Hyaluronidase Genes Associated with Risk for Canine T Zone Lymphoma

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Background

- T zone lymphoma (TZL) is a rare histologic variant of human peripheral T cell lymphoma NOS. It is comparatively common in dogs, representing about 12% of all canine lymphomas

- In dogs, this disease follows an indolent course with an average survival of >2 years regardless of treatment (vs. <1 year with most other subtypes)

- TZL appears to have a striking predilection for Golden Retrievers, which represent over 40% of cases. This finding suggests a genetic risk factor for this disease

- Because of the relatively recent development of dog breeds, there is reduced genetic variation within breeds of dogs, leading to an enhanced ability to study genetic risk factors

Study objective
To identify genes associated with TZL in Golden Retrievers using a genome-wide association study with a case-control design

Methods

Cases (n=100)
Recruited from Golden Retrievers that had peripheral blood samples submitted to CSU-CI lab due to suspicion of a lymphoproliferative disorder (LPD)

Cases are uniquely identified using immunophenotyping by the loss of CD45 expression on T cells (homogeneous expansion of CD5+CD45- T cells)
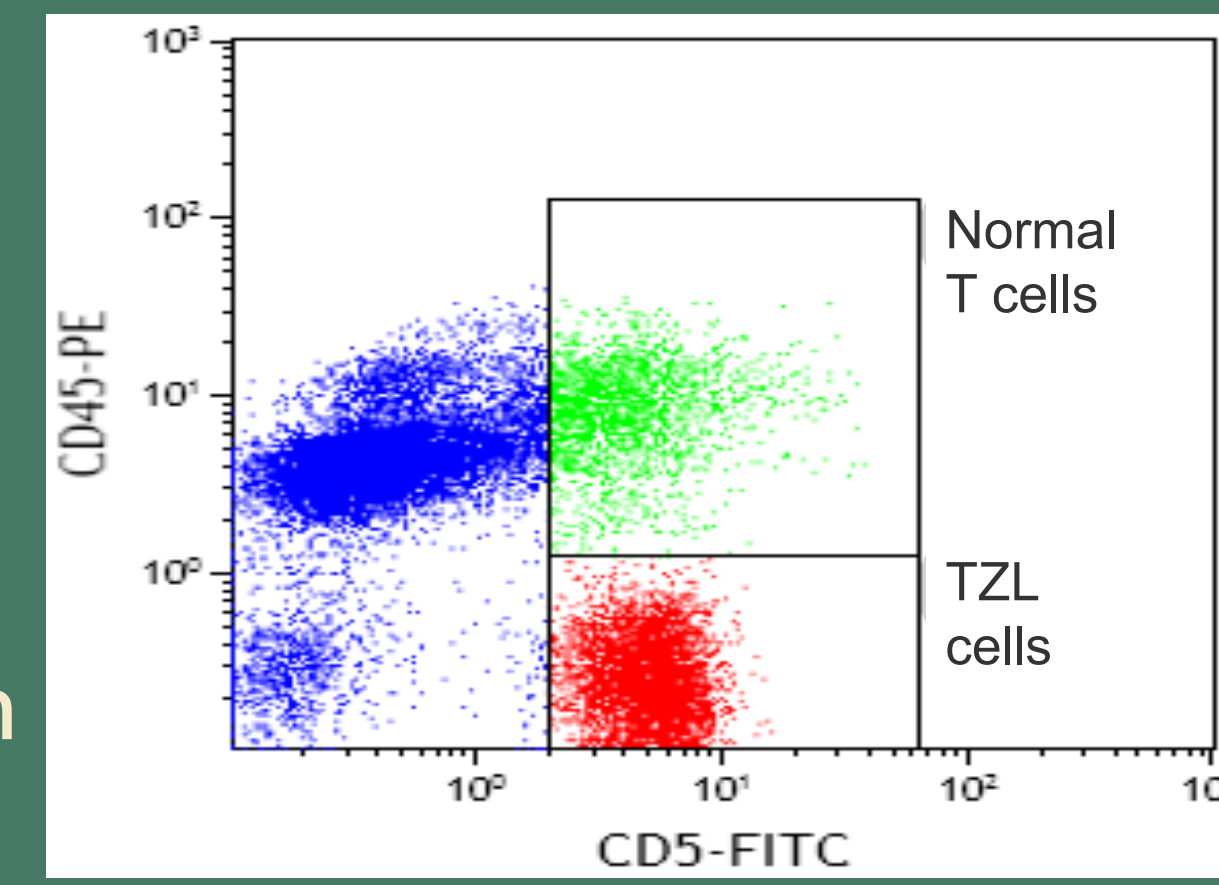
Controls (n=238)
Golden Retrievers aged 9+ years with no history or suspicion of a LPD. Recruited from the submitting clinic of cases or owners who expressed interest in the Canine Lifetime Health Project.

Controls were confirmed to be free of TZL using immunophenotyping (no CD5+CD45- T cells)
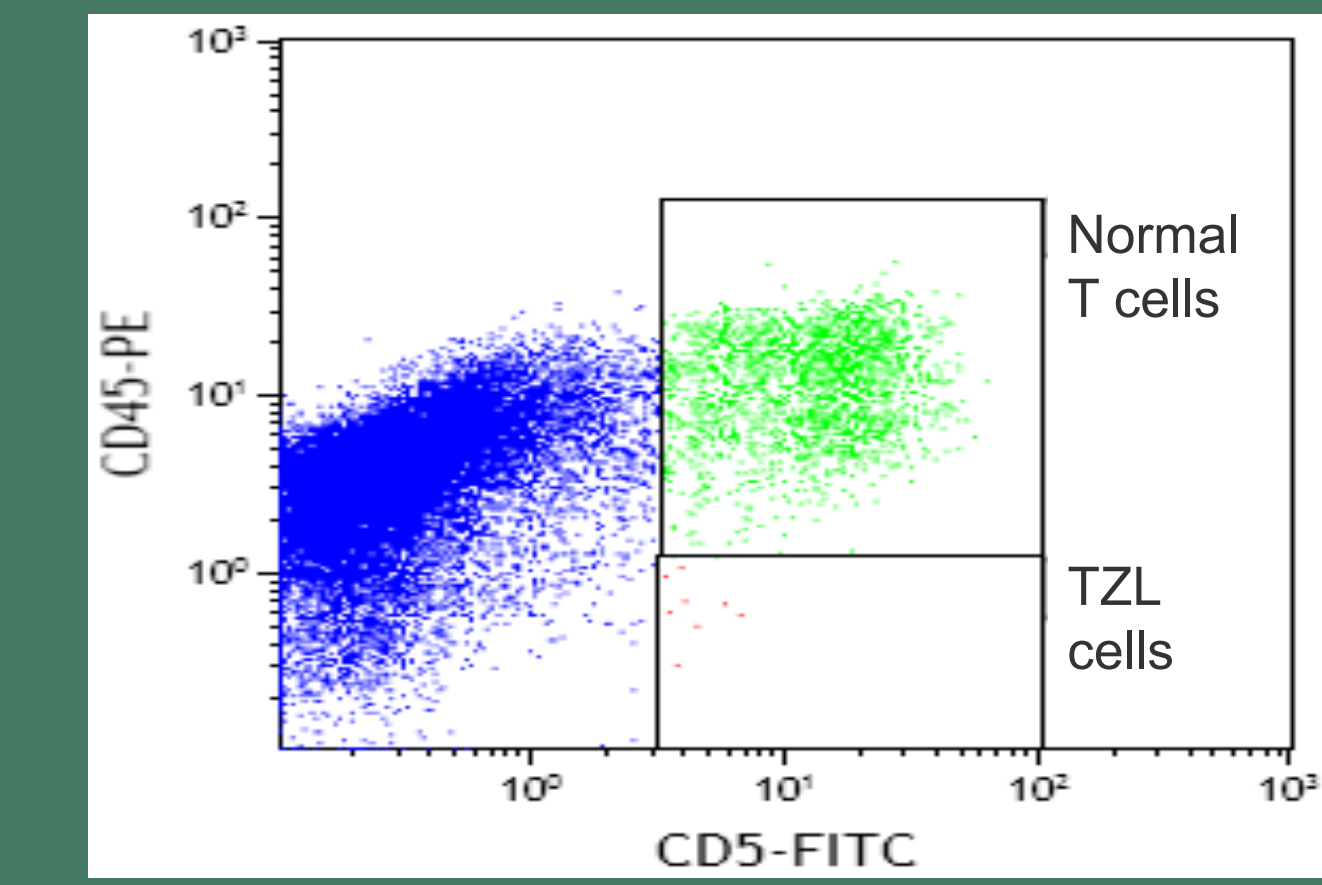
1. Dogs were enrolled from November 2013 through May 2015
2. DNA was extracted from peripheral blood samples using the QIAGEN Midi kit
3. Genotyping was conducted with the Illumina CanineHD BeadChip (170k SNPs)
4. Quality control measures were implemented to filter SNP and individual call rates $\leq$97.5% and minor allele frequencies $\leq$5%
5. A mixed linear model was used to calculate odds ratios and p-values adjusting for population stratification using GCTA software
6. A significance level of 10^{-4} was chosen based on QQ-plots

Discussion

- 5 SNPs on **chromosome 8** spanning 53.7–53.9 Mbp were significantly associated with TZL, with ORs ranging from 1.35–1.43

  - This region includes genes involved in endocytotic trafficking and thyroid function

  - Preliminary data suggests hypothyroidism, a relatively common finding among Golden Retrievers, is significantly less likely among cases than controls

- 9 SNPs on **chromosome 14** spanning 10.4–11.9 Mbp were significantly associated with TZL, with ORs ranging from 1.18–1.20

  - This region includes multiple genes encoding hyalonidases, including SPAM1 and HYAL4

  - SNPs in the same region were found to be associated with mast cell tumors in Golden Retrievers, suggesting a potential shared susceptibility

Results

The final analytic dataset included 92 cases, 229 controls, and 110,371 SNPs. The average age at enrollment was 11 years and 56% of participants were female.
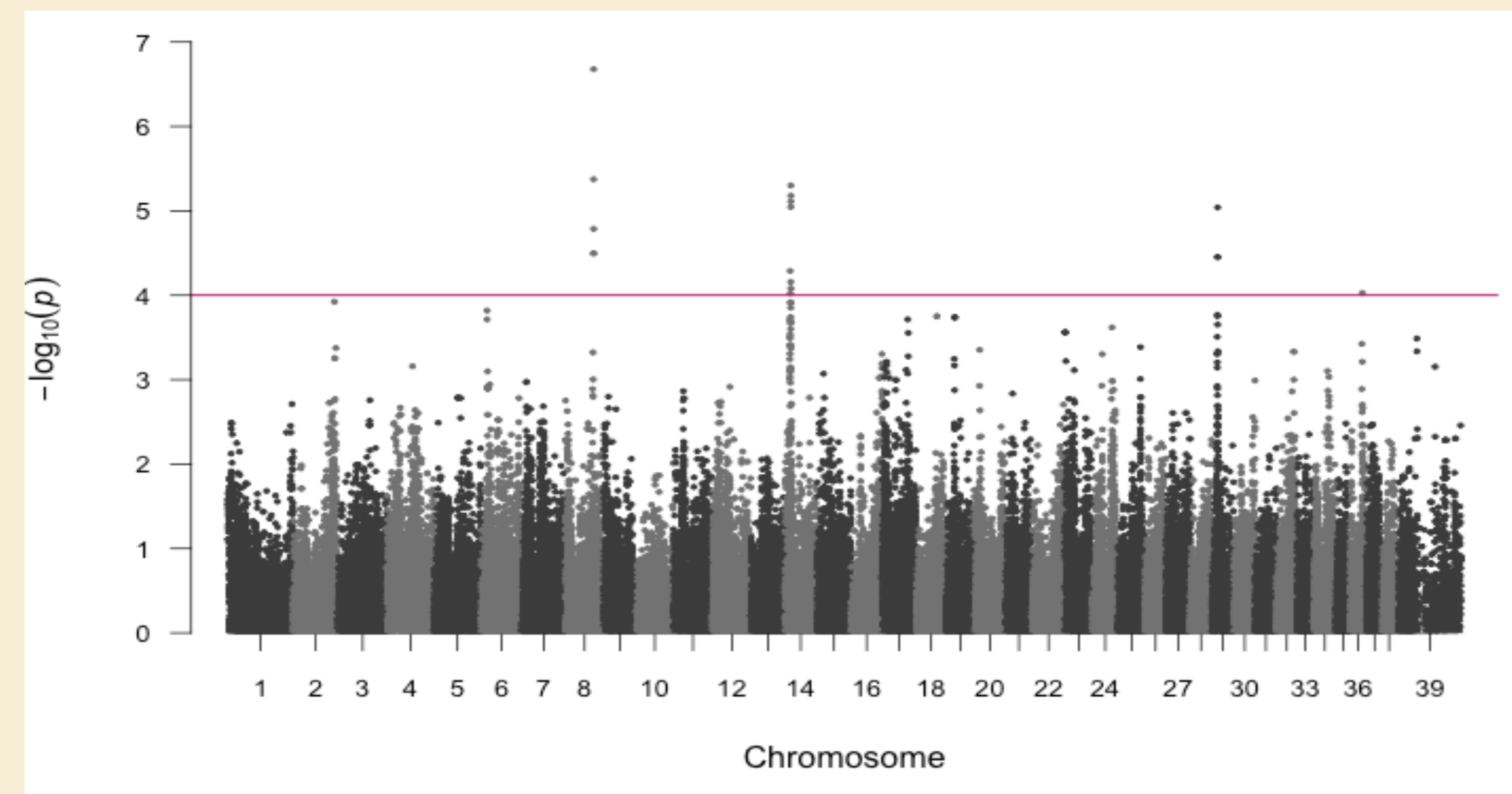

Figure 1. Manhattan plot showing p-values for the association of each SNP and TZL, adjusting for population stratification. Chromosomes are shown in alternating colors along the x axis.
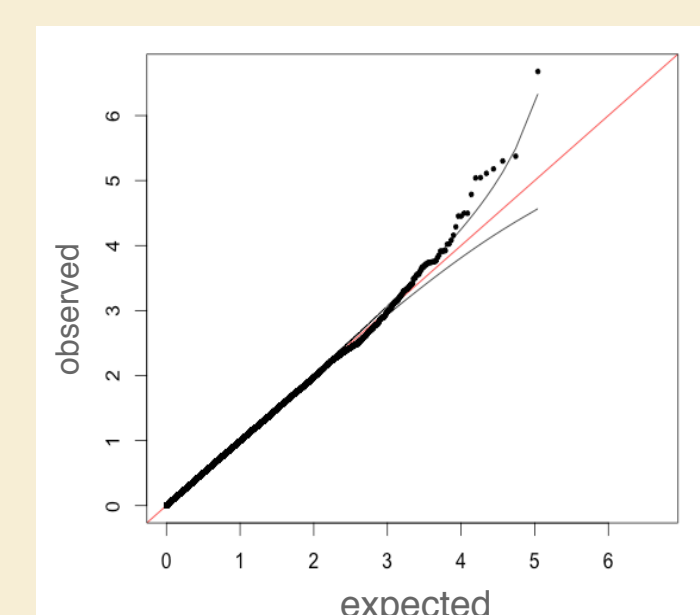

Figure 2. QQ-plot comparing observed and expected $-\log_{10}(p)$. The curve departs from expected around 4, supporting our 10^{-4} significance threshold.

Table 1. SNP name, location, p-value, odds ratio (OR), and minor allele frequencies (MAF; %) for all SNPs meeting the 10^{-4} significance level threshold.

Figures 3 and 4. Scatter plots of chromosomes 8 and 14 p-values, respectively, zoomed in to the region of significance. Points are colored based on R^2 from the SNP with the smallest p-value (Chr8: BICF2P378684, Chr14: TIGRP2P186605). Below each plot the region with $R^2 \geq 0.4$ is aligned to CanFam3.1 genes.
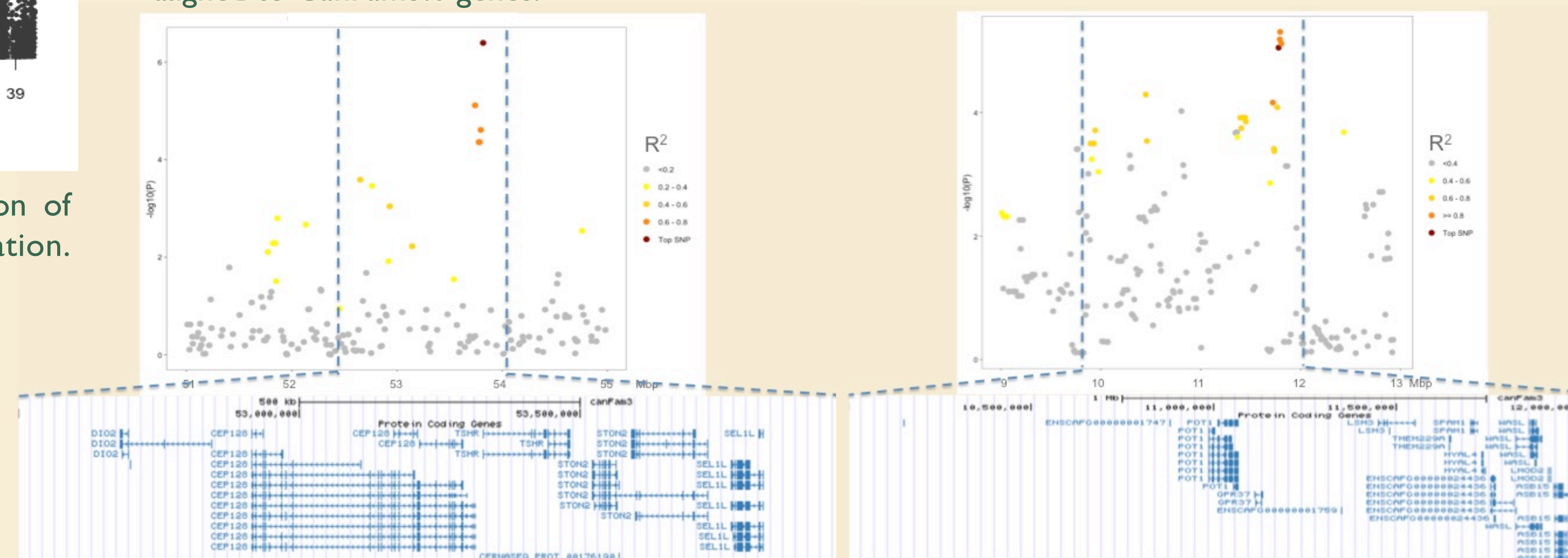
				MAF	
SNP	Base Pairs	P-value	OR	Case	Ctrl
Chromosome 8					
BICF2P378684	53,742,667	7.6E-06	1.43	14	4
BICF2P1080535	53,778,185	4.4E-05	1.35	15	5
BICF2P1048848	53,785,948	4.4E-05	1.35	15	5
BICF2P184533	53,796,442	2.5E-05	1.36	15	5
BICF2P948919	53,818,371	4.1E-07	1.40	20	7
Chromosome 14					
BICF2S23335956	10,450,765	1.8E-05	1.19	62	38
BICF2S23442141	10,462,378	9.9E-05	1.18	63	42
BICF2G630521189	10,806,759	2.2E-05	1.21	43	24
BICF2P65550	11,362,199	7.7E-05	1.20	41	23
BICF2G630521572	11,721,433	4.4E-05	1.18	32	54
TIGRP2P186605	11,778,977	4.6E-06	1.20	32	57
BICF2G630521678	11,791,385	8.0E-06	1.20	32	56
BICF2G630521681	11,794,735	5.9E-06	1.20	32	56
BICF2G630521696	11,807,161	1E-05	1.20	32	56

Conclusion

- The pathogenesis of canine TZL may be related to hyaluronan breakdown. Hyaluronan stimulates the inflammasome, which favors Th2 responses. The cell of origin of TZL is hypothesized to be Th2.

- The link to hypothyroidism, an autoimmune disease, is not yet clear.

- We plan to resequence the associated regions on chromosomes 8 and 14 to determine the exact location of mutations.