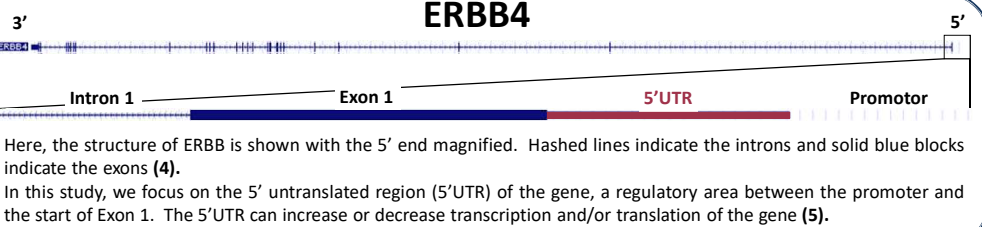


A Haplotype of the ERBB4 5'-Untranslated Region is Associated with Death from Cancer in Golden Retrievers

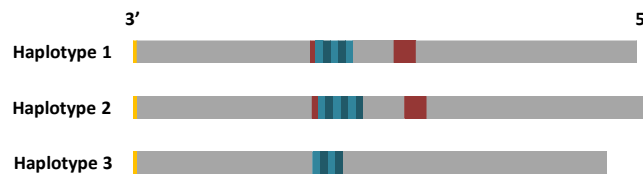
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Introduction

Although cancer is the most common cause of death in the majority of dog breeds (1), Golden Retrievers appear to be particularly hard-hit by cancer, with one study finding cancer was the cause of death in 65% of Golden Retrievers who underwent necropsies at the UC Davis Veterinary Medical Teaching Hospital (2). A previous GWAS study comparing long-lived Golden Retrievers with Golden Retrievers who died of cancer before old age found a region of genome-wide significance directly over ERBB4, a large 1MB gene that is part of a family of growth factor receptors that appear to be involved in several different cancers (3). Whole genome sequencing of 7 Golden Retrievers from the GWAS study revealed a regulatory region of ERBB4 with haplotypes that segregate with cancer status, the 5' untranslated region (5'UTR). An initial study by Dr. York identified three common haplotypes (versions) of the 5'UTR by Sanger sequencing of 26 Golden Retrievers. This study seeks to determine whether there is any association between 5'UTR haplotype and death from cancer in a larger sample of Golden Retrievers.



Haplotypes of the ERBB4 5'UTR

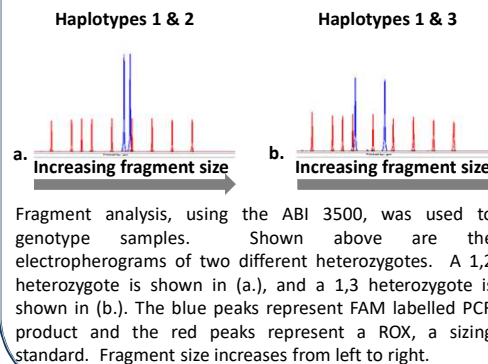


Three 5'UTR haplotypes were previously identified in Golden Retrievers and are shown in Figure 2. The start codon is shown in yellow, and common sequences among all haplotypes are shown in grey. A region containing variable numbers of 6 base pair repeats is shown in blue. Red represents the sequence regions missing in Haplotype 3.

Methods

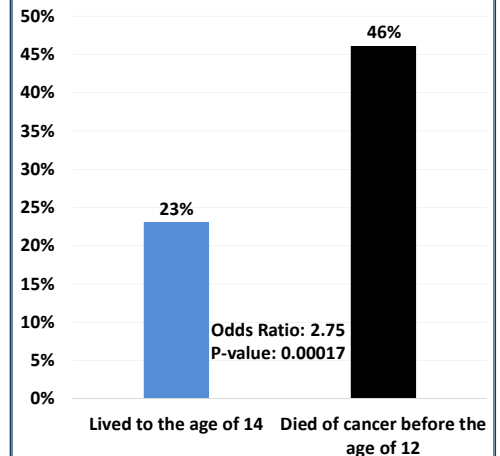
The ERBB4 5'UTR was genotyped in 143 Golden Retrievers who were separated into two study groups: dogs that lived to the age of 14, regardless of cancer status (n=62), and dogs that died of cancer before the age of 12 (n=81). This group of 143 Golden Retrievers consists of the dogs from the GWAS study, and includes the initial 26 dogs who were previously sequenced to define the 5'UTR haplotypes. In this study, individuals were genotyped by the fragment size resulting from PCR amplification of the 5'UTR using one fluorescently labeled primer. Fragment size was assessed using fragment analysis on the ABI 3500 Series Genetic Analyzer to allow the clear separation of the three Haplotypes based on size. Chi-square tests with Pearson P-values were used to compare the presence of haplotypes between study groups (6).

Fragment Analysis



Results

The Frequency of Haplotype 3 Differs Significantly Between Study Groups



Conclusions

The frequency of Haplotype 3 was significantly different between study groups, with a frequency of 46% in the dogs that died of cancer before the age of 12 versus only 23% of the dogs that lived to 14 (OR = 2.75, P-value = 0.00017). This association is higher than our most associated SNP from the GWAS (OR = 2.34, P-value = 0.0056), so Haplotype 3 could be a causative variant. Furthermore, homozygosity for Haplotype 3 differed by an even larger magnitude (21% vs 3%), indicating the effect of Haplotype 3 may be additive. It's possible that Haplotype 3 isn't causative but is simply in high linkage disequilibrium with the causative variant, so functional analyses will be necessary to determine whether Haplotype 3 has an effect on ERBB4 transcription or translation. Preliminary work in other breeds has demonstrated the presence of a modified form of Haplotype 3 in both the Labrador Retriever and the Flat-Coated Retriever, with both breeds differing from the Golden Retriever Haplotype 3 sequence by only 1 base pair. Fragment length analysis indicated that Haplotype 3 may also exist in the Boxer, Newfoundland, and Nova Scotia Duck Tolling Retriever.

References

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Acknowledgements

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Haplotype 3 Homozygosity Varies the Most Between Study Groups

