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Purpose of the research:

Purebred dogs have become an invaluable research tool by providing populations for mapping genes for morphological and behavioral traits as well as genetic disorders. The diversification and inbreeding of dog breeds has led to an enrichment of certain genetic disorders in particular breeds. In addition, many common, inherited human disorders have been identified in the purebred dog population. Dog populations are similar to an extreme version of geographically isolated human populations. Theoretically, this allows complex diseases to be more easily mapped in dog rather than human families In the past five years, enormous advancements have been made in canine genomics, beginning with complete Radiation Hybrid (RH) maps and culminating in a draft assembly of the full genome sequence. The combination of this data, with the development of new high throughput genotyping technologies, makes it now feasible to conduct comprehensive scans across the entire canine genome for cancer susceptibility genes.

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Segregation studies from the 19`90s suggest a multigenic mode of inheritance. We are collaborating with a French research laboratory (Dr. Catherine Andre's group at the University of Rennes), and the segregation analysis of their BMD pedigrees supports this mode of inheritance.

Our goal is to define the precise genetic variants that cause this devastating disease. Our initial work has been in the BMD where a cooperative breed club has provided substantial number of high quality samples. This work has implications for all breeds affected by this disease. Our long -term goal is to produce the information needed for genetic tests to be developed. Though rare, histiocytic disorders are also diagnosed in humans, and it will be important to determine if the genetic variant found in the BMD is similar in human patients affected by this disorder.

Scientific program:

Samples Collected to Date by Ostrander Labs

To find genes affecting susceptibility to MH, the Ostrander lab has undertaken a whole genome association study (WGAS) using a large population of affected BMD cases and unaffected, aged BMD controls. Both cases and controls are selected so as to be unrelated at the grandparent level. To date, the lab has collected DNA from more than 700 BMD, including 190 affected dogs and 160 unaffected controls. We are collecting current health status reports yearly for all dogs to determine if they will be placed in the affected or control groups. There is no overlap between dogs used by our group and the other collaborative group.

Whole Genome Association Study Results to Date:

We initially did a 5 cM genome scan using 55 validated cases and 120 aged controls and a set of nearly 500 microsatellite markers. Cases were any BMD dog <7.5 years of age with a validated clinical diagnosis including histological examination. Controls were any BMD dog >10 years of age or older that had not been diagnosed with any form of cancer. Markers were initially selected based on existing datasets of markers that span the genorne, and then "fili in" was done for regions not well represented in the initial scan by selecting markers from the 7.5x whole genome assembly. The data were analyzed by using the Clump, Structure and Strat software package, to take into account the effect of population substructure. These experiments allowed us to identify 3 major regions of association in the genome.

The Andre group also completed a microsatellite genome wide scan on a family of 300 BMD that includes 110 confirmed, affected MH dogs. In addition to this microsatellite scan, a second genome wide scan was done on the French family using a subset of the samples (50 dogs) with the Affymetrix version 1 64K single nucleotide polymorphism SNP chip, in collaboration with the Ostrander lab. Data were analyzed using the FastLink and Linkage

programs as well as non-parametric analysis using Genehunter and plink software. The analysis produced several statistically significant results on the same regions. Thus, both the Ostrander and Andre Labs report statistically significant results that largely overlap.

As shown in Figure 1, we recently completed a new genome wide scan using the Illumina 26K Canine whole genome SNP array. This resource provides higher fidelity and better coverage then previous marker sets and allowed us to interrogate regions of interest with greater refinement. We genotyped 115 affected dogs and 123 unaffected controls. Data were analyzed using plink and Eigenstrat software, the latter taking into account the population substructure. We obtained a very strong signal on one of the three previous identified regions, thus confirming our initial results. From this data, it appears that there is one major locus responsible for malignant histiocytosis in the generai BMD population. While the other two luci may stili play a role, particularly in familial forms of the disease, our data sugests that the encoded genes are more likely to act secondary to the primary locus, at least among affected dogs in the generai population.

Figure 1. Association Mapping for Cases and Controls, Chromosomes 1-38

Genotyping of 115 affected dogs and 123 unaffected controls using the illumina 26K Canine whole genome SNP array. Data were analyzed with plink software. Figure 1A: The\ horizontal axis represents genotyped SNPs in linear order from the top of chromosome 1 to the bottom of chromosome 38 and chromosome X across the whole genome. The -log(pValue) on vertical axis represents statistical calculation of association between allelic variant and the affected phenotype for each SNP marker. The arrow indicates the genomic location of the primary MH locus, as indicated by the most significant p-value we observed. Our best value of 10.5 means there is a probability of 1 out of 10 billion to obtain this result by chance. Figure 1B: Zoom in on the region of the genome with the major MH locus as indicated by high level of statistical significance. Several SNPs are informative and show the same trend, indicating further confidence in the result.

Our new objectives focus on finding the exact mutation responsible for the primary locus first, checking to see if this and other loci are relevant for other breeds, and reporting information to the breed clubs. That will be useful in developing tests that can ultimately be incorporated into breeding strategies aimed at reducing the incidence of MH.

Detailed Schedule of Work:

Aim I) Develop haplotypes across the chromosome regions of interest. Haplotypes are collections of alleles from sequential markers that are segregating on the same chromosome. This objective will be met by optimizing and then genotyping large numbers of SNPs selected from the whole genome assembly across regions of interest. Standard statistical programs will be used to assemble haplotypes. The Ostrander lab has extensive experience in this arena.

Aim II) Determine haplotype associated with disease status. Data will be analyzed by comparison of differential allele and haplotype frequencies to identify which haplotype is likely to contain the disease mutation. We can then compare data from affected dogs throughout the world, such as those being provided by Dr. Gerard Rutteman from Utrecht, to determine a minimal region of interest where candidate genes will be further investigated.

Aim III) Test candidate genes of interest for mutations by direct sequencing of exons, intron/exon boundaries, known regulatory elements, and multiply conserved regions. This complete analysis will allow us to identify any mutation associated with the disease. As second order experiments, Northern and Southern Blot analyses may be undertaken as needed to scan for large genomic deletions and presence, absence and size of transcripts from candidate genes. In addition, gene expression profiling may be done if necessary.

Aim IV) Extend mutation/haplotype analysis to other breeds of dog. MH ocurrs in a variety of other breeds. In some cases the presentation is slight different, and we don't yet know if the same mutations or the same genes will be responsible for the disease in breeds like the Flat Coated Retriever, Rottweiler and Golden Retriever. We will collect and test samples from affected dogs to determine this. In addition we plan to sample a cohort of young dogs to determine the frequency of the mutation in the population.

Summary:

Working with collaborators, particularly Dr. Catherine Andre (Rennes-France) and Dr. Gerard Rutteman (Utrecht-Netherlands) we are using complementary but distinct approaches to tackle the problem of malignant histiocytosis. Dr. Rutteman is providing samples, while our lab and that of Dr. Andre are working on the genetics. Dr. Andre had collected a high risk French BMD family and completed genome wide scans using both microsatellite markers (In the Andre lab) and SNPs, done in Ostrander lab. I have led the efforts to do a whole genome association study in the Ostrander lab using BMD cases and controls collected largely in the United States. This study is distinct in that it samples the general population to determine what locus or loci are responsible for the disease in the breed population at large. We have used both microsatellites and SNPs- based markers for our work.

Our initial results from the whole genome association study identified a subset of the same genomic regions as did Dr. Andre's family study, providing statistical significance for these loci and ensuring that they are highly likely to contain MH susceptibility genes. Since then, we have used a new Illumina SNP genotyping technology to further refine our analysis and identified a single major susceptibility locus. Our new objectives focus on finding the exact mutation responsible at the primary locus first, checking to see if these loci are relevant for other breeds, and getting the information out to the breed clubs. Subseqeunt studies will focus on the minor loci.