**Report to Leonberger Health Foundation**

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**Expanding the Repertoire of Genetic Risk Factors for Osteosarcoma:**

**Mapping in Leonbergers and Golden Retrievers and Meta-Analysis Across Breeds**

**Original Research Goals:**

1. Perform the meta-analysis across osteosarcoma breeds including Leonbergers
2. Use resequencing and bioinformatics analysis to discover candidate mutations in associated regions
3. Genetically and functionally validate candidate variants in associated regions

**Current Progress**

We have been making progress towards these goals, primarily by generating data and quality controlling the data. Our progress is as follows:

**Sample Collection & data generation**

Again, very few new samples were submitted to the Broad during the past year. However, to compensate for this we have been working with our collaborators to assess phenotypes and put together the best joint data set between our and their samples. Between us we now have 128 Leos diagnosed with OSA and with GWAS data and 92 dogs with HSA as well as 156 old healthy controls with GWAS data. After having performed careful QC we are currently in the process of doing a Genome Wide Association Study (GWAS) analysis and would expect to get results in the next few months.

We have also generated whole genome sequencing data from 12 Leos 6 controls, 3 OSA dogs and 4 HSA dogs. This data is currently being processed and quality controlled.

**Genome Wide Association Studies**

1. **Osteosarcoma**

Our preliminary results suggest strong population stratification and unusual allele frequency patterns around several association signals. However, we hope to be able to resolve this now that we have more data at hand and are using only very strict phenotypes. By examining these results together with the whole sequencing data, we will try to understand these unusual patterns, to identify more OSA risk loci, and to gain a better understanding of the genome structure of the breed. A lot of this work will be done in the next few months.

1. **Hemangiosarcoma (HSA)**

Preliminary results indicate possible associated regions on two different chromosomes—but the results are still rather unstable and we would love to have more HSA cases to genotype and include in the analysis.

Preliminary analysis of minor allele frequency indicates areas of lower variability near several loci found to be associated with HSA in the Golden Retriever. This may indicate that some HSA risk factors are fixed in the Leonberger breed.

**Genome Wide Association Study Meta-analysis**

We are waiting to perform this analysis because of the strong population stratification identified in the separate GWAS analyses.

**Resequencing to find mutations in osteosarcoma and hemangiosarcoma GWAS regions**

We have whole genome sequenced 4 HSA cases, 3 OSA cases and 6 controls and have started to process the data. We expect to be able to use this data to identify candidate mutations in the associated loci.

Wish list:

To make our work easier two things are on our wish list:

* more well-diagnozed HAS cases would increase the power of the analysis
* some more funding to pay for the graduate student working on the project.