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Project PRA in Tibetan Terriers: Progress report

Personnel:

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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.

Previously, four pedigrees have been assembled; each producing at least one affected animal. These pedigrees suggest that the disease is inherited in a recessive manner, expecting affected animals to only have one distinct genotype associated with the disease phenotype, which differs from non-affected animals. Microsatellite markers near 14 genes known to contribute to early onset retinal degeneration in dogs or a comparable disorder in humans were screened for such association.

While microsatellite markers differ between individuals by size, single nucleotide polymorphisms (SNPs) are DNA point variations that vary between individuals and also can serve as markers. A chip became available through Affymetrix, containing 127,000 of these SNPs spread throughout the genome, and subsequently was used to perform a whole genome association (WGA) study rather than screening individual genes. Five affected, and ten non-affected control animals from these pedigrees were genotyped on the SNP chip to identify regions in the genome potentially harboring candidate genes for this disorder. Once such a gene is identified, a more time and cost consuming sequencing

approach can be used to identify causative changes for the early onset retinal degeneration in Tibetan Terriers.

Methods:

Microsatellite studies

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.

Whole genome association

DNA from 5 affected and 10 non-affected Tibetan Terriers was genotyped on the 127k SNP chip according to the manufacturer's protocol. After excluding uninformative markers and those that did not perform well, a total of 58,601 markers distributed throughout the genome was used to perform an association test between the observed genotype and phenotype.

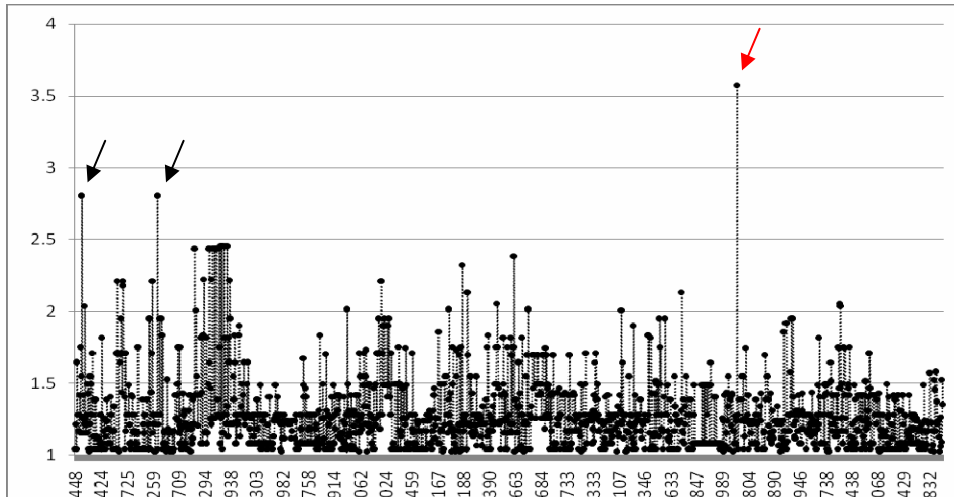
Results:

At this point, 12 candidate genes have been excluded from causative association with early onset retinal degeneration in Tibetan Terriers due to no segregation between disease and microsatellite marker alleles for one or two markers. Of the two remaining genes, the marker for one was not informative, while the markers for the other gene did not work with the chosen experimental method. Additional efforts in this direction were put on hold pending the outcome of the whole genome association studies.

For WGA, the first analysis of the SNP markers did not reveal association with any of the regions harboring previously selected candidate genes. Thus, we conclude that the retinal degeneration in the Tibetan Terrier may be caused by a gene not yet described for this disease phenotype. The highest signal is observed on one chromosome (Figure 1, red arrow), and additional peaks are located on two other chromosomes (Figure 1, black arrows), indicating at least four known genes within these regions. These genes are now investigated for their potential to contribute to the disease phenotype.

Figure 1

Log₁₀ *p-values* for WGA indicating genomic regions potentially associated with the retinal degeneration phenotype in Tibetan Terriers.



Perspectives:

No linkage could be established between known candidate genes or regions for early onset retinal degeneration and the disease in the Tibetan Terrier. Thus, likely this disease is caused by a gene not yet described to contribute to this disease phenotype.

A whole genome wide association study has been started, indicating regions potentially harboring such a gene. These genes, while not intuitively likely candidates, are now investigated for their potential to cause such a disease. Additional statistical methods will be applied, and any findings will be extended to all animals of the current pedigrees to narrow the search to the most likely candidate genes. Such genes will subsequently be sequenced for causative mutations leading to the Tibetan Terrier retinal degeneration phenotype.

Future direction and needs:

While an extensive data set has been accumulated, additional computational support is needed to perform more complex statistical analyses. These would require additional funds to support either a part time biostatistician or acquire the respective help

through a collaborative effort or core facility at the University of Pennsylvania or at another institution.

Verification and further refinement in the search for new candidate genes 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.

At this point, all funds donated for this project have been utilized in the costly GWA studies and preliminary bioinformatics analysis. We plan to pursue investigation of the indicated genomic areas, and extend analysis to additional disease-informative dogs as additional financial resources and samples become available.