Longitudinal follow-up and confirmation of the association of canine polymyositis in the Hungarian Vizsla with an MHC class II risk haplotype in a larger cohort and suggestive regions identified in a genome-wide association study.

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Introduction

- Polymyositis is an immune-mediated inflammatory myopathy characterised by muscle weakness.
- Previously profiled the Major Histocompatibility Complex (DLA) Class II haplotypes in 212 Hungarian Vizslas (29 cases and 183 controls).
- Identified a risk DLA haplotype.
- Continued to follow up all the dogs in this study, and have continued collecting samples from additional dogs.
- Now have used a genome-wide association study (GWAS) to search for other regions of the canine genome that could harbour risk variants for the disease.

Objective

- To characterise MHC haplotypes in a second cohort of Hungarian Vizslas.
- Identify other regions of the genome associated with polymyositis by GWAS.

Materials and Methods

- MHC genotyping using sequence-based typing and subsequent haplotype analysis was completed for an additional 286 Vizslas, (total n=498).
- Dogs from the original investigation were updated according to phenotypic status, and this, the new cohort, and the total cohort, were analysed separately.

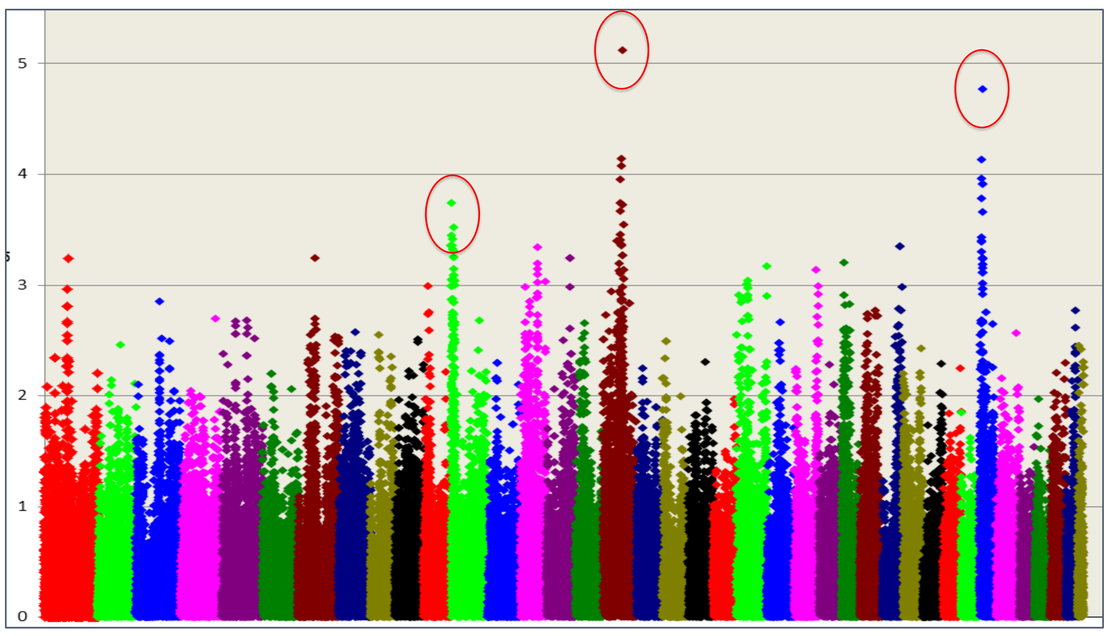
- We genotyped 51 cases and 93 controls on the Illumina HD canine SNP array.
- GWAS was conducted using GEMMA following SNP QC filtering (minor allele frequency (5% or above); call rate (97% or above); Hardy-Weinberg equilibrium (P-value threshold 5 x 10^{-5})).

Results

- Every time a dog is diagnosed with polymyositis, this affects not only its status, but also the status of its close relatives.
- Since publishing in 2013, 19% (41 of 212) dogs have changed phenotypic status
- Re-analysis strengthened the previous DLA association
- The second cohort and the combined dataset gave the same results
- See Figures 1 and 2

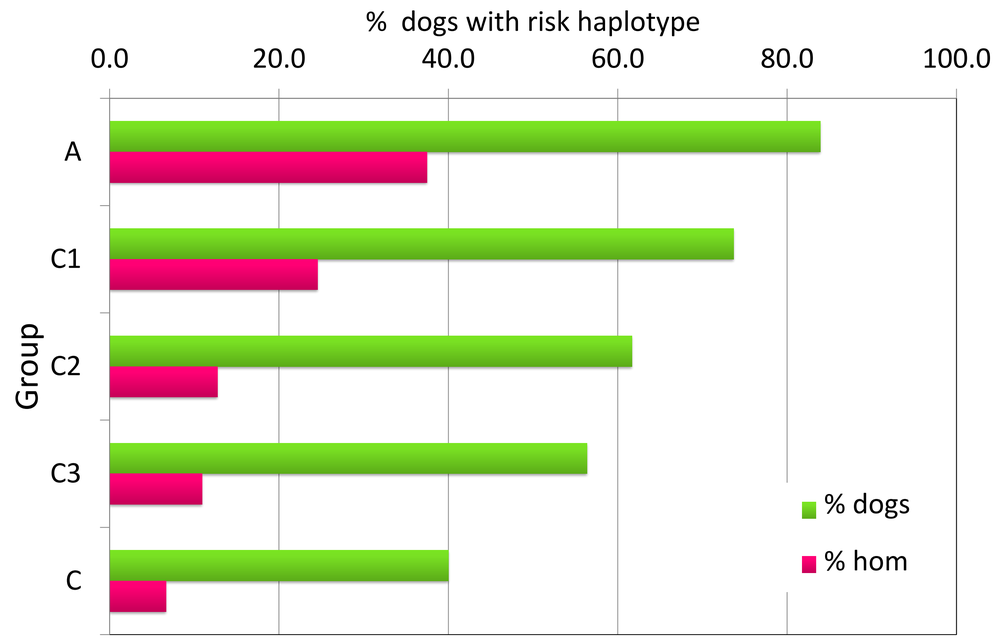
- GWAS analysis (after QC) using 49 cases and 85 controls revealed three regions of suggestive association with the disease ($P<5x10^{-4}$)
- On Chromsomes 12, 17 and 33. See Figure 3
- The MHC region is on chromosome 12, but this association may be with TNF rather than DLA class II

Figure 3. Manhattan plot for Hungarian Vizslas



Chromosomes

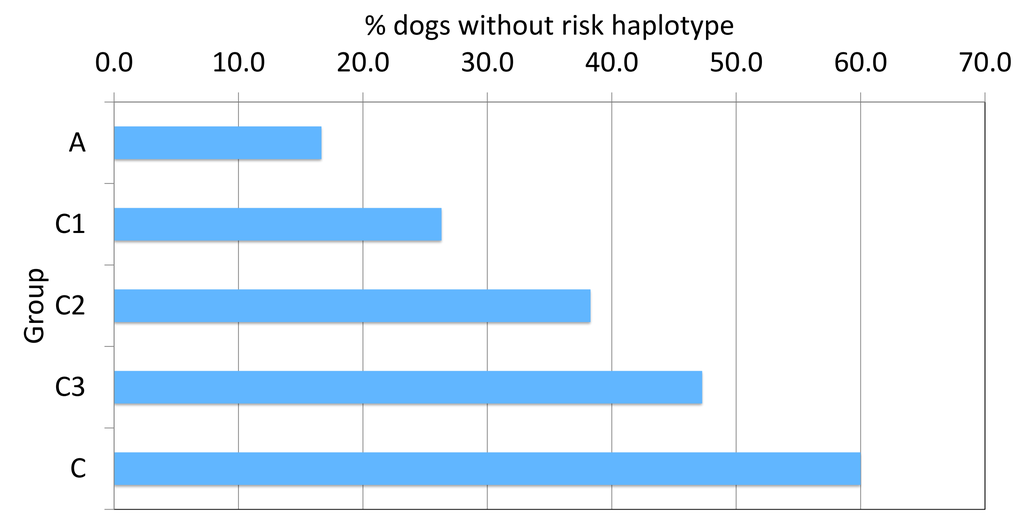
Figure 1. Percentage occurrence of risk haplotype (green) and risk haplotype homozygosity (red) in each group.



Risk haplotype: DLA-DRB1*02001/DQA1*00401/DQB1*01303

Name	n	Groups
A1	56	affected
C1	171	healthy with affected 1st degree relative
C2	141	healthy with affected 2nd degree relative
C3	55	healthy with affected 3rd degree relative
C	75	healthy with no affected close relatives

Figure 2. Percentage occurrence of non risk haplotype (green) in each group showing contrasting distribution with risk haplotype. Group designation as with figure 1.



Conclusions

- Longitudinal follow up of dogs is essential for accuracy of analyses.
- We confirm that the DLA class II risk haplotype shows a strong reproducible association with polymyositis, with an increased risk for homozygotes.
- The haplotype is more prevalent in dogs with close family relationships to affected dogs.
- The GWAS indicated suggestive regions of association that will be tested in the total cohort
- Next study will be whole genome sequencing to enable further characterisation of the genomic regions underlying these associations.

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Photos by Di Addicott