

Case Report

J Vet Intern Med 2017;31:539–544**Precision Medicine in Cats: Novel Niemann-Pick Type C1 Diagnosed by Whole-Genome Sequencing**

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State-of-the-art health care includes genome sequencing of the patient to identify genetic variants that contribute to either the cause of their malady or variants that can be targeted to improve treatment. The goal was to introduce state-of-the-art health care to cats using genomics and a precision medicine approach. To test the feasibility of a precision medicine approach in domestic cats, a single cat that presented to the University of Missouri, Veterinary Health Center with an undiagnosed neurologic disease was whole-genome sequenced. The DNA variants from the cat were compared to the DNA variant database produced by the 99 Lives Cat Genome Sequencing Consortium. Approximately 25× genomic coverage was produced for the cat. A predicted p.H441P missense mutation was identified in *NPC1*, the gene causing Niemann-Pick type C1 on cat chromosome D3.47456793 caused by an adenine-to-cytosine transversion, c.1322A>C. The cat was homozygous for the variant. The variant was not identified in any other 73 domestic and 9 wild felids in the sequence database or 190 additionally genotyped cats of various breeds. The successful effort suggested precision medicine is feasible for cats and other undiagnosed cats may benefit from a genomic analysis approach. The 99 Lives DNA variant database was sufficient but would benefit from additional cat sequences. Other cats with the mutation may be identified and could be introduced as a new biomedical model for *NPC1*. A genetic test could eliminate the disease variant from the population.

Key words: Feline; *Felis silvestris catus*; Lysosomal storage; *NPC1*; WGS.

The genetic and genomic resources available for health studies of the domestic cat are becoming

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Work: The cat was referred to the Veterinary Health Center at the University of Missouri, Columbia, Missouri, the genetic studies and analysis at the University of Missouri, Columbia, Missouri, the whole-genome sequencing was performed as fee-for-service at The McDonnell Genome Institute at Washington University in St. Louis, Missouri, and partial genome sequence analysis was conducted as fee-for-service at Maverix Biomics, Inc., San Mateo, California.

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Abbreviations:

ALP	alkaline phosphatase
ALT	alanine aminotransferase
bp	base pair
bpm	beats per minute
KIT	v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog
MRI	magnetic resonance imaging
<i>NPC1</i>	Niemann-Pick type C1
<i>NPC2</i>	Niemann-Pick type C2
<i>NPC</i>	Niemann-Pick type C
OMIA	Online Mendelian Inheritance in Animals
OMIM	Online Mendelian Inheritance in Man
PCR	polymerase chain reaction
qPCR	quantitative PCR
WGS	whole-genome sequencing

robust and cost efficient. The sequencing of a cat's entire genome can now be completed for about the cost of a magnetic resonance imaging (MRI) scan. In humans, rapid turnaround whole-genome sequencing such as the 26-hour genome efforts has demonstrated how genomic medicine can be applied to health management for acute care cats with time-critical morbidity and mortalities.^{1,2} Although the availability of the bioinformatics infrastructure and turnaround time are not yet as accessible in cats as for humans, the DNA variant database developed by the 99 Lives Cat Genome Sequencing Initiative has proven a valuable first step. Developed from a variety of cats comprising diverse populations and breeds, including those with and without known genetic health problems, the cat variant database supports the identification of DNA variants that are causal for health conditions suspected to have a genetic component. Two whole-genome sequencing (WGS) studies have already identified a

DNA variant in cats associated with progressive retinal atrophy in Persians, a variant causing the bobbed tail of the Japanese Bobtail breed,³ and one responsible for congenital myasthenic syndrome in Devon rex and Sphynx—related cats.⁴

Precision medicine is an emerging approach for disease diagnosis, treatment, and prevention that takes into account individual variability in genes, environment, and lifestyle. Most medical treatments have been developed for the “average patient”. *Precision medicine* gives clinicians tools to better understand the complex mechanisms underlying a cat’s health, disease, or condition, and to better predict which treatments will be most effective, or to determine polymorphisms requiring different drug dosages (pharmacokinetics).⁵ Overall, an individual’s specific genetic makeup will become an intricate part of their standard health care. This case report shows the potential to apply *precision medicine* to the diagnosis of neurologic disease in cats.

A 12-week-old silver tabby intact female cat of suspected American shorthair lineage presented for a new kitten examination. Abnormal physical examination findings included a pendulous abdomen, soft nonformed feces, and a shaky, unsteady, hypermetric gait causing the cat to fall over. A presumptive diagnosis of cerebellar hypoplasia was made. Two months later, she presented for ovariohysterectomy. During the presurgery examination, her neurologic signs had progressed and she was having difficulty ambulating. Although well hydrated, her weight gain was 0.05 kg in 4 weeks. Presurgery blood tests revealed hypoalbuminemia (1.7 g/dL, reference interval, 2.5–4.4 g/dL), elevated ALT (436 U/L, reference interval 10–118 U/L) and ALP activities (184 U/L, reference interval 20–150 U/L), hyperbilirubinemia (0.6 mg/dL, reference interval 0.1–0.6 mg/dL), and hyperphosphatemia (7.0 mg/dL, reference interval 2.9–6.6 mg/dL). Abdominal radiographs and ultrasonography showed generalized hepatomegaly and splenomegaly. The hepatic parenchyma was hypoechoic and homogenous. The hepatic vasculature was within normal limits. The gall bladder was mildly distended with anechoic bile. Although enlarged, the spleen was homogenous in echogenicity. The remainder of the abdominal ultrasound examination was unremarkable. Postprandial bile acids were elevated (42.1 μ mol/L, reference interval <20 μ mol/L). The surgery was canceled and referral recommended for further evaluation.

The kitten was referred to the University of Missouri, Veterinary Health Center at 24 weeks of age for evaluation of concurrent hepatic and neurologic disease. On presentation, she was alert, responsive, well hydrated, and weighed 2.0 kg. Abnormalities noted included bradycardia (120 bpm), hepatomegaly, and a distended bladder. Additional neurologic abnormalities included inability to walk without support but with good motor function and hypermetria in all 4 limbs. Palpebral reflexes were normal and the cat was visual, but menace responses were absent. She also had a horizontal nystagmus with the fast phase to the right.

A serum biochemical profile showed hyperphosphatemia (5.6 mg/dL, reference interval, 2–5.3 mg/dL)

consistent with a growing kitten; elevated ALT (326 U/L, reference interval 18–77 U/L); and ALP (159 U/L, reference interval 5–55 U/L) activities and total bilirubin (0.6 mg/dL, reference interval 0–0.3 mg/dL) most consistent with hepatocellular injury, cholestasis, and mild hepatic dysfunction. Serum creatine kinase was within the reference interval.

Based on the neurologic assessment, the lesion was localized to the cerebellum. Differential diagnoses included infectious disorders, such as toxoplasmosis, bartonellosis, feline infectious peritonitis encephalitis and others, a progressive developmental defect, and storage disorders. Although cerebellar hypoplasia caused by an intrauterine infection with feline parvovirus can produce cerebellar signs, the severity of signs seen in this cat would make that diagnosis very unlikely. Normally cats with cerebellar hypoplasia are ambulatory, and the disease does not progress. A storage disorder was considered the most likely diagnosis because of the combination of progressive hepatic and neurologic abnormalities, especially given there was hepatomegaly. Diagnosis of a storage disease would require a biopsy, for example, of the liver, which the owner declined.

When evaluated at 30 weeks of age, the cat was eating and drinking but had continued to lose weight. She was nonambulatory, but remained continent if carried to her litter box. She continued to decline and was euthanized at 38 weeks of age. Permission for necropsy was denied.

Methods

The remaining blood clots from the serum biochemical profile were processed for DNA isolation⁶ to aid diagnosis and to test the concept of *precision medicine* for cats, with owner informed consent and in accordance with an approved University of Missouri institutional Animal Care and Use Committee protocol (ACUC protocol # 8240). The DNA was submitted for whole-genome sequencing to the McDonnell Genome Institute at Washington University. The sample was assessed for quantity, and 1000 ng aliquots were distributed for library construction. A TruSeq PCR-free library (Illumina, San Diego, CA) was constructed with an average fragment size of 350–450 bp. Size selection was accomplished through a paramagnetic bead cleanup postfragmentation. The average insert size was expected to be approximately 350 bp. The concentration of the library was determined by qPCR according to manufacturer’s protocols (Kapa Biosystems, Inc., Woburn, MA) to optimize cluster counts for the Illumina HiSeq X (Illumina Inc, San Diego, CA). Library adapters were designed with 8-bp index sequences allowing for demultiplexing of samples after the sequencing process. Nine libraries from other cat samples from the 99 Lives project (<http://felinegenetics.missouri.edu/99lives>)^{3,4} were pooled together in equal molar ratios, based on the concentration determined by qPCR and run across a HiSeq X flow cell to generate ~1 Tb of data as 2 × 150 bp paired-end reads. This efficient pooling

scheme targets ~30× coverage for each sample. The Illumina HiSeq X Ten was used to generate sequences. Approximately 25× genomic coverage was generated for the case cat. Generated sequences were processed, and the variants were analyzed with active filters provided by Maverix Biomics, Inc. (Santa Cruz, CA) as previously described^{3,4} via comparison to the domestic cat reference genome⁷ and the genomes of 74 domestic cats and 9 wild felids of the 99 Lives Cat Genome Sequencing Initiative. Genes known to be involved with storage diseases were inspected for the presence of high and moderate impact variants. DNA variants were filtered by selecting only variants that were homozygous in the affected cat and not present in any of the other 73 domestic and 9 wild felid sequences in the 99 Lives sequence database.

Additional screening of the newly identified *NPC1* variant in random bred and breed cats has been performed with an MassArray system (Agena Bioscience, San Diego, CA) as previously described,³ and primers details are presented in Table S1.

Results

All data have been or will be submitted to the short-read archives in the United States and Europe upon publication. Current submitted sequences are under bioproject PRJNA308208 (<http://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA308208>). A predicted p.H441P missense mutation was identified in *NPC1*, the gene causing Niemann-Pick type C1 (NPC1) on cat chromosome D3.47456793 caused by an adenine-to-cytosine transversion, c.1322A>C. The cat of this report was homozygous for the variant, and no other cats in the dataset had the variant. The histidine at position 441 is highly conserved across species (Fig 1). PolyPhen-2 predicts the amino acid change to be benign with a damaging score of 0.43 (sensitivity: 0.89; specificity: 0.90).⁸ The *NPC1* variant is rare as it was not identified in 190 cats (Figure S1).

Discussion

Niemann-Pick type C (NPC) disease (NP-C; OMIM#257220 and OMIM#607625) is an autosomal recessive neurovisceral lysosomal storage disorder that results in defective intracellular transport of cholesterol. Greater than 95% of all human NPC cases are because of variants in the *NPC1* gene mapped to chromosome 18q11,⁹ which is homologous to cat

chromosome D3.^{7,10} *NPC1* in cats was first described as a lipid disorder in a Siamese.^{11–15} A feline model of *NPC1* (OMIA 000725-9685) is characterized and is phenotypically, morphologically, and biochemically similar to human *NPC1*.^{16–22} Affected cats show cerebellar and vestibular signs beginning as early as 6 weeks of age. The ataxia progresses to the point where cats fell frequently at 11.8 ± 1.9 weeks of age and were unable to take 1 step without falling at 17 ± 2.1 weeks age. By 19 ± 3 weeks of age, they were unable to stand without assistance, and they were euthanized at 20.5 ± 4.8 weeks of age (range 11–29 weeks).²¹ The cat in this study was not evaluated before 12 weeks of age but had clear ataxia by 12 weeks of age with difficulty walking by 20 weeks of age. When examined at 24 weeks of age, the cat could not walk without assistance and she was euthanized at 38 weeks of age. Thus, the onset and progression of neurologic signs in this cat are comparable to those observe in the feline *NPC1* colony. The cat in this report survived longer before euthanasia but that likely reflects additional nursing care provided to a pet versus cats in a research colony and reluctance to euthanize on the part of the owner. Serum ALP and ALT activities were elevated, and albumin concentrations decreased in the cat in this report comparable to *NPC1* colony cats.²² Histopathology and biochemical studies would be necessary to confirm the diagnosis of *NPC1* and demonstrate an effect of the variant identified on function. Unfortunately, the owners declined permission for biopsy or necropsy. Thus, we cannot prove the diagnosis of *NPC1*, which is a limitation of this study.

Complementation studies using cultured fibroblasts from NPC-affected cats and *NPC1*-affected humans support that the gene responsible for the NPC phenotype in this colony of cats is orthologous to the gene responsible for the major form of human NPC, *NPC1*.²³ A single base substitution (c.2864G>C) is identified in these *NPC1*-affected cats and in silico predicts a cysteine to a serine change (p.C955S)²⁴ (GenBank # AF503633 and AF503634). The known cat *NPC1* variant is predicted to be probably damaging using PolyPhen-2 with a score of 0.981 (sensitivity: 0.75; specificity: 0.96).⁸ Heterozygous cats also have metabolic manifestations.¹⁹ The parents of the cat could not be obtained for evaluation. In addition, a published intronic c.82+5G>A mutation, which leads to a splicing error in another gene associated with NPC in humans

NPC1 HUMAN	QPYPGADVPF GP PLDIQIL <u>HQ</u> VLDLQIAI EN ITASYDNETVTLQDICLAPLSPYNTNCT
NPC1 MOUSE	EPYPAGADVPF GP PLNKEIL <u>HQ</u> VLDLQIAIESITASYNNETVTLQDICVAPLSPYKNCT
NPC1 PIG	HPYPAGADVPF GP PLSRDIL <u>HQ</u> VLDLQTAIENITASYNNETVTLQDICLAPLSPYKNCT
NPC1 BOVIN	EPYPGADVPF GP PLAVDIL <u>HQ</u> VLDLQTAIESITASYNNETVTLRDICVAPLSPYNQNT
NPC1 FALCA	QPYPGSDVPF GP PLDLAIL <u>HQ</u> VLDLQTAIEINITASYNNETVTLQDICVAPLSPYKNCT

Fig 1. Protein alignment of *NPC1* in cats and other species within critical region for the cat variant H441P. The mutation is within the luminal topological domain that includes amino acids 372 to 620. The conserved variant site for this case (p.H441P) is presented in bold italics and underlined. *NPC1* in *Felis catus* has two amino acids shorter compared to human. In humans, three known variants at codon positions 433, 434, and 451 are associated with a disease phenotype and are bold in the alignment.^{33–35}

(*NPC2*), has been identified in cats with similar presentation,²⁵ but no significant variants were identified in *NPC2* in the cat of this report. However, as presented in Orphanet (<http://www.orpha.net/consor/cgi-bin/index.php?lng=EN>), the mutations in *NPC1* occur in 95% of the families with NPC type disease and more than 230 mutations have been identified.

The identified *NPC1* variant in this cat is novel and predicts a nonconservative missense mutation, a histidine-to-proline substitution at position 441 of the *NPC1* feline protein. The histidine is highly conserved in several species, and prolines generally cause significant disruption to the normal protein structure. The newly identified variant is not known in human patients, but several proline changes and a terminal variant are within the region of codons 421–474 (<http://www.hgmd.cf.ac.uk/ac>).²⁶ Although the PolyPhen-2 prediction was suggested as a benign effect on the protein, other benign predictions are associated with phenotypic effects in cats, such as the *Gloving* variants associated with *KIT* that are common to Birmans.⁷ The *NPC1* variant is rare as it was not identified in 190 cats or the other 73 domestic cats and 9 wild felids in the 99 Lives sequence database. However, the population of origin of the affected cat could not be established for further screening of a specific breed.

Precision medicine by WGS has proven to be useful for diagnosis and rapid intervention and treatment for critically ill children.^{1,2} Although the treatments for *NPC1* are not ready for clinical trials in cats and would not have assisted the present cat, other undiagnosed diseases in cats could benefit from genomics techniques. A second *NPC1* biomedical model in cats could be established if other afflicted or carrier cats could be identified, which would further support the development of potential treatments for the disease. Currently, cyclodextrin and miglustat have shown improvement in Purkinje cell survival, thereby preventing cerebellar dysfunction in cats.^{27–30} Assuming the cat of this report is representative of a specific breed, its *NPC1* variant could be segregating in a breeding population and other homozygous recessive cats may be produced which will succumb to the same condition if a genetic test is not implemented to avoid matings between carriers.

To date, over 40 genes with approximately 70 DNA variants have been documented to cause phenotypic, disease, or blood type variations in the domestic cat (for review).³¹ The clinical descriptions and phenotypes of each of these diseases and traits have been curated at the Online Mendelian Inheritance in Animals (OMIA) website (<http://omia.angis.org.au/home/>), which is an invaluable resource comparison of the phenotypes across 2216 animal species.³² These known and any newly identified DNA variants can be genotyped rapidly and cost-effectively in panels appropriate for breeds, populations, or in cats as part of wellness care. The vigilance of veterinarians and their collaboration with geneticists could lead to the rapid discovery of undiagnosed genetic conditions in cats, which lead to more effective and proactive treatments and preventative strategies. Whole-genome sequencing of rare and

undiagnosed feline cases may be resolved using the precision medicine approach.

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Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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Appendix 1. 99 Lives Consortium (83 cat analysis)

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. Genotyping primer sequences for cat *NPCI*.

Figure S1. Cartesian plot of genotypes detected by mass spectrometry genotyping. Plot representing the genotyping results of 96 cats, only the affected Niemann-Pick type C cat (upward triangle) was homozygous for the identified variant (c.1322A>C). One sample (circle) was excluded for low genotype quality.