

Case Study: Hyperparathyroidism in the Keeshond

Richard Goldstein, DVM
Cornell University

Dr. Richard Goldstein said his case study was less about a particular disease of a particular breed than an example “of what it’s possible to do today with a very involved breeding community, a health club, and researchers with today’s technology.” The experience of isolating the gene for primary hyperparathyroidism (PHPT) in the Keeshond points the way to “something that we can all be doing with all of our breeds.”

The four parathyroid glands are situated on the thyroid itself, and the role of parathyroid hormone is to restore calcium levels when they drop. Calcium is also activated by Vitamin D, and 99% of it is stored in the skeleton.

Only six canine diseases lead to high calcium, and PHPT is the second most common, after malignant tumors. The relatively simple diagnosis makes the condition easier to identify than more complex diseases like hip dysplasia. However, the average onset at 11 years of age makes genetic testing more challenging than with diseases that appear in younger dogs.

A further challenge is that PHPT symptoms like weakness, trembling, and bladder or kidney stones may be missed, or attributed to a dog’s age. The condition is almost always linked to a single, non-malignant tumor, and the resulting hormonal shifts can lead to kidney damage when unattended. With early diagnosis, PHPT is cured in 90% of cases.

PHPT tumors can be removed surgically, injected with ethanol, or treated with a needle heated at the tip. With any treatment, Dr. Goldstein said, aftercare is the biggest challenge. In the three to seven days following surgery, calcium levels drop sufficiently to show clinical signs of low calcium, and sometimes low enough to cause death. Treating the calcium deficiency is a simple matter if a veterinarian knows to look for it.

For some years, studies had shown that the Keeshond was over-represented among dogs with PHPT—one university calculated an odds ratio of 50:1 for a Keeshond to develop the condition compared to the average canine. After several “very prominent dogs in the Keeshond world” developed PHPT, individual breeders and the Keeshond Club of America approached Dr. Goldstein to study the condition. “When you look at a lot of success stories, there are always people in the breed who are very helpful in getting the samples and getting the studies done,” he said.

Establishing ties with the breed club was probably the most important step, Dr. Goldstein said, but it was followed by a sustained effort to collect pedigree data and determine the mode of inheritance. From there, the “biggest stepping stone” was to

collect samples from healthy and affected dogs—a task that ran into definitional issues, since a healthy 10-year-old might still develop the late-onset disease at a later date. Researchers assembled a larger collection of samples to deal with possible errors in classification.

In the end, he said, the entire research project took only two years. With funding from a genetic research consortium at Cornell University, Dr. Goldstein's team collected 180 samples, including 35 confirmed positives, and created a database of 1,647 dogs as a basis for designing pedigrees.

Although the pedigrees went back three to five generations, the late onset of the disease meant it would be difficult to get samples from live parents, and virtually impossible to get grandparents. However, from the available data, it became clear that the PHPT trait was autosomal dominant, not recessive, with partial, age-dependent penetrance. While the data showed a number of dogs listed as healthy, Dr. Goldstein said the easiest explanation was that they died of other causes before the onset of PHPT.

The other anomaly was that all the affected dogs showed one bad allele, but not two, leading Dr. Goldstein to conclude that the combination of two PHPT genes is lethal *in utero*.

Once PHPT was characterized as a dominant genetic disease, the team identified three candidate genes based on their links to comparable human syndromes. After all three of them were ruled out, the next step was to undertake a genome-wide scan, made possible by a two-year grant from CHF and sponsored by the Keeshond Club of America

Genome-wide scans can be based on linkage mapping within families, or association mapping across an entire population. While linkage mapping looks for shifts in relatively unstable markers likely to change within a few generations, association mapping relies on more stable markers with greater longevity. The limited number of generations available for the Keeshond PHPT study dictated association mapping of a particular marker allele at the population level, which meant studying dogs as unrelated as possible.

The timing of the study was fortunate, Dr. Goldstein said, since it coincided with the introduction of a new SNP chip technology for genotype analysis. After the SNP system was adapted for canines by the Broad Institute at the Massachusetts Institute of Technology, the research team used it to test genetic differences between 27 affected and 42 unaffected animals.

Somewhat to the surprise of the research team, the analysis revealed one chromosome with a statistically significant difference between affected and unaffected subjects, where most or all of the markers were shared by the affected dogs but different for the unaffected ones. The relevant chromosome contained three candidate genes, two of which were easily ruled out by sequencing.

When the researchers studied the remaining gene more closely, they found that the relevant mutation occurred in the promoter region. The result was that tissue RNA was normal, but the quantity of RNA was a problem—and upon further analysis, Dr. Goldstein said, the mutation showed up in every affected dog in the study.

The mutation was also present in some of the unaffected animals, indicating that they were likely to develop PHPT later in life. Dr. Goldstein said that kind of test result can be difficult for a breeder, but he recommended careful monitoring rather than drastic action: the disease may never appear, and if it does, it is treatable.

At the end of the two-year process, the research team had developed a genetic test for PHPT and made it available to the Keeshond community. Dr. Goldstein said the key success factors were “the ability to get samples from a variety of owners and not just from Ithaca, New York; the ability to get seed funding from CHF for a study of dogs for dogs; and the availability of the technology to run the study. It all came together at the perfect time.”

Discussion

A participant asked whether the PHPT mutation is present in younger dogs. Dr. Goldstein said tests of affected 10-year-old dogs had determined that their blood and muscle tissue were heterozygous, but that their PHPT tumors had shown only affected copies. Similarly, when surgeons remove tiny samples of parathyroid gland and surrounding fat cells, they find normal and affected tissue a millimeter apart. The same phenomenon is quite common in human endocrine cancers. The theory is that an individual might be carrying the gene, but cancer develops when the gland receives two defective copies of the chromosome.