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Genetic Variation of Hemoglobin and Ganglioside Monooxygenase in Korean Sapsarees (*Canis familiaris*)

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Abstract Genetic variation of hemoglobin and erythrocyte ganglioside monooxygenase were analyzed in Korean nature dog Sapsarees by horizontal starch gel electrophoresis and thin layer chromatography. Hemoglobin has three phenotypes A, B and AB, which are controlled by two codominant alleles, Hb^a and Hb^b, at one autosomal locus. Gene frequencies of Hb^a and Hb^b were 0.537 and 0.4625. Ganglioside monooxygenase has two phenotypes, dominant and recessive. They are controlled by a dominant allele Gmo^a and a recessive allele Gmo^g. Frequencies of Gmo^a and Gmo^g were 0.5477 and 0.4523.

Key words: Genetic Polymorphism, Hemoglobin, Ganglioside monooxygenase, Sapsaree

Introduction

The dog is one of the oldest domesticated animal in the preagricultural age by human [2] and the ancestor of domesticated dogs is believed to be wolf [4]. Sapsaree is now believed one of the oldest domesticated dog in Korea was designated as a natural monument 368th in March, 1992. But we don't know when Korean began to raise Sapsaree and how to immigrate to Korea. Moreover, some people doubt that Sapsaree is a Korean native dog [1].

Polymorphisms in isozymes and other protein in the blood and other tissues of domesticated animals enable us to elucidate the original and immigration route [13].

In the point of this view, there have been a number of studies on the electrophoresis variation in protein of dog [5,9,8,3].

These studies showed that specific genes on specific loci control polymorphisms of isozymes and proteins of the blood of dogs. Among various isozymes and proteins of dogs, erythrocyte hemoglobin and *Gmo* reveal the most prominent differences of gene frequency and clean gene flow [12].

In this study we examined erthyrocyte hemoglobin and *Gmo* of Sapsarees, and compared with other reports.

Materials and Methods

5 ㎖ of blood samples were taken from each the foreleg vein of 40 Sapsarees which are raising in Daegu farm. Blood was separate into plasma and blood cell fractions by centrifugation at 3000 rpm for 10 min. Blood cell fraction was centrifuged after being washed twice in isotonic saline. Horizontal gel electrophoresis was carried out for hemoglobin. Erythrocyte hemoglobin was detected by staining Amide Black 10 B after 12% starch gel electrophoresis of pH 8.6 at constant voltage of 20 V/㎝ for 3 hours [5].

Ganglioside monooxygenase was detected by staining sialic acids after thin layer chromatography(TLC) [3]. The frozen erythrocytes were treated with an equal volume of distilled water, and then lipid was extracted with 20 volumes of an propanol-chloroform mixture (11:7). The extracted lipids were subjected to TLC analysis to determine the type of sialic acid of hematoside. TLC analysis was performed on a precoated plate (silica gel 60, Merck) with a solvent system of chloroform-methanol-5N ammonium hydroxide-0.4% $CaCl_2$ in water (60 : 40 : 4 : 5 ; by vol.). Spots of gangliosides were visualized by heating the plate at 95℃ after spraying with resorcinol reagent. N-Glycolyhematoside and N-acetylhematoside were prepared from horse and dog erythrocytes, respectively [14].

Results and Discussion

Polymorphism of hemoglobin and erythrocyte ganglioside monooxygenase also were observed in Sapsarees. Hemoglobin has three kinds of phenotypes A, B and AB (Fig. 1). They are controlled by two codominat alleles Hb^a and Hb^b at one autosomal locus such as other studies [11,12]. The

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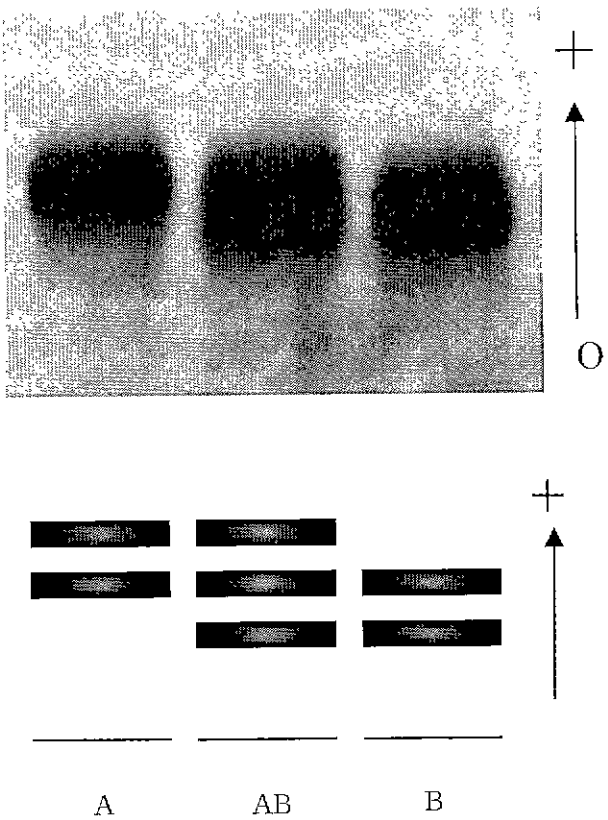

Fig. 1. Hemoglobin phenotypes on starch gel electrophoretogram and diagramatic representation of hemoglobin from starch gel electrophoresis.

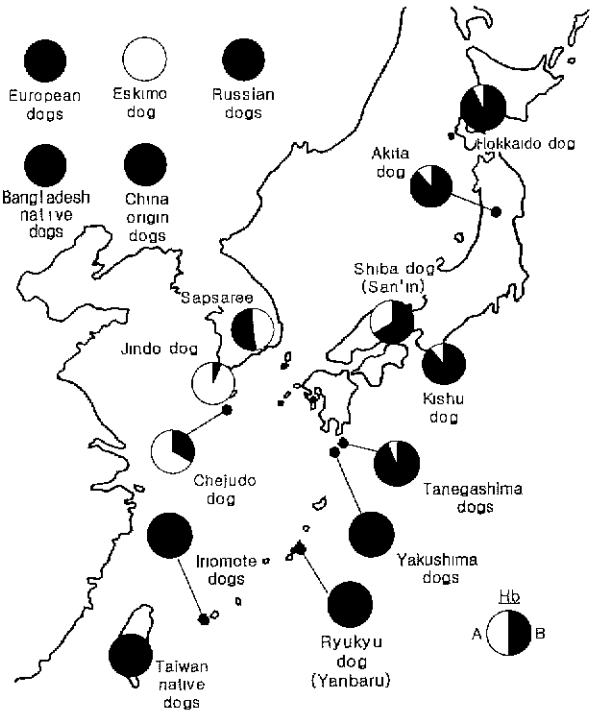

Fig. 2. Geographical distribution of dog erythrocyte hemoglobin (Hb) variants in Korea and its adjacent areas.

frequencies of Hb^a and Hb^b were 0.5375 and 0.4625 (Table 1). Erythrocyte ganglioside monooxygenase has two kinds of phenotypes and are controlled by a dominant allele Gmo^a and a recessive allele Gmo^g at one autosomal locus [3]. The frequencies of Gmo^a and Gmo^g are 0.5477 and 0.4523 (Table 1).

These frequency of Hb^a were lower than Jindo dog and Jejudo dog (Table 1).

Preview study showed that Hb^a was found only in Asian dog breeds, but not in European dog [10,12]. Hb^a frequencies of Korean native dog are lower than Eskimo dog and northern Chinese dogs but higher than Japanese dog and Taiwanese native dog. European dogs have only Hb^b gene and Gmo^a was not found in European dogs, Gmo^g was found only in Asian dog breeds and population [3]. These frequencies suggest that Hb^a and Gmo^a genes of Sapasrees and other Korean native dogs might inflow to Korea from northern China and Eskimo dogs.

These gene frequencies of hemoglobin and ganglioside monooxygenase clearly show that Sapsaree is a Korean native dog because European dogs don't have Hb^a and Gmo^g alleles. Also, these data show Hb^b gene from South Asian islands onto Korean through Japanese islands. However, Hb^a gene seems to flow from northern Asia onto Japanese island through Korean peninsula, similar tendency was observed in gene frequencies of ganglioside monooxygenase [14].

The patterns of *Hb* and *Gmo* genes flow were also observed on the incidence of canine tongue spot in studies [6,7] and the distribution of mtDNA haplotypes in mice [15]. The patterns of the distributional mtDNA haplotypes was very similar between the mice in southern and central Japan and those in Korea [15].

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Table 1. Genc frequencies of hemoglobin and ganglioside monooxigenase in korean native dogs and foreign dogs

Breed	Hemoglobin		Ganglioside monooxigenase	
	Hb ^{A}	Hb ^{B}	Gmo ^{a}	Gmo ^{g}
Korean native dogs				
Sapsaree	0.5375	0.4625	0.5477	0.4523
Jindo dog	0.9692	0.0308	0.4975	0.5025
Jejudo dog	0.6600	0.3400	0.6387	0.3613
Japanese dogs				
Hokkaido-dog	0.056	0.944	1.000	0.000
Akita-dog	0.115	0.885	0.906	0.094
Kai-dog	0.061	0.939	0.686	0.314
Kishu-dog	0.074	0.926	0.736	0.264
Shiba-dog				
(Shinshu)	0.060	0.940	0.818	0.182
(San'in)	0.290	0.710	0.861	0.139
(Mino)	0.020	0.980	0.775	0.225
China origin dogs(north)				
Chow Chow	0.000	1.000	1.000	0.000
Pug	0.000	1.000	1.000	0.000
Shih Tzu	0.000	1.000	1.000	0.000
China origin dogs(south)				
Chin	0.000	1.000	0.707	0.292
Pekingese	0.000	1.000	0.000	1.000
European and western dogs				
Pointer	0.000	1.000	1.000	0.000
Maltese	0.000	1.000	1.000	0.000
Doberman Pincher	0.000	1.000	1.000	0.000
German Shepherd	0.000	1.000	1.000	0.000
Shetland Sheepdog	0.000	1.000	1.000	0.000
Beagle	0.000	1.000	1.000	0.000
Taiwanese native dogs	0.000	1.000	0.948	0.052
Eskimo dogs	1.000	0.000	0.973	0.027

※The datum about canine hemoglobin of foreign dogs are from Tanabe's [10], and One about *Gmo* are from Hashimoto's [3].

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