Breed Differences in the Phenotype and Gene Frequencies in Canine D Blood Group System

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ABSTRACT. The D system of canine blood groups was studied in 3,191 dogs of many different breeds. The frequencies of the D system phenotypes and genes were measured. These frequencies varied considerably between the breeds native to Japan. The frequency of the D1 phenotype was higher in breeds native to Japan than in those of non-Japanese origin. Conversely, non-Japanese breeds generally had the D2 phenotype. The dogs described as mongrel in Japan had D system frequencies intermediate between native Japanese and non-Japanese breeds. One of the most interesting findings was that in the Afghan hound the frequency of the D1 gene (0.3333) was the same as in the Shiba, though only the Shiba was native to the Japanese isles. Another Japanese breed was the Tosa, and its D1 gene frequency was 0.063, a value even lower than that for the non-Japanese Maltese (0.097).—KEY WORDS: blood group D system, breed, canine, population genetic, red cell antigen.

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Research on canine red blood cell types has been done by many investigators [1, 2], and has also been pursued by an international workshop [12]. The latter has led to the standardization of the Dog Erythrocyte Antigen (DEA)-1, -2, -3, -4, -5, -6, -7, and -8 systems. There are many other studies of blood group systems [3–5, 7–9], but these efforts have not yet resulted in international standardization.

This multiplicity of canine red cell antigens includes one blood group reported to occur only in Japan [5, 8]. This system (the "D" system) uses heterogenous antibodies obtained from the immune reaction of dog red cells in rabbits and it is totally different from blood group systems that use naturally occurring antibodies, allo-immune antibodies, or lectin. The D system uses two antibodies, anti-D1 and anti-D2. Dogs can be divided into three types, depending on whether they react only to the former (D1), only to the latter (D2), or to both (D1D2). Any one dog will necessarily fall into one of these three groups. The genotype of the D1 phenotype is D1/D1, that of the D2 phenotype is D2/D2, and that of the D1D2 phenotype is D1/D2. Furthermore, the D1 and D2 genes is codominant as shown in Table 1 [5, 8, 11].

We have maintained antibodies that allow us to classify dogs based on the D system. In the earlier studies of the D system, we showed that the specificity of our anti-D1 antibody is identical to that of the anti-DEA 3 which has already been internationally standardized [3]. In the context of research designed to find out how long canine ova can be maintained in the fertilizable state, we used the D system to identify specific paternity from the red cells of puppies born by superfecundation after mating with two fathers [11]. We also prepared monoclonal antibodies specific for the anti-D1 antibody [6]. Studies on the frequency of occurrence and the genetics of the D system have been carried out mainly in beagles and mongrel dogs native to Japan [3–5]. The many other canine breeds have not been studied in Japan or in other countries either.

Table 1. D system in the dog

Phenotype	Genotype	Reaction with	
		Anti-D1	Anti-D2
D1	D1/D1	+[a)]	−
D2	D2/D2	−	+
D1D2	D1/D2	+	+

a) Hemagglutination.

MATERIALS AND METHODS

Antibodies: Anti-D1 and anti-D2 antibodies were used [5]. The anti-D1 antibody was a heterogeneic-immune antibody, and was obtained by immunization of rabbits with D1 red cells. These heterogeneic-antibodies were absorbed by D2 red cells, which produced allo-specific antibodies. Thus they were specific anti-D1 antibodies. The anti-D2 antibody was also heterogeneic-immune antibody, and was obtained by immunization of rabbits with D2 red cells. These heterogeneic-immune antibodies were absorbed by D1 red cells, which produced allo-specific red blood cell antibodies. They were therefore specific anti-D2 antibodies.

Red blood cells: Red blood cells taken from the dogs were used as the antigens. The red blood cells were used after they were washed three times with physiological saline and suspended in a 4% red cells solution with physiological saline.

Breeds: Dogs of 30 breeds were studied, along with mongrels, dogs of mixed breed, and some of unknown breed. At least ten dogs of each kind were tested. The total sample was 3,191 animals. Table 2 shows how many dogs of each kind were tested. Among those of known

Table 2. Kinds and numbers of dogs tested

Origin	Kind	Number tested
Japanese	Akita	45
	Kishu	37
	Mongrel	862
	Shikoku	20
	Shiba	157
	Tosa	27
	Subtotal	1148
Non-Japanese	Afghan hound	12
	Beagle	757
	Chihauhau	10
	Collie	12
	Dachshund	11
	Doberman pinscher	14
	English pointer	37
	English setter	50
	German shepherd	68
	Golden retriever	15
	Labrador retriever	15
	Maltese	80
	Mixed	65
	Pomeranian	27
	Poodle	11
	Pug	10
	Saint Bernard	10
	Shetland sheepdog	60
	Shih tsu	45
	Siberian husky	28
	Toy poodle	17
	West-highland white terrier	10
	Yorkshire terrier	26
	Subtotal	1390
Unidentified		653
	Total	3191

Table 3. Differences in phenotype frequencies of D system

Kind[a)]	Number Tested	Phenotype (%) D1	D2	D1D2
Shikoku	20	66.7	0.0	33.3
Akita	45	61.5	11.5	27.0
Shiba	157	16.7	50.0	33.3
Afghan hound	12	16.7	50.0	33.3
Kishu	37	10.0	60.0	30.0
Mongrel	862	7.9	63.1	29.0
Mixed	65	7.7	61.5	30.8
Doberman pinscher	14	7.1	71.4	21.5
Unidentified	653	5.9	79.6	14.5
English setter	50	2.0	94.0	4.0
Maltese	80	1.4	81.9	16.7
Beagle	757	0.7	97.4	1.9
West-highland white terrier	10	0.0	66.7	33.3
Tosa	27	0.0	87.5	12.5
Collie	12	0.0	91.7	8.3
Poodle	11	0.0	90.9	9.1
Labrador retriever	15	0.0	93.3	6.7
Toy poodle	17	0.0	94.1	5.9
English pointer	37	0.0	96.9	3.1
German shepherd	68	0.0	97.0	3.0
Shetland sheepdog	60	0.0	98.1	1.9
Chihauhau	10	0.0	100.0	0.0
Dachshund	11	0.0	100.0	0.0
Golden retriever	15	0.0	100.0	0.0
Pomeranian	27	0.0	100.0	0.0
Pug	10	0.0	100.0	0.0
Saint Bernard	10	0.0	100.0	0.0
Shih tsu	45	0.0	100.0	0.0
Shiberian husky	28	0.0	100.0	0.0
Yorkshire terrier	26	0.0	100.0	0.0
Total	3191	4.7	80.7	14.6

a) Arranged in order of D1 phenotype frequency.

breeds, most were not native to Japan.

Determination of red blood cell type: A 0.1 m*l* sample of antibody and 0.1 m*l* of the 4% red cell-physiological saline solution mentioned above were mixed gently in a small test tube (0.9 mm × 100 mm). The mixture then stood for 20 min at room temperature. Using only a concave-surface mirror for magnification, an observer checked whether the agglutination reaction took place or not [3].

Calculation of phenotype and gene frequencies: The phenotype frequency was calculated by dividing the number of positive individuals by the total number of samples, and was expressed as a percent. The gene frequency was calculated with the formula proposed by Tanaka [10].

RESULTS

Phenotype frequency: The D system phenotype frequencies are shown in Table 3. The Shikoku and Akita breeds had all three D system phenotypes (D1, D2, and D1D2). The most common phenotype was D1; its relative incidence of occurrence was over 60%. In the Shiba, Afghan hound, and Kishu breeds the most common phenotypes were D1, D2, and D1D2, respectively. The English setter, Maltese, and Beagle had all three D system phenotypes, but the most common by far was D2. The breeds listed in Table 2 from the Tosa to the Shetland sheepdog did not have D1, but almost all of them had D2. None of the breeds listed in Table 2 from the Chihuahua to the Yorkshire terrier had either D1 or D1D2, that is, all of them had the D2 phenotype. The relative frequencies of D system phenotypes in mongrels and Doberman pinschers were intermediate between those in native Japanese and non-Japanese breeds.

In these 3,191 dogs as a whole, the relative frequencies of D system phenotypes were: D1, 4.7%, D2, 80.7%; and D1D2, 14.6%.

Gene frequency: The relative frequencies of the D1 and D2 genes were calculated from the frequencies of the D phenotypes. The results are shown in Table 4. The breeds native to Japan, particularly the Shikoku and Akita breeds, had high D1 gene frequencies. Next came the

Table 4. Differences on gene frequencies of D system

Kind[a)]	Number Tested	Gene frequencies D1	D2	χ^2
Shikoku	20	0.8333	0.1667	0.7826(0.30<P<0.50)
Akita	45	0.7500	0.2500	3.4489(0.05<P<0.01)
Shiba	157	0.3333	0.6667	9.8596(P<0.01)
Afghan hound	12	0.3333	0.6667	0.7536(0.30<P<0.50)
Kishu	37	0.2500	0.7500	1.4357(0.20<P<0.30)
Mixed	65	0.2308	0.7692	1.1690(0.20<P<0.30)
Mongrel	862	0.2244	0.7756	24.0360(P<0.01)
Doberman pinscher	14	0.1786	0.8214	0.9877(0.30<P<0.50)
West-highland white terrier	10	0.1667	0.8333	0.3993(0.50<P<0.70)
Unidentified	653	0.1313	0.8687	88.9425(P<0.01)
Maltese	80	0.0970	0.9030	0.2524(0.50<P<0.70)
Tosa	27	0.0630	0.9370	0.1193(0.70<P<0.80)
Poodle	11	0.0450	0.9550	0.0253(0.80<P<0.90)
Collie	12	0.0420	0.9580	0.0254(0.80<P<0.90)
English setter	50	0.0400	0.9600	9.0065(P<0.01)
Labrador retriever	15	0.0333	0.9667	0.0172(0.80<P<0.90)
Toy poodle	17	0.0290	0.9710	0.0158(0.90<P<0.95)
Beagle	757	0.0162	0.9838	117.2243(P<0.01)
English pointer	37	0.0160	0.9840	0.0097(0.90<0.95)
German shepherd	68	0.0150	0.9850	0.0158(0.90<P<0.95)
Shetland sheepdog	60	0.0090	0.9910	0.0101(0.90<P<0.95)
Chihauhau	10	0.0000	1.0000	0.0000(0.99<P)
Dachshund	11	0.0000	1.0000	0.0000(0.99<P)
Golden retriever	15	0.0000	1.0000	0.0000(0.99<P)
Pomeranian	27	0.0000	1.0000	0.0000(0.99<P)
Pug	10	0.0000	1.0000	0.0000(0.99<P)
Saint Bernard	10	0.0000	1.0000	0.0000(0.99<P)
Shih tsu	45	0.0000	1.0000	0.0000(0.99<P)
Siberian husky	28	0.0000	1.0000	0.0000(0.99<P)
Yorkshire terrier	26	0.0000	1.0000	0.0000(0.99<P)
Total	3191	0.1199	0.8801	316.4610(P<0.01)

a) Arranged in order of D1 allele frequency.
The χ^2 value is for the Hardy-Weinberg test of equilibrium of the phenotypes, with 1 degree of freedom.

Shiba, Afghan hound, and Kishu breeds, with values from 0.2500 to 0.3333. Non-Japanese breeds such as the Doberman pinscher, West-highland white terrier, Maltese, Poodle, Collie, and English setter had D1 gene frequencies of 0.1786 or less, which was much lower than that for breeds native to Japan. The Chihauhau, Dachshund, Golden retriever, Pomeranian, Pug, Saint Bernard, Shih tsu, Siberian husky, and Yorkshire terrier, all non-Japanese, had no D1 gene. In all these breeds, the D system was represented only by the D2 gene. An interesting finding is that the mongrel dogs had gene frequencies intermediate between the Japanese and non-Japanese breeds.

The Afghan hound, a breed that originated in Afghanistan, had the same relatively high D1 gene frequency as the Shiba (0.3333), a Japanese breed. The Tosa, another Japanese breed, had a D1 gene frequency of 0.063, which was very low. It was even lower than that of the Maltese (0.097), a non-Japanese breed.

There was no detectable relationship between sex and the frequency of D system genes.

DISCUSSION

The D system can be studied with two types of antibodies; anti-D1 and anti-D2. The specificity of the anti-D1 antibody is identical with that of the anti-DEA 3 antibody, which had been internationally standardized [3]. However, anti-D2 has a different specificity. None of the internationally standardized antibodies used to study canine red cells has been assigned a symbol. Studies of genetic pedigree suggest that the D system does not consist of independent D1 and D2 systems. Rather, it seems to consist of two codominant allels, the D1 and D2 genes. The genotypes are D1/D1 for the D1 phenotype, D2/D2 for the D2 phenotype, and D1/D2 for the D1D2 phenotype [4, 8]. We have observed (unpublished results) that repeated transfusion of D2 type blood into a D1 type patient, or conversely, the transfusion of D1 type blood into a D2 type patient, causes a severe acute transfusion

reaction. This indicates how important the D system is in red cell typing for clinical blood transfusion. As there are many varieties of indigenous dog breeds and large populations in Japan, there is a higher risk of D system incompatibility than in countries with less variety and smaller populations of Japanese dog breeds.

According to Iseki and Terashima [8], the incidence of D system phenotypes has been estimated as 5% for D1, 60% for D2, and 35% for D1D2. The present study, however, shows that these frequencies vary considerably with the canine breed. In general, the breeds native to Japan tended to have a higher incidence of the D1 phenotype than the non-Japanese dog breeds, and the non-Japanese breeds tended to have a higher incidence of the D2 phenotype than the native Japanese breeds. Another interesting finding was that those dogs classed as mongrels had D system frequencies intermediate between the native Japanese and non-Japanese breeds.

The results of the present study show that the Afghan hound has a D1 frequency identical to that of the Shiba, a breed native to Japan. It was of great interest to see that this aspect of the Afghan hound genotype was very different from its counterpart in other non-Japanese breeds. The difference in the place of origin might well account for this difference in genotype. The findings for the Tosa lead to a similar conclusion. The Tosa is a native Japanese breed, but its D1 gene frequency was lower than those of other Japanese breeds and also the non-Japanese breed Maltese. This suggests that the origin of the Tosa is different from those of other native Japanese breeds. These results show that the frequency of D system genes in Japanese breeds differs according to breed.

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