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CANINE PITUITARY DEPENDENT HYPERADRENOCORTICISM SERIES

Part 1: Comparative Epidemiology & Etiology in Dogs & Humans

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Canine pituitary dependent hyperadrenocorticism (PDH), also known as **Cushing's disease**, is a common endocrine disorder in older dogs. This disorder is caused by a pituitary adenoma (PA) that secretes inappropriate amounts of adrenocorticotropic hormone (ACTH), which results in bilateral adrenal hyperplasia and disorderly and excessive production of cortisol by the adrenal gland.

CLASSIFICATION

Basics of Classification

As with all central nervous system tumors, the Tumor–Node–Metastasis system used by the World Health Organization (who.int) does not apply. Current classification systems for PAs in veterinary patients are based primarily on secretory characteristics of the tumor. However, in humans, PAs are currently classified based upon:

- Tumor size and degree of invasiveness (**Table 1**)[1]
- Tumor endocrine activity (hormone secretion), or *functional* classification based on immunohistologic findings, such as ACTH and thyroid- and follicle-stimulating hormones.

In both humans and dogs, pituitary corticotroph adenomas that are responsible for Cushing's disease (ie, PDH in dogs) are classified as **functional ACTH-secreting PAs** (ACTH-PAs).

Further Classification

The World Health Organization classification system for PAs in humans has been refined to include designations for benign adenoma, atypical adenoma, and pituitary carcinoma on the basis of proliferation indices (p53 immunoreactivity, MIB-I Index, mitotic activity) and the absence/presence of metastases.[2]

More comprehensive molecular classification systems based on relevant gene expression have not been systematically used to further characterize pituitary tumors. Similar work to classify canine pituitary tumors both morphologically and functionally is currently underway.

TABLE 1.
Classification of Pituitary Tumors in Humans by Size & Anatomic Location

CLASSIFICATION BY SIZE BASED ON RADIOLOGIC FINDINGS	
Microadenomas	Less than 10 mm diameter
Macroadenomas	Equal to or greater than 10 mm diameter
CLASSIFICATION BASED ON RADIOANATOMIC FINDINGS	
Stage I	Microadenomas (< 1 cm) without sella expansion
Stage II	Macroadenomas (≥ 1 cm); may extend above the sella
Stage III	Macroadenomas with enlargement and invasion of the floor or suprasellar extension
Stage IV	Destruction of the sella

PREVALENCE

The study of pituitary tumors in both humans and animals is important because new discoveries regarding pathogenesis and treatment options in one species may cross over to the other, resulting in an example of One Medicine.

Humans

In humans, PAs are common tumors, with an overall prevalence in the general U.S. population estimated at 16.7%.[2]

- Corticotroph adenomas, comprising functional ACTH-PAs and silent corticotroph adenomas, represent approximately 10% to 15% of all PAs.
- Functional ACTH-PAs are the most common cause of Cushing's syndrome (hypercortisolemia from any source), accounting for an estimated 70% of all cases.
- Prevalence of Cushing's syndrome is estimated to be 1.2 to 2.4 per 1 million people, and it affects approximately 12,000 people in the U.S. This number, however, may be much higher, given that Cushing's syndrome is frequently misdiagnosed and diagnosis is often delayed.[2]

Dogs

Functional ACTH-PAs have a reported incidence of 0.2% in all dogs (1–2 cases/1000 dogs/year), with approximately 100,000 dogs affected yearly in the United States.[3,4] PDH accounts for approximately 85% to 90% of cases of hyperadrenocorticism, with the remainder of cases resulting from functional adrenal tumors, meal/food-induced cases, occult, or atypical disease.[4]

- *Meal or food-induced Cushing's syndrome* is thought to occur as the result of a congenital defect resulting in aberrant expression of the gastric inhibitory polypeptide (GIP) receptor in the adrenal cortex. Stimulation of this receptor by GIP, normally released by the stomach in response to a meal, leads to clinical signs of hyperadrenocorticism in younger dogs.[5]
- *Occult hyperadrenocorticism* refers to presence of elevated adrenal steroid concentrations in the absence of clinical signs, and has been best described in Scottish terriers with elevated serum alkaline phosphatase and vacuolar hepatopathy.[6]
- *Atypical hyperadrenocorticism* refers to animals with clinical signs of classic hyperadrenocorticism secondary to excess sex steroid production rather than excessive cortisol secretion.[7]

PATHOGENESIS

The pathogenesis of pituitary tumors that produce ACTH is becoming more evident based on ongoing studies evaluating gene and protein expression in both humans and dogs. This information will aid our understanding of tumorigenesis and point us toward targeted specific therapies.

Two theories have been put forward to explain the development of an ACTH producing pituitary tumor (ACTH-PA, corticotrophinoma):[8]

1. Hypothalamic theory
2. Monoclonal theory.

Hypothalamic Theory

In this theory, the hypothalamus stimulates corticotrophs through enhanced secretion of corticotropin releasing hormone (CRH) and vasopressin.[9] In addition, concurrent defects in the pituitary glucocorticoid receptors (GRs) on the corticotroph cells lead to decreased negative feedback by cortisol on CRH and ACTH synthesis.[10]

Gene Mutation. Karl and colleagues[11,12] and Lamberts[10] described a mutation in the gene in humans that encodes the GR, with a reduction in the sites of DNA binding, while maintaining affinity for cortisol. This *de novo* mutation promotes GR resistance that precedes the formation of the corticotrophinoma.

Recent studies by Teshima and colleagues,[13,14] using trilostane to decrease cortisol, demonstrated pituitary tumor growth as a consequence of a reduction in negative feedback. Their studies on canine ACTH tumor cells suggest that reduced negative feedback might first lead to corticotroph hyperplasia followed by a subsequent somatic mutation that could lead to tumor development.[9]

Dopaminergic Actions. Other possibilities for a hypothalamic theory of PDH include:

- Dopaminergic neurodegeneration in aged individuals[15-17]
- Decreased expression of the D_2 dopaminergic receptor on the corticotroph cells, resulting in decreased dopaminergic inhibition and subsequent hyperplasia.[18-20]

With this possibility, adenomas would evolve secondary to somatic mutations in hyperplastic cells.

The hypothalamic theory is reinforced by the recurrence of ACTH producing tumors following surgery or in patients in which no tumor was found on MRI or when exploring the sella. Both scenarios suggest stimulation or lack of inhibition of ACTH producing cells from higher centers (hypothalamus or hippocampus). This hypothesis is also supported by the fact that individuals with chronic stress and greater activation of the hypothalamic–pituitary–adrenal axis show corticotroph hyperplasia.[21]

TABLE 2.
Genes & Proteins Expressed in Pituitary Tissue in Humans with Functional ACTH-PAs[2]

GENES	
NEUROD1 + hPTTG1	*Overexpressed* in 3 studies
HIGD1B + HSD11B2	*Overexpressed* in 2 studies
CDKN1B	*Underexpressed* in 4 studies
CDKN2A	*Underexpressed* in 2 studies
let-7	*Underexpressed* in 2 studies
PROTEINS	
c-myc	*Overexpressed* in 2 studies
p27Kip1	*Underexpressed* in 4 studies
p16	*Underexpressed* in 2 studies

Monoclonal Theory

The main evidence against the hypothalamic theory is presence of tumor clonality in the majority of the adenomas studied in humans.[9,22] The monoclonal theory argues that the adenoma occurs in the pituitary outside of other influences and arises through the somatic mutation of a corticotroph cell, resulting in a tumor clone. This mutation precedes the clonal expansion of the tumor.[23]

Unknown are *which* mutation(s) result in the development of a tumor. Taking into account microadenomas and macroadenomas, the existence of a variety of corticotrophinomas is suggested. The majority of microadenomas in dogs do not progress to become macroadenomas. Macroadenomas can display a variety of behaviors, from limited growth and indolent course to more aggressive behavior as seen in human patients with Nelson's syndrome in which the pituitary tumor grows rapidly following bilateral adrenalectomy or suppressive medical therapy.[24]

Gene Origin. In humans with functional ACTH-PAs, studies have identified 43 genes and 22 proteins as overexpressed and 58 genes and 15 proteins as underexpressed compared with normal pituitary tissue (**Table 2**).[2]

In dogs, we are just beginning to learn more about genes and protein expression in patients with PDH. The recent demonstration of expression of somatostatin receptor subtypes and dopamine receptor subtype 2 (D_2) in canine corticotroph adenomas offers the possibility for novel medical treatment of PDH with somatostatin analogs and dopamine agonists (see Part 3 of this series in a future issue of *Today's Veterinary Practice*).[19]

Cancer Stem Cell Origin. Another possible origin of pituitary adenomas is found in cancer stem cells. In a recent study in dogs, the expression of melanotroph specific transcription factor *paired box protein 7* (Pax7) and stem cell marker and reprogramming factor *sex determining region Y-box 2* (Sox2) was determined and correlated to parameters, such as tumor size. This study suggested that Pax7 and Sox2 remain interesting targets for molecular investigations into their roles in pituitary tumorigenesis, but were unsuitable as clinical prognosticators in dogs.[25]

Pituitary Size & Proliferation Markers

The ratio between pituitary height and area of the brain (P/B) has been used to evaluate pituitary size:

- A **P/B ratio > 0.31** indicates an **enlarged** pituitary
- A **P/B ratio ≤ 0.31** indicates a **nonenlarged** pituitary.

A recent study investigated the expression of proliferation markers Ki-67 and minichromosome maintenance-7 (MCM7) in canine corticotroph adenomas in enlarged and nonenlarged pituitaries, and evaluated their relation to the size of canine pituitary corticotroph adenomas:[26]

- Canine corticotroph adenomas in enlarged pituitaries showed greater proliferation potential compared with adenomas in nonenlarged pituitaries.
- MCM7 expression was significantly greater than Ki-67 expression in canine pituitary corticotroph adenomas.

Thus, MCM7 may be superior to Ki-67 as a proliferation marker in canine pituitary tumors.

Polymorphism Versus Mutation

A **polymorphism** is a DNA sequence variation that is common in the population, and no single allele is regarded as the standard sequence. Instead, there are 2 or more equally acceptable alternatives.

In contrast, a **mutation** is defined as any change in a DNA sequence away from normal, implying that there is a normal allele prevalent in the population and the mutation changes this to a rare and abnormal variant.

The cut-off point between a mutation and polymorphism is 1%: To be classified as a polymorphism, the least common allele must have a frequency of 1% or greater in the population. Once the frequency is less than 1%, the allele is regarded as a mutation.

A **missense mutation or polymorphism** (compared to a nonsense mutation or polymorphism) is a single nucleotide change that results in a code for a different amino acid, leading to disease.

Corticotroph & Melanotroph Cell Marker: Tpit

In the pituitary glands of humans and mice, corticotrophs and melanotrophs have a specific marker in common, the *T-box transcription factor* (Tpit, or Tbx19), which regulates the late differentiation of corticotrophs and melanotrophs and, therefore, may contribute to the pathogenesis of corticotroph adenomas.

A recent study in 14 dogs with PDH examined the expression and mutation analysis of Tpit in normal canine pituitary and corticotroph adenomas:[27]

- Tpit was expressed in corticotroph and melanotroph cells of normal and adenomatous canine pituitaries, and remained present in nonadenomatous corticotrophs of pituitaries from PDH dogs.
- No tumor-specific mutation in Tpit cDNA from corticotroph adenomas was found; however, a missense polymorphism (see **Polymorphism Versus Mutation**, page 41) in the highly conserved DNA-binding domain, the T-box, was discovered in one dog.

The study concluded that Tpit can be used as a reliable marker for corticotroph and melanotroph cells in canine pituitary tissue, but that mutations in the Tpit gene are unlikely to play a major role in pathogenesis of canine corticotroph adenomas.

Corticotroph Differentiation Markers: LIF & LIFR

Leukemia inhibitory factor (LIF) is a cytokine of the IL-6 family that activates the hypothalamic–pituitary–adrenal axis and promotes corticotroph differentiation during development. LIF and leukemia inhibitory factor receptor (LIFR) expression were studied in pituitary glands of control dogs and specimens of corticotroph adenoma tissue collected from dogs with PDH.[28]

Their results demonstrated that:

1. LIFR was highly co-expressed with ACTH and alpha-melanocyte-stimulating hormone in the control canine pituitary gland and corticotroph adenomas.
2. There was a strong co-expression of LIFR and ACTH 1-24 in the cytoplasm of cells in the pars distalis and pars intermedia of control pituitary tissue. In pituitary glands harboring an adenoma, cytoplasmic expression of LIFR followed that of ACTH 1-24.
3. Nontumorous cells of the pars distalis showed no cytoplasmic staining but did demonstrate nuclear to perinuclear immunoreactivity for LIFR in 10 of 12 tissue specimens from PDH dogs.
4. This nuclear immunoreactivity was not observed in the control pituitary tissues or in the pituitary cells with corticotrope hyperplasia.

Role of ACTH Production & Glucocorticoids

As mentioned earlier, a characteristic biochemical feature of corticotroph adenomas is their relative resistance to negative feedback by glucocorticoids. In a recent study, gene expression related to ACTH production and secretion, and the negative feedback by glucocorticoids in canine corticotroph adenoma, was evaluated in pituitary tumors in 10 dogs with Cushing's disease. The results demonstrated increased ACTH production and resistance to negative feedback by glucocorticoids in canine corticotroph adenomas.[14]

Therapeutic Role of EGFR

Since tumors in dogs and humans express epidermal growth factor receptor (EGFR), in another study we examined whether EGFR might provide a therapeutic target for Cushing's disease.[29]

- In cell cultures from surgically resected human and canine corticotroph tumors, blocking EGFR also suppressed expression of proopiomelanocortin (POMC), the ACTH precursor.
- In mouse corticotroph EGFR transfectants, ACTH secretion was enhanced and POMC promoter activity was increased.

In mice, blocking EGFR activity with gefitinib, an EGFR tyrosine kinase inhibitor:

- Attenuated POMC expression
- Inhibited corticotroph tumor cell proliferation and induced apoptosis
- Decreased both tumor size and corticosterone levels
- Reversed signs of hypercortisolemia, including elevated glucose levels and excess omental fat.

These study results indicate that inhibiting EGFR signaling may be a novel strategy for treating Cushing's disease.

DIAGNOSIS

Diagnosis of PDH requires incorporating information from the history, physical examination, and routine laboratory tests.

Clinical Signs

Clinical signs, as well as laboratory abnormalities, seen in patients with PDH are secondary to the effects of steroid excess, well recognized and similar in scope to those seen with exogenous glucocorticoid supplementation (**Tables 3 and 4**).

- *Polyuria and polydipsia* occur as the result of excessive cortisol interfering with pituitary release

TABLE 3.
Common Clinical Signs of PDH in Dogs

- Polyuria and polydipsia
- Polyphagia
- Abdominal distention
- Bilaterally symmetric endocrine alopecia
- Panting
- Hypertension
- Urinary tract infections
- Additional dermatologic signs:
  - » Thin skin
  - » Pyoderma
  - » Calcinosis cutis

of antidiuretic hormone (ADH) or binding of ADH to receptors in the renal tubules.

- *Abdominal distention and thinning of skin* occur due to the catabolic effects of cortisol on tissues, such as muscle and connective tissue. Hepatomegaly due to steroid-induced vacuolar hepatopathy also contributes to the "pot-bellied" appearance of these patients.
- *Endocrine alopecia* mirrors the known distribution of sex hormone receptors in the skin, with endocrine alopecias often sparing the head and extremities.
- *Panting* occurs in both dogs and humans and is believed to result from steroid-induced stimulation of ventilator centers in the brain stem.
- *Pyoderma and urinary tract infections* reflect the immunosuppressive effects of glucocorticoids.
- The mechanism(s) behind the steroid induction of *calcinosis cutis* is poorly understood, though the condition does occur with both iatrogenic and spontaneous hyperadrenocorticism, and can take months to clear following resolution of hyperadrenocorticism or withdrawal of exogenous steroid.

Awareness of PDH has increased over time, resulting in the presentation of patients with only mild clinical signs, clinical signs affecting only one organ system (eg, polyuria and polydipsia or alopecia), absent clinical signs, or unusual isolated manifestations of the disease (**Table 5**).

Laboratory Analysis

Specific endocrine tests and imaging modalities are available to diagnose PDH and distinguish between the various causes of hyperadrenocorticism. No single test is perfect and, if the initial screening test is negative and high clinical suspicion of PDH exists, additional tests should be performed to rule it in or out. Endocrine evaluation of patients with PDH and nonadrenal illness can be difficult; it is important to

TABLE 4.
Common Laboratory Findings of PDH in Dogs

HEMATOLOGIC ABNORMALITIES

- "Stress" leukogram:
  - » Neutrophilic leukocytosis
  - » Lymphopenia
  - » Eosinopenia
- Mild thrombocytosis
- Mild erythrocytosis

SERUM BIOCHEMICAL ABNORMALITIES

- Increased serum alkaline phosphatase
- Milder increase in alanine aminotransferase
- Hypercholesterolemia
- Hypertriglyceridemia
- Hyperglycemia

URINALYSIS

- Decreased urine specific gravity < 1.018
- Proteinuria
- Urinary tract infection (even in absence of pyuria and bacteriuria)

TABLE 5.
Atypical Presentations of PDH in Dogs

- Thromboembolic disease
- Myotonia
- Pancreatitis
- Cranial cruciate ligament injury
- Facial nerve paralysis
- Gall bladder mucocele
- Reproductive abnormalities
- Hypertension

eliminate or manage the concurrent illness before undertaking adrenal function tests.

Further information about diagnosis of canine PDH will be discussed in Part 2 of this series, to be published in the next issue of *Today's Veterinary Practice*.

ACTH = adrenocorticotropic hormone; ACTH-PA = adrenocorticotropic hormone-secreting pituitary adenoma; ADH = antidiuretic hormone; CRH = corticotropin releasing hormone; EGFR = epidermal growth factor receptor; GIP = gastric inhibitory polypeptide; GR = glucocorticoid receptor; LIF = leukemia inhibitory factor; LIFR = leukemia inhibitory factor receptor; MCM7 = minichromosome maintenance-7; PA = pituitary adenoma; Pax7 = paired box protein 7; PDH = pituitary dependent hyperadrenocorticism; POMC = proopiomelanocortin; Sox2 = sex determining region Y-box 2; Tpit = T-box transcription factor

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