Cystinuria and DNA Research

Ian MacDonald – Health and Research Chair

CYSTINURIA IN IRISH TERRIERS

Cystinuria (CU) in Irish Terriers appears to be on the rise in North America. It is an inherited disease that impairs cystine reabsorption in the kidney which can lead to the formation of cystine stones in the bladder, blockage of urine flow, kidney failure and death. Because it is a genetic recessive trait, it remains mostly hidden in the population and although only a few dogs may exhibit symptoms, far more are carriers, passing the gene mutation on to future generations. At present, there is no genetic test for the disease, so there is no way to identify carriers.

New technology has been developed which makes the development of a genetic test quicker and less expensive. It does depend, however, on having DNA samples from a sufficient number of affected dogs and close relatives. The DNA can be analyzed to identify the mutation responsible so a genetic test can be developed. Once we have a DNA test, we can breed any dog to an appropriate mate with no risk of producing diseased puppies. This way we can maintain good dogs in the gene pool and eventually eliminate the disease entirely.

Test development is done in the lab of Dr. Gary Johnson, a lifetime member of the ITCA, and he has offered to start things off by covering the cost ($7,500) of testing samples from three selected dogs. What we have to do is provide DNA samples from as many affected dogs and close relatives as possible. We can do this by submitting blood and urine samples to the Canine Health Information Center.

The following article describes in some detail our current understanding of cystinuria in Irish Terriers and how we can address the problem.

THE BAD NEWS

In late March 2016, a comprehensive report on the incidence of cystinuria in Irish Terriers was submitted to the Board of the ITCA. This included an up to date description of the disease, its causes and the incidence of the gene mutation (CU gene) causing the disease in Irish terriers, based on reported incidents and pedigrees of about 1300 Irish Terriers obtained from the book of champions and recent show catalogs. The following is a summary of the findings of that March report.

In Irish Terriers, cystinuria appears to be due to a recessive mutation of an as yet unidentified gene that interferes with the normally complete reabsorption of cystine in the kidney. Symptoms are characteristic of type-III cystinuria which is testosterone dependent, so females and immature males are unlikely to have signs of the disease even if they have 2 CU genes. In mature affected males, cystine levels can be variable depending on testosterone levels, diet or state of hydration. At low levels, cystine can be completely dissolved in the urine but detectable by the nitroprusside or COLA (cystine, ornithine, lysine, arginine) tests. At higher concentrations characteristic hexagonal crystals can form which will pass with the urine and can be seen by microscopy during urinalysis. If larger stones form, the urethra
can be blocked and serious damage or death can occur. At present there is no DNA test so the presence of the CU gene in females or immature males and carriers cannot be detected.

Analysis of dogs in the database born since 2006 indicate that on average they have a probability of about 3.5% of carrying 2 CU genes and 29% of being carriers while there is a 67% probability of being clear. As only males are affected and symptoms can be variable, only about 1% of the population may actually suffer from the disease. However, there is a nearly 19% probability of passing on the CU gene to offspring.

Recommendations:

Until a DNA test has been developed, avoid breeding affected dogs (which will have 2 CU genes), or their parents or offspring (carriers) or siblings (which could have 2, 1 or 0 CU genes). Test young males periodically to detect cystinuria before symptoms occur. The nitroprusside test detects elevated dissolved cystine levels in the urine while at higher levels, characteristic hexagonal cystine may be seen by microscopy. In this way unwanted breedings can be avoided and dogs can be treated before serious damage can occur. Neutering (or even chemical castration which significantly reduces testosterone levels) appears to alleviate the symptoms. A positive test should be followed up with blood samples from the dog and close relatives sent for DNA research. Consider collecting and freezing semen before castration. If a DNA test does become available, the semen can be used to breed safely to known clear dogs.

RECENT DEVELOPMENTS – THE GOOD NEWS!

Over the past year, new technology has been made available for genetic research in dogs. This equipment, which had formerly been reserved only for human research, does whole genome sequencing so the entire DNA sequence (see below) from an individual animal can be determined. This equipment makes the process much faster and less expensive (down to $2,500) than previous methods. To develop a DNA test for the mutation responsible for cystinuria in Irish Terriers, the DNA sequences of a number of Irish Terriers with a confirmed diagnosis for cystinuria will be required.

Dr. Gary Johnson, a lifetime member of the ITCA, does DNA research looking for inherited diseases in dogs at his lab at the University of Missouri where the Canine Health Information Centre DNA data bank is located. He is now using the whole genome sequencer and has offered to pay for analysis of three Irish Terriers with cystinuria as a contribution to the development of a DNA test for the disease. Liz Hansen, his project coordinator, gave a comprehensive presentation of their DNA research at the ITCA hospitality evening for the 2016 Montgomery weekend. The following is based on her talk.
Chromosomes, DNA and Genes: (the simplified version)

Dogs have 38 pairs of chromosomes plus the X and Y chromosomes. These are long DNA molecules containing strings of hundreds of millions of small molecules known as nucleotides. There are four nucleotides used (adenine, cytosine, guanine and thymine (usually shown as A C G and T) and it is the sequence of these four letters that makes up the genetic code. On the chromosomes there are regions that make up about 19,000 pairs of genes that act as “blueprints” for constructing the proteins that determine the structure of the dog or control its metabolic processes. Many genes are the same or similar to those of other mammals including humans. The sequence of nucleotides (A C G and T) is grouped to form three letter words called codons. Most of the 64 possible codons code for one of the 21 amino acid that make up proteins (e.g., CTT = Leucine, GCT = Alanine, AGT = Serine, AGC also = Serine etc.). Other codons mark the start or end points of the genes on the DNA sequence that makes up the chromosome.

Mutations can occur (e.g., due to carcinogens or radiation) where one of the nucleotides is changed for another. This is called a SNP (single nucleotide polymorphism) which may or may not affect the protein produced. For example, if AGT is changed to AGC, serine is still produced, but if it is changed to AGA, it codes for arginine so the protein the gene produces will be changed and may not be able to do its job. Mutations can also be due to missing or added sections of the DNA that change the gene. Fortunately, there are two chromosomes in each cell so the normal copy of the gene does the job provided the mutation is recessive and doesn’t override the normal gene. A dog with one recessive mutation will be unaffected, but it is a carrier and can pass the mutation on to its puppies.

Because the mutation can be passed on by unaffected carriers, a puppy can receive the mutation on both genes (one on each chromosome from mother and father). Thus, when normal function is lost and the effects are observed, we know the mutation is there and that both parents must be carriers. This is where the whole genome sequencing comes in.

The New Research:

Blood (or tissue) samples are submitted to the CHIC DNA Repository where it is stored and can be accessed for genetic research. In the lab, DNA is extracted from the blood sample. This includes DNA from lots of cells so there are multiple copies of DNA from both sets of chromosomes from the animal. The entire genome is too big to deal with at once (about 3 billion nucleotides for humans) so the DNA is chemically split into smaller fragments (several hundred nucleotides). These are separated and the order of the nucleotides along each fragment is “read” by the sequencing equipment and recorded. Because the fragments originally come from many chromosomes, there is a lot of overlap in the fragments so the records of their sequences can be fit together by computer to form the entire original sequence.
The sequence is then compared with the standard dog sequence to identify the locations on each of the chromosome pairs where variations in the nucleotide sequence occur (SNPs, extra bits (insertions), missing bits (deletions) inverse repeats etc.). Except for identical twins, there are usually millions of these variants. The problem is to determine which one causes the disease and which are responsible for all the other traits (hair color, size, other diseases etc., or no effect at all). This is where the expertise of the researchers really comes in.

As an example, in one Standard Schnauzer with Dilated Cardiomyopathy (DCM) there were over 7,000,000 sequence variants. 101 other Schnauzers that did not have DCM also had many of these variants so those could not be the cause of the disease, narrowing it down to 321,000 candidate genes. As the disease was recessive, heterozygous variants (occurring only on one of the chromosome pairs) could be eliminated reducing it to 8,000. By examining the effect of the resulting codon change on the protein produced, only eight were found to inactivate the gene. When these genes were checked against other animals, only one was related to DCM, so it was the most likely candidate. A test for this variant was developed and found to be positive for nine other schnauzers with DCM. For this disease the abnormality turned out to be a large deletion with 22 missing nucleotides and the DNA test could be used to detect the mutation on carriers. Amazing detective work!

_How can We Contribute to Developing a DNA Test for Cystinuria in Irish Terriers?_

The Canine Health Information Center (CHIC) has two separate functions. The first is provide breed clubs with a system where they can identify health problems in the breed and recommend tests for these problems. Individual dogs can then be tested, given a CHIC number and the results of these tests can be posted. This information can then be used by the public to assess potential breedings or puppy health. Owners with healthy dogs are happy to participate in this system. Those that don’t may be treated with suspicion. At present, the ITCA has not participated in this system because 1) ITs do not have any significant problems for which there are tests (the nitroprusside test for cystinuria does not detect carriers or homozygous recessive females, so results can be deceptive), and 2) a test requirement for rare conditions in ITs, e.g., hip dysplasia, is an unnecessary expense and may suggest to the public that there is a problem where there isn’t. Although the requirement for testing is useful for many breeds, for Irish Terriers it would be counterproductive until DNA tests can be developed for significant diseases.

However, the second function provided by CHIC is the DNA repository. This is a completely separate confidential system for storing DNA samples from healthy dogs as well as from those with inherited diseases. These samples are used to develop DNA tests and any individual results are confidential and go only to the owner of the dog. Samples are kept in perpetuity and portions are provided only to accredited researchers holding approved research funding. The ITCA fully supports contributing to the CHIC DNA repository and some other clubs even require participation for dogs, e.g., to receive awards or be listed for puppy referral.

DNA samples from all Irish terriers are encouraged but those with confirmed cystinuria are particularly important, along with their close relatives. For those dogs, pedigrees to show the relationships and full clinical/diagnostic information should be submitted along with the sample. For individual submission instructions, go directly to the CHIC DNA repository website (http://www.caninehealthinfo.org/dnabank.html). Don’t worry about any of the other CHIC information for now. The samples can also be submitted through an organized collection at a national specialty or
other event. Fill out the application form to get a blood collection kit. The sample will go directly to Gary Johnson’s lab.

From the submissions, Dr. Johnson will select three dogs most likely to provide candidate genes for developing a DNA test for cystinuria. The availability of dogs from different family trees will help reduce the number of common variants to eliminate. The more DNA samples we can provide, the easier it will be to develop a DNA test for cystinuria. Once the test is developed, we will be able to breed with confidence so we can maintain the size of our gene pool and keep desirable traits while avoiding the risk of producing affected dogs and eventually eliminating the disease.