STUDY OF MHC CLASS II GENES OF BEARDED COLLIES REVEALS NARROW DIVERSITY

Like humans, dogs have many heritable diseases. However, because of breed histories, the incidence of diseases can be much higher compared to humans. Closely related animals are often used for breeding and matadors are not rare. Inbreeding present in closed breeding populations increases the number of carriers of breed-specific diseases, and consequently the number of individuals that are affected increases.

An important region in the animal genome is MHC, Major Histocompatibility Complex. Genes in this region play many roles and they are responsible e.g. for the recognition of the individual's own tissue as well as the recognition and destruction of foreign elements like bacteria, viruses, and pathogens. The diversity of the genes in the MHC region is important for its functionality. Higher diversity gives better protection against harmful intruders. The dog MHC region is also known as DLA, Dog Leukocyte Antigen.

About this study

A research program studying the canine genome was started in 2006 at Helsinki University and Folkhälsan's research center under the leadership of professor Hannes Lohi. Most projects concentrate on various heritable diseases, with the aim of identifying the underlying genetic defects. The results are often applicable to human diseases, as the genetic causes of many diseases are common between humans and dogs. The genetic diversity of small dog populations has also been studied, because loss of genetic diversity is associated with many diseases, such as autoimmune diseases.

The Finnish Bearded Collie Club decided to invest in a healthier future for the breed by funding a study of genetic variability. By the end of 2009, a total of 260 Bearded Collies living in Finland had given genetic samples to the research group. Dogs selected in the study were chosen to be as unrelated as possible, although some relatedness was unavoidable because of the breed history. The total number of individuals was 77, of which 29 had been imported into Finland. Eleven dogs originated in Sweden, five in Great Britain, three in France, three in Belgium, two in Norway, and single dogs in USA, the Czech Republic, Poland, Spain, and Germany. Of the 48 Bearded Collies bred in Finland, 32 had at least one imported parent. The study was completed in January 2010, and its results are reported here.

Canine purebreeding has decreased the diversity of the dog MHC genes. This may increase the risk for autoimmune diseases such as diabetes, rheumatism, polyarthritis, hypothyroidism, anemia (AIHA), Addison's disease, and perianal fistula. SLO (Symmetrical Lupoid Onychodystrophy) is a breed-specific disease for Bearded Collies affecting the development of claws, and it is also suspected to have an autoimmune origin. Many autoimmune diseases in dogs have already been linked to genes in the MHC region. Testing and monitoring of these genes is important in fighting against diseases, and maintaining DLA diversity in one's own lines and the breed as a whole. It is now possible to test the diversity of MHC genes.

The MHC region is located in chromosome 12, and it consists of more than hundred genes. The genes can be categorized into three classess (I-III) according to their function and location. The MHC diversity is typically measured by studying the variability in the alleles (variants) of the MHC class II genes DRB1, DQA1, DQB1, as well as the variability of the combinations (haplotypes) of the three genes. The genes code for proteins that recognize foreign elements (e.g. bacterial protein fragments), and present them for the rest of the immune system for destruction. Higher number of alleles and haplotypes indicates better diversity in the breed, and likely for better chance for the proper function of the MHC II immune system.

By testing the MHC II diversity, the genetic information can be taken into an account in breeding plans by mating a bitch to a dog that has a different MHC structure. This increases the number of possible combinations in the litter and increasing the genetic diversity of the breeding line and possibly the whole breed if systematically followed. Some studies indicate that a dog who become homozygous for a particular MHC II alleles or haplotypes or homozygous for the MHC II region in general is more susceptible to autoimmune diseases. However, it is important to remember that although some alleles or homozygosity in the MHC region may increase the disease risk, they usually do not alone explain the mechanisms of autoimmune diseases. Other genetic and environmental factors may be responsible in other parts of the genome.

DLA Diversity of Bearded Collies

The DLA diversity of 77 Bearded Collies was studied by Genoscoper Ltd. The dogs were selected from different lines, mainly from the samples donated to the canine DNA bank maintained by the Canine Genomics Research Group in the University of Helsinki. The study cohort included also 16 known SLO cases in order that researchers were able to test if there is any correlation between SLO and the MHC II genes. For practical reasons, the tested population may not reflect a proper random pool of samples from the breed and some of the SLO dogs were closely related to the other dogs in the study. However, this cohort should give a reflection of the diversity present in the current Finnish Bearded Collie population.

The results of the study are presented in Table 1 and Figure 1. Four DRB1 alleles (i.e. four different variants of the same gene), three DQA1 alleles, and six DQB1 alleles were found. Since these genes are inherited as a single group different combination of three alleles can be formed as distinct haplotypes. Altogether seven different haplotypes were present in the tested samples. All the haplotypes have already been encountered in other breeds. The haplotypes are denoted as Parta1, Parta2, etc.

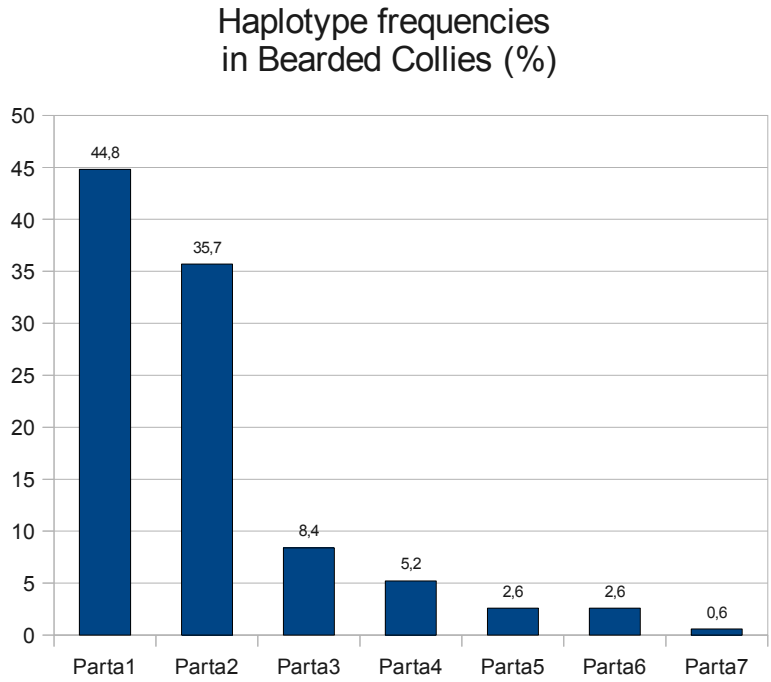
Table 1. Summary of the DLA diversity of 77 Bearded Collies in January 2010. Two most common haplotypes are found in over 80% of dogs.

Haplotype	DRB1	DGA1	DQB1	Frequency (%)
Parta1	01801	00101	00201	44,8
Parta2	01801	00101	00802	35,7
Parta3	00901	00101	00802	8,4
Parta4	01501	00601	00301	5,2
Parta5	01501	00601	02301	2,6
Parta6	00201	00901	00101	2,6
Parta7	00501	00601	02201	0,6
Different alleles	4	3	6	

One of the goals of the DLA study was to find out how many dogs are homozygous for each haplotype (the dog has inherited identical haplotypes from both parents). Most Bearded Collies are heterozygous, that is, they have different haplotypes in the two corresponding chromosomes. However, 30 individuals (39%) were homozygous, so about one third of the dogs carry identical haplotypes in their chromosomes. The distribution of haplotypes among homozygous dogs is as follows: Parta1 60%, Parta2 36,7%, and Parta3 3,3%. Homozygosity is thus present in the three most common haplotypes as expected.

In earlier studies homozygosity has been found to increase the risk of autoimmune diseases. The sixteen SLO dogs in the study had only the two most common haplotypes Parta1 and Parta2. Moreover, eleven out of sixteen SLO dogs were homozygous for either haplotypes. Of the 30 homozygous individuals in the study, 11 had SLO. On the other hand, only 24,7% (19/77) of dogs without SLO were homozygous. Therefore it preliminarily looks like homozygosity in the MCH II locus increases the risk for SLO in Bearded Collies. SLO is a subject of further study and will be reported separately in more detail.

Figure 1: Relative frequencies of haplotypes in the study



Each breed has a breed typical DLA profile reflecting the origin of the breed, development and breeding practices. Most breeds have 3-5 dominant haplotypes, so in this respect the Bearded Collie is fairly typical. For comparison, Table 2 lists the number of alleles and haplotypes found in some other breeds that we have studied. The Bearded Collie is at about the average or below it. When comparing breeds, it is important to look at the frequency and distribution of the haplotypes in the population. The fact that ~80% of the Bearded Collies (in Finland) carry the two most common haplotypes indicates very narrow gene pool and that should be carefully addressed by the breed club in their future breeding plans.

It should also be noted that the MHC region is not a neutral locus and there may be selective pressures connected with the diversity and vitality. The MHC genes play an important role in immune response, and all combinations may not be necessarily beneficial. This may explain the absence or rareness of some haplotypes in the breed. However, this study could not address this question enough for conclusions. Besides the MHC locus, it is recommended to check and measure the variability in other parts of the genome by other neutral markers available (microsatellites) and compare those results for the MHC II diversity. This could be a future experiment for consideration.

Table 2. Results of DLA profiling of some other breeds.

Breed	Number of dogs studied	Average number of alleles per gene	Number of haplotypes	Number of dominant haplotypes (>75% of dogs)
Finnish Hound	50	7	8	3
Kromfohrländer	40	4,3	5	3
Icelandic Sheepdog	58	6,3	10	4
Löwchen	72	6	8	3
Nova Scotia Duck Tolling Retriever	176	5	5	2
Whippet	100	9	13	3
Bearded Collie	77	4,3	7	2

About Testing DLA Diversity

In general, it is important to maintain the genetic diversity and heterozygosity of genes in the MHC II region. This study provided a DLA profile for Finnish Bearded Collies and gives an opportunity to monitor, maintain, and even improve the diversity in the breed by better breeding choices in future. It is recommened that all breeding lines are used as much as possible, and the Club and the breeders should possibly favor dogs with less common haplotypes to balance the frequencies of haplotyes and therefore also increases the chance for heterozygosity. DLA testing could be utilized as follows:

1. A breeding bitch and 2-3 candidate males are tested for their DLA profile.

2. The male whose profile differs most from the bitch is chosen for mating. This ensures that the puppies will get different combinations of genes. If the bitch and the dog represent the same haplotype the puppies would inherit only the same haplos from their parents. That would narrow the genetic variability and increases the risk of homotsygosity in the litter.

The results of the DLA testing have been sent to the owners of the dogs selected for the study and the breeding committee of the Finnish Bearded Collie Club.

Inquiries about DLA testing and testing for alleles related to diseases in dogs and other species can be sent to Genoscoper, www.genoscoper.com.

Original text written by Prof. Hannes Lohi, Vet cand. Niina Ryynälä and Genoscoper Ltd. was published in the Finnish Bearded Collie Club's magazine Partis, 1/2010. English translation: Pertti Kellomäki