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Analysis of *POU1F1* gene *Dde*l polymorphism in Chinese goats

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ABSTRACT. As a member of the POU-domain family, the POU1F1 is a positive regulator for growth hormone, prolactin and thyroid-stimulating hormone β, by binding to target DNA promoters as a dimer in mammals. This study described the polymorphisms at the goat *POU1F1-Dde*l locus and analyzed the distribution of alleles in 15 indigenous Chinese goat breeds. The PCR-RFLP analysis showed a predominance of the D_1D_1 genotype and the D_1 allele, with average frequencies of 0.550 and 0.790, respectively, irrespective of goat utility type. The D_1D_2 genotype was the second most frequent, with a mean frequency of 0.371. The distributions of genotypic and allelic frequencies at this locus were found to be significantly different among populations based on a Chi square test ($P < 0.001$), suggesting that the breed factor significantly affected the molecular genetic character of the *POU1F1* gene. The genetic diversity analysis revealed that Chinese indigenous populations had a wide spectrum of genetic diversity at the goat *POU1F1-Dde*l locus. However, an ANOVA analysis revealed no

significant differences in gene homozygosity, gene heterozygosity, effective allele numbers, or polymorphism information content among meat, dairy, and cashmere utility types (P > 0.05). This suggests that the goat utility types had no significant effect on the spectrum of genetic diversity.

Key words: Goat; *POU1F1* gene; Polymorphism; PCR-RFLP

INTRODUCTION

As a member of the POU-domain family, POU1F1 (also called PIT-1 or GHF-1) is a positive regulator for growth hormone (GH), prolactin (PRL), and thyroid-stimulating hormone β. It does this by binding to target DNA promoters as a dimer in mammals (Bodner et al., 1988; Lin et al., 1992; Tuggle and Trenkle, 1996). The inhibition of POU1F1 synthesis has been associated with a marked decrease of both GH and PRL expression and proliferation of somatotropic and lactotropic cell lines (Castrillo et al., 1991). POU1F1 mutations are associated with the Snell dwarf and Jackson dwarf mutations in mice, as well as dwarfism in human (Li et al., 1990; Pfäffle et al., 1992; Reynaud et al., 2004). Moreover, polymorphisms in the *POU1F1* gene are associated with important quantitative traits in cattle (Renaville et al., 1997a,b; Zhao et al., 2004; Huang et al., 2008; Khatib et al., 2009) and in pig (Yu et al., 1995; Stanceková et al., 1999; Sun et al., 2002). Recently, polymorphisms in the sheep *POU1F1* gene were reported by Bastos et al. (2006). All these efforts have been concentrated mainly on mice, humans, cattle, pigs, and sheep. The RFLP within the goat *POU1F1* gene, with respect to the *Dde*I restriction enzyme (*POU1F1-Dde*I), was first identified by Lan et al. (2007). However, no comprehensive information on the status of the genetic diversity at this important gene locus is available in indigenous Chinese goat breeds.

The goat industry constitutes an important part in the so-called big domestic animals sector in China. The estimated extent is more than 157,361,000 animals, belonging to more than twenty native breeds (e.g., dairy, meat, and wool breeds) that are mainly reared in the northern China. Therefore, the present study was planned with the objective to reveal the distribution pattern of known variants at the goat *POU1F1-Dde*I locus using a PCR-RFLP approach. We did this in various Chinese goat breeds from different regions, with the aim of providing information on the present goat genetic resources.

MATERIAL AND METHODS

Genomic DNA isolation

Genomic DNA samples were obtained from 709 goats belonging to six genetic types: Nanjiang cashmere goat (N = 247), Xinjiang goat (N = 175), Shaanbei White cashmere goat (N = 150), Boer goat (N = 84), Haimen goat (N = 33), and Xuhuai goat (N = 20) reared in the Chinese provinces of Xinjiang, Shaanxi, and Jiangsu, respectively. DNA samples were extracted from leucocytes and ear tissue according to Sambrook et al. (1989).

PCR conditions

The primer pairs reported by Lan et al. (2007) (F: 5'-CCATCATCTCCCTTCTT-3' and R: 5'-AATGTACAATGTGCCTTCTGAG-3') were synthesized to amplify the goat *POU1F1* gene in-

cluding exon 6 and its flanking region. The PCR was performed in a 25-μL reaction mixture containing 50 ng genomic DNA, 0.5 μM each primer, 1X buffer (including 1.5 mM $MgCl_2$), 200 μM dNTPs, and 0.625 U *Taq* DNA polymerase (Dingguo, Beijing, China). The cycling protocol was 4 min at 95°C, 35 cycles of denaturing at 94°C for 45 s, annealing at 54.5°C for 45 s, extending at 72°C for 1 min, with a final extension at 72°C for 10 min.

Genotyping of *POU1F1-Dde*I allele using PCR-RFLP

Aliquots of 20 μL PCR products of *POU1F1* gene were digested with 10 U *Dde*I at 37°C for 5 h. The digested products were detected using 12.0% PAGE electrophoresis and stained with 0.1% silver nitrate.

Statistical analysis

Genotypic and allelic frequencies were calculated directly. Differences in these frequencies at the *POU1F1-Dde*I locus among Chinese populations were analyzed using Chi square tests, which were performed using SPSS (v. 13.0). The population genetic indices, such as gene homozygosity (H_O), gene heterozygosity (H_E), effective allele numbers (N_E), and the polymorphism information content (PIC), were calculated using the methods of Nei and Roychoudhury (1974). For these analyses, we included not only the six breeds analyzed in the present study, but also the nine breeds reported by Lan et al. (2007).

RESULTS AND DISCUSSION

When screening the *POU1F1-Dde*I for polymorphisms in 709 indigenous Chinese goat individuals, two alleles (D_1, D_2) and three genotypes (D_1D_1, D_1D_2, and D_2D_2) were identified. The genotype and allele frequencies of *POU1F1-Dde*I variants in each of the 15 Chinese goat breeds analyzed are listed in Table 1. The DNA restriction fragments corresponding to the D_1D_1, D_1D_2, and D_2D_2 genotypes were: 200, 118, 102, 20, and 11 bp; 200, 118, 113, 102, 20, and 11 bp; 200, 118, 113, and 20 bp, respectively. Because the two small bands (20 and 11 bp) were not visible on the 12.0% PAGE electrophoresis, only three bands (200, 118, and 102 bp), four bands (200, 118, 113, and 102 bp), and three bands (200, 118, and 113 bp) were visible for genotypes D_1D_1, D_1D_2, and D_2D_2, respectively (Figure 1).

Most of the studied goat breeds demonstrated a high proportion of the D_1D_1 genotype that ranged from 0.200 (Laoshan) to 1.00, with an average frequency of 0.550 ± 0.177 (Table 1). In the Guizhou Black goat population, 100% of the animals were homozygous for the D_1D_1 genotype. The D_1D_2 heterozygotic genotype was the second most frequent with a mean frequency of 0.371 ± 0.203. Interestingly, only four of the 15 goat breeds examined exhibited the homozygous D_2D_2 genotype. Similar distribution trends were observed for the D_1 and D_2 alleles in all the Chinese goat breeds irrespective of their functional type. The D_1 allele was found to be the predominant allele with an average gene frequency as high as 0.790 ± 0.117. The frequency distribution of the D_1 allele was significantly higher than the D_2 frequency distribution in all goat breeds and ranged from 0.581 (Nanjiang) to 1.00, with the Guizhou Black goat breed showing the highest frequency (Table 1). On the other hand, the D_2 allele was found to be distributed at a relatively low frequency (average: 0.210 ± 0.116). However, none of the Guizhou Black individuals were found to possess the D_2 allele.

Table 1. Genotypic and allelic frequencies at the *POU1F1-Dde*I locus in 15 indigenous Chinese goat breeds.

Breeds	N	Genotype frequencies			Allele frequencies	
		P_{D1D1}	P_{D1D2}	P_{D2D2}	D_1	D_2
Nanjiang	247	0.324	0.514	0.162	0.581	0.419
Xinjiang	175	0.663	0.263	0.074	0.794	0.206
Shaanbei	150	0.460	0.400	0.140	0.660	0.340
Boer	84	0.774	0.226	0.000	0.887	0.113
Haimen	33	0.576	0.333	0.091	0.742	0.258
Xuhuai	20	0.700	0.300	0.000	0.850	0.150
InnerMongolia*	452	0.750	0.250	0.000	0.875	0.125
XinongSannen*	74	0.770	0.230	0.000	0.885	0.115
Laoshan*	80	0.200	0.800	0.000	0.600	0.400
Guanzhong*	62	0.694	0.306	0.000	0.847	0.153
GuizhouBlack*	21	1.000	0.000	0.000	1.000	0.000
Matou*	22	0.455	0.545	0.000	0.728	0.272
Banjiao*	25	0.840	0.160	0.000	0.920	0.080
GuizhouWhite*	31	0.355	0.645	0.000	0.706	0.294
Leizhou*	34	0.412	0.588	0.000	0.777	0.223
Mean	1510	0.550 ± 0.177	0.371 ± 0.203	0.031 ± 0.055	0.790 ± 0.117	0.210 ± 0.116

The genotypic and allelic frequencies of breeds marked with asterisk are cited from Lan et al. (2007).

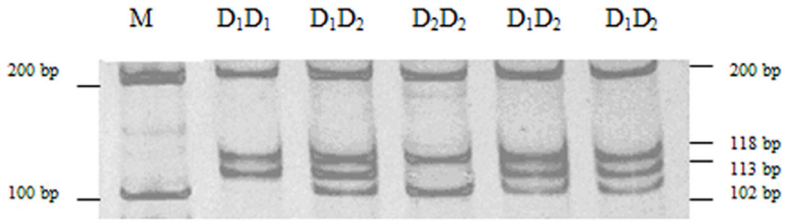

Figure 1. *Dde*I-digested *POU1F1* PCR products on 12% PAGE.

The genotypic frequencies for the various polymorphisms at the *POU1F1-Dde*I locus were found to be significantly different among the 15 populations, based on the chi-square test (χ^2 = 345.410, d.f. = 28, P < 0.001). Significant differences in allelic frequencies among the Chinese goat populations were also revealed (χ^2 = 258.213, d.f. = 14, P < 0.001). Thus, there were significant differences in both genotypic and allelic frequencies at the goat *POU1F1-Dde*I locus in indigenous Chinese goats. The breed factor significantly affected the distribution of the genotypic and allelic frequencies at the goat *POU1F1-Dde*I locus.

The H_O, H_E, N_E, and PIC values for the studied populations are shown in Table 2. Comparisons of genetic diversity in the studied goat breeds demonstrated that the Nanjiang goat had the lowest H_O and the highest PIC. This result indicates that the Nanjiang population is not in a good homozygous status, which is consistent with its breeding background. The Nanjiang goat comes from the Liaoning cashmere and Xinjiang native goats. The Nanjiang goat breed thus originated from two distinctly different breeds and the resulting genetic variability in this breed provided the Xinjiang province with a much needed genetic improvement. Meanwhile, the Boer goat, a meat producing breed famous across the world, had a relatively higher homozygosity and lower a PIC. This breed was imported to improve meat production and growth in the indigenous goat breeds in the Jiangsu Province. However, the ANOVA analysis revealed that there were no significant differences in the four genetic indices in meat utility, dairy utility, or cashmere utility (P > 0.05), respectively (data not shown). This implies that the goat utility types had no significant effects on the spectrum of genetic diversity at the *POU1F1-Dde*I locus in Chinese goats.

Table 2. Genetic diversity at the *POU1F1-Dde*I locus in 15 indigenous Chinese goat breeds.

Breeds	Types	H_O	H_E	N_E	PIC
Nanjiang	Cashmere	0.513	0.487	1.949	0.368
Xinjiang	Meat	0.673	0.327	1.485	0.273
Shaanbei	Cashmere	0.551	0.449	1.814	0.348
Boer	Meat	0.799	0.201	1.251	0.181
Haimen	Meat	0.618	0.383	1.619	0.309
Xuhuai	Meat	0.745	0.255	1.342	0.223
InnerMongolia*	Cashmere	0.781	0.219	1.280	0.195
XinongSannen*	Dairy	0.797	0.203	1.255	0.183
Laoshan*	Dairy	0.520	0.480	1.923	0.365
Guanzhong*	Dairy	0.741	0.260	1.350	0.226
GuizhouBlack*	Meat	1.000	0.000	1.000	0.000
Matou*	Meat	0.603	0.397	1.658	0.318
Banjiao*	Meat	0.853	0.147	1.173	0.136
GuizhouWhite*	Meat	0.563	0.44	1.776	0.342
Leizhou*	Meat	0.585	0.415	1.710	0.329

The genetic diversity of breeds marked with an asterisk are cited from Lan et al. (2007).

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