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Canine DLA diversity: 3. Disease studies

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Abstract

There are many millions of dogs worldwide, and these dogs have many different functions. The most obvious use is providing companionship, but there are also many working dogs, including guide dogs for the blind, hearing dogs, guard dogs and farm dogs, to mention a few. The health and welfare of these dogs is of great concern to dog owners, dog breeders and to those who use dogs in their work. Dogs spontaneously develop many diseases that are very similar to their human counterparts. Dogs may, therefore, provide exceptional animal models for such diseases. Identifying genetic markers in the dog may be easier than in humans, and may then provide useful information about genes that can be transferred to humans. This study looked for associations between DLA and two autoimmune diseases of the dog, diabetes and hypothyroidism. DLA associations were found for both of these diseases.

Introduction

There are an estimated seven million dogs in the UK alone, which means that, on average, one in ten people in the UK owns a dog. As well as providing companionship for many people, there are also many working dogs. These include guide dogs for the blind (seeing-eye dogs), hearing dogs, sniffer dogs, farm dogs and guard dogs. Dogs are also used to help locate earthquake and avalanche victims, and in mountain rescues. The health and welfare of these dogs represents a large investment for many companies (e.g. the police, the Guide Dog association), and there is great interest in improving the health of dogs.

Research into human immune mediated diseases has revealed strong associations with major histocompatibility complex (MHC) alleles and haplotypes. Similar work in dogs is starting to reveal MHC associations for canine polyarthritis (1), (the equivalent of human rheumatoid arthritis), canine diabetes (2), (the equivalent to late onset Type I human diabetes), canine hypothyroidism (3, 4) (the equivalent to human Hashimoto's disease) and canine immune-mediated haemolytic anaemia (5).

Materials and methods

For the disease component, we suggested collecting DLA data from dogs of different origins with the following diseases: diabetes mellitus, hypothyroid disease, exocrine pancreatic insufficiency, anal furunculosis and systemic lupus erythematosus. In the event dogs were only submitted with diabetes or hypothyroidism. There were 221 diabetic dogs (17 from CRO, 189 from MAN and 15 from UQU), and 186 hypothroid dogs (27 from AKA, 6 from CRO and 153 from MAN).

Results

Diabetes mellitus

In man, MHC class II alleles are strongly associated with human type 1 diabetes (6) accounting for over half of the genetic risk (6). Initial human studies demonstrated a strong association with human leukocyte antigen (HLA)-DR3 and -DR4 in Type 1 diabetes (7). Subsequently, diabetes susceptibility was shown to be associated

with the presence of an amino acid other than aspartic acid at position 57 of the HLA-DQ beta-chain (DQB non-Asp57) (6) and also with the presence of arginine at position 52 of the HLA-DQ alpha-chain (DQA Arg52) (8). However, the association of diabetes with MHC susceptibility alleles appears to differ in humans dependent upon ethnic origin (9), which is likely to be an important consideration when attempting to identify MHC susceptibility alleles in different dog breeds.

Diabetes mellitus occurs spontaneously in domesticated dogs, with an estimated prevalence of 0.32% (10). Diabetic dogs usually present with polydipsia, polyuria and weight loss, associated with hyperglycaemia and glucosuria. Diabetes typically affects dogs aged between 5 and 12 years, and it is uncommon in juvenile animals. Oral hypoglycaemic drugs are ineffective in canine diabetes and virtually all dogs are dependent upon insulin therapy to manage their hyperglycaemia. Some breeds are known to be more susceptible to developing diabetes including the Samoyed, Tibetan terrier and Cairn terrier. In contrast, other breeds such as the Boxer, German shepherd dog and Golden retriever are under-represented in the diabetic dog population (10, 11).

Table 1 shows the breakdown by breed of the 221 dogs submitted with diabetes. Analysis of all the diabetic dogs compared with 202 breed matched controls showed a significant increase of one haplotype DLA-DRB1*009/DQA1*001/DQB1*008 in cases. Within this cohort there are too few dogs of individual breeds to perform separate analyses. DQA1 alleles carrying an arginine at codon 55 (which may be analogous to Arg52 in human DQA1) also showed a significant increase. One DQ haplotype, DQA1*004/DQB1*013, was significantly reduced in cases (Table 2). These results confirm previous results, and indicate that the MHC has a strong influence on susceptibility and resistance to canine diabetes.

Table 1 Numbers of dogs by breed for the diabetes cohort

Breed	Cases	Breed matched controls	Breed	Cases	Breed matched controls
Australian Cattle dog	1	1	Samoyed	8	7
Australian Shepherd dog	1	1	Schnauzer (Miniature)	4	4
Beagle	3	3	Setter (English)	1	1
Bichon Frise	3	3	Setter (Gordon)	1	1
Collie	3	3	Sheepdog (Old English)	1	1
Collie (bearded)	1	1	Sheepdog (Shetland)	1	1
Collie (border)	10	10	Shih Tzu	2	2
Collie (rough) X	1		Spaniel (American Cocker)	1	
Collie X	1	1	Spaniel (CKCS)	8	6
Collie × Corgi	1	1	Spaniel (CKCS) X	1	
Corgi	3	1	Spaniel (Cocker)	5	
Dachshund	2	3	Spaniel (English Cocker)	1	8
Dachshund (miniature)	1	1	Spaniel (English springer)	1	5
Dalmatian	1		Spaniel (Springer)	3	
Doberman	4	12	Spitz (German)	1	
German Langhaar	1		Terrier (Border) X	1	2
Husky (Siberian)	1		Terrier (Bull)	1	
Labrador	25	26	Terrier (Cairn)	7	6
Labrador X	3		Terrier (Cairn) X	1	
Lhasa Apso	1	1	Terrier (Jack Russell)	8	10
Lurcher	1	1	Terrier (Jack Russell) X	2	
Pinscher (miniature)	1		Terrier (Maltese)	1	
Polish lowland sheepdog	1	1	Terrier (Scottish)	1	1
Pomeranian	1	1	Terrier (Staffs Bull)	2	2
Poodle	1	1	Terrier (Tibetan)	2	2
Poodle (miniature)	2	1	Terrier (West Highland White)	16	16
Poodle (toy)	2	2	Terrier (Yorkshire)	15	16
Pudl (medium)	3		Terrier (Yorkshire) X	1	
Pudl (mini)	2		Terrier X	2	
Retriever (Chesapeake Bay)	1		Weimeraner	1	1
Retriever (Golden)	2	5	X-Crossbreed	30	26
Rhodesian Ridgeback	1	1	z-unknown	4	1
Rottweiler	2	2	Totals	**221**	**202**

Table 2 Susceptible and protective haplotypes for canine diabetes

	221 Cases		202 Breed matched controls					
Alleles/haplotypes	No	%	No	%	χ^2	Odds ratio	95% CI	*P*
DRB1*009	29	13.1	13	6.4	3.81	2.13	1.01-4.63	0.05
DQA1*004/DQB1*013	39	17.6	53	26.2	7.51	0.5	0.3-0.92	0.006
DQA1 Arg55 alleles	191	86.4	155	76.7	6.02	1.93	1.13-3.3	0.01

CI, confidence interval.

If the data are analysed in three separate groups according to the countries of origin of the dogs, only the data from the UK shows a significant association with DRB1*009 haplotypes. However, we have shown that in a large cohort of diabetic dogs, with larger numbers per breed, that different MHC haplotypes are associated with susceptibility in different breeds. The dogs submitted from CRO and UQU were from breeds that do not normally have DRB1*009 haplotypes.

The frequency of DQA1 Arg55 positive alleles is high in both the Croatian (80.0%) and Australian (94.1%) diabetic samples, similar to that previously seen in the UK samples. The frequency of the protective haplotype is lowered, 20.0% (Croatian) and 23.5% (Australian), but this does not reach a level of significance. The lack of strong associations in these two groups is not surprising given the low sample numbers. Overall, these data confirm previous findings of both susceptible and resistant haplotypes.

It is particularly intriguing that an association has been found in canine diabetes with a similar region of the MHC as that seen in the human disease [DQA1 Arg52 (human) *vs* DQA1 Arg55 (canine)]. Interestingly, polyarthritis in the dog is also associated with the same DR "shared epitope" as human rheumatoid arthritis. These data suggest that there are similar genetic factors associated with diabetes and that the shared environment between dogs and humans may be contributing to the disease.

Table 3 Numbers of dogs by breed for the hypothyroid cohort

Breed	Cases	Breed matched controls	Breed	Cases	Breed matched controls
Basset hound	6	6	Retriever (Golden)	3	3
Beagle	1	1	Rhodesian Ridgeback	26	15
Bernese Mountain Dog	1	1	Rottweiler	1	1
Boxer	12	12	Schnauzer (German)	1	1
Collie (bearded)	2	2	Schnauzer (Miniature)	1	1
Collie (border)	1	2	Setter (English Blue)	1	
Collie (rough)	1	2	Setter (English)	17	17
Collie (rough) X	1		Setter (Gordon)	2	2
Dalmatian	1	1	Setter (Irish)	2	2
Doberman	32	30	Sheepdog (Old English)	1	2
German Shepherd dog	3	3	Sheepdog (Shetland)	2	2
Great Dane	1	1	Spaniel (American Cocker)	1	1
Hovawart	4	3	Spaniel (Clumber)	1	
Japanese Akita	1	1	Spaniel (Cocker)	3	3
Labrador (Retriever)	12	12	Spaniel (English springer)	2	2
Lagotto Romagnola	1		Spaniel (Springer)	2	2
Leonburger	1	1	Spaniel (Springer) X	1	1
Lhasa Apso	1	1	Spaniel (Welsh Springer)	1	1
Malamute	1		St Bernard	1	2
Petit Basset Griffon Vendeen	1		Terrier (Dandie Dinmont)	1	
Pointer (German short haired)	1		Terrier (Soft Coated Wheaten)	1	1
Poodle (miniature)	1	1	Terrier (Staffs Bull)	2	2
Poodle (standard)	1	1	Terrier (West Highland White)	2	2
Poodle (toy)	1		X-crossbreed	7	13
Pyrenean Mountain Dog	1	1	z-unknown	10	9
Retriever (flatcoat)	1	1	Totals	**186**	**169**

Table 4 Susceptible haplotypes for canine hypothyroid disease

	186 Cases		169 Breed matched controls					
Alleles/haplotypes	No	%	No	%	χ^2	Odds ratio	95% CI	*P*
DQA1*001	108	58.1	75	44.4	6.1	1.74	1.11-2.7	0.01

CI, confidence interval.

Hypothyroid disease

Primary hypothyroidism is a common endocrinopathy in dogs (12, 13), and is considered to be an immune mediated disease based on its clinical and histological similarities to Hashimoto's thyroiditis in man (14), and because of the prevalence of autoantibodies to thyroglobulin (15). Antibodies to circulating tri-iodothyronine and/or thyroxine also may be present (16).

We have previously published a study on Doberman Pinschers and hypothyroidism (3), which showed an association of the disease with a haplotype carrying DQA1*00101.

Table 3 shows the breed breakdown of the 186 hypothyroid dogs submitted for this study, together with a cohort of breed matched controls. Analysis showed that DQA1*00101 was significantly increased in the cases when compared with breed matched controls (Table 4). Further analysis of these samples has been published elsewhere (4).

We had hoped to get groups of dogs from other countries to compare with the Manchester dataset, but it has proved difficult to collect samples, and the workshop cohort of samples is therefore similar to the original Manchester cohort (5).

Analysis by breed shows that some breeds are over-represented in the patient group (Doberman pinscher, English Setter, Boxer and Rhodesian Ridgeback). It appears that Rhodesian Ridgebacks are so susceptible to hypothyroidism that we found it difficult to collect any control dogs of this breed. Some other breeds are not common, or are absent from the affected group (Siberian Husky, Shih Tzu and Yorkshire Terrier). Interestingly these breeds have much lower frequencies of DQA1*00101 than many of the other breeds represented in the patient group. There is some evidence that different breeds have different MHC associations. This is similar to previous findings in human immune mediated thyroid disease where a number of different associations both within (17) and between ethnic groups (18, 19) have been found.

Both of these disease studies highlight the merit of studying autoimmune diseases in the dog. If we can show that dog diseases are associated with similar genetic markers to the human disease, then it is worthwhile looking for other genetic markers in the dog. The dog has much larger blocks of linkage disequilibrium across the genome than man, because of the intense inbreeding that the breeds have been subject to. This means that genetic markers do not have to be very close together to detect linkage, making the dog an efficient and powerful model for human disease. If further genetic markers are identified in the dog, synteny can be used to identify areas to search in the human genome.

Conflict of Interest Statement

BC has received funding from Masterfoods for research carried out in this work. All other authors have declared no conflicts of interests.

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