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*Dde*I Polymorphism in Coding Region of Goat *POU1F1* Gene and Its Association with Production Traits

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ABSTRACT : *POU1F1* is a positive regulator for GH, PRL and TSHβ and its mutations associate with production traits in ruminant animals. We described a *Dde*I PCR-RFLP method for detecting a silent allele in the goat *POU1F1* gene: TC*T* (241Ser)>TC*G* (241Ser). Frequencies of D_1 allele varied from 0.600 to 1.000 in Chinese 801 goats. Significant associations of *Dde*I polymorphism with production traits were found in milk yield (*p<0.05), litter size (*p<0.05) and one-year-old weight (*p<0.05) between different genotypes. Individuals with genotype D_1D_1 had a superior performances when compared to those with genotype D_1D_2 (*p<0.05). Hence, the *POU1F1* gene was suggested to the potential candidate gene for superior milk performance, reproduction trait and weight trait. Genotype D_1D_1, characterized by a *Dde*I PCR-RFLP detection, was recommended to geneticists and breeders as a molecular marker for better performance in the goat industry. (**Key Words :** Goat, *POU1F1* Gene, Polymorphism, Association)

INTRODUCTION

The goat industry is an important part among the so-called big domestic animals sector in China. The estimated size is more than 157,361,000 mainly reared in the northern China, which belonged to more than twenty native breeds (e.g. dairy, meat and wool breeds). Dairy-goat farming is significant to the economics of the western China with the characteristics of under-development and poverty. Goat's meat from young or adult animals has been consumed throughout the country for recent ten years. Moreover, the wool of goat is used in many ways, e.g. for the wrapping of the dead, the making of clothes (Boyazoglu et al., 2005). Large exports of wool and its related products give great chance for rural and western families to improve the economic situation (Dubeuf et al., 2004). Therefore, the further improvement and increase of the quantity and quality in goat dairy, meat and wool will better contribute to the Chinese society, particularly in economy, nutrition, tradition and religion. This issue can be resolved by culturing more and better goat breeds. However, it is difficult to culture excellent goat breeds by the traditional genetic and breeding method. So, many breeders mainly focus on DNA markers for animal selection and breeding through marker-assisted selection (MAS).

As a member of the POU-domain family gene, POU1F1 is a positive regulator for growth hormone (GH) (Zhou et al., 2005), prolactin (PRL) (Li et al., 2006) and thyroid-stimulating hormone β (TSHβ) by binding to target DNA promoters as a dimer in mammalian animals (Jacobson et al., 1997). POU1F1 mutations associated with Snell dwarf (dw) and Jackson dwarf (dw-J) in mice and dwarfish in human (Li et al., 1990; Pfaffle et al., 1992; Revnau et al., 2004). Moreover, polymorphisms of *POU1F1* gene associated with important production traits in cattle (Renaville et al., 1997a; Renaville et al., 1997b; Zhao et al., 2004) and in pig (Yu et al., 1995; Stancekov et al., 1999; Sun et al., 2002). Recently polymorphisms of sheep *POU1F1* gene were firstly reported (Bastos et al., 2006). Few polymorphisms of goat *POU1F1* gene and their associations with production traits had been described.

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Table 1. The primer sequences and their information of goat *POU1F1* gene

Gene	The primer sequences	Size	Annealing temperature	Location
POU1F1 gene	Forward:5'-CCATCATCTCCCTTCTT -3' Reverse:5'-AATGTACAATGTGCCTTCTGAG-3'	450 bp	54.5°C	exon 6 and partial intron 5, 3' UTR

Therefore, it was an interesting and important work to study polymorphisms in *POU1F1* gene and their associations with production traits in goat, which could provide useful genetic markers for animal selection and breeding through marker-assisted selection (MAS). In this paper, we reported the identification of *Dde*I polymorphism at coding region of goat *POU1F1* gene and evaluated its effects on production traits.

MATERIALS AND METHODS

DNA samples

Genomic DNA samples were obtained from 801 goats belonging to nine genetic populations: Inner Mongolia White Cashmere (IMWC, 452), Xinong sannen dairy (Sa, 74), Laoshan dairy (LS, 80), Guanzhong dairy (GZ, 62), Guizhou Black (GB, 21), Matou (MT, 22), Banjiao(BJ, 25), Guizhou White (GW, 31), and Leizhou goat (LZ, 34), which were reared in the province of Inner Mongolia, Shaanxi, Shandong, Guizhou, Hubei, Sichuan and Guangdong (P. R. China). They were all unrelated animals. 1,512 records of milk yield for 216 dairy goats (Sa, LS, GZ), 3,010 records of litter size, 2,004 records of weight traits and one-year-old body sizes for 216 dairy goats (Sa, LS, GZ) and 452 IMWC goats, 4,500 records of lana length, lana thickness and lana yields for 452 IMWC goats were collected, respectively. DNA samples were extracted from leucocytes and ears tissues according to Sambrook et al. (2001).

PCR conditions

Based upon the sequences of sheep *POU1F1* gene (AJ549207) and bovine *POU1F1* gene (Zhao et al., 2004), a pair of primers was designed to amplify the goat *POU1F1* gene (Table 1). The 25 μl volume contained 50 ng genomic DNA, 0.5 μM of each primer, 1×Buffer (including 1.5 mM $MgCl_2$), 200 μM dNTPs and 0.625 units of *Taq* DNA polymerase (MBI). 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.

Single stranded conformation polymorphism (SSCP) and sequencing

Aliquots of 5 μl PCR products were mixed with 5 μl denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanole and 0.025% bromophenol blue), heated for 10 min at 98°C and chilled on ice. Denatured DNA was subjected to PAGE (80×73×0.75 mm) in 1×TBE buffer and constant voltage (200 V) for 2.5-3.0 h. The gel was stained with 0.1% silver nitrate (Kim et al., 2005; Hang et al., 2006; Zhou et al., 2006). The seventeen PCR products from different SSCP patterns in different breeds were subcloned to T-vector (Promega) and sequenced in both directions in ABI PRIZM 377 DNA sequencer (Perkin-Elmer).

Genotyping of *Dde*I *POU1F1* allele by 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 by 12.0% PAGE electrophoresis and stained with 0.1% silver nitrate (Zhou et al., 2006).

Statistical analysis

The chi-square test was used to analyze the Hardy-Weinberg equilibrium, which was performed by SPSS software (version 13.0). Statistical analysis was performed on the basis of records of production traits in 216 dairy goats and 452 IMWC goats, respectively. All analyses were done in two steps, first using a full animal model and then using a reduced animal model. The full animal model included fixed effects of marker genotype, birth year, season of birth (spring vs. fall), age of dam, sire, farm, sex, breed and random effects of measurements and animal. The reduced model was used in the final analysis. (1) Repeated measurements of the milk yield of 216 dairy goat (74 Sa, 80 LS, 62 GZ) were analyzed by the use of the statistical software SPSS (version 13.0) with the mixed linear model. (2) The adjusted Linear Model I with fixed effects was used to analyze the relationships between genotypes and weight traits and litter size in 668 goats (74 Sa, 62 GZ, 80 LS and 452 IMWC). linear model I: $Y_{ijklm} = \mu+S_i+D_{ij}+A_k+G_l+(SG)_{il}+E_{ijklm}$, where Y_{ijklm} was the trait measured on each of the ijklmth animal, μ was the overall population mean, S_i was the fixed effect associated with the ith sire, D_{ij} was the fixed effect associated with jth dam with sire i, A_k was fixed effect due to the kth age, G_l was the fixed effect associated with lth genotype (*POU1F1*/genotype D_1D_1 and D_1D_2), $(SG)_{il}$ was interaction between the ith sire and the lth genotype and E_{jiklm} was the random error. (3) The adjusted linear model with fixed effects was used to analyze the relationship between genotypes and lana traits in 452 IMWC goats. Lack associated of farm, sex, and season of birth (spring vs. fall) with variability of traits indicated that these factors were not into linear model in IMWC

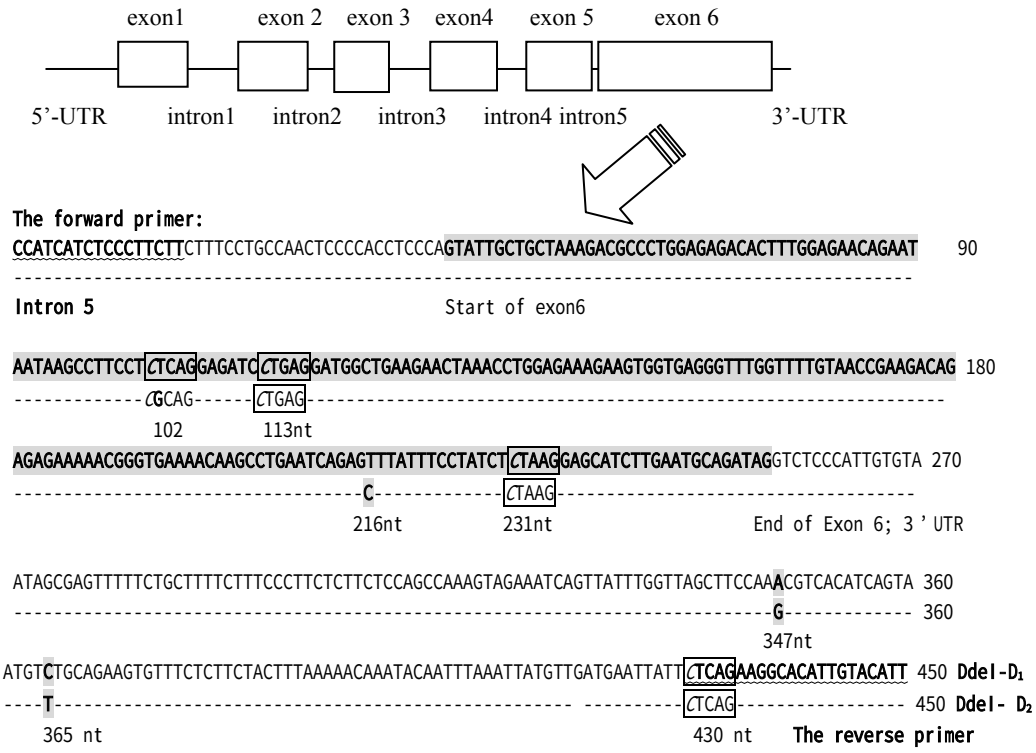

Figure 1. The structure of goat *POU1F1* gene and SNPs location for exon 6 and the *Dde*I enzyme site.
CTNAG: the enzyme site of *Dde*I endonuclease; ~~~~: the forward primer and reverse primer; **Shade**: the exon 6 of goat *POU1F1* gene.

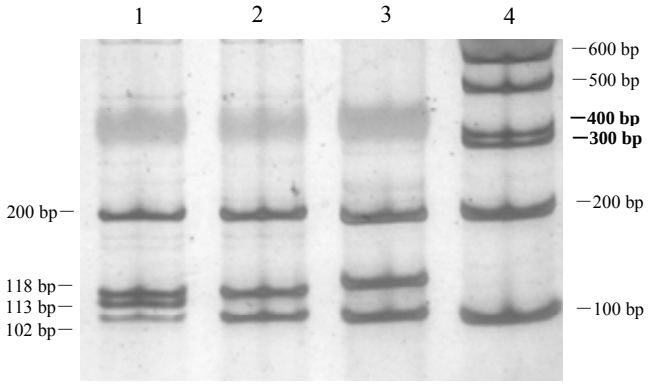

Figure 2. The DNA electrophoretic patterns on 12.0% PAGE after digestion with *Dde*I endonuclease of the DNA region of the goat *POU1F1* gene. Lane 4: DNA Marker (Tianwei times, China), ladder 100 bp, 200 bp, 300 bp, 400 bp, 500 bp and 600 bp; Lane 1: genotype D_1D_2; Lane 2, 3: genotype D_1D_1. Genotypes D_1D_2 and D_1D_1 had 6 bands (200 bp, 118 bp, 113 bp, 102 bp, 20 bp and 11 bp) and 5 bands (200 bp, 118 bp, 102 bp, 20 bp), respectively. As two small bands (20 bp and 11 bp) were invisible on 12.0% PAGE electrophoresis, only 4 bands (200 bp, 118 bp, 113 bp and 102 bp) and 3 bands (200 bp, 118 bp and 102 bp) were visible for genotypes D_1D_2 and D_1D_1, respectively. Moreover, it was obvious that 113 bp and 102 bp fragments could clearly classify genotypes D_1D_2 and D_1D_1.

populations. The least square means estimates (LSM) with standard errors and multiple range tests for two *POU1F1* genotypes and production traits were used.

Synonymous codon bias analysis

According to the high homology among bovine, sheep and goat *POU1F1* gene, the exon 6 of goat *POU1F1* gene was analyzed by www.ncbi.nlm.nih.gov/Blastn and www.ebi.ac.uk/clustalw online software. Then, ORF finder was used to identify the amino acid sequence of exon 6 of goat *POU1F1* gene. According to the online software (www.kazusa.or.jp/codon/countcodon.html), the codon frequencies of exon 6 of goat *POU1F1* gene was calculated. Finally, the codon usage frequency was analyzed by the following formula (Kurland et al., 1991; Eyre-Walker et al., 1996; Lavner et al., 2005; Esley et al., 2006; Liu et al., 2006): F = m×k/n, among them, "F" meant codon usage frequency, " "m" meant the number of synonymous codon for specific amino acid, "n" meant the number of specific amino acid in analyzed sequences, "k" meant the number of usage for specific synonymous codon.

RESULTS

Exon 6 and its flanking region of goat *POU1F1* locus demonstrated polymorphic patterns in nine populations by

PCR-SSCP. Then seventeen DNA amplification fragments including the exon 6 were sequenced (DQ826397-DQ826413). The comparisons among these sequences revealed four mutations (Figure 1). According to the high homology among bovine, sheep and goat *POU1F1* gene, one DQ826397:g.102T>G mutation in No.60nt of the exon 6 identified a silent allele: 241Ser (TC**T**)>Ser (TC**G**) of POU1F1 protein (291 aa). Interestingly, this mutation can be detected by *Dde*I endonuclease. We named the allele characterized by the presence of T as *POU1F1*-D_1, as well as G for *POU1F1*-D_2 allele. The DQ826397:g.102T>G mutation (C***T***CAG-to-C***G***CAG) of the exon 6 region removes a *Dde*I endonuclease restriction site (***CTNAG***) (Figure 1). Therefore, the amplified DNA fragment with *Dde*I endonuclease digestion showed five fragments (200 bp, 118 bp, 102 bp, 20 bp and 11 bp) for *POU1F1*-D_1 allele and four fragments (200 bp, 118 bp, 113 bp and 20 bp) for *POU1F1*-D_2 allele. Correspondingly, genotype D_1D_1 had five fragments (200 bp, 118 bp, 102 bp, 20 bp and 11 bp) and genotype D_1D_2 had six fragments (200 bp, 118 bp, 113 bp, 102 bp, 20 bp and 11 bp). It was obvious that 113 bp and 102 bp fragments could clearly classify the genotypes by 12.0% PAGE (Figure 2).

Frequencies of *POU1F1*-D_1 allele were 0.875, 0.885, 0.600, 0.847, 1.000, 0.727, 0.920, 0.706 and 0.777 for IMWC, Sa, LS, GZ, GB, MT, BJ, GW, LZ populations reared in China, respectively (Table 2). The genotype distributions of Sa, GZ, GB, MT, BJ populations were in agreement with Hardy-Weinberg equilibrium ($p>0.05$) except LS, GW and LZ populations.

The establishment of relationships between genotype D_1D_1 and D_1D_2 and production traits was attempted (Table 3). Significant statistical results were founded in milk yield ($*p<0.05$), litter size ($*p<0.05$) and one-year-old weight ($*p<0.05$) between genotypes.

The Serine (Ser/S) has 6 synonymous codon (namely, UCU, UCG, UCC, UCA, AGC and AGU). From Table 4, there were 5 Serines in analyzed region. The codon usage frequency for UCU codon meant 2.400, while the codon usage frequency for UCG, UCA) meant 0.000, the codon usage frequency (AGC, AGU and UCC) meant 1.200 (Table 5).

DISCUSSION

Goat, sheep and bovine *POU1F1* gene locate in 1q21-22 of chromosomes (Woollard et al., 2000). Chormosome 1 q in ruminant is highly conserved at the gene order and

Table 2. Genotype distribution and allelic frequencies at goat *POU1F1* locus

Breeds	Observed genotypes		Total	Allelic frequencies	
	D_1D_1	D_1D_2		D_1	D_2
Inner Mongolia White Cashmere (IMWC)	339	113	452	0.875	0.125
Xinong Sannen dairy (Sa)	57	17	74	0.885	0.115
Laoshan dairy (LS)	16	64	80	0.600	0.400
Guanzhong dairy (GZ)	43	19	62	0.847	0.153
Guizhou Black (GB)	21	0	21	1.000	0.000
Matou (MT)	10	12	22	0.727	0.272
Banjiao (BJ)	21	4	25	0.92	0.080
Guizhou White (GW)	11	20	31	0.706	0.294
Leizhou (LZ)	14	20	34	0.777	0.223

Table 3. Associations of *Dde*I PCR-RFLP with production traits at goat *POU1F1* locus

Production traits	Genotypes of *Dde*I PCR-RFLP genotyping		p value (two-tailed)
	D_1D_1 (Mean±SE)(number)	D_1D_2 (Mean±SE) (number)	
Milk yields (kg)	574.12^{b}±13.14 (n = 116)	528.46^{a}±3.89 (n = 100)	* p<0.05
Litter sizes (lamb)	1.87^{b}±0.09 (n = 455)	1.39^{a}±0.15 (n = 213)	* p<0.05
Birth weight (kg)	3.36±0.12 (n = 455)	3.01±0.15 (n = 213)	p>0.05
Nine-month-old weight (kg)	46.93±1.52 (n = 455)	41.00±1.00 (n = 213)	p>0.05
One-year-old weight (kg)	51.70^{b}±3.21 (n = 455)	45.12^{a}±1.23 (n = 213)	* p<0.05
One-year-old stature (cm)	68.40±1.32 (n = 116)	66.00±2.08 (n = 100)	p>0.05
One-year-old body size (cm)	78.90±0.77 (n = 116)	76.33±0.88 (n = 100)	p>0.05
One-year-old heart girth (cm)	84.87±1.12 (n = 116)	85.67±1.86 (n = 100)	p>0.05
One-year-old shank girth (cm)	8.31±0.15 (n = 116)	7.67±0.33 (n = 100)	p>0.05
Lana thickness (cm)	5.92±0.17 (n = 339)	5.75±0.17 (n = 113)	p>0.05
Lana length (cm)	18.27±0.61 (n = 339)	18.00±1.32 (n = 113)	p>0.05
Lana yield (g)	604.03±18.44 (n = 339)	549.46±15.03 (n = 113)	p>0.05

[a, b] Means of traits with different superscripts were significantly different (LSD test, * p<0.05).

Table 4. Codon frequencies of exon 6 of goat *POU1F1* gene

Codon frequency (Number)	Codon frequency (Number)	Codon frequency (Number)	Codon frequency (Number)	Codon frequency (Number)	Codon frequency (Number)
UCU 28.2 (Ser,2)	ACG 0.0(0)	GCU 14.1(1)	UUU 42.3(3)	UAU 14.1(1)	UGU 14.1(1)
UCC14.1 (Ser,1)	GAC 0.0(0)	GCC 14.1(1)	UUC 0.0(0)	UAC 0.0(0)	UGC 42.3(3)
UCA 0.0 (Ser,0)	AUG 14.1(1)	GCA 0.0(0)	UUA 14.1(1)	UAA 14.1(1)	UGA 0.0(0)
UCG 0.0 (Ser,0)	GUU 14.1(1)	GCG 0.0(0)	UUG 0.0(0)	UAG 14.1(1)	UGG 14.1(1)
AGU 14.1 (Ser,1)	AAG 28.2(2)	CCU 28.2(2)	CUU 14.1(1)	CAU 14.1(1)	CGU 0.0(0)
AGC 14.1 (Ser,1)	GAU 0.0(0)	CCC 0.0(0)	CUC 0.0(0)	CAC 14.1(1)	CGC 0.0(0)
CCA 0.0(0)	AGG 28.2(2)	GUC 0.0(0)	CUA 14.1(1)	CAA 0.0(0)	CGA 14.1(1)
CCG 0.0(0)	GGU 0.0(0)	GUA 0.0(0)	CUG 56.3(4)	CAG 70.4(5)	CGG 14.1(1)
ACU 0.0(0)	GGG 0.0(0)	GUG 42.3(3)	AUU 0.0(0)	AAU 28.2(2)	GAA 84.5(6)
ACC 0.0(0)	AGA 70.4(5)	GGC 0.0 (0)	AUC 28.2(2)	AAC 28.2(2)	
ACA 14.1(1)	AAA 42.3(3)	GGA 14.1(1)	AUA 0.0(0)	GAG 56.3(4)	

Table 5. The codon usage frequencies for Serine in the exon 6 of *POU1F1* gene

Synonymous codon	Number of synonymous codon	Number of animo acid (n)	Usage number of codon (k)	Codon usage frequency (F)
UCU	6	5	2	2.400
UCG	6	5	0	0.000
AGC	6	5	1	1.200
AGU	6	5	1	1.200
UCC	6	5	1	1.200
UCA	6	5	0	0.000

cytogenetics levels. Interval mapping to detect QTL revealed significant effects on milk and protein yield associated with chromosome 1 in the region of bovine *POU1F1* (Renaville et al., 1997). Moreover, the POU1F1 regulates expression of GH, PRL, TSHβ gene and itself (Sun et al., 2002). Hence, *POU1F1* gene was considered to have effects on production and will benefit for the goat industry, whose DNA markers will contribute to animal selection and breeding through marker-assisted selection (MAS).

We were aware of few research related to the polymorphisms of goat *POU1F1* gene and their association with production traits. In this paper, four mutations were revealed in the exon 6 of goat *POU1F1* gene by PCR-SSCP and DNA sequencing method. One DQ826397:g.102T>G mutation identified a silent allele (p.S241S) and formed the *Dde*I polymorphism. The frequencies of *POU1F1*-D_1 allele varied from 0.600 to 1.000. Interestingly, we observed that D_2D_2 genotype which was not detected in genotype analysis. We presumed that the absence of genotype D_2D_2 associated with "major codon bias". According to the previous papers, the frequencies with which individual synonymous codons were used to code their cognate amino acids was quite variable from genome to genome and within genomes, from gene to gene. One particularly well documented codon bias was that associated with highly expressed genes in bacteria as well as in yeast; this was the so-called major codon bias (Kurland et al., 1991; Lavner et al., 2005; Esley et al., 2006; Liu et al., 2006). As the complete CDS sequence of goat *POU1F1* gene was not available, the exon 6 region was used to analyze the codon bias. The analysis of codon usage frequency (F) revealed that different synonymous codons for Serine showed codon bias phenomenon. If F (codon)> 2.0, this codon was regarded as "high frequency codon"; while the codon was regarded as "low frequency codon" or "rare codon" if F (codon) = 0.000. Hence, "UCU" was regarded as "high frequency codon", while the "UCG" was called for "rare codon". From Figure 1, the genotype D_1D_1 linked to "T" mutation and complied with the high frequency codon (UC**U**) which was called for major codon, while the genotype D_2D_2 linked to "G" mutation and complied with the low frequency codon (UCG) which was called for rare codon. Major codon bias (UCU vs. UCG) was not an arrangement for regulating *POU1F1* gene expression. Instead, the similar data suggested that this codon bias, which was correlated with a corresponding bias of tRNA abundance, was a global arrangement for optimizing the growth efficiency of cells (Kurland et al., 1991; Eyre-Walker et al., 1996; Coghlan et al., 2000; Archetti et al., 2004; Lavner et al., 2005; Esley et al., 2006; Liu et al., 2006). Moreover, we presumed that the rare tRNA abundance for UCG may greatly decrease the translation speed from mRNA to animo acid, and seriously restricted the synthesis efficiency of protein and others, then resulted in the absence of D_2D_2 individual.

The statistical results revealed significant relationships between some traits and genotypes (*$p<0.05$). The individuals with genotype D_1D_1 had better performance (e.g. milk yield, litter size and weight) than those of the individuals with genotype D_1D_2. Although this silent

mutation didn't change amino acid sequence, it possibly resulted in the change of Serine synonymous codon usage frequency. The DQ826397:g.102T>G mutation changed the frequency of synonymous codon from 2.400 ("UCU", high frequency codon) to 0.000 ("UCG", rare codon). So, we presumed that the codon bias associated with expressed level of POU1F1. The geontype D_1D_2 with rare codon (UCG) possibly associated the less expression level of POU1F1 which regulates expression level of GH, PRL and TSHβ gene, thus the genotype D_1D_2 showed junior performance. This presume complied with the previous descriptions (Kurland et al., 1991; Eyre-Walker et al., 1996; Coghlan et al., 2000; Archetti et al., 2004; Lavner et al., 2005; Esley et al., 2006; Liu et al., 2006). Hence, we revealed that *Dde*I polymorphism of coding region of *POU1F1* gene associated with milk performance, reproduction traits and weight traits, which preliminarily implied that *POU1F1* gene has positive effects on them.

In this study, genotype D_1D_2 of *POU1F1* locus characterized by a *Dde*I PCR-RFLP detection was suggested to be molecular marker for junior milk yield, lambs and weight, as well as genotype D_1D_1 for superior performances.

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