

Mitral Valve studies in Norfolk Terriers.

Dear Fellow Norfolk enthusiasts,

In the last 2 years we have sampled nearly 100 Norfolk's for a study to see if a gene mutation could be identified that would link to dogs affected with Mitral Valve disease. For this study dogs were ultrasounded and identified as normal or affected based on the ultrasound study. Blood samples were collected for DNA analysis. DNA sequencing is a time consuming and expensive process and looking for subtle gene differences has been likened to searching for a needle in a haystack. Some diseases have simple dominant and recessive modes of inheritance and these would have a more obvious difference in gene sequencing. Unfortunately no individual gene was identified as different bringing us to the conclusion that the disease has multiple genes that are inherited to express the disease.

DR. OYAMA'S REPORT

The study's primary objective was to identify a putative autosomal dominant gene with a high likelihood to be associated with myxomatous mitral valve disease (MMVD) in Norfolk terriers. The Norfolk Terrier Club of Northern California initiated this project in partnership with the AKC-CHF. As such, the current project was carried out under a Memorandum of Understanding between the AKC-CHF and the investigators.

The mode of inheritance of MMVD is unknown. Given the high prevalence of MMVD in small breed dogs, including the Norfolk terrier, an autosomal dominant trait is possible, however, other modes such as autosomal recessive or polygenic cannot be ruled out. Based on previous recommendations (Karlsson E, Lindblad-Toh. Nat Rev Gen 2008;9:713-725), approximately 50 case and 50 control should be used in canine GWAS studies involving suspected autosomal dominant traits.

Methods specific to this project included recruiting Norfolk terriers >5 yrs of age at various dog shows and clinics. Presence or absence of MMVD was determined by cardiac auscultation and performance of a color flow Doppler echocardiogram by a board-certified cardiologist. Whole blood samples were drawn and DNA samples were extracted for genome-wide association study (GWAS). GWAS seeks to identify areas of DNA that are different between healthy and affected dogs. If DNA in these regions are different enough, they might signal that nearby genes are involved in the development of MMVD. Due to the fact that literally thousands of gene regions are evaluated by the GWAS test, the criteria used to determine if DNA areas are different are very stringent. In our case, the pre-specified definition of a chromosomal region of statistical significance was $p < 5 \times 10^{-5}$, or $P < 0.00005$. This differs from the normally used P value of $P < 0.05$.

A first batch of 72 samples, including 36 healthy dogs and 36 affected dogs was sent for GWAS in the middle of last year, and results became available late in 2015. The results of the GWAS study did not find any areas of DNA that were different enough to signal the

location of a candidate gene. These results suggest that the genetic cause of MMVD in Norfolk terriers might be more complex than a single dominant gene.

Based on the findings from the GWAS study in the first 72 dogs, it is highly unlikely that performing GWAS on additional samples would yield an area of interest. Based on our results, it is less likely that MMVD in the Norfolk breed is associated with a simple autosomal dominant trait, and other modes of inheritance, such as polygenic should be considered. The results of our study indicate that identification of a single causative gene and reduction in the incidence of MMVD in the Norfolk breed through a simple genetic screening tool and breeding program is unlikely. Next steps in identifying genetic causes of MMVD in the breed might be to continue to collect DNA samples from affected dogs that could be used for techniques including whole genome analysis that might assist with understanding a polygenic trait.

The message to us as owners, breeders and lovers of the breed is still that this disease while important to select against will not be simple to eradicate. We still need to be vigilant about testing our dogs to identify affected individuals. The obvious answers are not to breed close relations of dogs that are severely affected.

As owners there are some steps we can take to avoid cardiac disease from developing. Dental care as a lifelong commitment starting with our puppies is one of the single most important things we can do to help prevent heart disease. Bacteria from infected gums and teeth are a constant source of infection to the body which can in turn affect the valves of the heart. We should all begin tooth brushing early and make it part of our daily routine. Secondly, we all know how much our dogs enjoy eating. Make sure you discuss proper weight management with your veterinarian and keep your dog trim and fit!

Thank you to all who contributed to the cost of the study and also those who made the effort to include their dogs in the study.

Marian Shaw D.V.M.