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 5×10^{-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 ($P<5\times10^{-4}$)
- 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

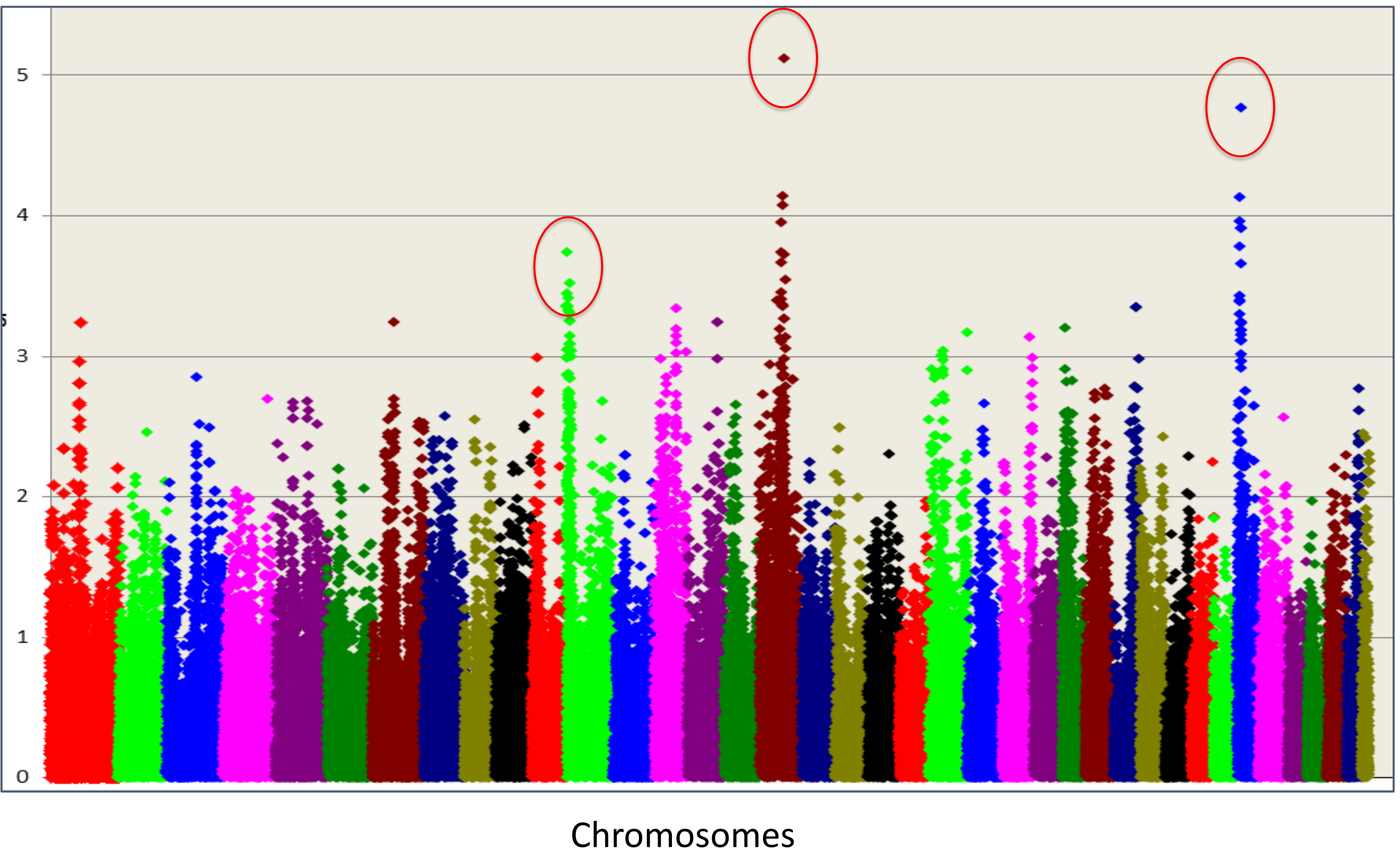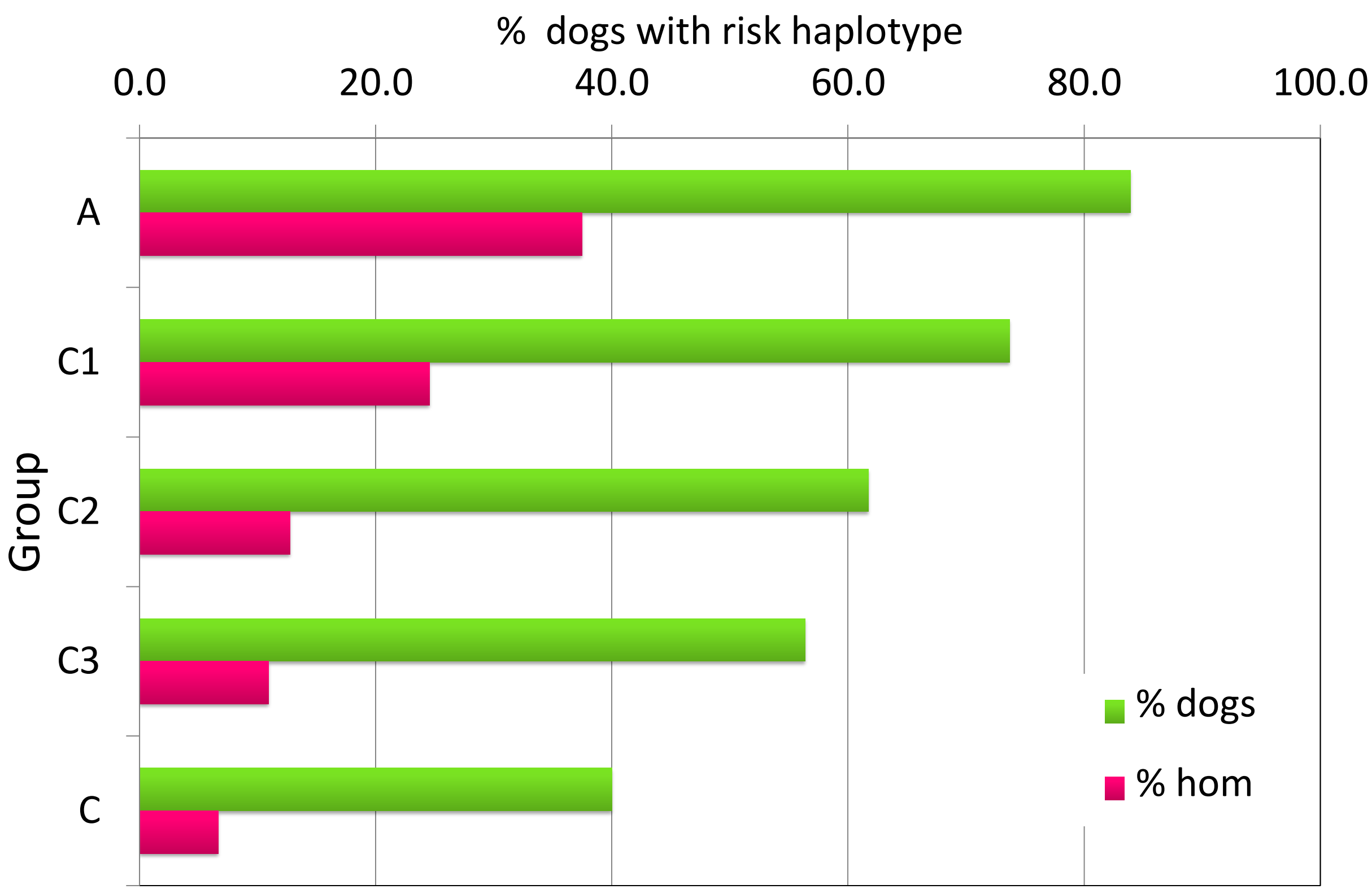Figure 3. Manhattan plot for Hungarian Vizslas

Chromosomes

Figure 1

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

% dogs with risk haplotype

- % dogs
- % hom

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.

% dogs without risk haplotype
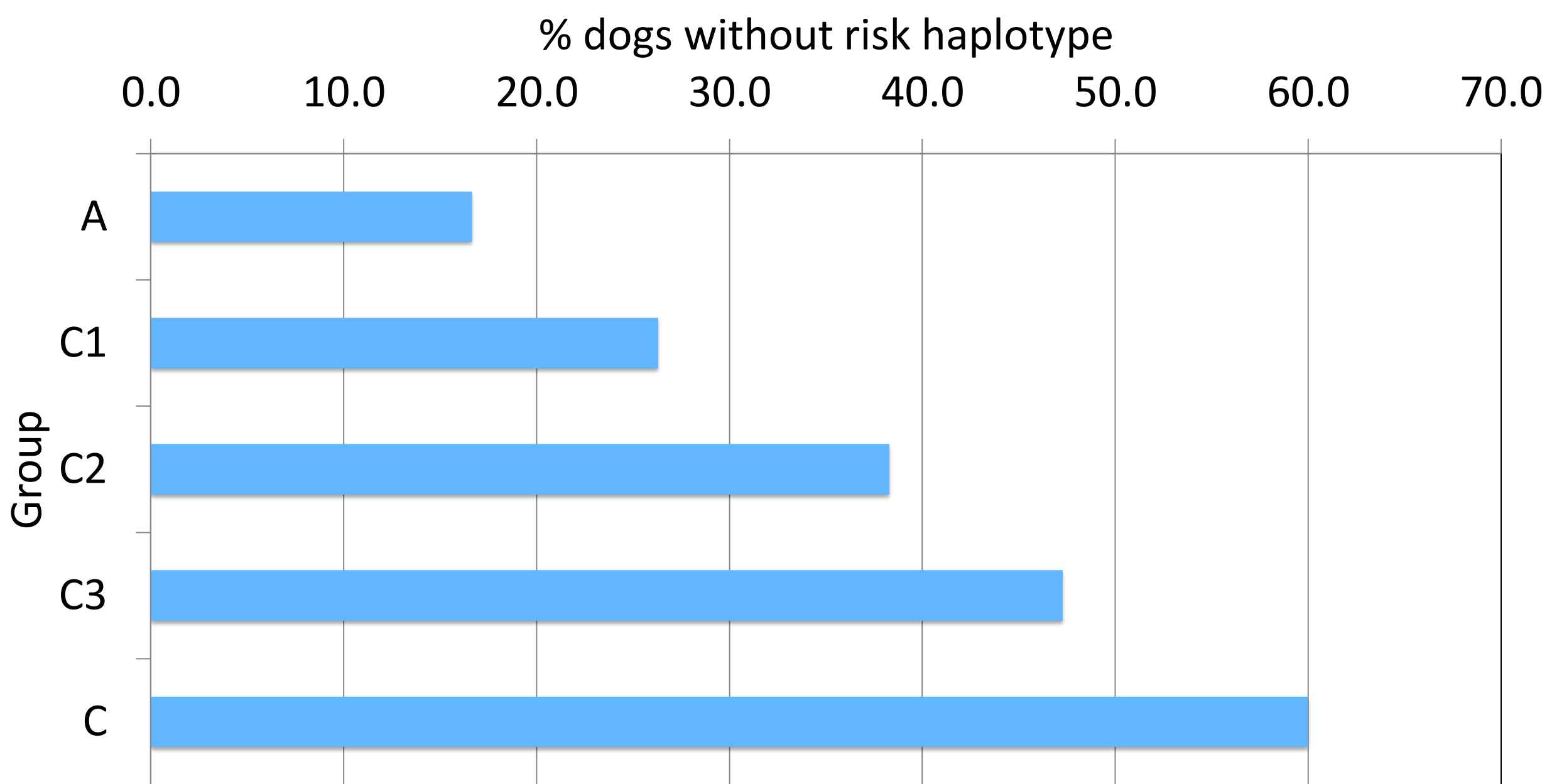
Conclusions

- 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