in results.
Answer to the reviewer: We apologize for this point. We have edited this point in the revised manuscript in the discussion part. As in Figure 4, the results of matrix correlation show a negative correlation between the number of cysts and body weight (r=0.08) and age (r=0.44).
Nevertheless, our study indicated a negative correlation between the age and the number of cysts in recruited cats. Moreover, the cats in this study have a negative correlation between cyst number and body weight. This could potentially result from the manifestation of PKD symptoms in cats with *PKD1* mutation.

"The feline Polycystin-1 (PKD1) gene was examined for the point of 85% of causal mutations" – not sure what this means.
Answer to the reviewer: We apologize for this point. We have edited this point in the revised manuscript in the introduction part.
The polycystin-1 (*PKD1*) gene mutation was indicated for 85% of human autosomal dominant polycystic kidney disease (ADPKD). The mutation of the *PKD1* gene in cats carried heterozygous CA transversion at c.10063 in exon 29, resulting in impaired renal function.

Best regards
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