Genetic Polymorphism of Plasma Vitamin D-Binding Protein (Gc) in Some Asian Sheep

K. Tsunoda[1], K. Doge, M. A. Hasnath[2], H. B. Rajbhandary[3], W. Xu[4], T. Zhanchiv[5] and B. L. Chau[6]
Department of Legal Medicine, Showa University School of Medicine, Tokyo 142, Japan

ABSTRACT : Using polyacrylamide-gel isoelectric focusing followed by immunoblotting, genetic polymorphism of plasma vitamin D-binding protein (Gc) was examined in Asian sheep. The Gc polymorphism was revealed in the Khalkhas sheep of Mongolia, consisting of F, S and W variants, and the Yunnan native sheep of China, consisting of F and S variants. In particular, W was a new variant. The V variant detected in European sheep up to now was not observed in these sheep. The Bhyanglung, Baruwal, Kagi and Lampuchhre sheep of Nepal and local sheep of Bangladesh and Vietnam were monomorphic for the S variant.

Family data and population genetic data supported the hypothesis that these variants were controlled by codominant alleles. In these Asian sheep, distribution of the Gc^S allele was predominant (0.9571 - 1) and was seen as well in European sheep (Suffolk, Corriedale, Cheviot and Finnish Landrace) raised in Japan. Gc^W allele was detected only in the Khalkhas sheep with the low frequency of 0.0025. The Gc^V allele was detected in the Suffolk and Corriedale sheep (0.0080 and 0.0682), but not in any of the Asian sheep studied.

(**Key Words**: Sheep, Vitamin D-Binding Protein, Gc Protein, Genetic Polymorphism, Isoelectric Focusing)

INTRODUCTION

Genetic polymorphism of plasma vitamin D-binding protein (Gc) has been reported in humans (Cleve and Constans, 1988) and many domestic animals, such as horses (Juneja et al., 1978; Weitkamp, 1978), cattle, water buffaloes (Gahne and Juneja, 1978; Masina et al., 1978), pigs (Ljungqvist and Hyldgaard-Jensen, 1983), llamas and alpacas (Penedo and Juneja, 1989).

In sheep, the Gc polymorphism was not found at first (Juneja and Gahne, 1980; Van de Weghe et al., 1982), but Tsunoda and Shimaoka (1985) observed the polymorphism of Gc protein as post-albumin in some British and Finnish sheep breeds raised in Japan; these consisted of two variants (F and S). The Gc polymorphism has also been reported by Kalab et al. (1990), who observed Gc variants of F, S and/or V in various sheep breeds raised in Czechoslovakia. These variants were detected by starch-gel or polyacrylamide-gel electrophoresis with the usual protein staining or immunoblotting.

No information on the Gc polymorphism in local breeds or populations of Asian sheep is available. Thus, this study was conducted to determine the genetic variants of Gc in local sheep breeds or populations of Nepal, Bangladesh, Vietnam, China and Mongolia using polyacylamide-gel isoelectric focusing (IEF) with immunoblotting, and to compare the results with those of some European sheep breeds.

MATERIALS AND METHODS

Blood samples

Blood samples were taken in heparinized test tubes by venipuncture from the following local breeds and populations of sheep: Bhyanglung, Baruwal, Kagi and Lampuchhre breeds of Nepal, Bangladeshi native sheep, Vietnamese sheep, Yunnan native sheep of China and the Khalkhas breed of Mongolia. Of 497 samples in total, there were 41 from Bhyanglung sheep (27 and 14 samples were obtained in the Kodari and Kali Gandaki

[1] Address reprint requests to K. Tsunoda.
[2] Department of Animal Breeding and Genetics, Faculty of Animal Husbandry, Bangladesh Agricultural University, Mymensingh, Bangladesh.
[3] Planning Division, Ministry of Agriculture, Singha Durbar, Kathmandu, Nepal.
[4] Yunnan Agricultural College, Kunming, Yunnan, China.
[5] Institute of General and Experimental Biology, Mongolian Academy of Sciences, Ulaanbaatar, Mongolia.
[6] Faculty of Animal Husbandry and Veterinary Medicine, Cantho University, Cantho, Haugiang, Vietnam.
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areas, respectively); 43 from Baruwal sheep (15, 17 and 11 in Solu, Kodari and Kali Gandaki); 41 from Kagi sheep (20 and 21 in Kathmandu and Chitlang); 28 from Lampuchhre sheep (6, 16 and 6 in Narayangath, Somnath-Parasi and Lumbini); 76 from Bangladeshi sheep (34, 10, 16 and 16 in Jessore, Khulna, Mymensingh and Noakhali); 35 from Yunnan sheep (15 and 20 in Lufeng and Lunan); 34 from Vietnamese sheep in Ninh Son; 199 from Khalkhas sheep (100 and 99 in Kharkhorin and Ulaanbaatar). These samples were obtained from unrelated adult sheep and from 2-4 flocks in an area, except for the Yunnan and Vietnamese sheep.

Blood samples of four European sheep breeds (Suffolk, Corriedale, Cheviot and Finnish Landrace) raised in Japan were used for comparison. The blood was collected from 409 unrelated adult sheep, consisting of 189 Suffolk (15, 136 and 38 in Saitama, Iwate and Hokkaido), 132 Corriedale (30 and 102 in Saitama and Iwate), 43 Cheviot (23 and 20 in Iwate and Hokkaido) and 45 Finnish Landrace sheep in Hokkaido. They were obtained from 2-3 flocks in Iwate except for Saitama and Hokkaido. Apart from these, 146 samples from 60 families selected, based on breeding records and parental determination by 20 blood protein types from Suffolk, Corriedale and Cheviot populations were tested to examine the inheritance of Gc variants.

Plasma samples were separated by the usual method and stored in a freezer (−40℃) until use.

Electrophoresis

Polyacrylamide-gel (120 × 235 × 0.5 mm) for IEF was prepared as follows: 4.2 ml of 20% acrylamide-bisacrylamide solution (19.4% acrylamide and 0.6% bisacrylamide), 6.9 ml of glycerol-sucrose solution (10 ml glycerol and 20 g sucrose per 100 ml of distilled water), 3.0 ml of distilled water and 840 μl of pharmalyte (pH 4.5-5.4, Pharmacia) were mixed. After de-airing, 120 μl of 1.2% anmonium sulfate and 30 μl of TEMED were added to the mixture. Plasma was diluted 1:9 with distilled water and the sample was absorbed on filter paper wicks (Whatman No. 3MM). The anolyte and catholyte filter paper wicks (Advantic, No. 585) were rinsed with 0.04 M DL-glutamic acid and 0.1 M NaOH, respectively. Pre-focusing was conducted at a constant voltage of 1,000 V for 30 minutes. After 90 minutes, the sample wicks were remove and focusing was continued at the same voltage for 120 minutes.

Polyacrylamide-gel for horizontal electrophoresis (PAGE) was prepared according to the modified method of Gahne et al. (1977) described by Yokohama et al. (1987). Electrophoresis was carried out for 6 hours at a constant current of 0.85 mA/cm of the gel. During electrophoresis, the gel was kept at 7-8℃ with a cool pump.

Immunoblotting

A durapore (GVHP) filter (Millipore Type GV, 0.22 μm) presoaked in phosphate-buffered saline (PBS, pH 6.8) was laid on the gel surface after IEF or PAGE. The durapore filter was removed after 30 minutes and incubated with a blocking solution (0.1% gelatin, 0.05% Tween 20) for 15 minutes. The filter was washed three times at 5-minute intervals in PBS containing 0.1% Tween 20 (T-PBS). The filter was then exposed overnight at 4℃ to rabbit anti-human Gc-globulin (Dako) diluted 1:400 in T-PBS followed by three washes in T-PBS at 5-minute intervals. The filter was also incubated for two hours at room temperature in goat anti-rabbit IgG conjugated horseradish peroxidase (Bio-Rad) followed by three washes in T-PBS at 5-minute intervals. The filter was stained histochemically using 6 mg of 3.3'-diaminobenzine tetrahydrochloride, 120 μl of 30% H_2O_2 in 12 ml PBS followed by washing in running water.

Estimation of allele frequency and genetic variability

Allele frequency was calculated by the simple gene counting method. Genetic variability of the Gc locus in the breed population was quantified in a terms of heterozygosity.

RESULTS AND DISCUSSION

Using PAGE with immunoblotting, four plasma Gc variants were revealed as shown in figure 1. The electrophoretic patterns of three variants, except for the slowest-moving variant, were closely similar to those of the F, S and V variants observed by Kalab et al. (1990). Our three variants were considered to be identical to them.

These variants were revealed more clearly by IEF than PAGE with immunoblotting (see figure 2). Each of the Gc variants consisted of three components: one major and two minor components. Figure 3 shows a diagrammatic representation of four Gc variants, which were designated as F, S, V and W in the order of decreasing mobility from the cathode. The corresponding Gc component of W is a new variant, which has not been detected by other workers. This was discovered in only

one Mongolian ewe raised in the Ulaanbaatar area. However no W variant was found in the Kharkhorin area in Mongolia. The distribution of the W variant in the other areas of Mongolia has not yet been surveyed.

Five phenotypes, FS, S, SV, V and SW were observed in the Asian or European sheep studied (figure 2).

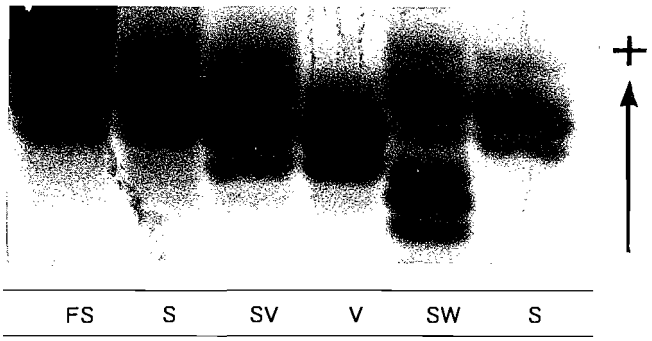

Figure 1. PAGE-immunoblotting patterns of sheep Gc protein phenotypes.

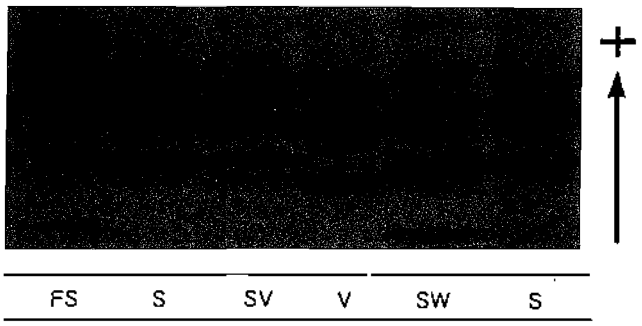

Figure 2. IEF-immunoblotting patterns of sheep Gc protein phenotypes on polyacrylamide-gels, pH, 4.5-5.4.

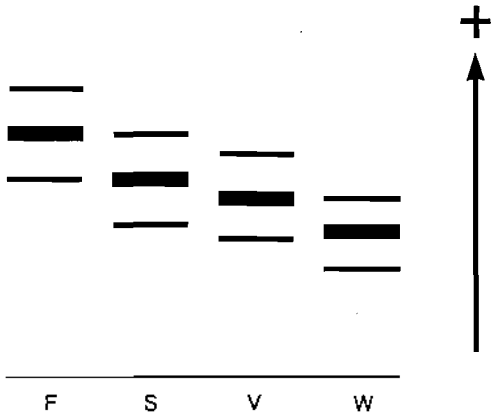

Figure 3. Diagrammatic representation of banding patterns of four sheep Gc variants obtained on polyacrylamide IEF gels.

The distribution of phenotypes in offspring from 60 different matings in shown in table 1. Segregation of phenotypes in the offspring indicated that they were controlled by three codominant alleles at a single genetic locus; these alleles were designated as Gc^F, Gc^S and Gc^V.

Table 1. Inheritance of plasma Gc phenotypes in sheep

Mating ♂ × ♀	Number of matings	Number of rams	Phenotype of offspring		
			FS	S	SV
S × S	49	11	0	61	0
S × FS	6	3	3	5	0
S × SV	5	3	0	4	2

The codominant mode of inheritance was verified from genetic analysis of populations showing the Gc polymorphism, as shown in table 2. In the distribution of phenotypes in Yunnan, Khalkhas, Suffolk, Corriedale and Finnish Landrace sheep, the oberved values were equal to the expected values, calculated according to Hardy-Weinberg's law under the condition of genetic control by four codominant alleles, adding an allele controlling the W variant, designated as Gc^W. No family data on the W variant could not be obtained in this study. In view of the population genetic analysis of the Khalkhas sheep, the mode of inheritance of the W variant was supposed to be the same as in the other variants.

In all the Asian sheep tested, local breeds of Nepalese sheep, such as Bhyanglung, Baruwal, Kagi and Lampuchhre and local populations of Bangladeshi or Vietnamese sheep (probably of Indian derivation) were monomorphic for the Gc^S allele at this locus. In a local breed of Mongolian sheep, such as Khalkhas and local populations of Yunnan sheep, the three alleles, Gc^F, Gc^S and Gc^W, were detected with the Gc^S allele showing the highest frequency. The Gc^V allele was not detected in any of the Asian sheep tested. In particular, the Gc^W allele was seen only in Khalkhas sheep with the low frequency of 0.0025. Thus the genotypes of all the Asian sheep studied consisted predominantly of the Gc^S allele.

The Suffolk, Corriedale and Finnish Landrace sheep were polymorphic for the Gc^F, Gc^S and/or Gc^V alleles. However, the Cheviot sheep were monomorphic for the Gc^S allele. This Gc^S allele was predominant in all the European sheep tested and was seen as well in the Asian sheep. The Gc^V allele was found in the Suffolk and

Table 2. Distribution of Gc phenotypes and allele frequencies in Asian local sheep breeds or populations and some European sheep breeds raised in Japan

Breed or population	Number of animals		Phenotype								Allele frequency			
			F	FS	FV	FW	S	SV	SW	V	Gc^F	Gc^S	Gc^V	Gc^W
Bhyanglung	41		0	0	0	0	41	0	0	0	0	1	0	0
Baruwal	43		0	0	0	0	43	0	0	0	0	1	0	0
Kagi	41		0	0	0	0	41	0	0	0	0	1	0	0
Lampuchhre	28		0	0	0	0	28	0	0	0	0	1	0	0
Bangladeshi	76		0	0	0	0	76	0	0	0	0	1	0	0
Yunnan	35	ob.	0	3	0	0	32	0	0	0	0.0429	0.9571	0	0
		[1]ex.	0.1	2.9			32.0							
Vietnamese	34		0	0	0	0	34	0	0	0	0	1	0	0
Khalkhas	199	ob.	0	4	0	0	194	0	1	0	0.0101	0.9874	0	0.0025
		[2]ex.	0.0	4.0		0.0	194.0		1.0					
Suffolk	189	ob.	0	5	0	0	181	3	0	0	0.0132	0.9788	0.0080	0
		[3]ex.	0.0	4.9	0.0		181.1	3.0		0.0				
Corriedale	132	ob.	0	8	0	0	107	16	0	1	0.0303	0.9015	0.0682	0
		[4]ex.	0.1	7.2	0.6		107.3	16.2		0.6				
Cheviot	43		0	0	0	0	43	0	0	0	0	1	0	0
Finnish Landrace	45	ob.	0	4	0	0	41	0	0	0	0.0444	0.9556	0	0
		[5]ex.	0.1	3.8			41.1							

ob.: observed value, ex.: expected value.
[1]: $x^2 = 0.10$, $p > 0.7$.
[2]: $x^2 = 0.03$, $p > 0.95$.
[3]: $x^2 = 0.08$, $p > 0.95$.
[4]: $x^2 = 1.01$, $p > 0.3$.
[5]: $x^2 = 0.10$, $p > 0.7$, compared to observed values.

Table 3. Heterozygosity at the Gc locus in different sheep breeds or populations in Asia and Europe

Bhyanglung	0
Baruwal	0
Kagi	0
Lampuchhre	0
Bangladeshi	0
Yunnan	0.0821
Vietnamese	0
Khalkhas	0.0100
Suffolk	0.0417
Corriedale	0.1817
Cheviot	0
Finnish Landrace	0.0849

Corriedale sheep with the frequencies of 0.0080 and 0.0682, respectively. The comparatively high frequency of the Gc^V allele in the Corriedale sheep has been due only to the high frequency of the allele (0.1429) in one flock in Iwate. The other flocks of this breed in Iwate and Saitama had no Gc^V allele.

To assess the genetic variability of the Gc locus in the Asian and European sheep tested, heterozygosity (H) values of each breed or population were calculated. The results are shown in table 3. The values for the Asian sheep ranged from 0 to 0.0821 and those of the European sheep from 0 to 0.1817. H values of 12 sheep breeds in Czechoslovakia, calculated from the allele frequency data (Kalab et al., 1990) were in the range of 0-0.2661. Though a slightly high H value was noted in the Corriedale sheep (0.1817) in this study and the improved Walachian sheep (0.2661) described by Kalab et al. (1990), no difference in the genetic variability of this locus between the Asian and European sheep was found.

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