Cardiomyopathy Genetics Project

By: Liz Hare, Ph.D. and Brandi Weaver

As has been discussed in other articles, Toy Manchester Terriers have been found to have a distinctive and unique form of cardiomyopathy. Cardiomyopathy is an inherent abnormality of the heart muscle tissue that is usually hereditary and can cause life-threatening disturbances of the circulation. The biological reasons TMT cardiomyopathy is unique have been outlined and will be explained again in more detail in a formal paper being prepared by members of the research team looking more closely at its physical causes and behaviour. The purpose of this article is to look at whether a genetic cause is likely, how it may be inherited and what strategies breeders can use to minimize the risk of producing affected dogs.

What We Have Done

The cause of cardiomyopathy in Toy Manchesters is unknown, but because it appears in only a single breed with a restricted gene pool, it is reasonable to investigate a possible genetic contribution to the development of the disease. With this in mind, over the past eight months, we have looked closely at the familial relationships between the 12 affected puppies for which pedigree information is available.

We began by compiling individual 10-generation pedigrees for each case. These pedigrees were then combined to form a single document containing all of the affected dogs and their ancestors; each animal in the pedigree was assigned a unique number. The final pedigree is far too large to publish here, but to give you an idea of what this process looked like we have prepared a smaller chart focusing on the five most closely related cases (Figure 1).

Inbreeding Coefficients

Table 1: The following list of inbreeding coefficients for affected puppies are based on ten-generation pedigrees.

- 0.081 (8.1%)
- 0.161 (16.1%)
- 0.062 (6.2%)
- 0.083 (8.3 %)
- 0.000
- 0.137 (13.7%)
- 0.096 (9.6%)
- 0.020 (2.0%)
- 0.250 (25%)
- 0.257 (25.7%)
- 0.087 (8.7%)
- 0.085 (8.5%)

Margin of Error: 0.11 +/- 0.08

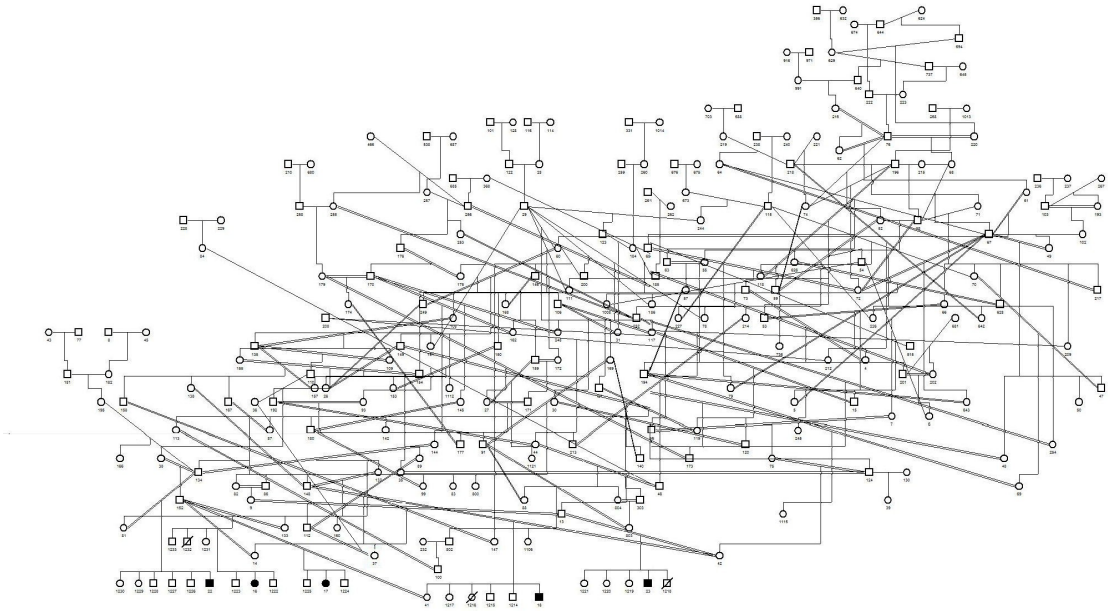

Figure 1: Sample of pedigree charts used in analyzing TMT cardioymopathy inheritance.

Next, we looked at the inbreeding coefficients of the affected pups (Table 1). The inbreeding coefficient is the probability that two alleles (copies) of a gene are "identical by descent"— meaning that they share the same origin. To give you a frame of reference, the highest values in this group of dogs are around 25%, which means the puppy's parents shared as many genes as full siblings would.

What We Know So Far

Although pedigree information was available for only 12 of the cases confirmed by necropsy, there are a few inferences we can easily make:

- Pedigree data tells us that TMT cardiomyopathy occurs in a large family of TMTs. All 12 of the cases studied are related to each other in some way.
- TMT cardiomyopathy is not a dominant trait because it is not passed directly from parent to offspring. In fact, none of the affected dogs in the study have lived long enough to be parents.
- Since male and female puppies are equally represented in the cases, it is unlikely to be a sex-linked trait.

With these points in mind, it is our opinion that the condition is most likely to be inherited as a recessive trait, that is, the affected pup has to receive two mutant genes, one from each parent. In this form of inheritance the parents must be carriers of the trait. It is also possible that TMT cardiomyopathy does not fit a simple inheritance pattern because it is the result of the actions of multiple genes, and/or because environmental factors affect its expression.

What Breeders Can Do To Minimize Risk

Manchester Terriers are a breed with a relatively small gene pool. This is compounded by the fact that TMTs were developed by more closely restricting the gene pool by selecting dogs based on body size. When genetic

diversity is reduced, the likelihood that a puppy will receive two recessive alleles (copies of the gene) is increased. It is especially difficult to breed recessive traits out of a population, because they are not always visible in the parents (unless or until genetic testing is available). As a result, carriers continue to be used for breeding, transmitting the TMT cardiomyopathy allele to approximately half of their offspring, who will in turn be carriers.

An important strategy for avoiding the accumulation of deleterious recessive alleles in a population is out crossing and maintaining as much diversity as possible in the population. This can be accomplished by using a variety of sires rather than only the most popular ones and breeding more bitches for fewer litters each. If dogs with affected relatives are bred to unrelated dogs, the odds that two recessive alleles will come together in a puppy will be reduced. With more inbreeding, the alleles are more likely to be identical by descent and, depending on the family of Toy Manchesters you are working in, they could be the genes related to cardiomyopathy.

Many breeders wonder how to use inbreeding coefficients to make decisions about which parents to use for the next generation, and how far back in the pedigree to use data when making the calculations. Most modern software can process as many generations of data as you are able to get, and you should use as many generations as possible. There are many common ancestors in the TMT pedigree further back than we were able to show in Figure 1. If you eliminate that data from your calculations, you are underestimating the inbreeding coefficient. There is no fixed number describing an optimal inbreeding coefficient. The most important thing to keep in mind is that as the inbreeding coefficient increases, so does the risk of producing puppies with a problem. The best way to reduce the risk is to choose breedings that result in puppies with the lowest possible inbreeding coefficients while keeping in mind your other goals for your breeding program.

Another frequent question concerns whether relatives of affected dogs should be used for breeding. They can be used with the understanding that their offspring will be at increased risk of developing or carrying TMT cardiomyopathy. So, for example, if this disease is, as we be-

Genetics 101

Simple Inheritance Patterns for a Trait Caused by a Gene at One Locus

When a trait is caused by a gene at a single locus, there are a limited number of ways inheritance can occur. Since TMT breeders are already familiar with von Willebrand's Disease (vWD), we'll use that as an example. Keeping in mind that one copy of each gene is inherited at random from each parent, we can think about ways in which the two copies of a gene can interact.

Dominant: A dominant trait will be expressed every time it is inherited, whether the individual receives one or two copies of the mutant or disease-causing allele (alleles are the different forms of a gene; usually we are talking about a normal version and a mutant, disease-causing version).

Recessive: A recessive trait will only be expressed if the individual inherits two copies of the mutant disease-causing allele, one from each parent. Each parent must have at least one copy of the disease causing allele (one copy would make them a carrier while two copies would make them affected with the disease). This is how vWD is inherited in TMTs. Each carrier has a 50% chance of passing the disease-causing allele on to his offspring. If two carriers are mated, about 25% of the puppies will be affected, 50% will be carriers, and 25% will be clear. (For more about eliminating recessive traits, see http://doggenetics.com/popgenforweb.html).

Sex-Linked: The inheritance of sex-linked traits depends on the sex of the individual. Each dog has 78 chromosomes in 39 pairs. 38 of these pairs contain the same genes in every individual, but the two sex chromosomes (X and Y) differ. Each male has an X and a Y chromosome, and each female has two X chromosomes. Males only get X chromosomes from their mothers, and since they only have one, they are affected more frequently by X-linked recessive traits than females, who would need two copies of a disease allele to be affected.

lieve, a recessive trait, each full sibling of an affected puppy has a 25% chance of being affected, a 50% chance of being a carrier and a 25% chance of being clear. We are unable to make definite statements about risks outside of the initial litter itself because we do not know the frequency of the mutant allele in the population, or whether it is isolated to one or more subpopulations (lines). However, if the frequency of the mutant allele is low in the population, for half siblings, the risk is approximately halved

In order for this strategy to work effectively, breeders must openly share information about the incidence of TMT cardiomyopathy. Before breeding to a sire, you should be able to find out whether the disease has occurred in his immediate/close family. As a general rule, the more closely he is related to an affected dog, the more likely it is that he is a carrier. Until cardiomyopathy can be identified early, such as with a genetic test, it is important to remember that the complexity of breeding means cardiomyopathy is just one risk factor -one piece of the puzzle- to consider when making a decision about breeding. Our goal is to reduce the occurrence of cardiomyopathy without eliminating good breeding stock or causing the emergence of another abnormal trait through excessively rigid selection. This is a balance that can be achieved with more research.

Next Steps

Further research is necessary to find the gene or genes for this trait, and the scientific community needs help from breeders. Since at this time TMT cardiomyopathy can only be diagnosed at necropsy, it is important that pups that die are examined to definitively determine whether they are affected with TMT cardiomyopathy. Blood or tissue samples from which DNA can be extracted are needed. Researchers at the University of Pennsylvania have indicated that when 10 such samples are accumulated they may be able to begin looking for genetic markers at specific locations on the chromosomes, bringing us closer to finding the gene or genes influencing this trait. When genes are identified, it will be possible to test potential sires and dams to determine whether they are carriers.

Dr. Liz Hare is a statistical geneticist working on the understanding of complex traits in humans and dogs that are the result of interactions of many genetic and environmental factors. She has studied psychiatric illnesses including schizophrenia and bipolar disorder, working ability in explosives detector dogs, and litter size in dogs at the University of Texas Health Science Center and Cornell University. Brandi Weaver holds a degree in Anthropology with a minor in biomedical sciences. She has performed pedigree work for psychiatric genetic grant research for over five years.

Don't Forget!

The American Manchester Terrier Club's Health Committee can provide assistance in paying for necropsies through their innovative **Breeder's Challenge Fund**. We recognize that necropsies can be expensive, however the information and genetic material necropsies provide is priceless. That's why we're here to help you help our breed.

For more information or to access the Breeder's Challenge Fund, please contact Kimberle Schiff, AMTC Health Chairperson (kimberle@oakwoodkennel.org) or Michelle Barlak, CMTC/AMTC Heart Study Chair (michelle@bleusprings.net) Users wishing to remain anonymous should direct inquiries to Dr. Shannon Martinson, Diagnostic Pathologist (smartinson@upei.ca)

Cardio Clearances

Please Note: There is currently NO heart clearance or examination that can identify the unique form of cardiomyopathy found in Toy Manchester Terriers. While health clearances are always encouraged, including examination by a cardiologist, clear test results should not be interpreted as indicating whether a dog has the disease or may produce it.

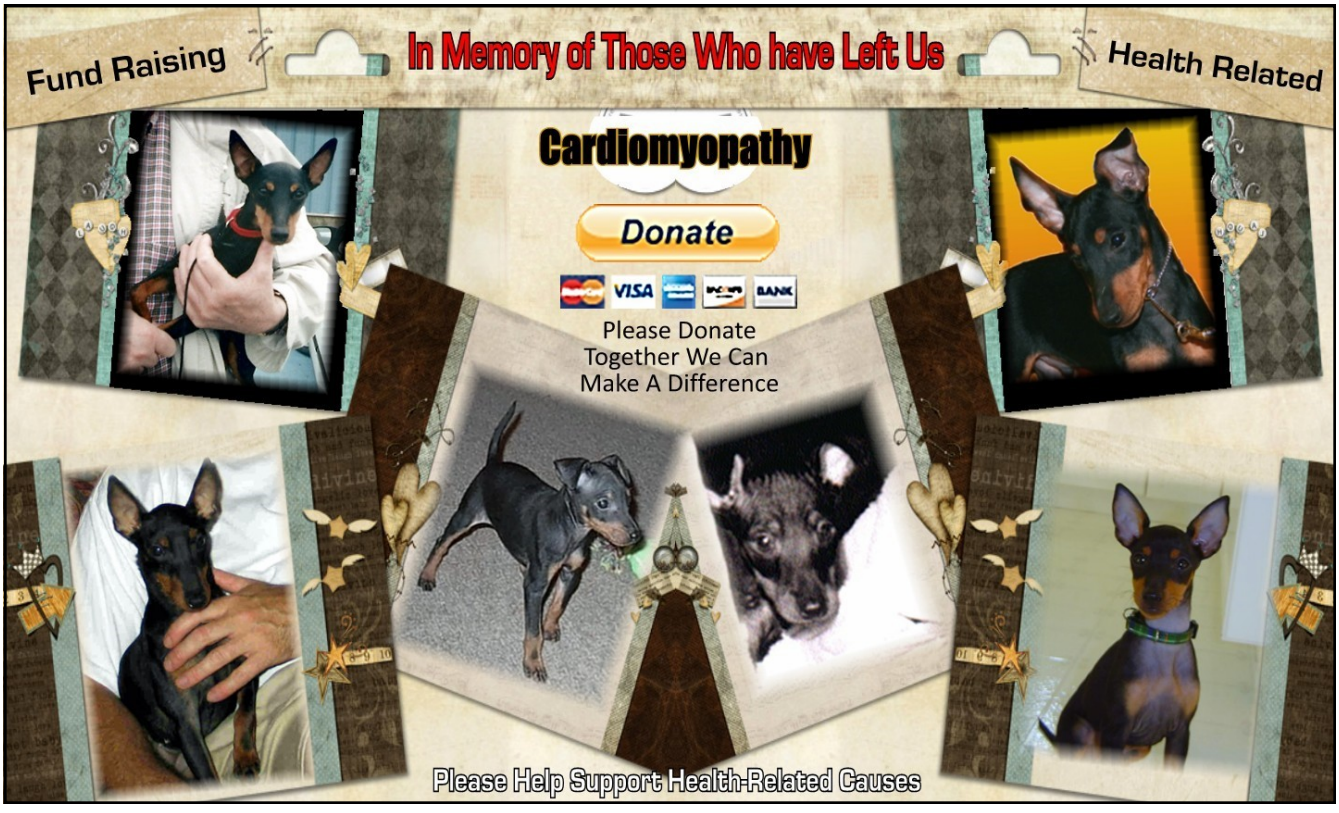

Photo montage by: Gene Caron

HEART STUDY UPDATE

By Amanda Kelly

This is a very exciting time for the heart study as we move toward publication of several important articles and papers while also transitioning to a brand new study coordinator. So, let's start there!

New Study Coordinator

We are extremely happy to welcome Michelle Barlak to the Study Team. Many of you will already know Michelle as she and her mother, Kris, have bred Toy Manchesters under the Bleu Springs prefix for many years. Professionally, Michelle is currently a Senior Public Relations
Associate with the Seeing Eye, a non-profit organization that breeds, raises and trains dog guides for people who are blind and visually impaired. With five years experience in public relations for the American Kennel Club, Michelle has also worked closely with organizations like the Canine Health Foundation.

Michelle will focus on (1) furthering ongoing work to collect genetic material from affected dogs and their close relatives and (2) strengthening available data on TMT heart function by increasing physical screening. Individuals wishing to submit information on deceased puppies have several options. They can contact Michelle at michelle@bleusprings.net or 585-329-4317. As always, necropsy results can also be submitted anonymously by contacting Dr. Shannon Martinson, Diagnostic Pathologist at the Atlantic Veterinary College (smartinson@upei.ca or 902-566-0864). Welcome, Michelle!

Genetic Analysis Complete

In this issue of Black & Tan magazine you will find the results of our eight-month study into the genetic basis of cardiomyopathy. Dr. Hare and Ms. Weaver's work has provided compelling evidence based on objective information that the disease is most likely genetic and they have also provided advice on useful breeding strategies. At the risk of editorializing, I believe one additional strategy we must employ is the maintenance of some "cardio-free" lines. While it is certainly smart to breed dogs who are related to cardio producers to non-cardio lines in order to reduce the risk of producing the problem, this approach will only work so long as there are such lines to go to. It may behoove us to think about how we might be able to accomplish both goals in a sustainable way.

Study Progress

Dr. Carolyn Legge is in the process of completing her thesis on "Dilated cardioymopathy in the Toy Manchester Terrier". Dr. Legge's Master's degree has been entirely devoted to a study of the tissues of Toy Manchesters that have died of cardiomyopathy. Her analysis of the disease at the tissue level will be the first complete description of this form of heart disease. Publication in a peer-reviewed scientific journal would represent the official discovery and recognition of the disease. This is the first step in understanding how a disease "behaves" and then how to go about detecting and preventing it.

In June, Dr. Etienne Coté, our lead cardiologist, presented some of his preliminary findings internationally at the American College of Veterinary Internal Medicine Forum in Anaheim, California. The forum was attended by approximately 3,000 veterinarians from around the world. Dr. Coté also completed work on the second edition of his widely used textbook, Clinical Veterinary Advisor: Dogs and Cats (St. Louis, MO: Mosby Elsevier, 2011). While mention of the disease is quite short, its presence is very significant given the popularity of the book as a reference tool among new and practicing veterinarians alike.

Over the coming months it is our hope to screen more relatives of dogs that had cardiomyopathy, as well as healthy, unrelated TMTs without sudden death in their family lines. Any dog can have a cardiac exam, which takes a couple of hours and is done on an outpatient (same-day) basis. The exam involves an ultrasound scan (echocardiogram) by a board-certified cardiologist, a blood sample, an EKG, blood pressure measurement, and a short exercise period conducted while the dog is wearing a portable heart monitor. These tests can be performed at a discount at the Atlantic Veterinary College (Charlottetown, PEI), and interested TMT owners and breeders should contact Michelle.

Fundraising

As many will know, we initiated an aggressive fundraising campaign this year in order to raise moneys needed to fund the genetic analysis completed by Dr. Hare. The response was amazing! In less than six months, we have succeeded in raising almost $5,000 — enough to not only pay for the initial project but to also support continued work on physical screening. You will find a small thank you on the following page, which was put together with great trepidation as my biggest fear is missing someone (if I have, sincere apologies in advance)!

With the above in mind, I will close with a thank you to those who have supported our work over the past few years. We have accomplished a lot!

- We are nearing completion of Phase I and will hopefully soon have a paper ready for publication that characterizes and firmly establishes the disease;
- We have completed an initial analysis of genetic influences;
- We have fully screened several TMTs and begun the process of establishing a foundation of knowledge regarding heart function in our breed;
- And, we have collected and stored several DNA samples from affected puppies, which will be invaluable in future work to identify a genetic test.

Most important of all, in my eyes, we have as a breed (mostly) moved from being skeptical about whether or not this condition exists to working together to address it. And that, in the end, is all we can really do. With Michelle's addition to the team I will be stepping back and enjoying a much needed rest—something I'm sure my dogs will enjoy!

“Have A Heart”
By Artist Beth Rutherford

Thank you!

Because of the generosity and support of Manchester Terrier and English Toy Terrier breeders around the globe, we were able to raise almost $5,000 this year to support research into Cardiomyopathy in Toy Manchester Terriers. These funds have already benefitted research into the genetic basis of the condition and will be used to support an expanded physical screening project in the coming months.

Please join us in thanking those who have generously contributed over the past few months!

Canadian Manchester Terrier Club
American Manchester Terrier Club
Shay Lockhart & Co-workers at Chintz & Company
Black & Tan Magazine
Cynthia Sytnyk & Bev Clark
Janna Morgan, Evrmor Toy Manchester Terriers
Dianna Texter & Karen Cornell, Bayside TMTs
Betty Hodges, Rosewood Toy Manchester Terriers
Rodney Herner, Renreh Toy Manchester Terriers
Blossom Scott-Heim, Blossom's TMTs
Jim Burrows & Pat Mackesey, Burmack MTs
Paige Saunders, Yurrugar English Toy Terriers
Karen & Rex Bristol, Aquarius TMTs
Pennyann Styles, McLean's Toy Manchester Terriers
Irene Hetrick, Trinity Toy Manchester Terriers
The Heisler Family
Patti Taylor, Blackrystal Manchester Terriers
Wendy & Amanda Kelly, Fwaggle TMTs
Carol Henning, Rumor's Toy Manchester Terriers
Ed & Jeanette Tonini, Di Bragano Manchester Terriers
Carolyn & David Horowitz, Rustic Lane MTs
Deanna & Ted Bettle, Deebet Toy Manchester Terriers
Kirsti & Eirik Kahrs, X-Pected Dine Mite MTs & ETTs
Aiko Shinseki, Aiko's Toy Manchester Terriers
Jes & David Kemp, Dixieland Manchester Terriers
Melissa Doldron
Michelle Barlak, Bleu Springs Toy Manchester Terriers
Robin Gates,
Judy & Gary Anderson, Oasis Manchester Terriers
Mary Gonzalez, Kismet Manchester Terriers
Bonnie Paul, Spirit Toy Manchester Terriers
Lisa Nonog, Jetcity Manchester Terriers
Don & Pat Reid, Reid Manchester Terriers
Jessica & David Kemp, Dixieland Manchester Terriers
Marianne Ona Hansen, Historical Diamond's ETTs
Dave Barrett, DXB Photos
Sarah Leigh, Mansiya Manchester Terriers
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Lorrie Jollimore
Diana & Bob Jones, LSKAM Manchester Terriers
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Yvonne Frecker
Jennifer Dugovich
Mary Munizza
Regina Allen, Regal Toy Manchester Terriers
Rachel Kanarek

Thanks are also extended to the staff and contributors of Black & Tan magazine, whose willingness to share has helped us not only raise needed funds but also disseminate important study information.