Canine DLA diversity: 2. Family studies

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Abstract

The canine Major Histocompatibility Complex is referred to as DLA (for dog leukocyte antigen). There are no published studies on DLA segregation in the dog, so this part of the DLA workshop aimed to collect DNA from multigeneration families of different breeds of dogs. Twenty-two families of dogs were submitted to the workshop, comprising 313 individuals, of which 247 had one or both parents available.

To date, there are no published data for segregation of the canine Major Histocompatibility Complex (MHC) DLA class II haplotypes in families. While it has been relatively straightforward to identify DLA haplotypes in the dog due to the generally high (>35%) homozygosity rates, it would be interesting to confirm these haplotypes in dog families.

There were 22 families submitted to the workshop, 11 two-generation and 11 multi-generation families, from 11 different breeds, making a total of 313 individuals (Table 1). There were 247 individuals who had one or both parents submitted to the workshop. Dog families tend to be larger and more complicated than human families, in that there

Table 1 Details of the dog families submitted

Number of dog families (total = 22)	Family type	Breed	Number of individuals in families (total = 313)	Number of puppies (total = 247)	Submitting laboratory
2	Multigeneration	Alaskan Malamute	14	10	UQU
1	Multigeneration	Australian Cattle dog	10	9	ADE
1	Two generation	Great Dane	3	1	ADE
2	Two generation	Husky (Alaskan)	11	8	AKA
1	Two generation	Labrador	15	13	MAN
3	Multigeneration	Newfoundland	78	53	MAN
1	Two generation	Retriever (Golden)	11	7	MAN
5	Multigeneration	Rhodesian Ridgeback	52	44	MAN
1	Two generation	Spaniel (Cocker English)	10	8	MAN
1	Two generation	Terrier (Staffs Bull)	11	7	MAN
4	Two generation	Mixed breed	98	87	MAN

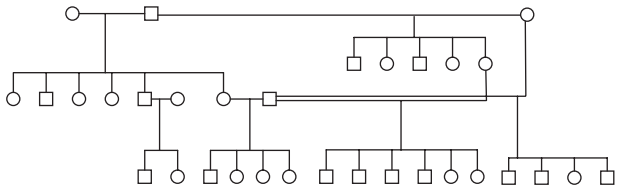

Figure 1 One of the multi-generation Newfoundland families submitted.

are often brother/sister or parent/child matings. Figure 1 shows one of the simpler family trees, a family of Newfoundland dogs collected by J Dukes-McEwan, and DLA typed in Manchester.

Table 2 lists the 29 haplotypes that were identified by segregation within families. Eight of these haplotypes were newly identified in this study, while the other 21 had previously been identified in homozygous dogs. Some of the new haplotypes have since been identified in other dogs.

In these 22 families and 247 offspring, we found one haplotype that represented a cross-over between DRB1 and DQA1 in a Labrador family. This family is shown in Figure 2, and both parents as well as 13 puppies, which were born in two separate litters, were typed for three DLA class

Table 2 Haplotypes inherited in the dog families

Number of haplotypes	DRB1	DQA1	DQB1	Previously known?
83	00101	00101	00201	
3	00101	00301	00501	
1	00101	00601	02301	
12	00103	00101	00201	New
29	00201	00901	00101	
54	00601	005011	00701	
36	00601	005011	02001	
14	010011	00201	01501	
2	01101	00201	01303	
49	01201	00401	013017	
9	01201	00401	01303	
42	01301	00101	00201	
1	01301	00301	00501	
5	01501	00901	00101	
6	01501	00601	00301	
18	01501	00601	02002	
75	01501	00601	02301	
6	01502	00601	02301	
2	01601	00101	00201	
13	01801	00101	00802	
14	02001	00401	01303	
6	03001	00601	00301	New
3	04001	01001	01901	
1	04701	00402	New	New
2	07001	01801	05001	New
42	07601	00601	02301	New
2	07801	00401	01303	New
4	08201	00601	05401	New
1	New	00101	00201	New

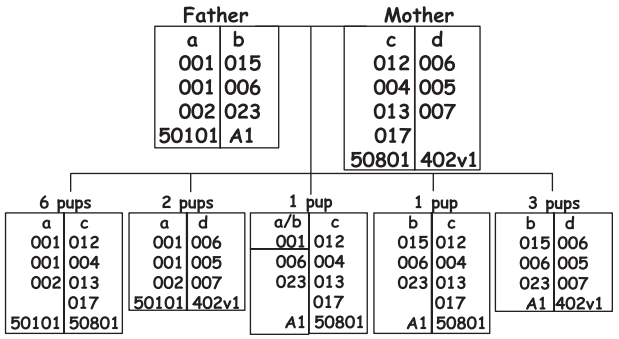

Figure 2 Labrador family showing DRB1/DQA1 cross-over. The alleles in the haplotypes are listed vertically in order: DRB1, DQA1, DQB1 (second DQB1, if present) and DLA-88.

II alleles. These were controlled matings, and there is no issue of paternity. The paternal haplotypes are labelled a and b, and the maternal haplotypes c and d. Six puppies inherited a+c, two a+d, one b+c and three b+d. The other puppy inherited the maternal c haplotype and an a/b paternal haplotype. The family also shows segregation of the DLA-DRB1*01201 haplotype (maternal c) that has two DQB1 alleles, mentioned in the previous report. The family was typed for DLA-88, a highly polymorphic class I gene in the major histocompatibility complex region. The segregation of DLA-88 fits with the haplotypes as shown in Figure 2. (N.B.: Not all the DLA-88 alleles have official names.)

It is interesting that a DRB1/DQA1 cross-over has been identified in these few dog families because there has not been a report of such a cross-over in all the thousands of human families that have been characterised for human leucocyte antigen (S. G. E. Marsh, Anthony Nolan Institute, London; personal communication).

Another interesting finding is the apparent creation of a new allele, the result of an intragenic cross-over within the maternal DRB1 locus. The allele was identified in a mixed breed family comprising both parents as well as 16 puppies (Figure 3). The presence of a new allele in the puppy that was not present in either parent was confirmed by

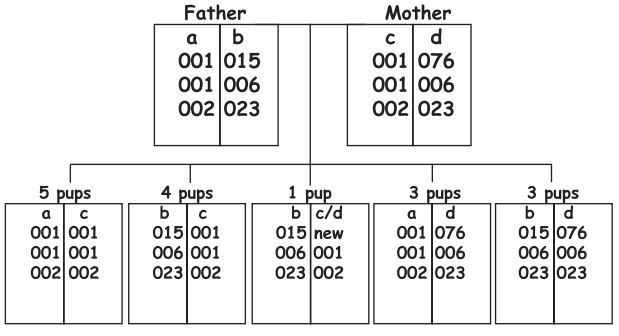

Figure 3 Mixed breed family with a new DRB1 allele in one puppy. The alleles in the haplotypes are listed vertically in order: DRB1, DQA1 and DQB1.

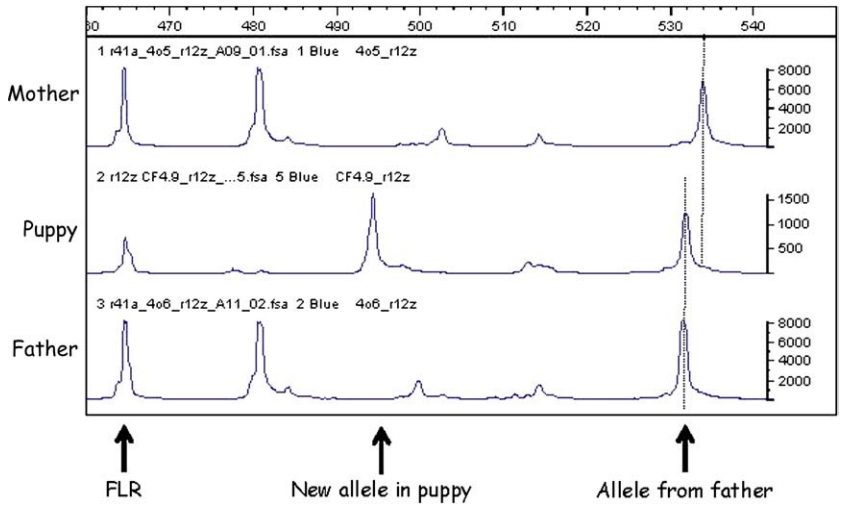

Figure 4 Reference strand-mediated conformation analysis (RSCA) results for new allele; FLR, fluorescent labelled reference.

Figure 5 Diagrammatic representation of the creation of the new allele.

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00101    *HFLEVAKSECYFTNGTERVRFVERYIHNREEFVRFDSDVGEYRAVTELGRPVAESWNGQKEILEQERATVDTYCRHNYGVIESFTVQRR*
new      ------------------------------------------------------------------------V-----------------
07601    -----MV-F--H---------LLV-D-Y-----------------------D--Y------L---R--E---V-----------------
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Figure 6 Amino acid sequence alignment of the new allele with the two maternal alleles; *, partial codon which has not been translated; – indicated a base that is identical to that in DRB1*00101.

reference strand-mediated conformation analysis (RSCA) (Figure 4). This Figure clearly shows that each parent has two allele peaks and that they have one peak in common (DLA-DRB1*00101). The other paternal allele (DRB1*01501) can be also seen in the puppy, whereas the other maternal allele (DRB1*07601) is not seen in the puppy, which has another peak altogether.

The presence of this new allele was confirmed by repeating the sequence based typing and RSCA from new polymerase chain reactions. It was also confirmed by DNA cloning and sequencing. Figure 5 shows a diagrammatic representation of the formation of this new allele. Figure 6 shows the amino acid sequence alignments for the new allele together with the two maternal alleles. Interestingly, a heterozygous crossbreed dog (submitted by UQU) appears to carry this new allele, together with DRB1*01501. This has yet to be confirmed by DNA cloning and sequencing.

While these data might suggest a high rate of cross-overs in dogs, this is at odds with other haplotype data from 3600 dogs (LJK, unpublished data), where there are few examples of unexpected DR/DQ combinations. The data provide a good evidence to support the existence of strong linkage disequilibrium between DR and DQ. If there were a high rate of cross-overs, then there would be evidence of the breakdown of the clear haplotypes detected to date. Indeed, we would not have been able to identify clear DR/DQ haplotypes in such a situation.

It may be pure serendipity that led us to find two interesting cross-overs in a relatively small study. We now have another large multigeneration family, with 64 members available for DLA typing. It will be interesting to see if we find any examples of cross-overs within this family.

This workshop component has shown some unexpected interesting findings and justifies this international collaboration.

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Conflict of Interest Statement

All authors have declared no conflicts of interests.