Risk for Symmetrical Lupoid Onychodystrophy (SLO) is Associated With Several Linked Genetic Variants in the Bearded Collie. Liz Gershony, DVM, PhD (UC Davis).

To remind the readers of the condition under study and what was previously reported, symmetrical lupoid onychodystrophy (SLO) is characterized by inflammation of the nail beds that causes the nails to slough off resulting in considerable pain and discomfort to affected dogs. After initial treatment, it is not uncommon for the condition to recur throughout a dog's life, thus requiring life-long management.

Although the exact cause of SLO remains unclear, it appears to be an autoimmune condition that is more prevalent among certain dog breeds, such as Bearded Collies, German Shepherds, Gordon and English Setters, and Giant Schnauzers. Health surveys conducted by the Bearded Collie Foundation for Health (BeaCon) have indicated an increasing prevalence of SLO over the years, affecting 3.7% of surveyed dogs in 2020. Beacon and the BCCA have generously supported research conducted in the Oberbauer Laboratory, in the Department of Animal Science, University of California, Davis, leading to the discovery of the genetic factors underlying SLO in Bearded Collies.[2] Here we provide an updated summary of the findings to date.

The studies confirm an association between the major histocompatibility complex (MHC) class II genes and SLO disease development in Bearded Collies. These immune genes are important for distinguishing between an individual's own healthy tissue and what is foreign, harmful or diseased. However, when an individual's immune system fails to recognize their own tissue as self, a condition referred to as autoimmunity can ensue. Autoimmunity occurs when one's own healthy tissue gets mistaken for a harmful agent and is consequently attacked by the immune system. In fact, MHC class II genes are often associated with autoimmune disorders in many species including humans and dogs.

The dog MHC is referred to as the dog leukocyte antigen (DLA). The DLA class II genes are highly diverse with many versions of these genes existing in the overall dog population. However, given the genetic history of dog breeds, only a handful of versions usually exist within a specific breed. Moreover, because these genes are so close to each other on the chromosome (i.e. neighboring genes), they tend to be connected to form what are called haplotypes. Haplotypes are the genotypes of a group of genes that are inherited together from a parent.

For canine disease studies, it is most informative to characterize a dog's DLA in terms of the specific combination of three DLA class II genes (designated DLA-DRB1, DLA-DQA1 and DLA-DQB1) generating haplotypes. More specifically, because the haplotype involves three DLA genes and each gene has a slightly different location (i.e. locus) on the chromosome, the combination haplotype is called a "three-locus haplotype".

A study[1] conducted by the Oberbauer Lab revealed five common DLA class II haplotypes in the Bearded Collie population, with four additional haplotypes seen in a very small number of dogs, thus considered rare. (See Appendix for Table). Two of the five common DLA class II haplotypes were associated with an increased risk for developing SLO in Bearded Collies. However, the two DLA class II risk haplotypes were not able to completely explain disease development in the breed. According to the study, having two DLA class II risk haplotypes accounted for up to 60% of the risk for SLO. This is consistent with the fact that SLO is likely inherited as a complex trait (i.e. involving more than one gene and possibly environmental factors that are yet unknown). To improve our understanding of SLO susceptibility, the research team sought to characterize additional genetic contributions underlying SLO in Bearded Collies by exploring the genetic characteristics of individuals that carried these DLA class II risk haplotypes for SLO in a genome-wide association study (GWAS).

A GWAS allows researchers to explore the natural genetic variation between individuals within a dog breed to determine if any particular variation (or genetic variant) is more frequently found among dogs that have a disease when compared to dogs that do not have the disease. When using this approach,

dogs are tested for several genetic variants that are distributed throughout the dog's genome (i.e. their DNA or genetic material), and each variant acts as a marker representing a wide region of the genome that may contain several genes.

Once the region is identified to be of interest (or associated with disease status), it is necessary to look at the individual genes within that region in detail to determine which one is responsible for causing disease. In the case of SLO, the study was conducted on 101 Bearded Collies (38 with SLO and 68 who were at least 8 years old and never developed SLO) and revealed two regions of association on different canine chromosomes, both of which contain genes that seem likely to be involved with SLO disease development. One of the regions also corresponds to the location of the DLA class II genes, which supports the initial observation of particular DLA class II haplotypes being strongly associated with SLO. However, dozens of other genes are also found in this wide region of association. To refine the association and identify the true gene (or genes) responsible for disease development, the researchers used whole genome sequencing of SLO affected and healthy Bearded Collies to target detailed genetic variants within each gene in the region.

The whole genome sequencing study revealed genetic variants in several genes other than the DLA class II genes that could be involved in SLO disease development. Many of the genetic variants seen in these genes are predicted to change the function of the protein that the gene encodes. Additionally, it was seen that every Bearded Collie with DLA class II risk haplotypes also had these genetic variants. This is not an unusual situation in genetics because genetic regions that are physically located close to each other on a chromosome are always inherited together and considered "linked". Because of this co-inheritance, it is often difficult, if not impossible, to separate the individual effects of linked genetic variants in a natural population.

The SLO studies completed so far have focused on genetic variation found within a gene proper. It is also known that genetic variation that is found in between genes (i.e. in intergenic regions) can also affect how genes function. Further exploration of the genetic variation in these regions is underway and may reveal additional genetic variants that contribute to SLO disease development in Bearded Collies. In addition, genetic studies in other dog breeds that are predisposed to SLO may help clarify which genetic variants can be reliably used to inform breeding decisions in the future.

Despite being unable to determine whether susceptibility for SLO is caused by one of the identified linked genes or the also linked DLA class II genes (or even a combination of the two), these research findings offer progress in understanding the genetics behind SLO disease development in Bearded Collies. Although a clear and strong association exists between the DLA class II risk haplotypes, the linked genetic variants and SLO, this only accounts for a portion of disease risk (at most 60%) and due to the multigenic nature of SLO, a small proportion of dogs without these haplotypes are still at risk.

Thus, while this information may be potentially useful as one of many criteria used to inform breeding decisions, extreme caution is warranted in attempting to use this information on its own for selective breeding since neither the DLA class II risk haplotypes nor the linked genetic variants are able to completely explain SLO disease development in the breed. In fact, many healthy Bearded Collies have these associated variants and the DLA class II risk haplotypes and never develop any nail problems. Moreover, these variants are relatively common in the Bearded Collie population so that extreme selection against these DLA class II risk haplotypes or the linked variants could result in significant loss of genetic diversity in the breed.

References

1. DLA class II risk haplotypes for autoimmune diseases in the bearded collie offer insight to autoimmunity signatures across dog breeds. Gershony, L.C., et. al. *Canine Genet. Epidemiol.* **2019**, *6*, 2 https://cgejournal.biomedcentral.com/articles/10.1186/s40575-019-0070-7

2. Whole Genome Sequencing Reveals Multiple Linked Genetic Variants on Canine Chromosome 12 Associated with Risk for Symmetrical Lupoid Onychodystrophy (SLO) in the Bearded Collie. L Gershony, et.al. *Genes* **2021**, *12*(8), 1265; **https://doi.org/10.3390/genes12081265**

Appendix

Table from Ref 1 with DLA haplotypes in study Bearded Collies

Code	Haplotype	Controls		AD				SLO			
	DLA-DRB1/DQA1/DQB1	2*n* = 244	%	2*n* = 122	%	OR (95%CI)	*p*-value†	2*n* = 100	%	OR (95%CI)	p-value†
1	009:01/001:01/008:02	24	9.8	**25**	**20.5**	**2.36 (1.29–4.34)**	**0.0058**	1	1.0	**0.09 (0.01–0.70)**	**0.0047**
2	015:01/006:01/003:01	31	12.7	18	14.8	1.19 (0.64–2.22)	0.6263	4	4.0	**0.29 (0.10–0.83)**	**0.0172**
3	015:01/006:01/023:01	33	13.6	23	18.9	1.49 (0.83–2.66)	0.2177	2	2.0	**0.13 (0.03–0.55)**	**0.0013**
4	018:01/001:01/002:01	73	29.9	27	22.1	0.67 (0.40–1.11)	0.1355	**47**	**47.0**	**2.08 (1.29–3.35)**	**0.0029**
5	018:01/001:01/008:02	70	28.7	27	22.1	0.71 (0.42–1.18)	0.2094	**46**	**46.0**	**2.12 (1.31–3.43)**	**0.0026**
6	015:01/006:01/022:01	5	2.0	2	1.6	0.80 (0.15–4.17)	1	0	0.0	N/A	
7	002:01/009:01/001:01	6	2.5	0	0.0	N/A		0	0.0	N/A	
8	023:01/003:01/005:01	1	0.4	0	0.0	N/A		0	0.0	N/A	
9	015:02/006:01/023:01	1	0.4	0	0.0	N/A		0	0.0	N/A	

			Oberbauer lab (peer review published)		Finnish Study*r
Finnish study code	Oberbauer study code	DLA haplotype	Frequency in the control dogs (total 122 dogs)	Frequency in all dogs (total 233 dogs)	Frequency (n=77)
Parta 3	1	009:01/001:01/008:02	9.8 %	10.7 %	8.4%
Parta 4	2	015:01/006:01/003:01	12.7 %	11.4 %	5.2%
Parta 5	3	015:01/006:01/023:01	13.6 %	12.4 %	2.6%
Parta 1	4	018:01/001:01/002:01	29.9 %	31.5 %	44.8%
Parta 2	5	018:01/001:01/008:02	28.7 %	30.7 %	35.7%
	6	015:01/006:01/022:01	2 %	1.5 %	
Parta 6	7	002:01/009:01/001:01	2.5 %	1.3 %	2.6%
	8	023:01/003:01/005:01	0.4 %	0.2 %	
	9	015:02/006:01/023:01	0.4 %	0.2 %	

*Original text written by Prof. Hannes Lohi, Nina Ryynalal, and Genoscoper Ltld was published in the Finnish Bearded Collie Club's magazine, partis, 1/2010.

The reason for differences in frequencies of some haplotypes between the earlier Finish study and Oberbauer's lab is not known.