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Extensive interbreed, but minimal intrabreed, variation of DLA class II alleles and haplotypes in dogs

Key words:
class II haplotypes, DLA, dog breeds, MHC

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We are very grateful to Dr Linda Parkin (Nottingham, UK) for providing dog DNA samples. We are also very grateful to Sue Bell for her help in collecting and cataloguing all the samples.

Abstract: The DLA class II genes in the dog major histocompatibility complex are highly polymorphic. To date, 52 DLA-DRB1, 16 DLA-DQA1 and 41 DLA-DQB1 allelic sequences have been assigned. The aim of this study was to examine the intrabreed and interbreed variation of DLA allele and haplotype frequencies in dogs, and to ascertain whether conserved DLA class II haplotypes occur within and between different breeds. One thousand and 25 DNA samples from over 80 different breeds were DLA class II genotyped, the number of dogs per breed ranging from 1 to 61. DNA sequence based typing and sequence specific oligonucleotide probing were used to characterize dogs for their DLA-DRB1, DQA1 and DQB1 alleles. The high frequency of DLA class II homozygous animals (35%), allowed the assignment of many haplotypes despite the absence of family data. Four new DLA alleles were identified during the course of this study. Analysis of the data revealed considerable interbreed variation, not only in allele frequency, but also in the numbers of alleles found per breed. There was also considerable variation in the number of breeds in which particular alleles were found. These interbreed variations were found in all three DLA class II loci tested, and also applied to the three-locus haplotypes identified. Within this data set, 58 different DLA-DRB1/DQA1/DQB1 three-locus haplotypes were identified, which were all found in at least two different animals. Some of the haplotypes appeared to be characteristic of certain breeds. The high interbreed, and relatively low intrabreed, variation of MHC alleles and haplotypes found in this study could provide an explanation for reports of interbreed variation of immune responses to vaccines, viruses and other infections.

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Developments in molecular genetics are now rapidly leading to the determination of full genomic sequence in man and other species. This is already having a major impact in comparative genetics and helping to establish the evolutionary relationship between closely and distantly related species. It is also likely to assist in our understanding of the genetic basis of normal and disease processes/phenotypes.

Genomic sequence and organization in different species can only be usefully translated into meaningful comparative data when the extent of both the inter– and intra–species polymorphism is determined.

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This will be particularly important for genes where polymorphism has had a fundamental impact on survival and natural selection.

The Major Histocompatibility Complex (MHC) presents an obvious example as this highly conserved region encodes genes of fundamental importance in regulating acquired immunity. Comparative studies of MHC gene function will require an appreciation of both genomic organization and gene polymorphism.

MHC genomic organization has only been fully determined for relatively few species. Considerable variation is already apparent both with respect to relative gene position and the number of duplicated gene homologs. For example in the chimpanzee, 11 duplicated DRB genes have been identified (1). The importance of comparative studies are also emphasized by observations in the rat where extensive polymorphism in the TAP genes involved in antigen processing appear to fulfill the role of determining the affinity by which peptides bind to and are presented by class I MHC molecules to the T cell receptor (2). This is in stark contrast to humans, where TAP genes are less polymorphic and variation in peptide binding affinity is determined by MHC class I polymorphism itself.

The dog represents an important species for comparative MHC studies. Its prominent position is largely based on the extensive veterinary health care knowledge that has already been gained about the spontaneous diseases which develop in this species. Furthermore the dog has provided an important model for experimental transplantation and therapeutic studies.

The genomic organization of the MHC region in the dog has not yet been fully established although studies have provided preliminary evidence for DLA-DRA, DRB, DQA, DQB and DPB class II genes (3–5). The tissue distribution of these molecules has yet to be fully determined although earlier reports have indicated the expression of class II molecules on all peripheral blood lymphocytes (6, 7) unlike the situation in humans.

Previous studies have indicated that DLA-DRB1, DQA and DQB genes are highly polymorphic with 52, 16 and 41 alleles, respectively, being identified to date (8). It also appears that linkage disequilibrium exists between certain polymorphisms forming preferential allelic associations (9).

Hundreds of different dog breeds have now been established although the majority have only come into existence in the last two centuries (10). These breeds have largely been generated by the selection of gross phenotypic attributes particularly suited for work or decorative purposes, many being encoded by single gene mutations. A consequence of such a severe selection and inbreeding history is that many breeds have come from a relatively restricted gene pool.

Given this situation it would be predicted that many breeds would have a relatively restricted distribution of MHC alleles and as a consequence a high level of homozygosity. Furthermore it would be predicted that MHC alleles are likely to be different between breeds. If interbreed variation of MHC exists, this would have important implications for vaccine response and susceptibility/resistance to infection. We have DLA class II typed a large number of dogs to determine the distribution of alleles and haplotypes in a range of dog breeds.

Materials and methods

Dog samples

One thousand and 25 dogs were available for study and came from a variety of sources. The majority were consecutive consultations at the University of Liverpool Small Animal Hospital. Excess blood taken from samples required for clinical diagnostic purposes was collected into EDTA and frozen until required. Clinical data, age, gender and breed were recorded for each animal. The Small Animal Hospital is a referral center for the whole of the UK, and therefore dogs recruited from there are unlikely to be closely related. The Brazilian mongrel dogs came from Belém and Marajó Island, Pará State, and were sampled during a study of canine leishmaniasis (11). Some dogs were from the same litters, but most dogs were unrelated. Other samples were collected by the authors at their Veterinary Faculties.

The panel of dogs tested included 82 different breeds, including groups of crossbreeds and dogs of unrecorded breed (unknown), with numbers per breed ranging from 1 to 61. Table 1 summarizes the numbers of dogs per breed included in this study.

DNA extraction

Some samples were received as DNA, whereas others were received as frozen blood. In the latter case, DNA was extracted using DNeasy kits (Qiagen, Crawley, UK).

Numbers of samples genotyped

The number of dogs genotyped were 886 for DLA-DRB1, 887 for DLA-DQA1 and 293 for DLA-DQB1. Seven hundred and fifty seven were typed for both DLA-DRB1 and DQA1, 255 were typed for both DLA-DQA1 and DQB1 and 225 were typed for all three loci.

DLA-DRB1, DQA1 and DQB1 allele assignment

DLA-DRB1 and DQA1 alleles were originally assigned using sequence specific oligonucleotide probing (SSOP) as described previously (12, 13). Later samples were characterized for their DLA-

The distribution of dog breeds and mongrels available for study

Breed	Number	Breed	Number
Basset Hound	5	Poodle	11
Beagle	61	Poodle (Miniature)	17
Bernese Mountain Dog	7	Pug	1
Bichon Frise	1	Pyrenean Mountain Dog	1
Bloodhound	1	Retriever (Flatcoat)	3
Borzoi	1	Retriever (Golden)	42
Bouvier	1	Rhodesian Ridgeback	5
Boxer	27	Rottweiler	22
Briard	4	Samoyed	5
Bull Mastiff	9	Schnauzer	2
Bulldog	3	Setter (English)	54
Chow Chow	7	Setter (Gordon)	2
Collie	11	Setter (Irish)	12
Collie (Bearded)	4	Sharpei	3
Collie (Border)	19	Sheepdog (Old English)	5
Collie (Rough)	7	Sheepdog (Shetland)	7
Corgi	3	Shih Tzu	14
Dachshund	2	Spaniel	4
Dalmatian	1	Spaniel (Cavalier King Charles)	20
Deerhound	1	Spaniel (Cocker)	28
Doberman	25	Spaniel (English springer)	4
Foxhound	3	Spaniel (Springer)	14
German Shepherd Dog	45	Spinone	4
German Spitz	1	St Bernard	3
Great Dane	5	Terrier (Airedale)	3
Greyhound	3	Terrier (Border)	1
Hovawart	6	Terrier (Bull)	1
Husky	12	Terrier (Cairn)	3
Irish Wolfhound	6	Terrier (Jack Russell)	9
Japanese Akita	56	Terrier (Manchester)	1
Japanese Spitz	1	Terrier (Staffs Bull)	8
Labrador	66	Terrier (Tibetan)	3
Leonberger	2	Terrier (Welsh)	2
Lhasa Apso	2	Terrier (West Highland White)	26
Lurcher	1	Terrier (Yorkshire)	11
Maltese	1	Vizsla (Hungarian)	5
Mastiff (Neapolitan)	3	Weimaraner	28
Mongrel Brazilian	113	Whippet	4
Newfoundland	5	Crossbreed	52
Papillon	4	unknown	44
Pharaoh Hound	1		
Pointer	5	Total	1025

Table 1

DRB1, DQA1 and DQB1 alleles by sequence based typing (SBT), as previously described (14, 15).

PCR samples were purified prior to sequencing as follows: 5 µL PCR product was vortexed with 2 µL Shrimp Alkaline Phosphatase and 1 µL Exonuclease-1 (both from Pharmacia), incubated for 1 h at 37°C and then for 15 min at 80°C.

Definition of haplotypes

Three-locus, DLA-DRB1/DQA1/DQB1, haplotypes were identified by following a sequential analytical process. Firstly, all dogs that were homozygous at all three loci were selected, and from these several different DLA-DRB1-DQA1-DQB1 haplotype combinations were identified. Dogs that were homozygous at only two loci were then selected. From these dogs, many of the previous haplotypes were confirmed and also several further haplotypes were identified. The remaining dogs were examined using the haplotype data already identified and haplotypes were assigned to each of these dogs. From these dogs further possible haplotypes were identified.

Two-locus haplotypes, DLA-DRB1/DQA1 and DLA-DQA1/DQB1, were identified using similar sequential analytical processes.

Results

DLA-DRB1

DLA-DRB1 data were generated for 886 dogs from 78 different breeds, including crossbreeds and mongrels, with numbers per breed ranging from 1 to 105. As more than half these data were generated by SSOP, 30 broad DRB1 types have been used in the analysis, as the SSOP system used could not distinguish all the individual alleles. Clustering of DLA-DRB1 subtypes in particular breeds was not apparent in the SBT data. The grouping of DRB1 alleles into broad types was therefore unlikely to bias the analysis. DLA-DRB1 allele frequencies were generated for the general dog population, and are shown in Table 2. However, such frequencies are of limited use, as an analysis by breed revealed a large amount of variation between breeds, not only in allele frequencies, but also in the number of alleles found per breed, and the number of breeds per allele. Table 2 also shows the DLA-DRB1 allele frequencies for 20 breeds where 10 or more dogs were tested. As expected, the mongrels have the highest number of different alleles (22 out of 30 possible alleles tested). While the Labrador is the breed with the most alleles (14/30), some breeds, such as Doberman and Rottweiler, have only two alleles despite 20 animals having been tested. In fact all the Dobermans tested carried DRB1*006, with 95% being homozygous for DRB1*006. Huskies had

Distribution of broad DLA-DRB1 alleles (%) in mongrels, crossbreeds and dog breeds (where n > 9)

DRB1*	All dogs	No. breeds in which alleleis present	Cross-breed	Mongrel Brazilian	Beagle	Boxer	Collie Border	Doberman	German Shepherd	Husky	Japanese Akita	Labrador	Poodle Miniature
n	886	78	40	105	60	26	19	20	42	12	52	61	23
001	18.1	36	13.8	10.0	40.0	3.8	5.3	–	15.5	8.3	11.5	23.0	15.2
002	5.1	24	5.0	1.4	13.3	–	15.8	2.5	7.1	–	13.5	–	–
003	3.6	25	7.5	5.7	–	–	2.6	–	1.2	–	1.0	0.8	2.2
004	1.7	10	2.5	0.5	–	38.5	–	–	–	–	–	–	2.2
005	1.5	14	3.8	2.9	–	–	5.3	–	–	–	–	0.8	–
006	15.0	37	10.0	7.6	7.5	9.6	5.3	97.5	–	12.5	1.0	9.0	4.3
008	1.3	4	3.8	0.5	12.5	–	–	–	–	–	–	3.3	–
011	4.5	11	6.3	6.2	1.7	–	–	–	32.1	–	–	0.8	–
012	7.7	24	6.3	1.0	0.8	3.8	–	–	2.4	4.2	1.0	31.1	–
013	4.3	19	3.8	8.1	1.7	23.1	13.2	–	–	–	10.6	0.8	4.3
014	0.2	1	–	–	2.5	–	–	–	–	–	–	–	–
015	20.9	55	26.3	26.7	16.7	21.2	34.2	–	38.1	25.0	–	22.1	65.2
017	0.4	2	–	2.9	0.8	–	–	–	–	–	–	–	–
018	2.5	16	5.0	1.4	–	–	13.2	–	–	–	–	1.6	4.3
020	4.6	26	5.0	3.3	–	–	5.3	–	3.6	12.5	1.0	4.1	2.2
024	3.3	1	–	–	–	–	–	–	–	–	55.8	–	–
025	1.0	4	–	1.4	–	–	–	–	–	–	–	–	–
026	0.2	3	–	–	0.8	–	–	–	–	–	–	0.8	–
029	0.2	4	1.3	0.5	–	–	–	–	–	–	–	0.8	–
032	0.3	1	–	–	–	–	–	–	–	–	4.8	–	–
033	0.3	1	–	–	–	–	–	–	–	–	–	–	–
039	0.1	1	–	–	–	–	–	–	–	4.2	–	–	–
040	0.3	1	–	–	–	–	–	–	–	20.8	–	–	–
046	0.3	2	–	1.0	–	–	–	–	–	–	–	–	–
047	0.2	1	–	–	–	–	–	–	–	12.5	–	–	–
048	0.2	1	–	1.9	–	–	–	–	–	–	–	–	–
050	0.1	1	–	1.0	–	–	–	–	–	–	–	–	–
051	0.2	1	–	1.4	–	–	–	–	–	–	–	–	–
052	1.5	1	–	12.9	–	–	–	–	–	–	–	–	–
new	0.6	5	–	1.9	1.7	–	–	–	–	–	–	0.8	–

Table 2

a high number of different DRB1 alleles, 8/30, considering that only 12 animals had been tested.

Seventeen of the DRB1 alleles had a relatively restricted distribution, being found in less than six of the breeds tested. Ten DRB1 alleles were found in more than 10, but less than 30, different breeds, while only three DRB1 alleles were found in more than 30 of the 78 breeds tested. However, even these three common alleles had widely varying interbreed frequencies. Thus DRB1*001 was found in 36 of the 78 breeds tested, with an allele frequency ranging from 3.8% in Weimaraners to 73.1% in West Highland White Terriers. Some alleles such as DRB1*04601 which previously appeared (LJ Kennedy, unpublished data) to be limited to a single breed (Papillon), have since been found in other groups: mongrels.

DLA-DQA1

DLA-DQA1 data were generated for 887 dogs from 81 different breeds, including crossbreeds and mongrels, with numbers per breed

Continued

DRB1*	All dogs	No. breeds in which allele is present	Retriever Golden	Rott-weiler	Setter English	Setter Irish	Shih Tzu	Spaniel Cavalier King Charles	Spaniel Cocker	Spaniel Springer	Terrier West Highland White	Terrier York-shire	Weimaraner
n	886	78	35	21	47	12	13	20	26	13	26	10	26
001	18.1	36	5.7	35.7	63.8	54.2	–	–	11.5	11.5	73.1	5.0	3.8
002	5.1	24	–	–	–	–	–	–	–	–	–	15.0	–
003	3.6	25	7.1	–	1.1	–	–	10.0	15.4	3.8	–	–	–
004	1.7	10	1.4	–	–	–	–	–	–	–	–	–	–
005	1.5	14	–	–	–	8.3	–	–	–	3.8	3.8	10.0	–
006	15.0	37	7.1	64.3	30.9	8.3	30.8	7.5	65.4	23.1	–	30.0	7.7
008	1.3	4	–	–	–	–	–	–	–	–	–	–	–
011	4.5	11	–	–	–	–	–	55.0	3.8	–	–	–	–
012	7.7	24	54.3	–	–	–	–	–	–	11.5	–	–	25.0
013	4.3	19	4.3	–	4.3	–	–	–	–	–	–	–	–
014	0.2	1	–	–	–	–	–	–	–	–	–	–	–
015	20.9	55	14.3	–	–	–	7.7	2.5	3.8	23.1	23.1	40.0	44.2
017	0.4	2	–	–	–	–	–	–	–	–	–	–	–
018	2.5	16	–	–	–	8.3	–	–	–	–	–	–	–
020	4.6	26	5.7	–	–	20.8	–	25.0	–	23.1	–	–	19.2
024	3.3	1	–	–	–	–	–	–	–	–	–	–	–
025	1.0	4	–	–	–	–	42.3	–	–	–	–	–	–
026	0.2	3	–	–	–	–	–	–	–	–	–	–	–
029	0.2	4	–	–	–	–	–	–	–	–	–	–	–
032	0.3	1	–	–	–	–	–	–	–	–	–	–	–
033	0.3	1	–	–	–	–	19.2	–	–	–	–	–	–
039	0.1	1	–	–	–	–	–	–	–	–	–	–	–
040	0.3	1	–	–	–	–	–	–	–	–	–	–	–
046	0.3	2	–	–	–	–	–	–	–	–	–	–	–
047	0.2	1	–	–	–	–	–	–	–	–	–	–	–
048	0.2	1	–	–	–	–	–	–	–	–	–	–	–
050	0.1	1	–	–	–	–	–	–	–	–	–	–	–
051	0.2	1	–	–	–	–	–	–	–	–	–	–	–
052	1.5	1	–	–	–	–	–	–	–	–	–	–	–
new	0.6	5	–	–	–	–	–	–	–	–	–	–	–

Newly defined alleles found in single animals are included in "new"

– allele tested but absent

Broad DLA-DRB1 types include the following subtypes: DRB1*001 = 00101, 00102; DRB1*002 = 00201, 00202; DRB1*003 = 00301, 00901; DRB1*004 = 00401, 010011, 010012, 02701; DRB1*006 = 00601, 02801; DRB1*012 = 01201, 01901; DRB1*013 = 01301, 01302; DRB1*015 = 01501, 01502, 01503, 01504

Table 2

ranging from 1 to 111. Overall allele frequencies were generated for the total dog population, and are shown in Table 3. Again, the overall frequencies are of limited value because of the high interbreed variation of allele numbers and frequencies. Table 3 summarizes the DLA-DQA1 allele frequencies for the 20 breeds where 10 or more animals were tested. There are fewer DLA-DQA1 alleles compared to DLA-DRB1, but the general pattern of interbreed variation is the same. Mongrels have the highest number of different alleles (13 out of a possible 17), with individual breeds having numbers varying from 2 to 9. Nine DQA1 alleles were found in less than 10 different breeds, three DQA1 alleles were found in more than 10, but less than 30 different breeds and five were found in more than 30 of the 81 breeds tested.

Distribution of DLA-DQA1 alleles (%) in mongrels, crossbreeds and dog breeds (where n > 9)

DQA1*	All dogs	No. breeds in which alleleis present	Cross-breed	Mongrel Brazilian	Beagle	Boxer	Collie	Collie Border	Doberman	German Shepherd	Husky	Japanese Akita	Labrador
n	887	81	48	111	51	20	11	19	20	34	12	51	56
00101	29.5	53	25.0	23.4	44.1	32.5	27.3	36.8	–	13.2	4.2	61.8	25.0
00201	6.4	25	5.2	10.8	2.0	22.5	–	–	10.0	14.7	4.2	–	1.8
00301	5.7	20	10.4	7.2	14.7	–	–	5.3	–	–	–	13.7	6.3
00401	14.3	38	10.4	5.4	1.0	5.0	4.5	5.3	85.0	10.3	–	–	32.1
00402	0.2	1	–	–	–	–	–	–	–	–	12.5	–	–
005011	10.7	37	7.3	6.3	4.9	10.0	–	5.3	2.5	–	20.8	1.0	9.8
00601	16.6	46	20.8	27.0	1.0	10.0	18.2	31.6	–	36.8	37.5	–	19.6
00701	2.9	18	5.2	–	1.0	20.0	–	–	–	16.2	–	1.0	1.8
00801	0.1	2	–	–	–	–	–	–	–	–	–	–	–
00901	9.7	35	10.4	1.8	31.4	50.0	15.8	2.5	8.8	–	19.6	3.6	
01001	0.3	2	1.0	–	–	–	–	–	–	–	20.8	–	–
01101	0.1	1	–	0.5	–	–	–	–	–	–	–	–	–
012011	1.6	2	–	12.2	–	–	–	–	–	–	–	–	–
012012	0.6	2	–	1.4	–	–	–	–	–	–	–	–	–
01401	0.8	6	4.2	2.7	–	–	–	–	–	–	–	2.0	–
01501	0.3	2	–	0.9	–	–	–	–	–	–	–	–	–
new	0.2	4	–	0.5	–	–	–	–	–	–	–	1.0	–

DQA1*	All dogs	No. breeds in which allele is present	Poodle	Retriever Golden	Rott-weiler	Setter English	Setter Irish	Shih Tzu	Spaniel Cavalier King Charles	Spaniel Cocker	Spaniel Springer	Terrier West Highland White	Weimaraner
n	887	81	10	35	17	48	11	10	16	23	13	21	28
00101	29.5	53	40.0	12.9	69.8	63.6	–	9.4	30.4	3.8	59.5	3.6	
00201	6.4	25	–	1.4	5.9	7.3	–	–	46.9	2.2	11.5	–	–
00301	5.7	20	–	4.3	26.5	2.1	9.1	20.0	–	–	3.8	4.8	–
00401	14.3	38	–	64.3	–	–	13.6	–	21.9	–	34.6	–	48.2
00402	0.2	1	–	–	–	–	–	–	–	–	–	–	–
005011	10.7	37	–	2.9	61.8	19.8	9.1	20.0	9.4	63.0	19.2	–	8.9
00601	16.6	46	60.0	10.0	–	–	4.5	5.0	–	2.2	7.7	23.8	32.1
00701	2.9	18	–	1.4	2.9	1.0	–	20.0	12.5	–	7.7	–	7.1
00801	0.1	2	–	–	2.9	–	–	–	–	–	–	–	–
00901	9.7	35	–	1.4	–	–	–	–	–	2.2	7.7	11.9	–
01001	0.3	2	–	–	–	–	–	–	–	–	–	–	–
01101	0.1	1	–	–	–	–	–	–	–	–	–	–	–
012011	1.6	2	–	–	–	–	–	–	–	–	–	–	–
012012	0.6	2	–	–	–	–	–	35.0	–	–	–	–	–
01401	0.8	6	–	–	–	–	–	–	–	–	3.8	–	–
01501	0.3	2	–	–	–	–	–	–	–	–	–	–	–
new	0.2	4	–	1.4	–	–	–	–	–	–	–	–	–

Table 3

DQA1*00101 was found in 53/81 breeds tested, but its allele frequency varied from 3.8% (Springer Spaniel) to 69.8% (English Setter).

DLA-DQB1

DLA-DQB1 data were generated for 293 dogs from 56 different breeds, including crossbreeds and mongrels, with numbers per breed ranging from 1 to 102. Overall allele frequencies were generated for the total dog population, and are shown in Table 4. Only five breeds with more than 10 animals were tested, and the highest number of alleles found in any one breed was seven, although the mongrels had 25 different DQB1 alleles. The majority, 24 out of 29, DQB1 alleles were found in less than 10 different breeds, while only five were found in more than 10, but less than 30 of the 56 different breeds tested. Although fewer animals and breeds have been tested for DLA-DQB1,

Distribution of DLA-DQB1 alleles (%) in mongrels, crossbreeds and dog breeds (where n > 9)

DQB1*	All dogs	No. breeds in which alleleis present	Mongrel Brazilian	Beagle	Labrador	Poodle (Miniature)	Retriever (Golden)	Spaniel (Cocker)
n	293	56	102	15	10	11	14	11
00101	6.1	14	2.0	13.3	–	–	–	4.5
00201	14.3	20	13.2	50.0	10.0	4.5	7.1	13.6
00301	3.6	8	4.9	–	–	–	–	4.5
00401	2.0	5	2.0	3.3	10.0	–	–	–
00501	3.6	8	5.4	6.7	–	–	3.6	–
00502	0.7	2	0.5	–	–	–	–	–
00701	11.3	18	5.4	13.3	15.0	9.1	–	59.1
008011	5.8	9	5.9	6.7	–	–	10.7	9.1
008012	0.2	1	0.5	–	–	–	–	–
00802	4.1	6	4.4	–	–	–	–	–
01201	0.2	1	–	–	–	–	–	–
01302	1.5	7	1.0	3.3	–	–	–	–
01303	13.1	24	9.8	3.3	10.0	–	28.6	9.1
01304	0.7	1	2.0	–	–	–	–	–
01501	1.9	5	1.0	–	–	9.1	–	–
01701	2.2	4	–	–	35.0	–	14.3	–
01901	0.5	2	–	–	–	–	–	–
02001	1.2	2	2.9	–	–	–	–	–
02002	1.4	7	1.0	–	–	4.5	–	–
02201	0.9	4	1.0	–	–	–	–	–
02301	10.9	20	9.8	–	15.0	50.0	14.3	–
02302	0.3	1	1.0	–	–	–	–	–
02601	3.1	3	7.4	–	–	4.5	–	–
02801	0.5	2	0.5	–	–	–	–	–
02901	0.5	1	1.5	–	–	–	–	–
03501	5.3	2	12.7	–	–	–	–	–
03601	1.0	2	1.0	–	–	18.2	–	–
03801	0.3	1	–	–	–	–	–	–
new	2.9	6	3.4	–	5.0	–	21.4	–

Newly defined alleles found in single animals are included in “new”.

Table 4

the pattern of interbreed variation appears similar to that seen in DLA-DRB1 and DQA1.

New DLA alleles

Four new DLA alleles were identified during this study: DRB1*04901, DRB1*05301, DQB1*03801 and DQB1*03901. Each of the alleles was found in several different animals. The Accession numbers for the new alleles are DRB1*04901; AJ316218: DRB1*05301; AJ316219: DQB1*03801; AJ316221: DQB1*03901; AJ316222.

Three locus haplotypes

Three-locus DLA class II haplotypes were identified using the sequential analytical process described in the methods. Firstly, 64 dogs that were homozygous at all three loci were selected, and in these, 28 different DLA-DRB1-DQA1-DQB1 combinations were identified, which occurred in varying numbers from 1 to 10. Dogs that were homozygous at only two loci ($n = 20$) or one locus ($n = 22$) were then selected. From these many of the previous haplotypes were confirmed, and also 19 further haplotypes identified. The remaining dogs ($n = 119$) were then examined using the haplotype data already identified, and haplotypes were assigned to each of these dogs. (In the total cohort of 225 dogs, only 6 haplotypes were unassigned ($< 1.3\%$). From these, a further 33 possible haplotypes were identified, making a total of 80 different haplotypes. However, 22 of these 80 haplotypes were only found in one heterozygous individual and these have been grouped together as ''others'' until such time as more animals are found with those haplotypes. This left 58 haplotypes of which 52 were found in at least two different animals, and six haplotypes found in single homozygous animals only. Forty-seven of these 58 haplotypes have been identified in at least one dog homozygous for one or more of the loci studied. Two-locus haplotypes, for DLA-DRB1/DQA1 ($n = 757$) and DQA1/DQB1 ($n = 255$) were identified using a similar sequential analysis method.

Table 5 lists the 58 three-locus haplotypes identified, together with their frequencies, and the number of breeds in which each haplotype was found. Only 4 haplotypes were found in 10 or more of the 50 breeds tested, and most of the rest showed a limited breed distribution. Some haplotypes appear to be breed specific at this time, these are indicated in Table 4. In human population studies, a minimum of 50 individuals is considered appropriate for generating HLA allele frequencies. However, due to the very restricted nature of the sets of alleles found within dog breeds, it is reasonable to use smaller sample sizes to predict haplotypes that are characteristic of a breed.

Some DLA-DRB1 alleles are found in haplotypic association with several different DLA-DQ pairs, e.g., DRB1*00101 and DRB1*01501, while others are found in only one combination, e.g., DRB1*002. In general, the higher frequency DRB1 alleles are found in several different haplotypic combinations, while the rarer DRB1 alleles tend to be found in single combinations. Within DLA-DQ, it would appear that only certain combinations of DLA-DQA1/DQB1 alleles are stable, in that not all possible combinations have been found. It has been demonstrated in human HLA-DQ transfection experiments that not all HLA-DQA1/DQB1 pairs form viable heterodimers (16). In general, the subset of DQB1 alleles that are found in combination with a particular DQA1 allele are closely related, with only a few base pair differences between them.

Homozygosity

The levels of homozygosity within these data were very high: 40.5%, 45.4% and 34.1% of dogs were homozygous at DLA-DRB1, DQA1 and DQB1, respectively. In total, 34.5% of animals were homozygous at DLA-DRB1 and DQA1 ($n = 757$), 31.4% were homozygous at DLA-DQ ($n = 255$), and 28.4% were homozygous at all three class II loci tested ($n = 225$). These data do not fit Hardy–Weinberg equilibrium ($P < 0.0001$), and, as anticipated, confirm that many breeds are very inbred with respect to their MHC region. Although some of these data were generated using SSOP, where it is possible to miss new alleles, most samples that were homozygous at one locus and heterozygous at another, were rechecked by SBT for the homozygous locus.

Animals tended to be homozygous within the rarer breeds such as Bichon Frise, Lhasa Apso, Leonberger, Samoyed and Italian Spinone, whereas animals from breeds with larger population bases such as German Shepherd dogs, Labradors, Border Collies and Golden retrievers tended to be heterozygous for their MHC.

Discussion

A large cohort of dogs was genotyped for their DLA-DRB1, DQA1 and DQB1 alleles and a series of three-locus haplotypes was established. Although family data were not available, the use of DLA homozygous animals allowed unequivocal assignment of most haplotypes. Unsurprisingly we found that there was a wide representation of all the DLA alleles and haplotypes defined to date in mongrels and crossbreeds. This is likely to be due to the greater genetic admixture present in these groups. However, a restricted representation of DLA alleles and haplotypes were found within most of the breeds tested and a generally higher degree of MHC homozygosity. There were some alleles, but more particularly haplotypes, that could be said to be almost characteristic of particular breeds, see Table 5. There was great variation between breeds, such that in some instances, there

DLA-DRB1/DQA1/DQB1 haplotype frequencies in dogs (n = 225)

DRB1*	DQA1*	DQB1*	Haplotype frequency (%)	No. breeds in which haplotype is present *n* = 50	DRB1*	DQA1*	DQB1*	Haplotype frequency (%)	No. breeds in which haplotype is present *n* = 50
00101	00101	00201	7.6	11	015	00601	02002	1.6	6
00101	00101	03601	0.4	1	015	00601	02201	0.9	3
00101	00201	01303	0.9	2[a]	015	00901	00101	1.6	5
00101	00301	00401	0.9	2[b]	015	01401	00301	0.4	1
00102	00101	00201	1.8	3[c]	01501	00401	00301	0.4	1
002	00901	00101	4.7	9	01501	00601	00301	2.7	6
003	00101	00801	5.1	7	01501	00601	02301	5.3	10
003	01401	00801	0.4	1	01501	00601	02601	2.7	2
00401	00201	01501	0.7	2	01502	00601	02301	3.1	7
00401	00701	01501	0.7	1[d]	01502	01401	02301	0.4	2
00501	00301	00501	0.7	3	01701	00201	01303	0.4	2
00601	00401	01303	2.2	3[e]	01702	00201	01304	0.9	1
00601	005011	00701	8.7	14	01801	00101	00201	0.4	1
00601	005011	02801	0.7	2	01801	00101	00801	0.4	2
00601	00701	00701	0.4	2	01801	00101	00802	1.1	3
008	00301	00401	0.9	3	02001	00401	01302	0.4	2
010011	00201	01501	0.7	3	02001	00401	01303	4.0	13
01101	00201	01302	1.3	5	02301	00301	00501	1.8	4
01101	00201	01303	3.6	4	02401	01401	00801	0.4	1[g]
01201	00401	01303	1.1	3	02501	012012	03501	1.3	2[h]
01201	00401	01701	2.2	3[f]	02801	005011	00701	0.4	1
01201	00401	new	0.4	1	02901	00301	00401	0.4	2
01301	00101	00201	2.4	4	03301	00301	03801	0.7	1[h]
01301	00301	00501	1.6	3	04601	01501	00802	1.1	2
01301	00301	00502	0.4	1	04801	00101	00802	0.4	1
01401	00101	00801	0.4	1	05001	00601	02302	0.4	1
015	00401	02301	1.1	4	05101	00201	02901	0.7	1
015	00601	01303	0.4	2	05201	012011	03501	4.9	1[i]
015	00601	01901	0.7	2	Other single haplotypes			4.9	10
015	00601	02001	1.1	1	Unassigned haplotypes			1.3	2

Some haplotypes appear to be predominantly breed specific. These are indicated on the table: a = English Springer; b = Rottweiler; c = Beagle; d = Boxer; e = Doberman; f = Labrador and Golden Retriever; g = Japanese Akita; h = Shih Tzu; i = Brazilian mongrels. Where there is more than one breed indicated, the other breed is usually crossbreed, mongrel or unknown.

Table 5

was no overlap in alleles and haplotypes when comparing some pairs of breeds, e.g., Doberman and West Highland White Terriers. A small number of breeds showed a wider range of alleles and haplotypes; these included German Shepherd dogs, Labradors, Golden retrievers and Huskies. Presumably these breeds originated either from a larger population base (and thus greater gene pool), or have been subjected to more continuous admixture from other breeds. The latter is certainly true for Huskies, whose breeders have continued to introduce genes from other breeds e.g., Saluki, to improve certain characteristics such as running speed.

As haplotype data continues to accumulate, especially if the haplotypes can be refined and extended to include class I and class III loci, it may be possible to use haplotype data to establish the genetic relationships between different dog breeds.

The biological importance and resulting consequences of the variation observed is likely to have a major influence in determining the

immune response of individual animals and could explain the variations observed in immune response between different dog breeds. The MHC of a dog will affect its susceptibility/resistance to infection, autoimmunity, the possible development of certain malignancies and the level of response to vaccination. There is likely to be a functional advantage for particular DLA allelic combinations, as preferential allelic associations have been demonstrated in humans (16, 17), and an allele from one locus may complement the function of an allele at another locus (18). The interbreed variation of MHC could explain the anecdotal reports of some breeds being more susceptible to certain diseases. One strain of English Setters appears to be highly susceptible to hypothyroid disease (L. Parkin, personal communication). Other data show that there is an increased prevalence of hypothyroid disease in certain breeds which may be due to the limited number MHC alleles (19). An increased incidence of thyroiditis has been reported in Beagles (20), Great Danes (21) and English Cocker Spaniels (21). Studies are required to examine such diseases and should take account of the restriction of MHC alleles in breeds, as one cannot compare disease groups to random healthy dog cohorts. We have recently shown that dogs with polyarthritis had an increased frequency of a five amino acid sequence motif in the third hypervariable region of DLA-DRB1 when compared to breed matched controls (22).

In a recent report from the American Kennel Club (AKC) on the major health issues in dogs (http://www.akcchf.org/top%20ten.htm), four out of the top 10 conditions had an immune component. These were hypothyroid disease, autoimmune diseases (e.g., canine rheumatoid arthritis, SLE, diabetes), cancer and allergic dermatitis. The Canine Health Foundation (part of the AKC), funds canine research (http://www.akcchf.org/research.htm) and also co-ordinates a list from the breed clubs of particular diseases in specific breeds that the breeders wish to be investigated (http://www.akcchf.org/parentclubs/parent.htm#Parent Club Partnership Program)

There may be consequences of the increased MHC homozygosity observed in some breeds, such as a polarization of the immune response depending on the antigen being presented. There are some data in humans to suggest that MHC homozygous individuals have a reduced fertility (23) and there are also reports of associations with repeated miscarriage (23). Similar effects may occur in dogs, especially in breeds that are very inbred. Some breeds certainly have much smaller litter sizes than others (24). MHC homozygosity has also been used to explain the total susceptibility of cheetahs to feline infectious peritonitis (25). Homozygosity may thus be a threat in some breeds in the future due to new emerging infections or mutations of current infectious agents.

Recently, renal transplantation has become a recognized veterinary therapeutic treatment available for dogs with end-stage renal failure. Quite apart from the ethical considerations, there are major implications for transplant survival with regards to donor selection. Currently there is no matching of MHC for canine renal transplantation, although we know from work in man that MHC matching has a dramatic beneficial effect on transplant survival. MHC matching can also significantly reduce the amount (and cost), of immuno-suppressive therapy that is required to maintain tolerance of grafts. Our data shows that a minimal strategy of transplanting within a breed might have a significant effect on graft survival, and complete matching could lead to less use of immuno-suppression.

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