Case Report
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Congenital Adrenal Hyperplasia Associated with Mutation in an 11β-Hydroxylase-Like Gene in a Cat

S.L. Owens, M.E. Downey, B.M. Pressler, A.J. Birkenheuer, D.W. Chandler, and J.C. Scott-Moncrieff

A 3-year-old indeterminate sex domestic medium-hair cat was presented to the Purdue University Veterinary Teaching Hospital for further evaluation of chronic polyuria and polydipsia, foul-smelling urine, and increased serum androgen concentrations. The cat had been surrendered to a shelter 4 months previously for undetermined reasons. Diagnostic tests performed before referral included a serum biochemistry panel, CBC, urinalysis, urine culture, and ACTH stimulation testing (cortisol, aldosterone, and sex hormone assay). Abnormalities included minimally concentrated urine (specific gravity. 1.018), decreased baseline and stimulated serum cortisol and aldosterone concentrations, increased baseline progesterone, and increased baseline and stimulated 17-OH progesterone and androstenedione concentrations.

The cat had a small body frame, thickened skin, gynecomastia, a fully formed penis with barbs, and an intact but empty scrotum. Initial indirect systolic blood pressure was 180 mm Hg, and remained persistently elevated during hospitalization. Clinicopathologic abnormalities included increased serum urea nitrogen concentration (41 mg/dL, reference range 15–35 mg/dL), hypernatremia (158 mmol/L, reference range 148–157 mmol/L), and hyperglobulinemia (5.2 g/dL, reference range 2.3–3.8 g/dL). Feline leukemia and feline immunodeficiency virus status were both negative, and a CBC did not reveal abnormalities. Urine specific gravity was 1.007, with no abnormalities on urine dipstick or sediment examinations. A repeated ACTH stimulation test including sex hormone assay confirmed the previously noted abnormalities (Table 1). Endogenous ACTH concentration was increased (>1,250 pg/mL, reference range 25–50 pg/mL).[1] Abdominal radiographs did not reveal abnormalities and abdominal sonographic examination revealed anatomically normal adrenal glands and no identifiable internal genitalia.

From the Department of Veterinary Clinical Sciences (Owens, Scott-Moncrieff), College of Veterinary Medicine, Purdue University, West Lafayette, IN; Department of Clinical Sciences, College of Veterinary Medicine (Downey, Birkenheuer), North Carolina State University, Raleigh, NC; Department of Veterinary Clinical Sciences (Pressler), The Ohio State University, Columbus, OH; and Endocrine Sciences (Chandler), Calabasas, CA.

Corresponding author: J. Catharine Scott-Moncrieff, Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Purdue University, 625 Harrison Street, West Lafayette, IN 47907; e-mail: scottmon@purdue.edu.

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Abbreviations:

11-DES	desoxycortisol
CAH	congenital adrenal hyperplasia
DOC	deoxycorticosterone
DHEA	dehydroepiandrosterone

Baseline serum deoxycorticosterone (DOC), 11-desoxycortisol (11-DES), dehydroepiandrosterone (DHEA), pregnenolone, and corticosterone concentrations were measured by high-performance liquid chromatography with tandem mass spectrometry by specific assays validated to bioanalytical standards, and compared with 4 age-matched healthy cats (2 neutered males, 2 spayed females).[a] Serum concentrations of DOC (1,177 ng/dL [healthy control cats, 4.6–18.0]), 11-DES (3,647 ng/dL [healthy control cats, 9–29]), DHEA (155 ng/dL [healthy control cats, 9–47]), and pregnenolone (552 ng/dL [healthy control cats, 115–311]) were markedly greater than results for any of the 4 healthy cats, whereas serum corticosterone concentration (9 ng/dL [healthy control cats, 164–550]) was lower. Exploratory laparotomy was performed with the aim to identify internal testes that would explain the cat's foul-smelling urine and physical examination abnormalities. The vas deferens and spermatic cords were bilaterally identified traversing through the inguinal rings and into the scrotum, consistent with prior castration; the identity of this tissue was confirmed histopathologically. The adrenal glands were free of gross lesions. The cat was confirmed as genetically male by DNA extraction of whole blood with identification of X and Y chromosome linked markers.[b]

The cat was complacent, easy to work with, and good natured during initial hospitalization. After evaluation the cat was adopted into a home with 2 other cats, and immediately demonstrated intercat and owner-directed aggression during play and handling. A diagnosis of intermale aggression was made, presumed secondary to excess androgen concentration or other hormonal imbalance. Behavior modification and fluoxetine (0.6 mg/kg PO q24h) failed to modify the undesired behavior.

A presumptive diagnosis of 11β-hydroxylase deficiency resulting in congenital adrenal hyperplasia (CAH) was made based on results of the physical examination abnormalities and adrenal steroid testing, and prednisone therapy (0.2 mg/kg, PO, q24h) was prescribed. Two weeks after initiation of prednisone, the cat's mammary glands had decreased in size and systolic blood pressure had decreased to 150 mm Hg. Because of the partial but incomplete response, after

Table 1. ACTH stimulation sex hormone profile results before and after treatment.

	Pretreatment		Post-treatment[a]		Reference Ranges	
	Pre-ACTH	Post-ACTH[b]	Pre-ACTH	Post-ACTH[b]	Pre-ACTH	Post-ACTH[b]
Cortisol (ng/mL)	7.7	10	9.6	6	9.8–59	95–183
Androstenedione (ng/mL)	>10	>10	0.24	0.36	0.07–0.55	0.52–2.8
Estradiol (pg/mL)	55.5	50.8	83.7	73	39–79	38–70
Progesterone (ng/mL)	1.98	3.92	<0.06	0.28	0.06–0.7	0.9–4.6
17-OH Progesterone (ng/mL)	1.45	3.64	<0.08	0.37	0.08–0.3	0.2–1.6
Aldosterone (pg/mL)	<11	<11	<11	n/a	11.3–294.3	n/a
Testosterone (ng/mL)	0.27	0.21	0.04	0.04	0.2–0.5	0.25–0.5

[a]9 months after beginning 0.5 mg/kg/d prednisolone administration.
[b]60 minutes after intravenous administration of 125 ug cosyntropin.

8 weeks the dose of prednisone was increased (0.5 mg/kg PO q24h). Complete regression of mammary tissue and gradual regression of the penile barbs were noted after 3 weeks at the increased prednisone dose: systolic blood pressure (165 mm Hg) and endogenous ACTH concentration (235 pg/mL), however, were still increased. The prednisone dose was increased further (0.8 mg/kg/d), which resulted in normalization of systolic blood pressure (120 mm Hg) and resolution of polyuria, polydipsia, and malodorous urine within 4 weeks. Secondary sex characteristics continued to resolve, with subjective reduction in skin thickening and regression of the penile barbs. However, despite resolution of clinical signs, repeat endocrine testing revealed persistently increased endogenous ACTH (617 pg/mL) and androstenedione (>10 ng/mL) concentrations. Therapy was changed to prednisolone (0.62 mg/kg PO q24h) because of increased bioavailability.[2]

The cat's systolic blood pressure (110 mm Hg) and endogenous ACTH (26 pg/mL) decreased to within reference range within 2 weeks of the change in therapy. Sex hormone assay revealed baseline concentrations of androstenedione (0.39 ng/mL), progesterone (<0.03 ng/mL), 17 OH progesterone (0.09 ng/mL), and testosterone (0.02 ng/mL) within or below reference ranges. Serum aldosterone concentration remained below reference range (<11 pg/mL).

Eight weeks after therapy was changed to prednisolone, the cat was diagnosed with Klebsiella pyelonephritis based on acute lethargy and inappetance, abdominal pain, an inflammatory leukogram, azotemia, and a positive urine culture, and successfully treated with 6 weeks of amoxicillin/clavulanic acid[c] (15.5 mg/kg/d PO divided q12h). After antibiotic treatment the prednisolone dose was decreased (0.5 mg/kg PO q24h).

Nine months later the cat remained normotensive (systolic blood pressure 140 mm Hg), endogenous ACTH was within reference range (11.6 pg/mL), DOC was 102 ng/dL, and sex hormone concentrations were markedly reduced pre- and post-ACTH stimulation (Table 1). Mild inte-male aggression persisted; however, owner-directed aggression was almost nonexistent at this time and the cat is more relaxed in his home environment.

Genetic mutations associated with 11β-hydroxylase deficiency in people have thus far all been identified within the coding regions of *CYP11B1,* the 11β-hydroxylase gene.[3] Alignment[d] of the 9 *Homo sapiens CYP11B1* exons (GenBank accession no. NG_007954) to the 2X *Felis catus* whole genome shotgun (GenBank accession no. PRJNA10762) identified a *CYP11B1*-like gene within 3 nonoverlapping feline genome contigs (GenBank accession nos. ACBE01524914.1, ACBE01524915.1, ACBE01524916.1). To determine whether 11β-hydroxylase deficiency in the cat of this report was likewise because of mutation of the *CYP11B1*-like coding region, genomic sequence analysis of this cat's and clinically healthy control cats' *CYP11B1*-like gene was performed.

The complete feline *CYP11B1*-like gene was amplified in 13 overlapping amplicons for the cat reported here and 1 healthy control cat with total DNA isolated[e] from 200 μL whole blood (Table 2). After PCR, appropriately sized amplicons were isolated, purified as needed,[f] and directly sequenced.[g] Amplicons with unresolvable chromatograms were presumed to be secondary to intronic variation; sequencing was repeated[g] after cloning into a plasmid vector,[h,i] Chromatograms were manually inspected for heterogeneity, and contigs assembled by commercially available software.[j] Intron-exon boundaries were annotated by alignment with human 11-β hydroxylase exons,[j] with putative exons translated in tandem *in silico*. The initial *CYP11B1*-like gene sequence from the cat of this report suggested that 1 or more mutations were present in the region homologous to exon 7 of human *CYP11B1* (Fig 1). The PCR amplification of the putative exon 7 region from the cat of this report and 5 healthy cats was performed using a proofreading polymerase (Table 2). Two exonic mutations within the putative *CYP11B1* coding sequence were found exclusively in the cat of this report, including a silent (ie, not associated with a change in amino acid) guanosine-to-adenosine mutation in exon 1, and a guanosine-to-adenosine mutation in exon 7 that results in an arginine to glutamine amino acid substitution; this latter mutation results in 11β-hydroxylase deficiency-associated CAH in people.[4]

CAH syndrome is because of 1 or more adrenal enzyme deficiencies resulting in inadequate cortisol

Table 2. Oligonucelotide primers used to amplify the presumptive feline *CYP11B1*-like gene, and expected amplicon base pair sizes.[a]

Amplicon	Forward Primer	Reverse Primer	Size
1	5′-GGACAACTTCTCCCATGACG	5′-ACCTCCAGGTGAAGGTTCTC	295[a]
2	5′-GGCAACAAGTGGATGAGGTT	5′-AGCAAGAACACGCCACATTT	649[b]
3	5′-GTATGACGTGGGAGGGACAC	5′-GGACACATATGATCCCCAAGG	302
4	5′-CGTGTTCTTGCTGTGAGAAA	5′-TTCCCACCCTGACCTGTAG	302
5	5′-TCTCCCTTCCTACAGGTCAG	5′-GGAGTTTGTTCACGCACACC	510
6	5′-TATCCTCTTGGGTGGACAGG	5′-CCATCTTCCTCGTCTCCAAGG	396
7	5′-CAGAAGTTCTGGGCTCAGC	5′-CACCATGGGCAGGTACTTC	320
8	5′-AGCTGAACCCAGACTTGCTG	5′-GAACATGAGCTGCGCAGTAG	418
9	5′-CTTTTTGGAGAGCGGCTGG	5′-GATGGATTTGGCCCGGATG	550
10	5′-TGCCATCCAGAAAATCTACCAGG	5′-AGCACGACATCACAGATGCTG	672
11	5′-GAGAACAGATGGCATGCAGC	5′-GTAGGGCCTGCTGCACAT	674
12	5′-CCCTGTTGATGACTCTCTTTGA	5′-GTGCAGCAACAGCAGCATC	960
13	5′-TGGTGCTGCAGAACTACCAC	5′-CTAGTTGATGGCCCGGAAG	668
EXON 7[c]	5′-AGGCTCAGGGAACTTTGTCTG	5′-GTTTCGACCCAGGGAGTAGAG	412

[a]Amplicons <550 bp: 5 μL of DNA template, 25 pmol each primer, 2× SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA), with initial denaturation at 95°C for 3 minutes, then 45 amplification cycles (95°C for 10 seconds, 58°C for 20 seconds, and 72°C for 30 seconds), and final melt curve-producing step (temperature increased from 65°C to 95°C, with 0.5°C stepwise increases every 5 seconds) for real-time PCR (MiniOpticon real-time PCR system; Bio-Rad Laboratories, Hercules, CA).

[b]Amplicons $\geq$550 bp: 50 μL total reaction volume containing 1 μL DNA template, 50 pmol each primer, 10 nmol dNTPs, 75 nmol $MgCl_2$, 2.5 U AmpliTaq Gold DNA polymerase, 1× concentration of GeneAmp PCR Gold Buffer (Applied Biosystems), with initial denaturation at 95°C for 5 minutes, then 45 amplification cycles (95°C for 20 seconds, 57.5–64°C for 30 seconds, 68°C for 1–2 minutes), and final extension at 72°C for 7 minutes for PCR thermal cycling (Techne Inc, Burlington, NJ). Annealing temperatures for each amplicon were optimized by use of a temperature gradient, and extension times modified according to amplicon size.

[c]Proofreading polymerase amplification of the exon 7 region with suspected mutations: 50 μL total reaction volume containing 1 μL DNA template, 50 pmol each primer, 10 nmol dNTPs, 1.755 U Expand High Fidelity Enzyme mix, 1× concentration Expand High Fidelity Buffer with $MgCl_2$ (Roche, Mannheim, Germany); initial denaturation at 94°C for 3 minutes, followed by 45 amplification cycles (94°C for 15 seconds, 68°C for 30 seconds, 72°C for 45 minutes) and final extension at 72°C for 7 minutes thermal cycling conditions (Techne Inc).

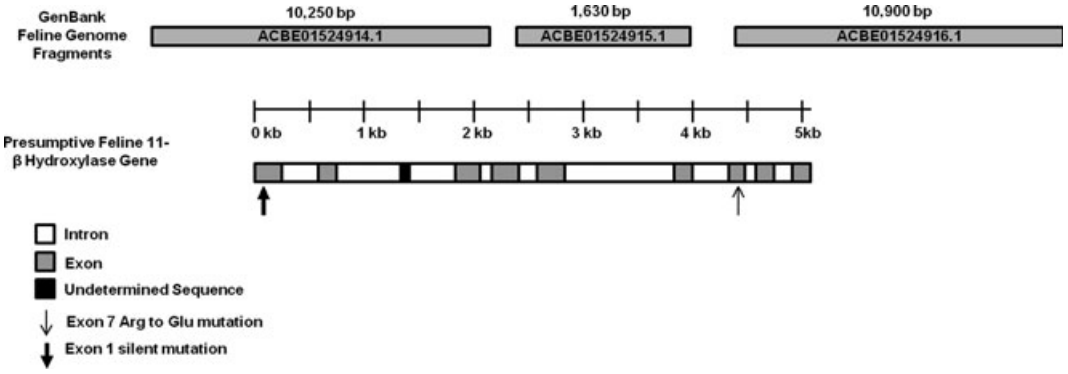

Fig 1. Putative feline 11β-hydroxylase gene.

biosynthesis and altered production of androgens.[5] CAH is the most common genetic endocrine disorder in humans.[6] While mutations associated with dysfunction of any of the 5 adrenal enzymes required for cortisol biosynthesis could result in CAH, mutations of *CYP21A* (90–95% of cases) and *CYP11B1* (5–8% of cases), the genes encoding 21-hydroxylase and 11β-hydroxylase, respectively, are most commonly implicated in people. Inheritance of all forms of CAH is autosomal recessive, with an overall worldwide prevalence of 1 in 15,000 live births.[7] CAH is rare in domestic animals, reported thus far in 1 female cat and 1 Great Dane (sex not reported), suspected to be secondary to 11β-hydroxylase deficiency in the cat and 17-hydroxylase deficiency in the dog.[8],[k]

11β-hydroxylase is a mitochondrial cytochrome P450 adrenal enzyme that converts 11-deoxycortisol to cortisol, and deoxycorticosterone to corticosterone.[5] 11β-hydroxylase deficiency results in insufficient cortisol production and secondary inadequate negative feedback to the hypothalamus and cranial pituitary gland, leading to overproduction of ACTH.[5] Uninhibited ACTH production continuously stimulates the adrenal cortex to produce steroid precursors, which

are inappropriately shunted toward production of sex hormones such as androstenedione. Clinical signs therefore result from increased circulating concentrations of androgens and mineralocorticoids.

The hallmark of common forms of CAH in people is virilism of females.[9] Human girls can be misclassified as males at birth because of ambiguous genitalia as excess androgen concentrations *in utero* can result in an enlarged penis-like clitoris, a common urogenital sinus replacing a divided urethra and vagina, and partial fusion of the labia majora, which may be mistaken for a scrotum.[9] Despite the presence of external male genitalia in the cat of this report, medical records before initial evaluation were unavailable, and it was unclear if he had been neutered or if the cat was a genotypic female with ambiguous genitalia; without this information definitive sexual classification as a male required histopathology and genotyping.

Additional phenotypic changes observed in people with 11β-hydroxylase deficiency include precocious puberty, gynecomastia, and benign testicular nodules in males,[5] hirsutism in females,[9] and premature physeal closure, stunted growth, acne, and infertility in both sexes.[5] Although these findings may be difficult to identify in domestic animals, the cat reported here did have gynecomastia, a greasy haircoat, and a small body frame. Whether or not precocious puberty, testicular nodules, or infertility would have been encountered is unknown because of the cat's age at presentation and prior castration.

21-hydroxylase deficiency, the most common cause of CAH in people, results in defective DOC production, absence of aldosterone, massive salt-wasting, and life-threatening systemic hypotension, whereas 11β-hydroxylase deficiency-associated CAH results in excess DOC, salt retention, volume expansion, and hypertension. Despite the weak mineralocorticoid effects (one-thirtieth the potency of aldosterone) of DOC, 10–100-fold increases in production of this hormone in 11β-hydroxylase deficient CAH patients often results in hypertension-associated adverse effects.[10] Treatment with corticosteroids reduces the production of DOC and may resolve hypertension. The 1 previously reported 11β-hydroxylase deficiency cat was too aggressive for accurate blood pressure measuring; therefore this is the first report in which hypertension, and successful therapy, is documented in a cat with CAH.[8] Consistent with reports of CAH in people, the systolic blood pressure of the cat in this report normalized synchronously with reduction of endogenous ACTH and serum androgen concentrations.[5]

Treatment of choice for human patients with 11β-hydroxylase deficiency is supplementation with corticosteroids, usually hydrocortisone because of its similarity to cortisol.[11,12] Differences in 24-hour cortisol demand, and at different life stages, frequently complicates therapy in people: for example, infants and adolescents routinely require higher doses of glucocorticoids than do adults.[13] A more aggressive alternative to medical therapy, particularly when adverse effects secondary to increased exogenous glucocorticoid administration become intolerable, is bilateral adrenalectomy and treatment for hypoadrenocorticism.[14]

Serum concentrations of renin, 17-OH progesterone, androstenedione, testosterone (in females), and DOC can be used in people as markers of adequate hormonal control.[5,15] We used multiple criteria to evaluate adequate adrenal suppression for the cat of this report, including endogenous ACTH concentration, serum androgen concentrations, systemic blood pressure, physical examination findings, and serum DOC concentration.

Psychological disorders are strongly associated with CAH in people, including higher aggression scores in females presumptively secondary to increased *in utero* androgen concentrations,[16] increased anxiety and depression, and overall impaired general quality of life.[6] The behavioral abnormalities noted in the cat of this report might be consistent with these findings, as standard behavior modification both with and without psychopharmacologic therapy was not effective until after the cat's hormonal status was equivalent to that of a castrated male cat, and therefore more consistent with intermale aggression rather than territorial aggression.

11β-hydroxylase is capable of catalyzing the terminal biosynthesis reactions of both aldosterone and cortisol.[17] *CYP11B1* encodes 11β-hydroxylase, which is responsible for cortisol biosynthesis in the zona fasciculata and zona reticularis, whereas *CYP11B2* encodes aldosterone synthase, the enzyme necessary for aldosterone biosynthesis in the zona glomerulosa.[18] These 2 unique genes have been identified in people and several rodent species,[17–21] whereas other animals, including frogs,[22] pigs,[8] and cows[22] have a single isozyme that catalyzes both reactions. It is unclear whether or not cats have 2 distinct enzymes or a single isozyme. In people, serum aldosterone concentration is decreased with 11β-hydroxylase deficiency because increased DOC concentration provides adequate and even excessive amounts of mineralocorticoid activity, and aldosterone production (which is stimulated by hyperkalemia and the renin-angiotensin system) is down-regulated.[5,23,24] When ACTH production decreases after corticosteroid supplementation, DOC production declines as well, eliminating the low-renin hypertension and returning the sodium-potassium balance to normal: serum aldosterone concentrations should consequently return to normal, precluding the need for mineralocorticoid supplementation.[5] It is assumed that reduction in DOC concentrations in species with a single isozyme would result in a state of mineralocorticoid deficiency, with life-threatening hyperkalemia and hyponatremia. Hyponatremia or hyperkalemia were never recorded in the cat of this report despite persistent hypoaldosteronism, likely because DOC was maintained at high enough concentrations. Although there is not sufficient clinicopathologic evidence to support 2 functional isoenzymes in this cat, it is still possible that 2 distinct isoenzymes are present but DOC concentration did not decrease low enough to allow stimulation of aldosterone biosynthesis.

Congenital adrenal hyperplasia is the most common genetic endocrine disorder in humans, yet this is just the 2nd report of the disease in a cat. Although a genetic disorder, clinical signs might not be clinically apparent until later in life and could vary considerably from neonatal hypovolemic shock to male precocious puberty and mild hypertension. While many patients with this disorder might not survive the neonatal period, many others may go unnoticed with subtle clinical signs. CAH should be a differential diagnosis for cats with unexplained hypertension, polyuria, polydipsia, presence of secondary sex characteristics postneutering, and behavioral abnormalities, including intercat aggression.

Footnotes

[a] Esoterix, Endocrine Sciences, Esoteric Division, LabCorp, Calabasas Hills, CA
[b] Veterinary Genetics Laboratory, University of California, Davis, CA
[c] Clavamox Tablets (62.5 mg), Pfizer, New York, NY
[d] BLAST (Basic Local Alignment Search Tool algorithm), National Library of Medicine
[e] QIAamp DNA Blood Mini Kit, Qiagen Inc, Valencia, CA
[f] QIAquick PCR purification kit, Qiagen Inc
[g] MCLAB, South San Francisco, CA
[h] pGEM-T Easy vector system, Promega Corporation, Madison, WI
[i] One Shot TOP10 Chemically Competent *E. coli*, Invitrogen, Grand Island, NY
[j] BioEdit Sequence Alignment Editor (ClustalW, full multiple alignment default settings), Ibis Biosciences, Carlsbad, CA
[k] Breitschwert EB, Vaden SL, Bailey EM, Lothrop CD. Congenital adrenal hyperplasia in a dog because of 17-hydroxylase deficiency, in: Research abstract program of the 7th annual ACVIM Meeting. J Vet Intern Med 1989;3:120 (abstract)

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