Case Study: Hyperparathyroidism in the Keeshond
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The skeleton contains approximately 98% of total body calcium. The remaining 2% circulate throughout the body. Calcium is regulated in the healthy mammalian body between approximately 2.20 and 2.60 mmol/L or 9-11 mg/dl. A complex relationship exists between the gastrointestinal uptake of calcium, calcium clearance by the kidney and calcium translocation from the small pool in the serum to the large storage area in the bone. Several factors are thought to be important in the regulation of serum calcium. Parathyroid Hormone (PTH) is an 84 amino acid polypeptide produced in the parathyroid glands. PTH secretion is controlled mostly by the level of serum ionized Ca2+. With decreased concentrations of serum Ca2+, PTH secretion is increased. With increased serum Ca2+ concentrations, PTH secretion is decreased. Increased vitamin D concentrations also inhibit PTH secretion. PTH-rp (PTH related peptide) is a PTH like factor with 139-173 amino acids. The N-terminus resembles that of PTH and is able to bind to PTH receptors in bone and kidney cells. PTH -rp is a normal fetal hormone but is also secreted by neoplastic lymphocytes, carcinoma (anal sac, mammary) and others in humans, dogs and cats. Thus PTH-rp plays an important role in hypercalcemia of malignancy. Vitamin D is a steroid hormone that is obtained through the diet or produced by the action of sunlight on vitamin D precursors in the skin. Calcitriol, the active form of vitamin D, is derived by hydroxylation of the precursor cholecalciferol, first in the liver (25-hydroxylation) and then in the kidney (1-hydroxylation). The principal target organ for vitamin D is the gut, where it markedly increases absorption of calcium and phosphorus. It also exerts negative feedback on PTH secretion. Calcitonin is a 32 amino acid hormone produced by the parafollicular C cells of the thyroid. Calcitonin is a weak inhibitor of osteoclastic activity and has some activity in apposing the effects of PTH on the kidney increasing calcium excretion. It is not clear how important these effects are and how efficient calcitonin really is in decreasing serum calcium during hypercalcemia.

Mild to moderate hypercalcemia may cause no clinical signs obvious to the owner, so this is commonly an incidental finding on geriatric or pre-anesthetic screening blood work. Other signs closely associated with hypercalcemia in dogs and cats include: Muscle weakness and lethargy, excessive drinking and urinating, calcium oxalate bladder stones, reduced appetite, weight loss, constipation and vomiting.

Primary Hyperparathyroidism (PHPT) is the 1st or 2nd most common cause of pathologic hypercalcemia in dogs, and relatively uncommon in cats. It is caused by an inappropriate secretion of PTH in the presence of ionized and total hypercalcemia. This results in most cases (80-85%) from a solitary parathyroid adenoma but may also be caused by malignant parathyroid neoplasia (very rare) or parathyroid hyperplasia. Older dogs (mean age 10.5 years) are affected by this condition with an over-representation of Keeshonden. When measured, PTH will be normal or high, iCa2+ will be high and PTHrp will be normal. Thoracic radiographs as well as abdominal ultrasound (US) or radiographs should be performed to rule out occult neoplasia. Cervical US often shows a singular (likely adenoma) or multiple (likely hyperplasia) enlarged parathyroid glands. Nuclear medicine scanning can be performed although is often unrewarding. For a solitary adenoma, surgical thyroidectomy is usually curative. Alternatives to surgery are percutaneous ethanol injection or heat ablation, which have a high success rate as well. Intensive
post-treatment monitoring (ideally hospitalization for 5-10) is a very important part of the treatment of this disease.

Prior to our study, little was known about the genetics of this condition in dogs despite obvious breed predispositions reported in the literature. The breed that is by far the most common in large surveys of dogs with this disease is the Keeshond. Despite being a relatively uncommon breed, Keeshonden represented 44 of 168 (26%) of the dogs in the UCD series and approximately 40% of the dogs diagnosed at the Cornell University Hospital for Animals in the last 10 years. In 2001, a review of cases of canine PHPT was published based on samples submitted to the Diagnostic Endocrinology Section, Animal Health Diagnostic Laboratory, Michigan State University. In this review the number of positive cases was reported for each breed, and was compared to the average number of dogs of each breed registered with the American Kennel Club over the years of the study. These data provided the basis for an estimation of an odds ratio for a positive result for each breed. The Keeshond was by far the most likely breed to be affected by PHPT with 214 positive samples and an average registration of 4,375 dogs yielding an odds ratio of 50,764.

The genetic portion of our study began with sample and pedigree information collection. This included an initial data base of over 600 Keeshonden. The PHPT status was known in over 200 of these dogs, with approximately 50 being known PHPT affected dogs. Based on the pedigrees assembled the node of inheritance was assessed to be autosomal dominant with age dependent penetrance. Three candidate genes were including the MEN 1 gene, the CASR gene and the HRPT-2 gene. These genes were chosen because of the resemblance of the human syndromes to the canine disease, as well as a similar mode of inheritance (autosomal dominant) in people. No meaningful mutations were identified within any of the 3 genes. No association was found between polymorphic markers within these genes and PHPT in Keeshonden. The combination of these two observations excluded these genes as candidates to include the causative mutation of PHPT in Keeshonden. The next step in the study was the genome wide scan.

In March of 2006 the AKC Canine Health Foundation agreed to fund a grant to enable the continued search for the gene that causes PHPT in Keeshonden. The defined goal of the study proposal was the development of a genetic test that would allow us to recognize Keeshonden predisposed to the disease. New technology was utilized which included looking at over 20,000 polymorphic markers spread throughout the DNA in affected and non-affected Keeshonden. The goal of the scan was to identify an association between a specific group of markers and the presence of disease. That would then define a region of the DNA that would be associated with the presence of disease. Genes known to be located in that area would then become new candidates for genes associated with PHPT.

Three known genes were present within that new region of interest. All 3 were investigated very thoroughly by assessing additional polymorphisms and their association with the disease, as well as assessing the sequence of these genes in affected vs. older, healthy Keeshonden. Two of the 3 genes were eliminated in this fashion and all of our efforts were focused on one of them. The name of this gene is still not being publicized so as not to jeopardize pending publications. We have identified a mutation in this gene and are currently working hard to discover the mechanism of how this abnormality causes the disease. Concurrently we decided to develop a test based on
the mutation we had found within the gene that we had proven to be highly associated with the disease. The technique was refined and found to be repeatable. Two validation processes were successfully performed on the PHPT genetic test. Following the validation of the test by the study samples, we were able to offer the test commercially to Keeshond owners.

Publications to date resulting from this study:

3. Genome-wide association mapping of three traits, including one cancer, utilizing 20,000 SNPs. 4th International Canine Cancer Conference, Chicago IL, September 2006.

We are very pleased that Keeshond breeders and owners now have the ability to test their dogs for the PHPT gene. This knowledge allows for informed breeding decisions as well as better monitoring of PHPT positive dogs, in an attempt to diagnose the clinical disease as early as possible if it should develop. We are also pleased that recently the Orthopedic Foundation of America (OFA) has added this test to the list of tests they sanction and Keeshond owners can now post the results of the PHPT test on the OFA website. We look forward to continuing to work closely with Keeshond breeders and owners as well as the KCA, and AKC Canine Health Foundation for the good of this wonderful breed.

Biographical Profile

Dr. Richard Goldstein graduated from the Koret School of Veterinary Medicine, in Israel in 1993. After completing an internship there, he pursued a residency in Small Animal Internal Medicine at the University of California, Davis, in 1995. Dr. Goldstein spent two years in a private specialty practice in California, joining the Cornell faculty in 2001. He is currently an Associate Professor of medicine at Cornell University. Dr. Goldstein is board certified in Small Animal Internal Medicine by the American and European Colleges of Veterinary Internal Medicine and his clinical and research interests are in infectious and genetic causes of small animal kidney disease and hypercalcemia.

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631: The Genome Wide Search for the Genetic Cause of Primary Hypoparathyroidism in the Keeshond
Case Study – conference notes

Calcium homeostasis – PTH
Increase calcium – parathyroid is next to the thyroid

Calcium physiology
Calcium normally tightly regulated between approx
High calcium in dogs causes:
  Malignancy – cancer of the kidneys
  PHPT
  Renal problems
  Addisons
  Vitamin D toxicity

PHPT
  2nd most common cause of pathologic hypercalcemia in dogs
  Inappropriate secretion of PTH in the presence of ionized and total hypercalcemia
  Occurs in older dogs – particularly keeshounds

Clinical signs:
  Can be none
  Increased drinking and urination
  Weakness
  Trembling
  Urolithiasis (calcium oxalate)

Diagnosis
  Usually not sick
  CBC, chem., UA
  Hypercalcemia
  Low to low normal serum phosphorus
  Occasional azotemia
  PHT – normal or high
  Calcium – high
  Ultrasounds/x-rays or Cervical ultrasound
  This is cancer but not malignant?

Definitive treatment
  Surgery
  Percutaneous ethanol injection
  Heat Ablation
  Intensive post-treatment monitoring for clinical hypocalcemia and if necessary treat

Why kees? Odds ration of 50 to 1 for a keeshond to develop PHPT compared to the average dog
Determined that this is a genetic disease and it is incomplete dominant. A dog with two affected alleles has not been seen – why? Because it is most likely lethal. Found four genes that were candidates for being the modifier genes. – unfortunately found nothing in those four genes.

Did a genome wide scan to search for the genes after receiving a CHF grant. With this, they were able to discern a strong association with one area of a chromosome and narrowed it down to three genes. Two of the genes were ruled out quickly. A mutation was found – an insertion was found within the gene. This mutation was present in 35/35 of the affected dogs; however it was also present in 11/144 of unaffected dogs. This concludes that the gene could be present but the condition is not expressed. But it is important to note that these dogs could pass the disease along to their offspring.

PHPT is hereditary in Keeshonded
Dominant
No known human genes
Successful association – canine SNP chip
Gene identified
Functional analysis ongoing
Genetic test currently offered commercially – http://www.vet.cornell.edu/labs/goldstein/