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Authors

Habacher, Gabriele

Malik, Richard

Lait, Philippa

et al.

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Feline precision medicine using whole-exome sequencing identifies a novel frameshift mutation for vitamin D-dependent rickets type 2

Gabriele Habacher[1], Richard Malik[2], Philippa JP Lait[3], Lyndon M Coghill[4], Rondo P Middleton[5], Wesley C Warren[6] and Leslie A Lyons[7]

Abstract

Objectives A 14-week-old female domestic longhair kitten presented with shifting lameness and disproportionately smaller size compared with a co-housed littermate.

Methods Hematology and serum biochemical testing were conducted to investigate causes for delayed growth, and radiographs of the appendicular skeleton were obtained.

Results The afflicted kitten had marked hypocalcemia, mild hypophosphatemia and substantial elevations in alkaline phosphatase activity, as well as pathognomonic radiographic findings consistent with rickets. Skeletal changes and hypocalcemia prompted testing of concentrations of parathyroid hormone (PTH) and vitamin D metabolites. Endocrine testing demonstrated significant increases in serum concentrations of PTH and 1,25-dihydroxycholecalciferol (calcitriol), supporting a diagnosis of vitamin D-dependent rickets type 2. Provision of analgesia, supraphysiologic doses of calcitriol and calcium carbonate supplementation achieved normalization of the serum calcium concentration and restoration of normal growth, although some skeletal abnormalities persisted. Once skeletally mature, ongoing calcitriol supplementation was not required. Whole-exome sequencing (WES) was conducted to identify the underlying DNA variant. A cytosine deletion at cat chromosome position B4:76777621 in *VDR* (ENSFCAT00000029466:c.106delC) was identified and predicted to cause a stop codon in exon 2 (p.Arg36Glufs*18), disrupting >90% of the receptor. The variant was unique and homozygous in this patient and absent in the sibling and approximately 400 other cats for which whole-genome and whole-exome data were available.

Conclusions and relevance A unique, heritable form of rickets was diagnosed in a domestic longhair cat. WES identified a novel frameshift mutation affecting the gene coding for the vitamin D3 receptor, determining the likely causal genetic variant. Precision medicine techniques, including whole-exome and whole-genome sequencing, can be a standard of care in cats to identify disease etiologies, and to target therapeutics and personalize treatment.

Keywords: Animal models; *Felis catus*; whole-exome sequencing; rickets; precision medicine

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[1]Raddenstiles Veterinary Surgery, CVS UK Ltd, Exmouth, UK
[2]Centre for Veterinary Education, The University of Sydney, Sydney, NSW, Australia
[3]Langford Vets, University of Bristol, Langford, Bristol, UK
[4]Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA
[5]Nestlé Purina Research, St Louis, MO, USA
[6]Division of Animal Sciences, College of Agriculture, Department of Surgery, School of Medicine, Institute for Data Science and Informatics, University of Missouri, Columbia, MO, USA
[7]Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO, USA

Corresponding author:
Leslie A Lyons PhD, Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, E109 Vet Med Building, 1520 E Rollins Street, Columbia, MO 65211, USA
Email: lyonsla@missouri.edu

Introduction

Rickets is a metabolic bone disorder resulting from inadequate serum concentrations of active vitamin D (calcitriol) or inadequate vitamin D receptor functionality, leading to failure of osteoid in the growth plate to mineralize, with subsequent growth abnormalities in mammals with an actively growing skeleton. Hypocalcemia causing defective endochondral ossification results from inadequate dietary intake of calcium, phosphorus and vitamin D (cholecalciferol), or genetic disorders of calcitriol biosynthesis or the structure of vitamin D receptors (VDRs) present on target organs. In normal cats, calcium homeostasis is stringently controlled within a narrow physiological range due to the interaction between parathyroid hormone (PTH), vitamin D metabolites and calcitonin, owing to the importance of normal calcium and phosphate concentrations to many electrophysiologic functions and enzymatic pathways.[1,2]

In contrast to humans and most mammals, which can synthesize vitamin D in the skin via ultraviolet light exposure, cats and dogs are exclusively dependent on the dietary intake of vitamin D prohormones, with all three species requiring a subsequent two-step process for activation.[3] As a fat-soluble vitamin, vitamin D is absorbed from the intestines and transported via vitamin D binding protein to the liver, where vitamin D hydroxylase (cytochrome P450 2R, *CYP2RI*) catalyzes the hydroxylation of vitamin D (cholecalciferol) to 25-hydroxycholecalciferol (calcidiol). A second hydroxylation step, under the control of PTH, proceeds in the proximal convoluted tubule of the kidney, catalyzed by 1-α-hydroxylase (cytochrome P450, subfamily 27, polypeptide 1, *CYP27B1*), converting calcidiol into the hormonally active form of vitamin D3 (1,25-dihydroxycholecalciferol; calcitriol). Fibroblast growth factor and a negative feedback loop further influence the activity of 1-α-hydroxylase.[4]

Calcitriol exerts its principal effects on the intestines, kidneys and bones, with the aim of increasing calcium and phosphate concentrations in serum. VDRs have also been detected in other tissues in dogs, prompting investigations into the role of vitamin D3 in non-skeletal health.[5] In the healthy gut, calcitriol binds and activates the nuclear VDR within enterocytes, promoting the expression of genes that encode calcium-binding protein, thus stimulating the active transport of calcium and phosphate across the intestinal mucosa and into the serum. In the kidneys, calcitriol increases calcium and phosphate tubular resorption, thus reducing calcium loss in urine. In the bones, calcitriol increases calcium and phosphorus resorption via direct and indirect effects on osteoclasts and PTH in times of hypocalcemia, which is counterbalanced by its negative feedback action on calcitonin. The pathologic effects of hypocalcemia are particularly detrimental during periods of increased demand, specifically during growth, when hypocalcemia and hypophosphatemia prevent normal mineralization of osteoid, leading to osteopenia and additional skeletal changes consistent with rickets.[1,2]

In cats, rickets can be classified further into both environmental (acquired) and heritable forms, including nutritional rickets, caused by dietary vitamin D3 deficiency combined with phosphorus/calcium imbalance (low calcium and high phosphorus) and accompanied by secondary nutritional hyperparathyroidism; and congenital vitamin D disorders (ie, vitamin D-dependent rickets [VDDR]). There are four subtypes of VDDR: (1) VDDR type 1A (*CYP27B1*; OMIA000837-9685) has been identified in cats and is an autosomal recessive trait caused by disruption of the second hydroxylation step (in the kidney) leading to impaired production of calcitriol;[6,7] (2) VDDR type 1B (*CYP2R1*; OMIA002221-9685) has been identified in cats and is an autosomal recessive trait caused by interference with the first hydroxylation step (in the liver);[8] (3) VDDR type 2 is caused by a *VDR* gene defect leading to a perturbed response of target organs to calcitriol, also known as hereditary VDDR;[2] and (4) VDDR non-type 1, non-type 2.[9]

Differentiation of VDDR type 1 and VDDR type 2 requires analysis of vitamin D metabolites to pinpoint the defect, with low calcitriol concentrations consistent with VDDR type 1 and high concentrations consistent with VDDR type 2. While some genetic defects have been identified in the cat for VDDR types 1A[6] and 1B,[8] so far none has been characterized for VDDR type 2.

Materials and methods

Case description

A 14-week-old female domestic longhair kitten presented for shifting lameness and reluctance to jump. The kitten was substantially smaller (approximately 50% the size) than its male littermate. Both siblings had been in the owner's possession since they were 8 weeks of age and were fed a nutritionally complete commercial diet, and were up to date with preventive parasite control and vaccinations. Despite good appetite, the discrepancy in size between the littermates became more apparent over the following weeks (Figures 1 and 2).

The kitten was bright, alert and responsive. Although small for its age, its growth was in normal proportion. Shifting lameness was present, with overall normal posture and ambulation. A reluctance to initiate movement suggested discomfort. Physical examination identified potential discomfort and palpable enlargement of the distal epiphyses of the long bones. Flexion and extension of the lumbosacral junction was resented.

A fasted blood sample was obtained for hematology and serum biochemical testing (Axiom Veterinary Laboratories). A substantial elevation in alkaline phosphatase (ALP) activity above the age-appropriate reference interval (RI) was noted.[10,11] While only mild ionized hypocalcemia and mild hypophosphatemia were evident

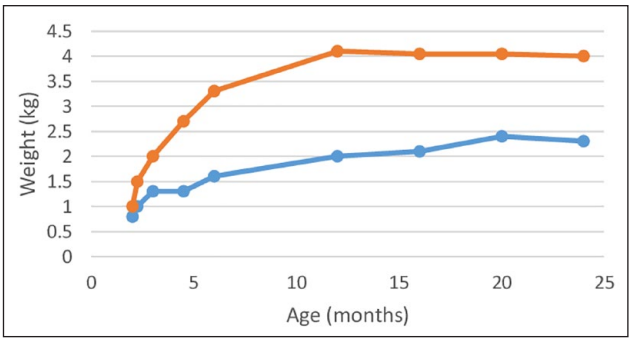

Figure 1 Growth chart of two domestic longhair kitten littermates. The orange line represents the male sibling demonstrating normal growth, plateauing at approximately 10 months of age. The blue line represents the female kitten (case), reaching maximum weight more than 2 months later than its normal sibling and approximately 50% of the male sibling's weight

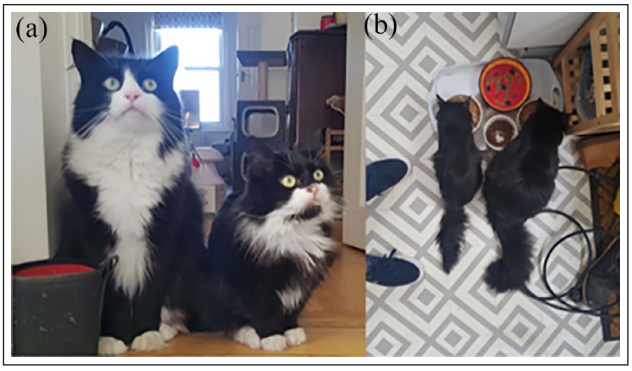

Figure 2 Images of two domestic longhair kitten littermates (a) view face on and (b) view from above. Normal male littermate on the left (a) and right (b) and affected female littermate at 20 months of age. The affected female littermate was markedly smaller in size and weight

when using an adult cat RI, interpretation in the light of established RIs for 4–6-month-old kittens[10] and a recent study on mineral metabolism in growing cats[11] confirmed the presence of marked ionized hypocalcemia, total hypocalcemia and mild hypophosphatemia (Table 1), with the remaining results, including total thyroxine and thyroid-stimulating hormone concentrations, being unremarkable.

An initial conscious lateral radiograph of the limbs identified reduced bone density, thin cortices and wide irregular growth plates with bulbous widening of the distal epiphyses of the long bones. The changes were most evident in the distal radius and ulna, the proximal humerus, the distal femur and the proximal tibia (Figure 3). Based on history, physical examination and imaging findings, a provisional diagnosis of rickets was made. Further endocrine testing to assess PTH (NationWide Specialist Laboratories) and vitamin D metabolite concentrations (Michigan State University, USA) demonstrated a substantially increased PTH concentration, indicative of secondary hyperparathyroidism (Table 2). Low ionized calcium concentration excluded primary hyperparathyroidism (Table 2). Secondary renal hyperparathyroidism and secondary nutritional hyperparathyroidism were both unlikely considering renal analytes were unremarkable and based on the normal growth of the littermate (fed the same 'balanced' nutritionally complete diet). Elevated PTH concentration resulting in decreased tubular phosphorus reabsorption explained the hypophosphatemia. Vitamin D analytes (measured by radioimmunoassay) further indicated adequate intake and intestinal absorption of vitamin D, with substantially increased concentrations of 1,25-dihydroxycholecalciferol (calcitriol), which, in combination with hypocalcemia, secondary hyperparathyroidism and consistent clinical signs, supported a diagnosis of VDDR type 2. Low concentrations of 25-hydroxycholecalciferol (calcidiol) were attributed to the increased conversion of calcidiol to calcitriol, and the subsequent calcitriol induced an increase of 24-hydroxylase promoting conversion of calcidiol into an inactive vitamin D metabolite for storage.[12]

Therapy and clinical course

Initially, analgesia was provided with the partially cyclooxygenase-2 selective non-steroidal anti-inflammatory drug meloxicam (0.05 mg/kg PO q24h; Metacam; Boehringer Ingelheim) while clinical results were pending. Based on treatment recommendations derived from

Table 1 Serum biochemistry of the affected kitten with rickets prior to therapy

Analyte	Patient	Reference interval		
		Adult	Kitten*	Kitten†
Phosphate (mmol/l)	2.19‡	0.70–2.10	2.7–3.2	2.45–2.55
Total calcium (mmol/l)	1.58‡	1.60–3.00	2.48–2.71	2.45–2.54
Alkaline phosphatase (IU/l)	706‡	11–67	92–137	

*Reference interval: Tomsa et al[10] data from seven healthy control kittens 4–6 months old
†Mineral parameters in growing kittens according to data from Pineda et al[11] from 14 healthy kittens 3–6 months old
‡Abnormal results

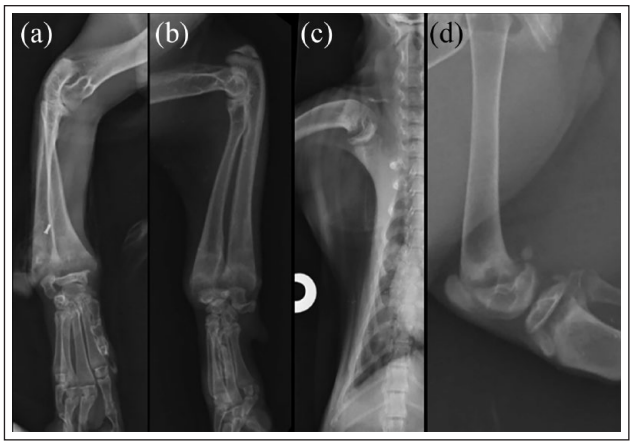

Figure 3 Representative radiographs of the affected kitten's limbs. Note the diffuse osteopenia, the mushroomed epiphyseal regions and the wide, irregular growth plates that are highly characteristic of rickets. (a) Craniocaudal view of forelimb; (b) lateral view of forelimb; (c) view of shoulder; (d) lateral view of stifle

human and feline cases,[13–16] calcium carbonate and vitamin D3 (ColloCal D 0.4 ml PO q12h; calcium carbonate 0.13 mg/ml and vitamin D3 70 IU/ml) and calcitriol (0.25 µg q24h PO for 2 weeks, then q12h from 10 weeks; Rocaltrol Roche) were administered, with doses titrated according to the ionized calcium concentration in plasma. Total and ionized calcium concentrations had normalized 15 months after commencing therapy and, accordingly, the dose was reduced. Treatment was discontinued after 24 months, as mild, persistent hypercalcemia suggested a substantial reduction in vitamin D requirements with skeletal maturity. The serum ionized calcium concentration has remained stable subsequently, and within the normal RI (Table 2).

Radiographs were repeated after 15 months of treatment and showed normal bony mineralization, with complete closure of growth plates and compensatory remodeling (Figure 4). Once fully grown, meloxicam was administered only intermittently to provide analgesia in relation to bone- and joint-related discomfort.

Genomic analyses

Genomic DNA from EDTA-anticoagulated whole blood of the affected kitten was isolated using a Chemagic 360 automated platform and blood nucleic acid extraction kit (Perkin-Elmer) and quantified by spectrophotometry. Approximately 2.9 µg genomic DNA was submitted to the McDonnell Genome Institute of the Washington University at St Louis for whole-exome sequencing (WES) and archived in accordance with the University of Missouri Institutional Animal Care and Use Committee study protocols 9056, 9178 and 9642.

WES was conducted as previously described using the domestic cat exome capture array (Roche Sequencing and Life Sciences) for hybrid-capture target enrichment

Table 2 Endocrine testing of calcium metabolism in a kitten with vitamin dependent-D rickets type 2

Time of diagnosis (T0) and treatment (weeks)	Kitten RI*	Kitten RI†	Adult RI	T0	T2	T10	T69	T88	T103	T108	T181	T195	T215
Age (weeks)				22	26	34	93	111	126	131	204	218	238
Weight (kg)				1.55	1.65	1.85	2.4	2.32	2.34	2.32	2.54	2.36	2.51
Calcium total (mmol/l)	2.48–2.71	2.45–2.54	1.60–3.00	1.64‡	1.73‡	NA	NA	NA	NA	NA	3.09	NA	2.72
Calcium ionized (mmol/l)	1.20–1.35	1.3–1.34	1.00–1.40	0.89‡	0.96‡	0.95‡	1.23	1.51‡	1.43‡	1.72‡	1.63‡	1.5‡	1.34
Phosphate (mmol/l)	2.70–3.20	2.45–2.55	0.7–2.10	2.19‡	NA	1.68	NA	NA	1.73	NA	1.36	NA	1.96
Calcidiol (nmol/l)	126–163	90–127	127–335	59‡	NA	NA	NA	NA	483‡	NA	NA	NA	NA
Calcitriol (pmol/l)	190–317	440–496	90–342	736‡	NA	NA	NA	NA	590‡	NA	NA	NA	NA
PTH (pg/ml)	3–28	10	<40	312‡	NA	NA	NA	NA	<10	NA	NA	NA	NA
ALP (IU/l)	92–137	NA	11–67	NA	NA	342‡	51	NA	30	NA	NA	NA	NA
Supplementation of calcitrol	0.25 µg	NA	NA	q24h	q24h	q12h	q24h	q48h	q7d	Discontinued			
Supplementation of calcium carbonate and calcitriol	0.052 mg and 28 IU	NA	NA	q12h	q12h	q12h	Discontinued						

*Reference interval (RI): Tomsa et al[10] data from seven healthy control kittens 4–6 months old
†Mineral measurements in growing kittens: Pineda et al[11] data from 14 healthy kittens 3–6 months old
‡Abnormal results
NA = not available; PTH = parathyroid hormone; ALP = alkaline phosphatase

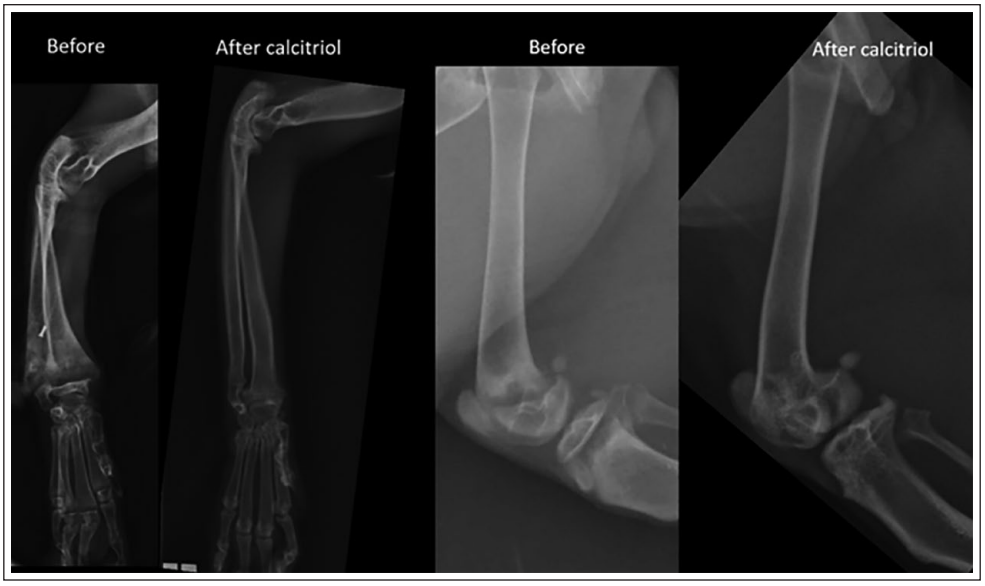

Figure 4 Radiographs of portions of the thoracic and pelvic limbs in a kitten with vitamin D-dependent rickets type 2 before and after calcitriol therapy. Note how the growth plates have 'filled in' with mineralized osteoid after months of calcitriol, although some bending deformity persists

of the domestic cat exons, which targeted 35.9 Mb, and sequence reads were produced using Illumina sequencing technology.[17] Processing of the sequencing data using the *Felis catus* genome assembly V9.0 followed Genome Analysis Toolkit best practices (https://software.broadinstitute.org/gatk/)[17] and Ensembl annotation.[18] The variant call file was combined with variants determined from 60 additional cats with WES data. The variant data were visualized and filtered using VarSeq software (GoldenHelix). The candidate variants were identified by assuming the affected cat was homozygous for a causal variant and no other cat in the dataset would have the variant present (ie, a unique homozygous variant). Single nucleotide variants were annotated as having high, moderate or low impacts on gene function depending on alteration to the predicted protein sequence.[19] The allele frequencies of the identified variants were also examined in the 99 Lives dataset that includes whole-genome sequence data from approximately 340 cats, also using VarSeq software (Golden Helix).

The identified candidate variant in *VDR* was validated in the affected cat and genotyped in the normal male sibling (DNA from buccal swab) by direct Sanger sequencing. PCR primers were designed using Primer3 (https://primer3.org) to flank the *VDR* variant (B4:76777621: ENSFCAT00000029466:c.106delC) (forward primer: 5′- GCCAAAGAAAATCCTGCTCAG-3′; reverse primer: 5′- TTACTCCCGGTGGCTGTAAG-3′) using cat sequence ENSFCAT00000029466 for *VDR*. The amplicon size was 429 base pairs (B4:76777405–76777833). PCR was conducted in a 25 µl reaction, using 1 U Choice-Taq DNA polymerase (Denville Scientific), 1× PCR buffer (includes 1.5 mM $MgCl_2$), 0.2 mM each nucleotide, 0.4 µM each primer and 20 ng genomic DNA. PCR product (5 µl) was visualized on 1.25% agarose gel via electrophoresis at 90 V for 90 mins using ethidium bromide staining. The amplicon was isolated using a QIAquick PCR Purification Kit (Qiagen) and eluted in 35 µl water, then submitted for direct Sanger sequencing on an ABI 3730XL instrument (Applied Biosystems) at the University of Missouri Genomics Technology Core.

The WES data set used for analysis is under the 99 Lives Consortium (Bioproject: PRJNA308208), including 50 published genomes (Bioproject: PRJNA627536) and WES from 10 additional cats (Bioproject: PRJNA844099),[20] presumably all domestic cats unaffected by rickets. The case sample is accession number SAMN33272255. The depth of exon coverage for the affected cat averaged approximately 60–70×. Using Ensembl 101 annotation, the affected cat was homozygous for 546 variants, of which 40 were unique within the WES data set (see Supplementary File 1). Eight loss-of-function (LoF) variants were homozygous within the case (Table 3; see also Supplementary File 1) and two were unique to the affected cat. A LoF variant caused by a frameshift was identified in *VDR*, a gene known to be affected in cases of rickets in other species. The variant had 65× read coverage and had a high-quality score of 99. The position of the variant is at B4:76777621 in cat genome assembly V9.0,[21] and is defined as a deletion of a cytosine (ENSFCAT00000029466:c.106delC; XM_019834851:c.106delC) and is expected to cause a disruption at exon 2, affecting >90% of all defined transcripts. The *VDR* variant was unique and homozygous, and it is predicted to cause a stop codon 18 amino acids

Table 3 Loss-of-function variants homozygous to the affected cat in exome sequence data

Gene acronym	Gene name	Sequence ontology	Heterozygous cats (n = 61)
CCDC105	*Coiled-Coil Domain Containing 105*	Stop gained	7
CFDP1	*Craniofacial development protein 1*	Frameshift	11
ENSFCAG00000029687	Unknown transcript	Frameshift	2
ENSFCAG00000030093	Unknown transcript	Initiator codon	0
ESPN	*Espin*	Frameshift	3
SIK3	*SIK family kinase 3*	Frameshift	3
TSNARE1	*T-SNARE Domain Containing 1*	Splice acceptor	6
VDR	*Vitamin D receptor*	Frameshift	0

downstream of the cytosine deletion (p.Arg36Glufs*18). The variant was confirmed as homozygous in the case by direct Sanger sequencing, although the variant was not present in the normal male sibling. This variant was not identified in the 99 Lives WGS dataset that included 340 cats, none of which had clinical signs of rickets (see Supplementary File 1).[22]

Discussion

Congenital vitamin D disorders are rare in companion animals, and this is the first report to identify a genetic variant predicted to be causal for VDDR type 2A in a kitten. The genetic basis for congenital rickets type 2A has been reported in a single Pomeranian dog,[23] and a dysfunctional VDR in skin fibroblasts was confirmed experimentally.[16,24] In this feline patient, the presumptive diagnosis was made based on characteristic clinical, laboratory and radiologic findings. The diagnosis was confirmed following endocrine testing, and further substantiated with molecular testing. Nutritional deficiencies were considered unlikely considering the balanced diet, and the normal growth of its littermate, and definitively excluded by vitamin D assays.

In this kitten, the novel frameshift mutation encoding the vitamin D3 receptor resulted in a dysfunctional receptor (VDR). In the absence of supraphysiologic concentrations of calcitriol, the defective VDR likely led to reduced calcium and inorganic phosphate absorption from the gut, with impaired skeletal development resulting in growth retardation accompanied by characteristic clinical signs of rickets including bowing of limbs, widened irregular cup-shaped growth plates and diffuse osteopenia. Other clinical signs of hypocalcemia such as constipation, pathologic fractures, tremors,[14] seizures and alopecia[2] were not observed in the patient in this report.

Extrapolating from humans and a small number of feline cases, treatment of VDDR type 2 is based on the administration of supraphysiologic doses of calcitriol.[13–16] The varying degrees of success of such therapy in human patients suggest the presence of a diverse range of LoF mutations with a spectrum of receptor dysfunction.[13] Stated another way, a response to pharmacologic doses of calcitriol hinges upon the VDR having at least some residual function.

Response to calcitriol supplementation was slow, and despite establishing normocalcemia, the kitten's development was limited, and some residual bowing of long bones persisted. The kitten remained of small stature, with normal mineralization of bones and closure of growth plates as documented by follow-up radiographs at 3 years of age. Normocalcemia was first documented at 19 months of age, much later than optimal, and yet calcitriol therapy appeared sufficient to establish physiologic concentrations of ionized calcium during growth sufficient to mineralize osteoid at the physes.

At some point in time between 19 and 24 months of age, the kitten's vitamin D requirement likely dropped, as the previously administered doses of calcitriol resulted in mild total and ionized hypercalcemia. With the benefit of hindsight, tapering of calcitriol dosing was probably undertaken too slowly, with mild hypercalcemia persisting for longer than ideal. Monitoring was suboptimal because of the COVID-19 pandemic, but normocalcemia was eventually achieved and maintained. Interestingly, once skeletally mature, the cat appeared to have no ongoing requirement for vitamin D supplementation. In humans, increased calcium absorption from the gut and decreasing requirements for skeletal growth, when combined with mild VDR receptor dysfunction, results in a net zero requirement for supplementation. In contradistinction, among those human patients with alopecia, about half fail to respond adequately to even very high doses of calcitriol.[13]

Long-term outcomes for VDDR type 2 in human patients are variable, with some patients suffering with alopecia, osteoarthritic changes and poor quality of life.[13] If the VDR variant resulted in dysfunctional receptor function but partial response to calcitriol supplementation, then early diagnosis and rapid instigation of therapy, such as in the present case, may prevent negative outcomes such as pathologic fractures or spinal deformities. Variable long-term outcomes likely reflect either

discrepancies in the timing of the diagnosis or potentially the presence of other, more severe variants with less residual receptor function.

In humans, VDDR type 2A[25] has causal variants in *VDR*.[26] The gene has 11 exons spanning approximately 75 Kb, including exons 1A, 1B and 1C, and eight additional coding exons 2–9.[27] Three unique mRNA isoforms are produced due to the differential splicing of exons 1B and 1C. Exons 2 and 3 of *VDR* are involved in DNA binding, while exons 7, 8 and 9 are involved in binding to vitamin D.[28] In the human ClinVar database, 228 single-gene variants in *VDR* have been catalogued, including 17 suspected as pathogenic variants.[29–31]

A variant (NM_000376.3 [VDR]:c.88C>T [p.Arg30Ter]) has been defined in humans, which is similar to the *VDR* cat case (XM_019834851:c.106delC; p.Arg36Glufs*18).[30] The identified homozygous 88C–T transition variant in exon 2 of human *VDR* results in an arg30-to-ter (R30X) substitution in the first zinc finger of the DNA-binding domain, truncating the VDR by 397 residues. The subject, a 12-year-old Brazilian boy born to first-cousin parents, had early-onset rickets, total alopecia, convulsions, hypocalcemia, secondary hyperparathyroidism and elevated 1,25-dihydroxyvitamin D3 (calcitriol) concentrations in serum. As previously noted in humans, pathogenic nonsense and missense mutations located in different domains of VDR have been identified in patients with similar clinical and biochemical features of hereditary VDDR. Interestingly, 6 of 15 hereditary VDDR variants involve the amino acid arginine (CGA).[32,33] The apparent trend for deleterious mutations at arginine residues in VDR is considered a consequence of deamination of methylcytosine or cytosine at CpG in genomic DNA, resulting in a 'mutational hotspot'.[33] The cat variant reported herein also occurs at an exon 2 arginine, supporting the hotspot hypothesis.

Conclusions

VDDR type 2 is a rare condition, often with a grave prognosis. We identified a unique, heritable form of congenital rickets in a domestic longhair kitten. Precision medicine techniques identified a novel frameshift mutation significantly impacting the function of the VDR. Contrary to previous reports in the feline literature, supraphysiologic calcitriol dosing was successful in achieving normocalcemia and permitted satisfactory mineralization of osteoid in growth plates of long bones, while at maturity supplementation with calcitriol was no longer required.

Identification of clinical cases with this VDDR type 2 receptor defect may therefore encourage owners and veterinarians to attempt treatment in some cases with a favorable long-term prognosis. Furthermore, precision medicine techniques should be considered as a diagnostic standard of care in cats to delineate inborn errors from environmental or other genetic causes of rickets in cats, such as *CYP21B1* variants. Regional shelters should be monitored as the obligate carrier parents and other carriers in the regional population could produce more afflicted kittens.

Acknowledgements We appreciate the laboratory assistance of Thomas R Juba, MS and Juan A Valencia, and the 99 Lives Cat Genome Consortium for sharing domestic cat variant frequency information. Figure 2 is used with the permission of the owner.

Supplementary material The following file is available online:
Supplementary File 1: Whole-exome sequencing and whole-genome sequencing data sets.

Conflict of interest Rondo P Middleton is an employee of Nestlé Purina.

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Ethical approval The work described in this manuscript involved the use of non-experimental (owned or unowned) animals. Established internationally recognised high standards ('best practice') of veterinary clinical care for the individual patient were always followed and/or this work involved the use of cadavers. Ethical approval from a committee was therefore not specifically required for publication in *JFMS*. Although not required, where ethical approval was still obtained, it is stated in the manuscript.

Informed consent Informed consent (verbal or written) was obtained from the owner or legal custodian of all animal(s) described in this work (experimental or non-experimental animals, including cadavers) for all procedure(s) undertaken (prospective or retrospective studies). No animals or people are identifiable within this publication, and therefore additional informed consent for publication was not required.

ORCID iD Rondo P Middleton https://orcid.org/0000-0001-5124-6703
Leslie A Lyons https://orcid.org/0000-0002-1628-7726

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