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Diagnosis of feline acute intermittent porphyria presenting with erythrodontia requires molecular analyses

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Abstract

Erythrodontia is the hallmark of human congenital erythropoietic porphyria (CEP), but is also a major phenotypic feature of acute intermittent porphyria (AIP) in cats. In this study, detailed biochemical and molecular analyses were performed on two unrelated cats with autosomal dominant AIP that presented with erythrodontia, yellow-brown urine and mild changes in erythrocytes. The cats had elevated concentrations of urinary 5-aminolevulinic acid and porphobilinogen, and half normal erythrocytic hydroxymethylbilane synthase (HMBS) activity. Two novel *HMBS* mutations were detected; one cat had a deletion (c.107_110delACAG) and one cat had a splicing alteration (c.826-1G>A), both leading to premature stop codons and truncated proteins (p.D36Vfs*6 and p.L276Efs*6, respectively). These studies highlight the importance of appropriate biochemical and molecular genetic analyses for the accurate diagnoses of porphyrias in cats and extend the molecular genetic heterogeneity of feline AIP. Thus, although erythrodontia is a classic sign of congenital erythropoietic porphyria in human beings, cats with erythrodontia may have acute intermittent porphyria, a hepatic porphyria.

Keywords

Acute intermittent porphyria; Hydroxymethylbilane synthase; *HMBS*; Feline; Anemia

The porphyrias are inborn errors of metabolism resulting from deficient activities of specific enzymes in the heme biosynthetic pathway. Acute hepatic porphyrias in human beings include autosomal dominant acute intermittent porphyria (AIP), hereditary coproporphyria (HCP), variegate porphyria (VP) and autosomal recessive 5-aminolevulinic acid dehydratase deficient porphyria (ADP). In these conditions, plasma and urinary concentrations of the porphyrin precursors 5-aminolevulinic acid (ALA) and porphobilinogen (PBG) are markedly increased during sudden, life-threatening neurological attacks. In contrast, human

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erythropoietic porphyrias present with erythrodontia and porphyrin-mediated cutaneous photosensitivity to sunlight, leading to erythema in autosomal recessive erythropoietic protoporphyria (EPP) and severe cutaneous blistering in congenital erythropoietic porphyria (CEP) (Anderson et al., 2001). Of the human erythropoietic porphyrias, only X-linked protoporphyria (XLP), CEP and hepatoerythropoietic porphyria (HEP) present with erythrodontia.

'Congenital porphyria' in domestic short haired cats with erythrodontia, fluorescent teeth and bones, and increased urinary PBG concentrations is inherited as an autosomal dominant trait (Tobias, 1964; Glenn et al., 1968). Siamese cats with erythrodontia had anemia, renal failure and high erythrocytic porphyrin concentrations, consistent with CEP, but the mode of inheritance was not reported (Giddens et al., 1975). A 10-year-old male domestic shorthair cat with recessively inherited erythrodontia had CEP (Clavero et al., 2010a), while two unrelated Siamese cats, 1 and 6 years of age, and a 1-year-old domestic shorthair cat, with dominantly inherited AIP, all had erythrodontia and were heterozygous for different erythrocytic hydroxymethylbilane synthase (*HMBS*) gene mutations (Clavero et al., 2010b). A 3-month-old domestic shorthair cat with erythrodontia had recessively inherited AIP and was homozygous for a missense mutation in *HMBS* (but had half-normal *HMBS* activity) (Clavero et al., 2010b).

All of the cats with AIP had elevated urinary concentrations of ALA and PBG, and a porphyrin isomer I:III ratio < 3, due to *HMBS* gene mutations, while the cat with CEP had normal urinary concentrations of ALA and PBG, and a porphyrin isomer I:III ratio >10, due to a mutant uroporphyrinogen (URO) synthase gene (*UROS*) (Clavero et al., 2010a and b). Here, we report two unrelated cats with erythrodontia, porphyrinuria and hematologic changes due to different novel *HMBS* gene mutations.

Case 1 was first presented as a 4-month-old spayed female domestic shorthair cat from Tennessee, USA. Case 2 was an 8-year-old spayed female domestic shorthair cat from Florida, USA. Both cats had erythrodontia and yellow-brown urine (see Appendix A: Supplementary Fig. 1) which fluoresced pinkish-red under a Wood's lamp. Neither cat had photoerythema. At 3-4 years of age, case 1 had normoblastosis, polychromasia, mild reticulocytosis and Howell-Jolly bodies on hematological examination, but no anemia, while case 2 had a moderate regenerative anemia with mild hypochromia and microcytosis (Table 1; see Appendix A: Supplementary Fig. 2). The urine was dipstick-negative for heme and bilirubin.

Concentrations of ALA, PBG and porphyrin isomers in urine and plasma, along with *UROS* and *HMBS* activities in erythrocytes, were determined according to Clavero et al. (2010b). With the exception of ALA in cat 1, concentrations of urinary metabolites of the heme biosynthetic pathway were increased in both cats (Table 2). Porphyrin metabolites were increased in both plasma and erythrocytes (Table 3).

Extraction of total leukocyte RNA and DNA, along with amplification by PCR and reverse transcriptase (RT)-PCR, were performed as described by Clavero et al. (2010b). Feline reference sequences were GenBank NC_018732 (16387785-16395224) for *HMBS* and NC_018733 (complement 83226715-83261885) for *UROS*. Novel *HMBS* mutations were present in both porphyric cats (Table 4), while no mutations were found in *UROS* in either cat. Case 1 had a splice site G→A transition at position -1 of the splice acceptor site of exon 14 (c.826-1G>A). Sequencing of cDNA revealed an aberrantly spliced RNA transcript with a 13 base pair (bp) deletion at the start of exon 14 (r.826_838del13) due to the use of the next available acceptor site (tgtacctgacagGA). RT-PCR and sequencing confirmed the predicted alternative splice site in 3/10 RT-PCR clones. Alternative splicing resulted in a

frameshift at codon 276, with a substitution of glutamic acid for leucine, followed by a premature stop codon (TAG) at codon 281 (p.L276Efs*6).

Sequencing of the *HMBS* gene from case 2 revealed a 4 bp deletion in a 4 bp direct repeat (ACAGACAG) in exon 4 (c.107_110delACAG). The resulting frameshift mutation started at codon 36, with the substitution of aspartate for valine, followed by five amino acids and a premature stop (TGA) at codon 41 (p.D36Vfs*6), thereby predicting a premature truncation after codon 40.

Sequencing also revealed a silent heterozygous polymorphism (c.348G>C; p.R112R) in exon 8 in both cases. The frequency of the C allele was 0.50 in 18 alleles (two normal cats and seven cats with AIP), including a C-homozygous and a G-homozygous normal cat, indicating that neither allele was causative for AIP.

At least five of the porphyrias seen in human beings have been diagnosed in veterinary species, including ADP, AIP, CEP, porphyria cutanea tarda and EPP (Online Mendelian Inheritance in Animals, OMIA¹). However, in many cases the diagnoses have not been based on comprehensive biochemical and/or molecular genetic analyses.

Both cats reported in the current study presented with erythrodontia, mild to moderate hematologic changes and yellow-brown urine, suggestive of a recessive erythropoietic porphyria (e.g. XLP, HEP or CEP). However, markedly elevated urinary PBG concentrations, low URO I:URO III ratios in urine, plasma and erythrocytes, intermediate HMBS activities in erythrocytes and heterozygosity for *HMBS* mutations established the diagnosis as autosomal dominant AIP.

The same splicing mutation seen in case 1 has been reported in human AIP, where it caused partial intron 13 retention, followed by premature termination, resulting in protein truncation (Martinez di Montemuros et al., 2001). In case 2, the 4 bp deletion of one member of a direct repeat presumably resulted from slipped-mispairing during replication (Levinson and Gutman, 1987). This mutation is predicted to result in a non-functional enzyme due to early termination of the polypeptide after only 40 residues of the normal 361 amino acid HMBS sequence.

The physiologic consequences in feline AIP differ substantially from those in human AIP. Although erythrodontia is a major feature of both feline AIP and CEP, it does not occur in human patients with AIP. Also, the acute abdominal and neurologic attacks seen in human patients with AIP have thus far not been observed in AIP cats. Therefore, the two feline porphyrias AIP and CEP can only be distinguished by their respective inheritance patterns, urinary and erythrocyte porphyrin profiles and, definitively, by mutations in different heme biosynthetic genes (Clavero et al., 2010a and b).

Feline erythrodontia has been investigated at the biochemical and molecular genetic level in seven unrelated cats, including the two reported in the present study; six cats had AIP and one had CEP (Clavero et al., 2010a and b). Mutations causing AIP appear to be more common in cats than those causing CEP, similar to finding in human beings. The two new feline cases of porphyria reported in the present study were presented with phenotypic manifestations similar to human CEP, while biochemical, enzymatic, and mutation analyses established the correct diagnosis of AIP.

¹See: <http://www.ncbi.nlm.nih.gov/omia>.

While erythrodontia is a classic sign of CEP in human patients, this report demonstrates that cats and other veterinary species with erythrodontia may have AIP, a hepatic porphyria, with precise diagnosis requiring analyses at the biochemical and molecular levels (see Appendix A: Supplementary Table 1). Similarly, porphyric pigs presenting with erythrodontia and porphyrinuria were initially reported as having autosomal dominant congenital porphyria (Jorgensen and With, 1963), but are most likely to have had AIP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Anderson, KE.; Sassa, S.; Bishop, DF.; Desnick, RJ. Disorders of heme biosynthesis: X-linked sideroblastic anemia and the porphyrias.. In: Scriver, C.; Beaudet, AL.; Sly, WS.; David, V.; Vogelstein, B.; Childs, B., editors. *The Metabolic and Molecular Bases of Inherited Disease*. McGraw-Hill; New York, USA: 2001. p. 2991-3062.
- Clavero S, Bishop DF, Giger U, Haskins ME, Desnick RJ. Feline congenital erythropoietic porphyria: Two homozygous UROS missense mutations cause the enzyme deficiency and porphyrin accumulation. *Molecular Medicine*. 2010a; 16:381–388. [PubMed: 20485863]
- Clavero S, Bishop DF, Haskins ME, Giger U, Kauppinen R, Desnick RJ. Feline acute intermittent porphyria: A phenocopy masquerading as an erythropoietic porphyria due to dominant and recessive hydroxymethylbilane synthase mutations. *Human Molecular Genetics*. 2010b; 19:584–596. [PubMed: 19934113]
- Glenn BL, Glenn HG, Omtvedt IT. Congenital porphyria in the domestic cat (*Felis catus*): Preliminary investigations on inheritance pattern. *American Journal of Veterinary Research*. 1968; 29:1653–1657. [PubMed: 5690689]
- Giddens WE Jr, Labbe RF, Swango LJ, Padgett GA. Feline congenital erythropoietic porphyria associated with severe anemia and renal disease. Clinical, morphologic, and biochemical studies. *American Journal of Pathology*. 1975; 80:367–386. [PubMed: 1231563]
- Jorgensen SK, With TK. Porphyria in domestic animals: Danish observations in pigs and cattle and comparison with human porphyria. *Annals of the New York Academy of Sciences*. 1963; 104:701–709. [PubMed: 13964925]
- Martinez di Montemuros F, Di Pierro E, Biolcati G, Rocchi E, Bissolotti E, Tavazzi D, Fiorelli G, Cappellini MD. Acute intermittent porphyria: Heterogeneity of mutations in the hydroxymethylbilane synthase gene in Italy. *Blood Cells, Molecules and Diseases*. 2001; 27:961–970.
- Tobias G. Congenital porphyria in a cat. *Journal of the American Veterinary Medical Association*. 1964; 145:462–463. [PubMed: 14215379]

Table 1

Hematologic parameters of two cats affected with acute intermittent porphyria.

Parameter (units)	Case 1 ^a	Case 2 ^b	Normal range
Hematocrit (%)	32-40	12-27	32-48
MCV (fL)	41-48	32-44	40-50
MCHC (g/dL)	27-32	32-36	30-35
RDW (%)	23-24	ND	16-22
Reticulocytes (/μL)	98,000-161,000	90,000-204,000	< 60,000
Normoblasts (/100 WBCs)	2-4	2-62	< 1

MCV, mean cell volume; MCHC, mean corpuscular hemoglobin concentration; RDW, red blood cell distribution width; ND, not determined.

^aData collected over a 1 year period.

^bData collected over a 3 year period.

Table 2

Urinary heme precursors and porphyrins in two cats affected with acute intermittent porphyria.

Precursor/porphyrin	Case 1	Case 2	Normal ^a
	Mean (Range) ^b <i>n</i> = 1 ^c	Mean (Range) ^b <i>n</i> = 1 ^c	Mean (Range) ^b <i>n</i> = 8 ^c
ALA (mmol/mol Cr)	1.86 (1.84-1.88)	4.62 (4.44-4.57)	2.4 (0.98-4.3)
PBG (mmol/mol Cr)	6.11 (5.99-6.21)	14.6 (13.1-16.6)	0.65 (0.20-2.2)
URO I (μmol/mol Cr)	205 (191-213)	636 (469-801)	0.40 (0.23-0.62)
URO III (μmol/mol Cr)	107 (97.8-113)	309 (227-391)	0.20 (0.10-0.35)
7-COOH (μmol/mol Cr)	8.29 (1.37-12.0)	28.4 (21.6-34.3)	0.39 (0.18-0.75)
6-COOH (μmol/mol Cr)	4.00 (2.69-6.05)	5.23 (4.61-6.17)	0.11 (0-0.23)
5-COOH (μmol/mol Cr)	13.3 (11.4-14.7)	22.0 (16.2-27.0)	0.27 (0-0.36)
COPRO I (μmol/mol Cr)	44.5 (41.8-46.0)	78.7 (60.0-93.6)	1.7 (1.4-1.9)
COPRO III (μmol/mol Cr)	5.12 (4.29-5.70)	5.68 (4.87-6.93)	0.72 (0.48-0.90)

	Ratio	Ratio	Ratio
URO I/III ratio	1.92	2.06	2.0
COPRO I/III ratio	8.69	13.9	2.36

ALA, 5-aminolevulinic acid; PBG, porphobilinogen; URO, uroporphyrin; 7-COOH, monodecarboxylated URO; 6-COOH, didecarboxylated URO; 5-COOH, tridecarboxylated URO; URO, uroporphyrin; COPRO, coproporphyrin, Cr, creatinine.

^a Normal values from Clavero et al. (2010a).

^b Mean of three replicates.

^c *n*, number of cats.

Table 3

Porphyrins in erythrocytes and plasma in two cats affected with acute intermittent porphyria.

Porphyrin	Case 1	Case 2	Normal ^a
	Mean (Range) ^b <i>n</i> = 1 ^c	Mean (Range) ^b <i>n</i> = 1 ^c	Mean (Range) ^b <i>n</i> = 1-3 ^c
Erythrocytes (pmol/g tissue)			
URO I	107 (85.6-129)	200 (175-225)	0.61 (0.0-1.22)
URO III	87.6 (67.8-107)	162 (86.6-237)	0.1 (0.0-0.1) ^d
7-COOH	13.2 (7.16-19.3)	20.2 (15.2-25.2)	0.28 (0.0-0.55)
6-COOH	2.42 (1.58-3.27)	2.52 (0-5.03)	0.47 (0.1-0.86)
5-COOH	1.36 (1.19-1.53)	2.22 (0-4.44)	0.76 (0.0-1.52)
COPRO I	33.2 (33.2-33.2)	17.9 (10.4-25.4)	0.07 (0.0-0.14)
COPRO III	22.0 (19.9-24.2)	13.8 (11.4-16.3)	0.1 (0.0-0.1) ^d
PROTO IX	55.5 (48.5-62.3)	379 (318-440)	1.46 (0.57-2.35)
Plasma (pmol/mL)			
URO I	54.2 (43.5-70.2)	76.6 (59.1-86.9)	0.24 (0.04-0.37)
URO III	31.4 (26.2-39.7)	24.1 (10.9-33.7)	0.19 (0.06-0.32)
7-COOH	16.6 (13.2-19.3)	8.98 (6.84-10.3)	ND ^e
6-COOH	7.98 (4.80-10.2)	2.94 (2.69-3.41)	ND
5-COOH	3.75 (2.41-5.95)	3.04 (2.80-3.50)	ND
COPRO I	22.4	19.0	0.36

URO, uroporphyrin; 7-COOH, monodecarboxylated URO; 6-COOH, didecarboxylated URO; 5-COOH, tridecarboxylated URO; COPRO, coproporphyrin, PROTO, protoporphyrin.

^aNormal values from Clavero et al. (2010a).

^bMean values are the average of three replicate assays per cat, except for erythrocytes, where the mean is the average of two replicates, and COPRO, where only one value was recorded.

^c*n* = number of cats.

^dLimit of detection of porphyrins is 0.1 pmol/g.

^eND, not detectable.

Table 4

Erythrocyte enzymatic activities and *HMBS* mutations in two cats affected with acute intermittent porphyria.

	Case 1	Case 2	Normal
HMBS activity (% of normal) ^a	2.4 (32)	4.6 (62)	7.4 (100)
Range	1.9-2.9	3.5-5.4	6.8-8.4
UROS activity (% of normal) ^a	2,700 (290)	3,700 (390)	940 (100)
Range	2,200-3,100	3,300-4,000	920-960
<i>HMBS</i> mutation	c.826-1G>A	c.107_110delACAG	None
RNA defect ^b	r.826_838delcuguaccgacag	r.107_110delacag	None
Predicted protein alteration	p.L276Efs*6	p.D36Vfs*6	None

HMBS, hydroxymethylbilane synthase; UROS, uroporphyrinogen synthase.

^a Means of two and three replicates for UROS and HMBS activities (pmol product/h/mg protein), respectively.

^b Reverse transcriptase PCR sequence.