THE CARDIOMYOPATHY CHRONICLES 1: THE SCIENCE NEWS AND GENETICS...

SCIENTISTS DETERMINE MODE OF INHERITANCE FOR DILATED CARDIOMYOPATHY IN DOBERMAN PINSCHERS

By Rod Humphries

While the hunt continues for the gene or genes which cause dilated cardiomyopathy (DCM) in Doberman Pinschers, a team of scientists led by one of America's leading geneticists, Dr. Kathryn Meurs, has determined the mode of inheritance which will dramatically impact the breed.

In a scientific study published in the September-October 2007 issue of the Journal of Veterinary Internal Medicine, Dr. Meurs announced that an eight year study involving 41 Dobermans in four generations of one extended family – a shocking 25 of 35 able to be fully diagnosed had DCM— proved that the mode of inheritance is **autosomal dominant**.

By definition, this mode expresses the disease in a dominant gene, not a recessive, and therefore has no classic "carrier" as in autosomal recessive mode. It needs only one affected parent to transmit the disease and without exception there are affected dogs of either sex in consecutive generations. Two affected parents can produce some unaffected offspring

The dramatic impact for Doberman breeders – many of whom believed DCM had "carriers" in the autosomal recessive mode — is that **a single animal affected with DCM will, regardless of the genetic status of its mate, transmit the disease to between 50 and 100 per cent of its offspring**.

As autosomal dominant needs only one dominant mutant gene to transmit a disease or trait, it is also expressed in the heterozygous state (Heterozygous means the gene pair for a disease or trait is not matching. In this case there is a mutant gene for DCM and a normal, clear gene). Therefore:

- **A heterozygous animal is affected with DCM and even when bred to an unaffected (clear) mate will transmit the disease to 50 percent of its offspring.**
- **An animal that is homozygous dominant (two matching mutant genes for DCM) will transmit the disease to 100 percent of its offspring regardless of the genetic status of its mate.**
- **If two heterozygous (affected) parents are mated, 75 per cent of the offspring will be affected and 25 per cent unaffected. (All the percentages are true over a large sample size. See the Punnett Squares and genetic breakdown later in the article).**

These high transmission rates clearly show why the Doberman has the highest incidence of DCM in the dog world. It has been estimated that Dobermans likely have more DCM than all other breeds combined.

Normally autosomal dominant traits are easy to eliminate because there is no "carrier" with a hidden recessive gene and the disease can be halted by simply not breeding affected animals. But adult late onset diseases such as DCM in Dobermans make control without a DNA test extremely difficult because almost all affected animals are not diagnosed until after the peak breeding years.

There are major complications because many affected dogs fail to be diagnosed at all when they die of some other cause, or sudden death at 10-plus years is misread as simply old age – factors which have hidden the true spread of the disease in Dobermans and have given a false sense of security for many breeders.

Over time there has evolved a dangerous level of acceptability – maybe even dodging responsibility –by some breeders who think it is alright if a dog dies of DCM at 10 or more years. But those which die at 10-plus years are producing more and more dogs which die at three, four and five years.

DCM deaths in Dobermans have been reported from 2.5 to 14.5 years with 70 percent in the range of 6 to 10 years. Dr. Meurs said the median age for diagnosis in her study was 7.5 years.

"An autosomal dominant mode of inheritance was defined by the appearance of the disease in multiple generations, equal gender representation, and evidence of male to male transmission. Finally, the mating of two affected animals produced unaffected dogs," the study reported as part of the evidence that nailed down the mode as autosomal dominant (see the published geneticist's pedigree chart from the study).

Meanwhile, Doberman breeders will still have to wait for discovery of the deadly gene or genes in the canine genome which would eventually lead to a DNA based screening test to help completely eradicate the disease. In concluding the study, Dr. Meurs

simply declared: "the causative gene(s) responsible for this condition remain unresolved."

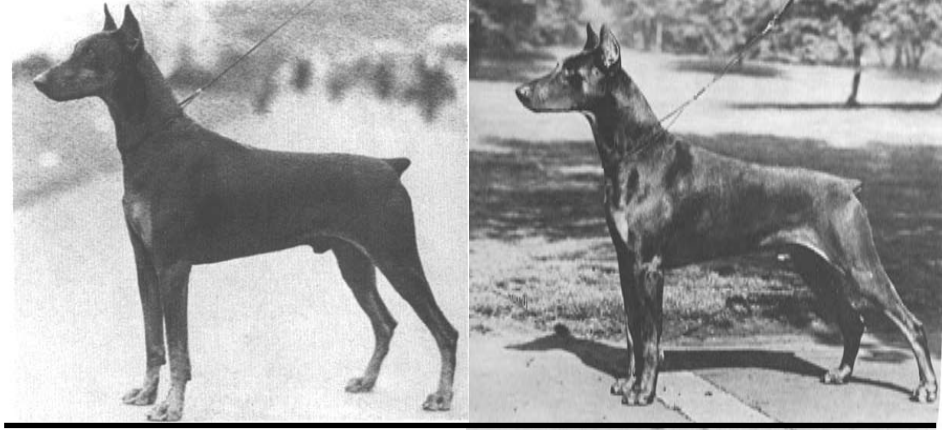
Dr. Meurs, who has a PhD in genetics and heads the Department of Veterinary Clinical Sciences at Washington State University, told me in an interview that she believed the eventual discovery would likely be a single primary gene with an unknown number of "modifiers." Modifiers are also genes which can change the expressivity of the disease and cause variable manifestations in affected dogs.

The study also noted that the "penetrance of DCM in the Doberman Pinscher is unknown, but it is incomplete in human beings." Incomplete penetrance means that a dog with the mutant gene (who would be classified as "affected") would not contract the disease. Because it does not conform to normal theory it can prove tricky for those trying to track a disease in a family of dogs.

So the undiscovered primary gene, undiscovered modifiers and the possibility of incomplete penetrance, underscores the daunting task in the search in the canine genome. (See genome sequencing later in this article).

From my telephone interview and an exchange of emails, it was obvious that the discovery of the gene(s) is unlikely in the foreseeable future. Obviously other scientific teams around the world are working on gene discovery, but when I asked Dr. Meurs about a timetable on finding the gene(s) responsible for dilated cardiomyopathy in Dobermans and Boxers, she was quite adamant that she could not, and would not, put a timetable on it.

"...I could make a breakthrough on any of these diseases tomorrow (hope so) or it could be a decade away (hope not)," she wrote. "This is the type of thing where we work on it every single day and it could be tomorrow that we find a mutation or it could be much longer…it is not the type of thing that you can see in the distance and say 'oh, we are really close, we need a few more months.' I know that it is very dangerous to tell people an estimated time since it is never correct and leads to disappointment," she added.



Ch. Emperor of Marienland and Ch. Dictator von Glenugel, two of the Seven Sires. According to the study, three of the Seven Sires are reported to have died of cardiac disease.

I reminded Dr. Meurs of one announcement in the scientific community in the late 1990s in which it was declared that the discovery of the DCM gene in Dobermans was less than two years away. "You noted a perfect example of why I do not tell people a date. I remember that as well in the 1990s and actually had been at some meeting in 1997 or 1998 when they said it would be two months! The fact that we both remember this so well is exactly why I NEVER give time frames until I find something," she wrote.

Dr. Meurs said that in a genetic search nothing is "black and white." In the study report she pointed out that it may be possible for different families of Dobermans to demonstrate a different mode of inheritance which is the case in humans. Obviously that statement was meant to leave the door cracked open in case of an obscure incidence somewhere in the breed.

But after speaking to Dr. Meurs and reading her study it is obvious that she and her team of six other experts have absolutely no doubt that the Doberman Pinscher has DCM inherited by an autosomal dominant mode of inheritance. She wrote in the study that an alternate mode "would seem unlikely given that the Doberman Pinscher is a pure bred dog with a closed gene pool at least in registered dogs. Therefore it is more reasonable to assume that most of the affected dogs in this breed would share a pattern of inheritance."

The study pointed out that the Doberman Pinscher is a relatively new breed established in the late 1890s in Germany and that the gene pool of the American breed mostly emanated from seven closely related sires in the 1940s (Ilena and the Seven Sires, author Peggy Adamson) which were immediate descendents of German stock prior to the Second World War. "Three of these dogs are reported to have died of cardiac disease," the study said.

I have written extensively in the past about Ilena and the Seven Sires and their dramatic effect on DCM in the breed (also see accompanying "Rod Humphries Writes" article) and it is understood that a fourth sire died of a heart attack at 10 years of age and that at least one other may have had the disease.

"The proband (the primary affected female studied) in the family presented here is a direct descendent of one of these dogs," the study said.

Dr. Meurs also noted that the familial nature of the disease and the autosomal dominant form of inheritance "supports the observation that genetic disease may occur with high frequency in populations with closed gene pools and in which breeding of close relatives is used to propagate desired traits. Breeds established from a small number of founders and expanded rapidly are thought to be particularly susceptible."

Historians know that the Doberman breed was established on a handful of dogs in Germany and inbreeding was an important part of the explosion in Germany and later in the United States. Dogs from these countries were exported internationally and the disease has a high incidence throughout the Doberman population worldwide.

Dr. Meurs underscored the major problem facing Doberman breeders: the late onset of the disease. "The determination of the affected and unaffected phenotype in the present study was challenging due to the adult onset nature of DCM in this breed," the report said.

"All dogs in the present study were prospectively evaluated on an annual basis by both echocardiography and ambulatory electrocardiography (Holter monitor). Some dogs died before reaching the age of disease onset and were unavailable for post mortem evaluation. These dogs were classified as indeterminate.

"The number of dogs whose clinical status could not be definitely defined is a limitation of this study as well as others that study adult onset diseases in natural animal populations. Although careful attention was paid to reevaluation of all dogs on an annual basis, it is impossible to control premature loss of animals (from the study) as a result of death from other causes."

"Early identification of affected animals would allow for exclusion of these dogs from breeding programs and would allow early medical intervention, although it is not yet known if this would have a significant impact on survival. There is significant interest in the identification of the causative gene for Doberman Pinscher DCM and the development of a DNA-based screening test," she said in the report.

The study noted that the findings could aid in the search for the DCM gene. The study said that, "narrowing the list of candidate genes might be accomplished by determining the mode of inheritance."

"This would allow the exclusion of certain genes associated with specific modes of inheritance. In addition, the list could be more focused by performing linkage analysis to identify a statistical relationship between the disease and specific genetic regions of a gene. The objectives of this study were to prospectively evaluate an extended family of Doberman Pinschers with DCM to determine a pattern of inheritance and to perform genetic linkage analysis to identify a region of the canine genome that is linked to the disease. Ultimately, the region linked to the disease could contain important cardiac genes that could be evaluated as possible causes of DCM," the study added.

The study also noted that a similar form of cardiomyopathy exists in humans. "Familial DCM in human beings can be inherited in an autosomal dominant, X-linked, autosomal recessive or mitochondrial pattern, but the autosomal dominant form is reported more frequently," she wrote.

"...genetic disease may occur with high frequency in populations with closed gene pools and in which breeding of close relatives is used to propagate desired traits."

"Although in many of these cases the specific genetic mutation has not been identified, causative mutations have been identified in 24 genes, including actin, dystrophin and desmin among others.

"The majority of these genes encode for cytoskeletal proteins that have important structural functions in the cell including maintaining structural integrity, preserving cell shape, organizing the contractile apparatus, and enabling the cell to withstand mechanical stress.

"It has been suggested that an abnormality of a cytoskeletal protein may be a common factor in the development of DCM and that without the structural support provided by these proteins, a dilated, dysfunctional heart develops. Mutations within different genes, or within different areas of the gene, may result in different clinical manifestations and survival times," the study said.

The study reported that information about the human form of familial DCM can provide insight into the Doberman Pinscher disease. "A candidate gene approach can be pursued by evaluating the genes known to cause the disease in human beings as candidates for the Doberman Pinscher disease. However, because there are now 24 different genes known to cause familial DCM in human beings, the candidate gene approach would be quite time consuming. Narrowing down the list of candidate genes might be accomplished by determining the mode of inheritance," the study said.

A total of 41 dogs over four generations starting with an affected bitch were evaluated annually with a physical examination, 24-hour ambulatory electrocardiogram (holter), and an echocardiogram (ultrasound). Postmortem cardiovascular evaluation was also performed where possible. The team evaluated the pedigree and collected DNA samples from all dogs to perform simulated linkage analysis using special software.

Of the 41 dogs, 25 were classified as affected, 10 as unaffected and six were undetermined.

Of the 25 affected with DCM, 14 were males (three castrated) and 11 were females (four spayed). The unaffected group included 8 females (five spayed) and two intact males, while the undetermined group included 4 females (2 spayed) and two males, one intact and one castrated.

Also involved in the study with Dr. Meurs were Drs. Philip R. Fox (Animal

Medical Center, New York), Michelle Norgard (Washington State University), Alan W. Spier, Allison Lamb, Shianne L. Koplitz and Ryan D. Baumwart (all from Ohio State University); Dr. Spier is now with the Florida Veterinary Specialists and Dr. Koplitz is now affiliated with Wisconsin Veterinary Referral Center. Funding was supplied by the AKC Canine Health Foundation and the Morris Animal Foundation.

A Close Look At The Disease In Dobermans

In medical terms, "dilated" means the enlargement of an organ; "cardio" means heart and "myopathy" refers to a disorder of any muscle or muscle tissues. Dilated cardiomyopathy is therefore a heart muscle disease. Dr. Meurs characterized it as "myocardial (heart muscle) dysfunction, cardiac arrhythmias (abnormal heart rhythm), and congestive heart failure." It begins with erratic heart rhythms and can be followed by enlarged heart chambers; leaking heart valves; weak contractions and inadequate pumping ability which all end in certain death.

There are two recognized manifestations of the disease which always proves fatal: (1) sudden death without clinical warning and (2) a slow deterioration with congestive heart failure. It has been reported that for every dog which dies of congestive heart failure, there are five or six which die suddenly.

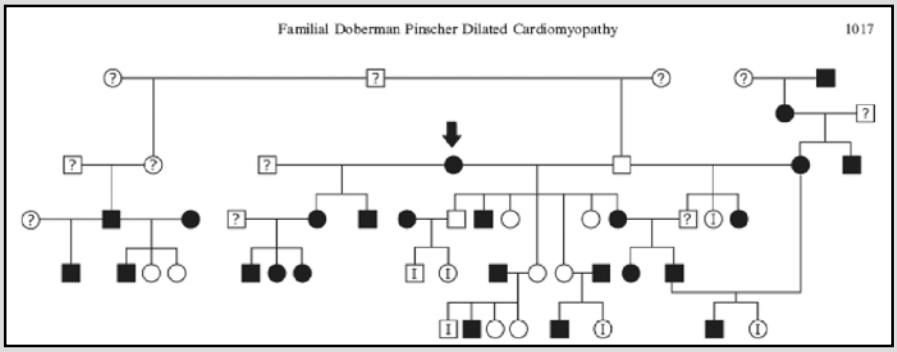
As mentioned earlier, medical reports have placed the death range at 2.5 to 14.5 years with the highest percentage – about 70 percent — from 6 to 10 years. Dr. Meurs said the median in her study was 7.5 years at diagnosis. I have read unscientific studies which place the Doberman's average life expectancy at between 8.5 and 9.2 years which fits the parameters of the DCM studies.

Dr. Clay Calvert, an award winning scientist who pioneered much of the research of dilated cardiomyopathy, especially in Dobermans, explained the two manifestations in a published article, saying that it all starts with a disturbance of the heart rhythm resulting from instability of the linings of individual heart muscle cells.

Dr. Calvert said that in some dogs the heart rhythm disturbance is severe from the beginning and results in sudden death. He said that the heart rate becomes very rapid – usually over 350 beats per minute – causing cardiac arrest.

When a dog collapses or faints the heart rhythm is severe, but not to the point of causing death. There is a third scenario called "occult," or undetected heart rhythm disturbance, which is less

The Genetic Pedigree Chart Published in the Study



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The text accompanying the chart included: "Pedigree from a Doberman Pinscher family with dilated cardiomyopathy and an autosomal dominant mode of inheritance. Mode of Inheritance was determined by the appearance of the disease in multiple generations, equal gender representation and evidence of male-to-male transmission. Finally, the mating of 2 affected animals produced unaffected dogs."

Dog breeders are accustomed to linear pedigrees but geneticists work exclusively with horizontal pedigrees to track diseases and traits. Breeders should also use these in their programs. Using the symbols and lines shown above and explained below, the pedigree can be built to personal specifications for any number of family dogs.

In this pedigree, the proband, the primary studied animal, is indicated with an arrow; circles represent females and squares represent males; horizontal lines link mated animals; vertical lines represent the offspring. Blackened symbols indicate a dog affected by DCM, while open symbols show clear animals. The letter "I" inside a symbol classifies that animal as "indeterminate." A question mark represents animals "not available for evaluation."

For example, the proband was bred to two males, one clear and the other unavailable for evaluation. The clear dog was also bred to an affected female.

In an autosomal recessive pedigree, symbols for a "carrier" would be half shaded or contain a simple "dot." Geneticists will often strike a line through a symbol to indicate a deceased dog. Others will number dogs; place the year next to mated animals; some place symbols within the symbols to represent structural parts, etc. If a pedigree overflows a page some will place a particular symbol for a female and link it with a similar symbol to start a following page.

severe and causes no immediate symptoms. Dogs which collapse or faint or have the occult form eventually die of congestive heart failure.

"Sudden death most often occurs in apparently healthy, active, vigorous dogs that have had no prior evidence of heart disease. Affected dogs drop dead, without warning, while exercising or while at rest. In some instances death occurs during sleep. Affected dogs may suddenly collapse and die while eating, walking in the house or yard, or while playing, retrieving, or running vigorously. In some instances, the dogs are observed to appear normal one minute and are then discovered dead a few minutes later. Affected dogs may cry out once when they collapse and then gasp a few times. It is important to note that affected dogs manifest no signs of disease prior to sudden death," he wrote.

"Congestive heart failure results from gradual deterioration of the heart muscle. This process of deterioration leading to enlargement and weakness of the heart occurs over an unknown period of time, but which is at least 15 months in duration and probably longer. Outward signs of heart weakness, however, occur only during the end stage of the disease. As the heart muscle becomes weaker, less blood is pumped into the system and a decrease in exercise tolerance eventually occurs. At the same time, since less blood is pumped into the system, blood begins to back up into the lungs, which leads to lung congestion, causing coughing and difficulty breathing – congestive heart failure."

It is also this writer's experience that there is a fluid buildup in the abdomen (ascites) and, because the heart can no longer provide sufficient blood to the brain, animals sometimes become disoriented, suffer fainting spells (syncope) and bump into objects.

Dr. Calvert said these clinical signs usually develop over a period of several weeks, "However, subtle decreases in exercise tolerance or activity, mild coughing, and mild difficulty breathing are not always observed by the owners…thus, the owners sometimes are aware of these problems only for one or several days prior to seeking help from a veterinarian. Weight loss of 5-15 pounds usually occurs within several weeks following the onset of coughing or difficulty breathing."

Dr. Meurs said in her study that the disease in the Doberman "appears particularly aggressive. There is no definitive treatment and therapy is, at best, palliative."

Traditional diagnosis of the disease has been done by stethoscope; chest X-rays; echocardiogram (ultrasound) and electrocardiograms (EKG or ECG which records electrical activity). In more recent years, the holter monitor which is strapped to the dog for a 24-hour EKG analysis during an animal's normal activity, has proven to be very effective in detection.

The problem for breeders has long been that all these methods – apart from being expensive — need to be performed every year because they do not necessarily pick up the early stages of the disease. Potential breeding stock may not have the disease diagnosed until four, five, six, or even more years down the road which can sometimes be way beyond the peak breeding years.

There is news of a blood test which the manufacturer claims has potential for early detection. It is called the "BNP test," which measures brain naturetic peptide. It was first used in humans and has been modified for canines.

Dr. Jeff Grognet, announcing the test in the October issue of the AKC Gazette, wrote: "If the heart is struggling to pump blood forward and the ventricle is enlarging beyond normal limits, the muscle stretches and releases BNP. Thus an elevated BNP level in the blood suggests the heart is stressed. A disease like cardiomyopathy can be detected in a high-risk dog (for example, a dog whose sire developed the disease), before the dog is used for breeding."

I raised the question of the BNP test when I interviewed Dr. Meurs and she was obviously not excited about its promise as a panacea for early detection of DCM. She said she had read all the information on the test and thought it had very limited application to help breeders in their battle with DCM.

Positively identifying affected dogs is a major problem, not only because affected dogs die of some other cause and the breeder is totally unaware that he or she had a problem dog; or that affected older dogs which die suddenly are filed away as dying of old age; but because necropsies are often "inconclusive." Most of the time it is because the veterinarian is not a cardiac specialist.

DCM in the Doberman "appears particularly aggressive. There is no definitive treatment and therapy is, at best, palliative"

When I asked Dr. Meurs about "inconclusive" cardiomyopathy necropsies she was quite aware of the problem and was adamant that the heart had to be dissected and diagnosed by a pathologist who is an expert in the field of cardiology because of the intricacies of the disease and its manifestations.

When a breeder suspects that a dog which died of say, cancer, also had DCM and may impact a breeding program with that disease, it will require sending the heart to an institution, maybe some distance away, to have it properly examined by a cardiac pathologist. Dr. Meurs told me that even in her study some owners were not prepared to do a post mortem on the heart of a beloved animal.