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Project PRA in Tibetan Terriers: Progress report

Personnel:

Gustavo Aguirre, VMD, PhD (5% effort)

Barbara Zangerl, DVM, PhD (10% effort)

Erika Braxton, BSc, MS (45% effort)

Objective:

Progressive Retinal Atrophy (PRA) is a hereditary retinal disease in dogs and is related to the human disease retinitis pigmentosa. There are many forms of PRA and it can differ broadly amongst various dog breeds. Tibetan Terriers (TT) are known to have an early onset form of PRA, beginning to show clinical signs around 6-8 months of age. However, at this time, it is not known which gene or genes are responsible for causing the disease in TT.

Based on our understanding of early onset retinal degeneration, 14 genes were selected that, if altered, are known to contribute to this phenotype in the dog or a comparable disorder in humans. These genes were screened utilizing linkage to nearby markers to circumvent high costs related with time consuming direct sequencing of all genes. Nearby microsatellite markers were selected and used to generate genotypes for affected and non-affected animals around the genomic location of each gene. If a gene was to cause PRA in the Tibetan Terriers, this genotype is expected to be the same in all affected animal and different from genotypes of non-affected animals.

Methods:

DNA from 33 Tibetan Terriers constituting four individual pedigrees was extracted and used for PCR amplification. Candidate genes are screened by 1-2 linked microsatellite markers. These markers are recorded based on the size of corresponding PCR products, as microsatellite alleles differ by length. Each locus was confirmed by direct sequencing.

Results:

At this point, 10 candidate genes have been excluded from causative association with early onset retinal degeneration in Tibetan Terriers due to no segregation between disease and marker alleles (See accompanying table). Three of these genes were excluded based on two markers; for another four a second marker will still be added to independently verify exclusion due to the large size of the corresponding genes. An additional two genes are currently being screened. Preliminary results do not suggest involvement of these genes with the disease; however, final results are not available at this point for analysis. Investigation of the remaining two candidate genes will continue after optimization and sequence confirmation of the corresponding markers.

Perspectives:

While no linkage between tested genes and disease in the Tibetan Terriers could be established yet, we still have 2 candidate genes left to test. In the event that any of them are linked, sequencing will immediately be extended to the complete gene in 2 affected and 2 non-affected dogs to identify the underlying mutation. The mutation then can be tested for segregation in all available animals.

If none of the candidate genes causes disease, it has to be expected that the responsible gene is not known, or not known as the cause for early retinal degeneration. In this case, the complete genome has to be scanned to identify the genomic area harboring the disease relevant gene.

Single nucleotide polymorphisms (SNPs) are DNA point variations that vary between individuals or groups of individuals and also can serve as markers. SNP chips are derived from whole genome sequencing of, in this particular case, the dog, and contain a catalogue of potential SNPs for the species. Several critical individuals for such an undertaking have already been identified from the available pedigrees, and DNA was purified for testing on the SNP chip. While the wealth of information accumulated through such an approach require considerable more specialized statistical analysis, the well kept background information on animal pedigree and disease status promises an excellent basis to identify new candidate genes, which subsequently will be sequenced.

Future direction and needs:

The most optimal approach to follow up on this project is a genome wide association using SNP chips. However, this is a costly procedure that has an approximate cost of ~\$400-500/chip/dog. This includes all the computational work needed for analysis. Use of this unique resource at the University of Pennsylvania would be greatly facilitated by having additional samples from unrelated affected dogs of the right age (diagnosis at 8m-1 year of age), plus samples from their unaffected parents. A minimum of 5 additional affected dogs would be optimal at this time.

Until the additional financial resources and additional affected dogs are available, we plan to continue the research by testing additional candidate genes known to cause early onset inherited retinal degeneration.