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CASE REPORT

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A novel missense variant in the *L2HGDH* gene in a cat with L-2-hydroxyglutaric aciduria and multicystic cerebral lesions

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Abstract

Case Description: A 9-month-old intact male domestic shorthair cat was evaluated for increasing frequency of generalized tonic-clonic seizures.

Clinical Findings: The cat was reported to have had episodes of circling between the seizures. Upon examination, the cat had bilateral inconsistent menace response but otherwise normal physical and neurological examinations.

Diagnostics: Magnetic resonance imaging (MRI) of the brain identified multifocal, small, rounded intra-axial lesions within the subcortical white matter containing fluid with similar characteristics as cerebrospinal fluid. Evaluation of urine organic acids showed increased excretion of 2-hydroxyglutaric acid. An XM_023255678.2: c.397C>T nonsense variant in the *L2HGDH* gene encoding L-2-hydroxyglutarate dehydrogenase was identified using whole genome sequencing.

Treatment and Outcome: Levetiracetam treatment was initiated at 20 mg/kg PO q8h, but the cat died after a seizure 10 days later.

Clinical Relevance: We report the second pathogenic gene variant in L-2-hydroxyglutaric aciduria in cats and describe for the first time multicystic cerebral lesions on MRI.

KEYWORDS

animal model, *Felis catus*, genetics, glutaric aciduria, neurology, precision medicine

1 | INTRODUCTION

L-2-hydroxyglutaric aciduria (L2HGA) is a rare neurometabolic disorder characterized by the presence of increased concentrations of L2-hydroxyglutaric acid in blood, urine and cerebrospinal fluid. It is caused by genetic variants affecting the *L2HGDH* gene encoding L-2-hydroxyglutarate dehydrogenase and has an autosomal recessive mode of inheritance.[1-7] Disease-causing variants have been reported in humans, dogs (Staffordshire bull terrier, Yorkshire terrier) and 1 cat.[1-5] The clinical signs and magnetic resonance imaging (MRI) findings in dogs and cats with L2HGA have been reported previously.[3-10] Our purpose was to describe a novel candidate disease-causing variant for L2HGA in a cat with previously unreported MRI findings and a fatal outcome.

Abbreviations: GATK, genome analysis toolkit; gVCF, genomic variant call format; L2HGDH, L-2-hydroxyglutarate dehydrogenase; mRNA, messenger ribonucleic acid; NCBI, National Center for Biotechnology Information; OMIA, Online Mendelian Inheritance in Animals; OMIM, Online Mendelian Inheritance in Man; PCR, polymerase chain reaction; WGS, whole genome sequencing.

Matthias Christen and Veronica Gonzalo-Nadal contributed equally to the manuscript.

2 | CASE DESCRIPTION

2.1 | Clinical presentation and diagnostic findings

A 9-month-old intact male domestic shorthair cat was evaluated for increasing frequency of generalized tonic-clonic seizures that had started 2 months earlier. The seizures were characterized by loss of consciousness, autonomic signs and tonic-clonic involuntary movements of all 4 limbs. They lasted 2-3 minutes and were accompanied by a post-ictal phase of variable duration characterized by ataxia and decreased mentation. A cluster of five seizures within 12 hours was reported 2 days before presentation. Just after this cluster, the cat

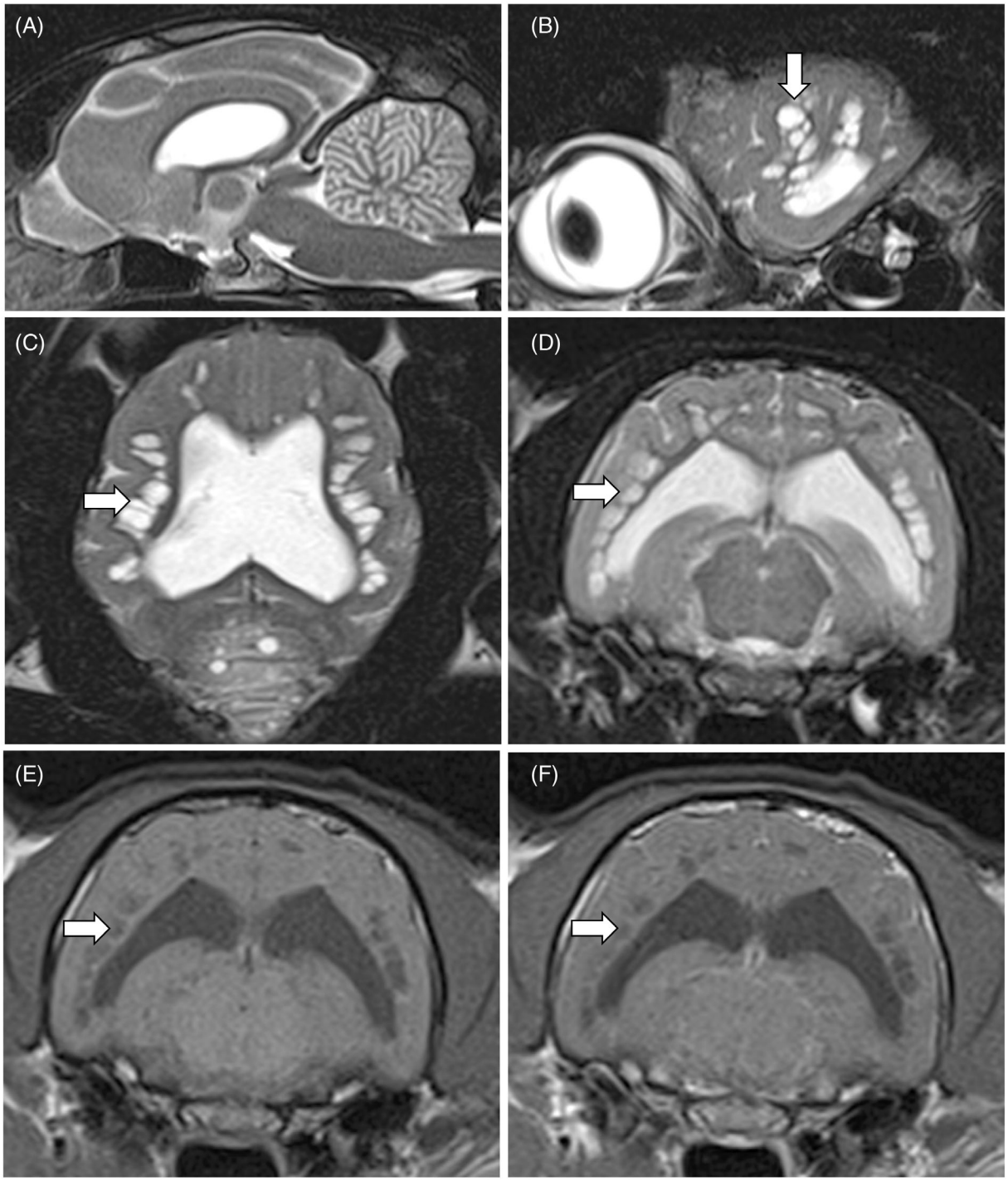

FIGURE 1 MRI of the brain. Sagittal T2WI (A) showing hyperintensity of the cerebellar white matter. Parasagittal T2WI (B), dorsal T2WI (C) and transverse T2WI (D), T1WI (E) and T1WI post contrast (F) at level of the mesencephalic aqueduct showing multifocal, bilateral, small, rounded intra-axial lesions within the subcortical white matter (arrows) containing fluid with similar characteristics to cerebrospinal fluid, hyperintense on T2WI, hypointense on T1WI with no contrast enhancement after gadolinium administration.

started straining to urinate and was treated for suspected urinary obstruction by catheterization before referral. The owner also mentioned a tendency to circle to either side in between the seizures. On presentation, physical examination was unremarkable. Neurological examination showed normal mentation and inconsistent bilateral menace response. Gait, postural reactions, and segmental spinal reflexes were normal. Neuroanatomical localization was the forebrain.

A CBC and serum biochemistry profile showed no clinically relevant changes. Urinalysis showed presence of hematuria. An MRI study of the brain using a 1.5T magnet (Magnetom Essenza, Siemens) identified multifocal, bilateral, small, rounded intra-axial lesions within the subcortical white matter surrounding the lateral ventricles containing fluid with similar characteristics as cerebrospinal fluid, hyperintense on T2WI, hypointense on T1WI with no contrast enhancement after gadolinium administration and suppression on fluid attenuated inversion recovery (FLAIR; Figure 1). Additionally, bilateral and symmetrical T2WI and FLAIR hyperintensity was present in the cerebellar cortex, cerebellar nuclei, thalamus, midbrain and pons with no contrast enhancement on T1WI. A cisternal cerebrospinal fluid sample was unremarkable, with normal nucleated cell count and protein concentration. Urine organic acid screening using gas chromatography-mass spectrometry showed markedly increased concentration of 2-hydroxyglutaric acid that, based on normal range in humans, was consistent with 2-hydroxyglutaric aciduria. No chiral differentiation was performed to determine if this was L-2-hydroxyglutaric acid or D-2 hydroxyglutaric acid.

2.2 | Whole genome sequencing and variant identification

Because the clinical signs resembled previous reports of L2HGA, and results of the urine organic acids evaluation confirmed increased concentration of 2-hydroxyglutaric acid, we hypothesized that the phenotype in this cat was caused by a biallelic loss of function of the *L2HGDH* gene. We sequenced the genome and searched for private homozygous variants that were not present in the genome sequences of 79 control cats (Tables S1 and S2). Genomic DNA was isolated from EDTA blood with the Maxwell RSC Whole Blood Kit, using a Maxwell RSC instrument (Promega, Dübendorf, Switzerland). An Illumina TruSeq PCR-free DNA library with approximately 400 bp insert size was prepared. We collected 178 million 2 × 150 base pair (bp) paired-end reads on a NovaSeq 6000 instrument (19 × coverage). Alignment to the F.catus_Fca126_mat1.0 reference genome assembly and variant calling were performed as previously described.[11] The sequence data were deposited under the study accession PRJEB7401 and sample accession SAMEA112203059 at the European Nucleotide Archive. To predict the functional effects of the called variants, SnpEff software[12] together with the F.catus_Fca126_mat1.0 reference genome assembly and NCBI annotation release 105, was used.

The bioinformatics analysis identified a single homozygous private protein-changing variant in the functional candidate gene *L2HGDH* (Table S2). It can be designated as chrB3:97881395 or XM_023255678.2:c.397C>T and leads to an early stop codon, truncating ~71% of the wild type *L2HGDH* open reading frame, XP_023111446.2:(p.Gln133*).

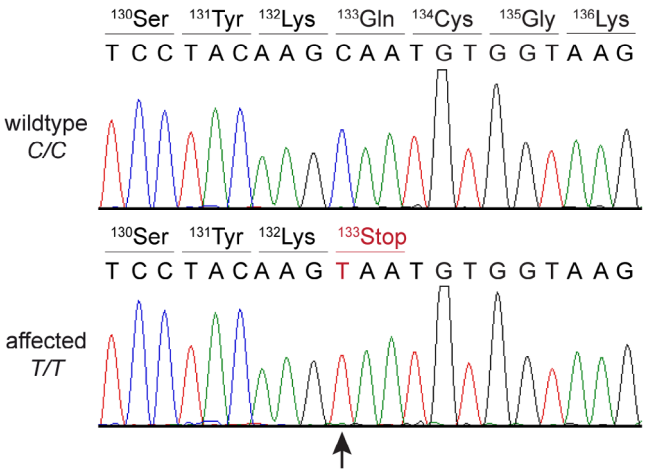

FIGURE 2 Details of the *L2HGDH*:c.397C>T variant. Sanger electropherograms derived from genomic DNA of a control and the affected cat are shown. Amino acid translations are also indicated.

The candidate variant was independently confirmed and genotyped by direct Sanger sequencing of PCR amplicons. A 320 bp PCR product was amplified from genomic DNA using AmpliTaqGold360-Mastermix (Thermo Fisher Scientific, Waltham, MA) and the primers 5′-TGT CCG GTA GCT ATT CAC CA-3′ (Primer F) and 5′-CGT GGA GTT AGT CAT ACC ACT TTT T-3′ (Primer R). The PCR was performed with an initial long denaturation of 10 min at 95°C, followed by 35 cycles of 30 s denaturation at 95°C, 30 s annealing at 60°C, and 60 s polymerization at 72°C. A final extension of 7 min at 72°C was performed at the end. After treatment with shrimp alkaline phosphatase and exonuclease I, PCR amplicons were sequenced on an ABI 3730 DNA Analyzer (Thermo Fisher Scientific). The sequencing reaction was performed with an initial long denaturation of 1 min at 96°C, followed by 25 cycles of 10 s denaturation at 96°C, 5 s annealing at 50°C, and 2 min polymerization at 60°C. Sanger sequences were analyzed using the Sequencher 5.1 software (GeneCodes, Ann Arbor, MI). The affected cat was homozygous for the mutant allele and 28 additional control cats all were homozygous wildtype (Figure 2).

2.3 | Treatment and outcome

Levetiracetam treatment was initiated at 20 mg/kg PO q8h with no more seizures observed during hospitalization. The cat was discharged but unfortunately died after a seizure 10 days later. No necropsy was performed.

3 | DISCUSSION

We identified a novel p.Gln133* nonsense variant in *L2HGDH* in a domestic shorthair cat with 2-hydroxyglutaric aciduria. It is the second

reported pathogenic variant for L2HGA in cats. This variant resulted in an early stop codon and is expected to completely abrogate expression of functional L2HGDH protein.[13]

2-Hydroxyglutaric acid exists in 2 enantiomeric forms (D and L) and, in human medicine, patients with D- and L-2-hydroxyglutaric aciduria have been reported with the majority having L-2-hydroxyglutaric aciduria and a well-defined clinical picture.[14] In our case, the urine organic acids showed a marked increase in 2-hydroxyglutaric acid, but we were unable to determine the enantiomeric form. The identification of a nonsense variant in *L2HGDH* helped us to confirm that this cat had the L-form.

The clinical phenotype observed in our cat was similar to the two previously reported cats. In all cases, seizure-like episodes were reported with either normal inter-ictal neurological examination findings or only decreased menace response. Age of onset was 7 months in our case compared to 14 months and 3 months in previously reported cases.[5,6] In humans, L2HGA causes only neurological signs, including psychomotor retardation, cerebellar ataxia, macrocephaly and seizures.[1,2] In Staffordshire bull terriers, the most common clinical signs include gait abnormalities and behavioral changes, with seizure-like episodes observed in two-thirds of the cases.[9] In the other dog breeds only small number of cases have been reported: the West Highland white terrier presented with gait abnormalities, impaired vision, behavioral changes and episodic head tremors, but no seizure-like episodes; and 2 of the previously reported Yorkshire terriers presented with generalized tonic-clonic seizures.[8-10]

Characteristic MRI changes have been reported previously in dogs and humans with L2HGA. In humans, the lesions primarily affect the white matter and are in the frontal and subcortical regions, although the dentate nuclei also are commonly affected.[15] In dogs, the lesions affect the gray matter causing a bilateral and symmetrical pattern affecting the cerebral cortex, cerebellar cortex, cerebellar nuclei, thalamus, hypothalamus, basal nuclei and dorsal brainstem.[3,4,7-10] In our cat as in the previously reported cats and dogs, the changes affected mainly the gray matter, cerebellar nuclei, cerebellar cortex, thalamus and colliculi in a bilateral and symmetrical manner.[5,6] Additionally, our cat had multicystic lesions affecting the subcortical white matter bilaterally, a feature that has not been reported in dogs or cats with L2HGA before. Subcortical cystic degeneration has been described in a few cases of L2HGA in humans, mostly as a late manifestation.[15-18] This finding could suggest that our cat had a more severe form of the disease and was presented with these changes at a very young age.

Unlike our cat, that died shortly after diagnosis, the two previously reported cats responded well to treatment with phenobarbital, having good seizure control.[5,6] The poor outcome combined with the multicystic lesions identified on MRI may indicate that the nonsense variant identified in our cat causes a more severe phenotype than the previously reported p.His434Arg missense variant.[5] In humans, limited phenotypic variation has been reported among the multiple genetic variants.[2] In general, the course of the disease in dogs and humans seems relatively slowly progressive.[2,9]

Our study had some limitations. First, the lack of pedigree data and the inability to obtain samples from relatives made it impossible to confirm genotype-phenotype co-segregation. Second, no necropsy examination was performed to further characterize the changes observed on MRI. Finally, no electroencephalography was performed to confirm that the episodes were seizures.

To our knowledge, our case report is the third description of L2HGA in a cat and the second report of a pathogenic *L2HGDH* variant. Interestingly, we report for the first time in veterinary medicine multicystic cerebral lesions on MRI, a rarely observed finding in human patients. The disorder L2HGA should be considered a differential diagnosis in young cats presenting with seizure-like episodes.

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CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

The cat in this study is privately owned and was examined with the consent of the owner. The Cantonal Committee for Animal Experiments approved the collection of blood samples from control cats that were used in this study (Canton of Bern; permit BE 71/19).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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