Genetic Test Development for Inherited Eye Diseases in the Poodle.

Personnel:

University of Pennsylvania

Gustavo D. Aguirre, VMD, PhD; Principal Investigator

Leonardo Murgiano, PhD; Senior Research Investigator and Project Scientist

Jessica K. Niggel, M.Sc., Research Scientist

Anil Sigdel, DVM, PhD, Postdoctoral Researcher

The aim of this research project is to characterize genetically and molecularly various ophthalmic disease affecting different varieties of Poodle breeds. Our strategy consisted in the evaluation whether a genetic cause is the most likely explanation for the phenotype, the elaboration of a hypothesis concerning the inheritance mechanism, and then the focus on the analysis of the population genetics and inheritance of dogs selected as cases and controls for such conditions.

Our aim is to use genetic markers (SNPs) technology and whole-genome sequencing to identify a gene and causative mutation for each of these conditions, and develop and implement DNA-based tests that can identify genetically normal, affected and carrier dogs for each condition. A quick identification of carriers is crucial for a better health of the Poodle population.

Standard Poodle: Day Blindness/Retinal Degeneration

Standard Poodles can be affected by a retinal condition that leads to visual deficits in daylight, day blindness, and ultimately *complete* blindness. Through support of the Poodle Club of America Foundation, we completed our research for the condition and identified the gene and mutation responsible for the disease. This genetic variant occurs in a retinal gene and its effect has been confirmed. A DNA-based test was developed for diagnosis of affected and carrier dogs, and the project can be considered a complete success!

Our initial mapping of the disease was refined by collecting additional samples, and a larger population of affected and unaffected Standard Poodles was tested. This allowed us to reconstruct the ancestry of the cases and build a family tree identifying a common female ancestor, born much further back than expected, in the year 1888. Fine genetic marker data analyzed in light of this discovery confirms this information. In further support of this finding, we have been able to trace the pedigrees on a large sample subset in our research archives of the so-called 'Doodle' crosses involving Labrador and Golden retrievers plus other breeds as well. In all of these cases, the same common female ancestor from 1888 shared by all affected dogs. Additional, functional molecular studies were carried out highlighting the pathological mechanism, which can further help in dealing with the disease. A scientific paper concerning this issue is under preparation.

Polymcrogyria

Polymicrogyria is an abnormal brain development characterized by altered “gyri” (the ridges of the brain). Not only the “gyri” differ in appearance as compared to their healthy counterparts, but they are smaller in size, irregularly formed, and have appear in multiples. The disease causes a number of effects related to abnormal brain functionality, such as: seizures and palsy. Dogs affected with the disorder are centrally blind.

Polymicrogyria in the Standard Poodle has been recognized since our first publication in 1994 (van Winkle, T., Fyfe, J., Dayrell-Hart, B., Aguirre, G., Acland, G. and Patterson, D.: Blindness due to polymicrogyria and asymmetrical dilatation of the lateral ventricles in standard poodles. Prog. Vet. Neurol. 5:66, 1994.). The first case we detected was decades old. Additionally, it was difficult to understand the inheritance mechanism, which we initially suspected to be complex. Gathering samples was a patient work of interaction with breeders and careful analysis of any phenotype. Our most successful strategy was to select several well-phenotyped cases and base our analysis on those. As with the day blindness, we thoroughly explored the ancestry of these selected cases and we again detected a common ancestor.

The marker analysis that followed ultimately led us to map reliably an associated candidate genomic region.

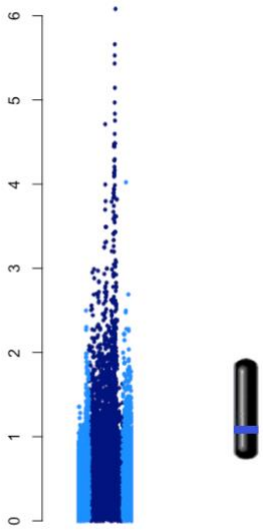

Figure 1 – the associated region detected with two different mapping methods, GWAS and homozygosity, pointed out at the same candidate canine chromosome and the same candidate region.

The region spans for approximately eight Mb and contains more than 40 genes. Whole genome sequencing was implemented to finally select a small number of functional candidate variants occurring in those genes to be tested in our population. Because of the small number of selected cases, we decided to do not exclude outright other regions. In parallel, we exploited our access to canine genomic databases to sequence the genome of additional affected dogs and added that information to our current dataset of variants to be tested and filtered.

We think that we are extremely close to the conclusion of this project and that we can soon propose a candidate marker for the condition that can, in the very least, explain severe cases and the old and sporadic nature of the condition.

Optic Nerve Hypoplasia

ONH is a congenital condition in which the optic nerve is abnormal/underdeveloped (hypoplasia) which primarily affects the Miniature Poodle variety. Depending on the degree of underdevelopment, the vision can be partially or fully impaired, leading to blindness. Almost all affected dogs are blind, and it is thought that ONH has a genetic cause in dogs (as in humans, in which the inheritance can be of variable complexity based on the specific disease form). Similarly, but less severely, Micropapilla (Mp) is characterized by a smaller than normal optic disc diameter, precisely at the point where the optic nerve enters the posterior part of the eye. Micropapilla does not appear to impair vision, at least in a clinically detectable manner. Our progress concerning this disease was more difficult than others. We suspect that the inheritance of the condition is complex as described in human and this contributes to the slower progress.

Nonetheless, our recent approach to the issue has given us new insights into the needed approach for the research. First and foremost, every time a breeder enthusiastically sent samples, we whole-genome sequenced one of those if it consisted in a severe ONH-affected dog, adding to our dataset a severely affected dog from a different family. This data is used in combination with available canine variant databases. In addition, we decided to re-genotype on a higher density (therefore higher information) SNP-chip all our well-phenotyped ONH and Micropapilla cases and most of the suitable controls. The new mapping that ensued allowed us to detect a suggestive association in a specific region.

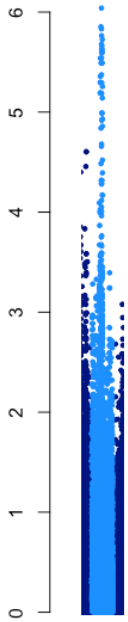

Figure 2 – ONH-associated region. The marker situation in the region is more complex than in Day Blindness/Retinal Degeneration or Polymicrogyria, but we detected a trend with a peak that is highly encouraging (that could be a part of the whole picture).

Our next move will be to (I) detect the best associated marker (or marker combination) in this region (II) carry out a whole-genome all-markers analysis looking for additional variants, in light of the fact that probably, different regions participate to the expression of this complex phenotype. The final aim is to end up with few reliable risk-factor variants suitable for testing.

Cataracts in Miniature Poodle

Early on in the project we put the cataract project on temporary 'hold' and focused on the other diseases. The main reason was that we had difficulty obtaining a suitably large population of affected dogs for study. During this 'hold' period, and thanks to the enthusiasm and contribution of the breeders, we gathered a sample pool of cataract cases in Miniature Poodle, and have started the GWAS analysis using the same mapping approach implemented for ONH. Because of the signal obtained was 'weak', we realize that additional samples and a stricter approach on our definition of the cataract phenotype will be necessary. Regardless, we are excited that we have restarted this phase of the project.