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Polymorphisms in Exon 2 of MHC Class II DRB3 Gene of 10 Domestic Goats in Southwest China*

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ABSTRACT : Polymorphism of the second exon of the caprine leukocyte antigen-DRB3 gene (CLA-DRB3*02) was investigated in this study. The 285 bp PCR product of 258 individuals from 10 domestic goat breeds in Southwest China was digested with restriction endonucleases *Pst*I and *Hae*III and then genotyped. Three alleles and 4 restriction digestion profiles were distinguished by digestion of the PCR fragment by *Pst*I, and 8 alleles and 13 genotypes by *Hae*III. For *Hae*III restriction enzyme sites, the Chi-square (X^2) test showed that all goat breeds in this study did not fit with the Hardy-Weinberg equilibrium ($p<0.01$ or $p<0.05$). The highly polymorphic nature of CLA-DRB3*02 was demonstrated and the ranges of gene heterozygosity (He) and polymorphism information content (PIC) were 0.36-0.63 and 0.32-0.55, respectively. Clustering analysis showed that the 10 goat breeds clustered into two groups and Dazu Black goat had a close genetic relationship with Chengdu Grey, Jintang Black and Nanjiang Yellow goats. (**Key Words :** Goat, Genetic Polymorphism, PCR-RFLP, DRB3 Gene Exon 2, Allelic Variation)

INTRODUCTION

The major histocompatibility complex (MHC) is a large genomic region or gene family found in most vertebrates that encodes MHC molecules. MHC molecules play an important role in the immune system and autoimmunity. There are two general classes of MHC molecules: Class I and Class II. Class I MHC molecules are found on almost all cells and present proteins to cytotoxic T cells. Class II MHC molecules are found on certain immune cells themselves, chiefly macrophages and B cells, also known as antigen-presenting cells (APCs) (Traherne et al., 2006). The MHC of the goat, also named the caprine lymphocyte antigen (CLA), has been shown to be similar to that of sheep and cattle. Class II MHC genes have been extensively characterized in sheep and cattle, whereas in goats only four goat class II genes (Cahi-DRA, Cahi-DRB, Cahi-DYA, Cahi-DIB) have been identified to date (Takada et al., 1998; Amills et al., 2004). DRB is the most polymorphic class II gene, with 58 sequenced alleles and six DR variants detected by isoelectric-focusing (Amills et al., 2004). Associations of alleles of the BoLA (Bovine leukocyte antigen) DRB3*02 with occurrence of disease and production traits have been reported widely in cattle(Machado et al., 2005; Sharma et al., 2005; Yoshida et al., 2009; Wojdak-Maksymiec et al., 2010).

It is well known that China has a long history of goat domestication, production and breeding. Goats have performed agricultural, economic, cultural, and even religious roles in China, and are important meat, hair, skin and dairy resources for the people. Southwest China contains numerous domestic goat breeds. This area has a diverse terrain, rich in rugged mountains, hills and deep basins. This area is also characterized as a humid subtropical monsoonal climate with a long, hot and wet summer, and a fairly mild, but damp and overcast winter (Zhao et al., 2010). This area has about one third of the indigenous Chinese goat breeds, thereby representing important genetic resources because of their special economic characteristics and differences in disease resistance (Sun et al., 2004; Li et al., 2006). However, many goat breeds are not characterized because most of them are

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in mountainous area under extensive production systems, and the population sizes of some domestic goat breeds are declining because of the introduction of exotic commercial goat breeds (such as Boer goat) and genetic improvement experiences (Galal, 2005).

In this study, the genetic variations of CLA-DRB3*02 in 10 domestic goat breeds of the subtropical monsoonal climate zone of Southwest China were investigated.

MATERIALS AND METHODS

Samples collection and DNA extraction

Blood samples were collected from 258 individuals representing 10 domestic Chinese goat (*Capra hircus*) breeds from three provinces of Southwest China. Sample size and locality for each population are listed in Table 1. DNA utilized in PCR amplification was extracted from blood according to the methods of Sambrook and Russell (Sambrook and Russell, 2001).

Molecular analysis

Amplification of CLA-DRB3*02 was conducted by using forward primer (5′-TATCCCGTCTCTGCAGCACA TTTC-3′) and reverse primer (5′-TCGCCGCTGCACACT GAAACTCTC-3′) described by Amills et al. (Amills et al., 1995). The primers were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. Each PCR sample consisted of 25 μl of solution containing 100-200 ng of template, 2.5 μl 10×Buffer, 2.5 μl $MgCl_2$, 0.5 μl dNTP, 0.5 μl of each primer, and 0.5 μl Taq polymerase (TaKaRa, Japan). PCR consisted of 35 cycles of denaturation at 95°C for 45 s, annealing at 63°C for 60 s, and extension at 72°C for 60 s, followed by 7 min extension at 72°C.

Eight microlitres of concentrated PCR products were digested for 4 h with *Pst*I and *Hae*III (Promega Biotech., Madison, WI, USA) according to the manufacturer's instructions. The restriction fragments were resolved by 12% polyacrylamide gel electrophoresis and were visualized with silver staining. PBR322/*Msp*I (TaKaRa, Japan) was used as a marker for determination of restriction fragment size (Li et al., 2006).

Statistical analysis

Frequencies of the alleles and the genotypes in the 10 Chinese domestic goat breeds were calculated. Homogeneity tests of allele frequency and genotype frequency among all the populations were determined using Fisher's exact test, which was implemented by SAS procedures (SAS Institute, Cary, NC, USA). Population genetic indices: gene heterozygosity (He), polymorphism information content (PIC) and effective allele numbers (Ne) were calculated according to Nei's methods (Nei and Roychoud.Ak, 1974; Nei and Li, 1979). Phylogenetic and molecular evolutionary analyses were conducted using MEGA (molecular evolutionary genetics analysis) version 4 (Tamura et al., 2007). The phylogenetic tree of the 10 domestic goat breeds was constructed based on genetic distances.

RESULTS

RFLP analysis

All the DNA samples yielded a 285bp amplification product. RFLPs (restriction fragment length polymorphisms) were detected with restriction digestion enzymes *Pst*I and *Hae*III. Sequence analysis revealed the existence of 4 different restriction patterns for *Pst*I: 241 bp/44 bp, 252 bp/33 bp, 158 bp/79 bp/48 bp and 252 bp/241 bp/44 bp/33 bp and three alleles: A, B and C. A total of 13 restriction patterns and eight alleles (A, B, C, D, E, F, G and H) were found for *Hae*III (Table 2). Allele frequencies and genotype frequencies of exon 2 of the CLA-DRB3 gene digested with *Pst*I and *Hae*III in the 10 Chinese domestic goat breeds are described in Table 1 and Table 3.

The results of Chi-square (X^2) test showed that 4 breeds (Banjiao, Yudong White, Yingshan Black and Qiandongnan

Table 1. The breed names, locality, sample sizes and allele frequencies (%) of exon 2 of CLA-DRB3 gene digested with *Pst*I and *Hae*III in 10 Chinese domestic goat breeds

Breed	Abbreviation	Locality	Number of individuals	With *Pst*I			With *Hae*III							
				PA*	PB	PC	HA	HB	HC	HD	HE	HF	HG	HH
Banjiao goat	BJG	Chongqing	20	40.00	60.00	0.00	17.50	25.00	20.00	30.00	2.50	0.00	5.00	0.00
Dazu Black goat	DZB	Chongqing	31	29.03	70.97	0.00	66.13	24.19	9.68	0.00	0.00	0.00	0.00	0.00
Yudong White goat	YDW	Chongqing	20	45.00	55.00	0.00	20.00	30.00	5.00	5.00	30.00	10.00	0.00	0.00
Chengdu Grey goat	CDB	Sichuan	19	23.68	76.32	0.00	65.66	13.03	21.05	0.00	0.00	0.00	0.00	0.00
Yingshan Black goat	YSB	Sichuan	27	64.81	35.19	0.00	22.22	33.33	22.22	11.11	0.00	11.11	0.00	0.00
Jintang Black goat	JTB	Sichuan	30	20.00	80.00	0.00	75.00	15.00	10.00	0.00	0.00	0.00	0.00	0.00
Lezhi Black goat	LZB	Sichuan	33	42.42	57.58	0.00	43.94	31.82	18.18	6.06	0.00	0.00	0.00	0.00
Nanjiang Yellow goat	NJB	Sichuan	24	29.17	70.83	0.00	70.83	12.50	16.67	0.00	0.00	0.00	0.00	0.00
Guizhou White goat	GZW	Guizhou	19	13.16	86.84	0.00	31.58	10.53	31.58	7.89	10.53	7.89	0.00	0.00
Qiandongnan Small goat	QDS	Guizhou	35	4.29	90.00	5.71	55.71	30.00	0.00	2.86	5.71	2.86	0.00	2.86

* Letter P and H are the abbreviations of *Pst*I and *Hae*III, the second letter is the allele.

Table 2. *Hae*III restriction patterns of the exon 2 of CLA-DRB3

Genotype	Restriction patterns
AA	146 bp/75 bp/64 bp
BB	163 bp/75 bp/47 bp
CC	146 bp/120 bp/19 bp
DD	154 bp/131 bp
EE	220 bp/65 bp
GG	134 bp/32 bp/19 bp
FF	154 bp/75 bp/37 bp/19 bp
HH	154 bp/75 bp/56 bp
AB	163 bp/146 bp/75 bp/64 bp/47 bp
AC	146 bp/120 bp/75 bp/64 bp/19 bp
AD	154 bp/146 bp/131 bp/75 bp/64 bp
BE	220 bp/163 bp/75 bp/65 bp/47 bp
DF	154 bp/146 bp/120 bp/75 bp/37 bp/19 bp

Small goats) significantly ($p<0.01$ or $p<0.05$) differed from the Hardy-Weinberg equilibrium law for *Pst*I restriction enzyme sites, whereas for *Hae*III, all breeds were not found in Hardy-Weinberg equilibrium ($p<0.01$ or $p<0.05$).

Genetic diversity

The highly polymorphic nature of the caprine DBR3 gene exon 2 was demonstrated by PCR-RFLP. The range of gene heterozygosity of the 10 domestic goat breeds was 0.36-0.63, and PIC was 0.32-0.55. The range of observed allele numbers was 5-9, and the range of effective allele numbers was 3.15-6.30 (Table 4).

Clustering analysis

The genetic variation of the 10 domestic goat breeds is shown in Table 5. Based on matrix genetic distance, the phylogenetic tree of the 10 domestic goat breeds was constructed (Figure 1). Clustering analysis showed that the goat breeds clustered into two groups. Dazu Black goat had a close genetic relationship with Chengdu Grey, Jintang Black and Nanjiang Yellow goats.

DISCUSSION

Genetic polymorphism

The highly polymorphic nature of the caprine DBR3 gene exon 2 in 10 domestic goat breeds has been demonstrated in this study. The results suggested that there were significant differences in both allele frequencies and genotype frequencies of exon 2. This finding was similar to other genetic diversity studies on Chinese domestic goat breeds (Sun et al., 2004; Li et al., 2006). In Sun's paper, only two goat populations in Northern China, Mongolian

Table 3. RFLPs genotype frequencies (%) of exon 2 of CLA-DRB3 gene digested with *Pst*I and *Hae*III in 10 Chinese domestic goat breeds

Breed	With *Pst*I				With *Hae*III												
	AA	BB	CC	AB	AA	BB	CC	DD	EE	GG	FF	HH	AB	AC	AD	BE	DF
BJG	0.00	20.00	0.00	80.00	15.00	20.00	20.00	30.00	0.00	5.00	0.00	0.00	5.00	0.00	0.00	5.00	0.00
DZB	9.68	51.61	0.00	38.71	58.06	16.13	9.68	0.00	0.00	0.00	0.00	0.00	16.13	0.00	0.00	0.00	0.00
YDW	40.00	50.00	0.00	10.00	10.00	30.00	0.00	0.00	30.00	0.00	10.00	0.00	0.00	10.00	10.00	0.00	0.00
CDB	5.26	57.89	0.00	36.84	63.16	10.53	21.05	0.00	0.00	0.00	0.00	0.00	5.00	0.00	0.00	0.00	0.00
YSB	59.26	29.63	0.00	11.11	11.11	33.33	11.11	0.00	0.00	0.00	0.00	0.00	0.00	22.22	0.00	0.00	22.22
JTB	3.33	63.33	0.00	33.33	70.00	10.00	10.00	0.00	0.00	0.00	0.00	0.00	10.00	0.00	0.00	0.00	0.00
LZB	18.18	33.33	0.00	48.48	36.36	24.24	18.18	6.06	0.00	0.00	0.00	0.00	15.15	0.00	0.00	0.00	0.00
NJB	8.33	50.00	0.00	41.67	70.83	12.50	16.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
GZW	5.26	78.95	0.00	15.79	31.58	10.53	31.58	0.00	10.53	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.79
QDS	0.00	85.71	5.71	8.57	37.14	11.43	0.00	0.00	5.71	0.00	0.00	2.86	37.14	0.00	0.00	0.00	5.71

Table 4. The genetic variation of the 10 domestic goat breeds

Breed	Observed allele numbers	Heterozygosity (He)	Polymorphism information content (PIC)	Effective allele numbers (Ne)
BJG	8	0.63	0.55	6.30
DZB	5	0.45	0.38	3.69
YDW	8	0.63	0.55	6.24
CDB	5	0.43	0.37	3.58
YSB	7	0.61	0.54	6.19
JTB	5	0.36	0.32	3.15
LZB	6	0.58	0.49	4.97
NJB	5	0.43	0.37	3.52
GZW	8	0.49	0.46	5.43
QDS	9	0.39	0.36	3.67

Table 5. The genetic distance of the 10 domestic goat breeds

Breed	BJG	DZB	YDW	CDB	YSB	JTB	LZB	NJB	GZW	QDS
BJG	0.000	0.604	0.426	0.627	0.429	0.724	0.369	0.646	0.512	0.699
DZB		0.000	0.610	0.176	0.705	0.181	0.319	0.144	0.510	0.361
YDW			0.000	0.676	0.451	0.745	0.420	0.675	0.603	0.698
CDB				0.000	0.770	0.155	0.397	0.103	0.417	0.385
YSB					0.000	0.868	0.405	0.749	0.785	0.922
JTB						0.000	0.485	0.154	0.520	0.337
LZB							0.000	0.386	0.517	0.554
NJB								0.000	0.501	0.435
GZW									0.000	0.465
QDS										0.000

and Kazakh, were studied by digestion of the 285 bp PCR fragment with *Hae*III, and a total of 7 alleles and 17 genotypes were found (Sun et al., 2004). Another report on allelic variations in the CLA-DRB3*02 gene in 12 Chinese indigenous goat populations found 6 alleles and 18 genotypes (Li et al., 2006). Southwest China is rich in goat types, breeds and populations, but sufficient targeted research is rare. The data presented in our paper contain informative findings on the biodiversity of Chinese domestic goat breeds in this area.

The extensive polymorphism observed at the CLA-DRB3 locus in our study shows the characteristics of MHC genes, i.e. i) multiple nucleotide substitutions between alleles, and ii) a large number of alleles (Snibson et al., 1998). The high genetic diversity observed in a breed could also be explained by overlapping generations, mixing of populations from different geographical locations, natural selection favoring heterozygosis or subdivision accompanied by genetic drift (Naderi et al., 2007). This finding also indicated that Southwest China is rich in goat breed resources that vary in their genetic potential for the production of meat, milk and fiber, disease resistance, heat tolerance and fecundity (Zhao et al., 2011).

Genetic relationships among the breeds

The genetic approach degree method and phylogenetic relationship clustering method were used, and indicated that the goat breeds in Southwest China could be clustered into two groups. Dazu Black goat is a domestic breed from the narrow region of subtropical monsoonal climate in Chongqing. The breed characters are large body size and black in color. Does are polyestrous, and they reach sexual maturity at 4 months of age. This breed also has a high rate of reproduction. This characteristic, as well as its resistance to disease, has resulted in interest in this breed for research on breeding and genetic engineering uses. Clustering analysis showed that the Dazu Black goat had a close genetic relationship with Chengdu Grey, Jintang Black and Nanjiang Yellow goats. This genetic relationship displayed a high consistency with its geographic distribution and previous studies (Yang et al., 2008).

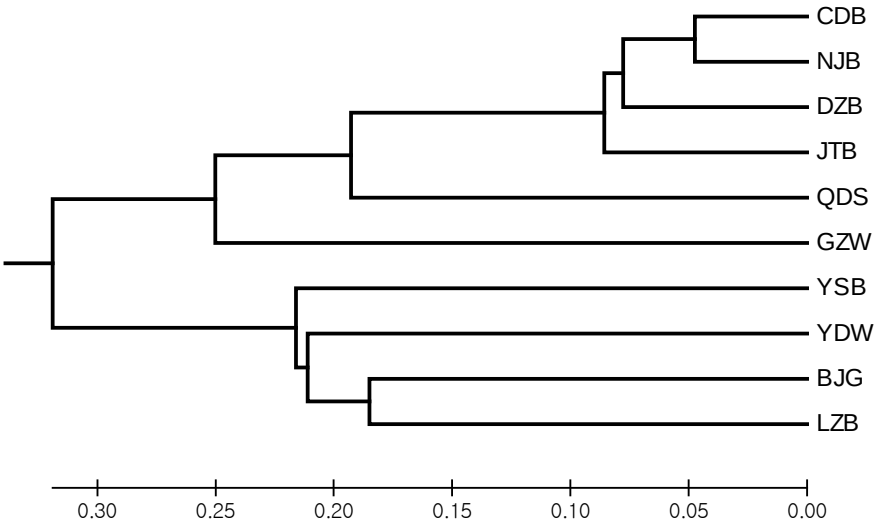

Figure 1. Phylogenetic tree of the 10 domestic goat breeds based on genetic distances.

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