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MOLECULAR ANALYSES FOR GENETIC POLYMORPHISMS OF THE *LPL* GENE AND THEIR ASSOCIATIONS WITH INTRAMUSCULAR FAT CONTENT IN GOATS

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ABSTRACT

The experiment was conducted to determine the genetic relationship between polymorphism of lipoprotein lipase(*LPL*) gene and intramuscular fat content in goat. 337 goats were collected from 6 goat populations including Anhui white goats, Boer goats, Linqu dairy goats, Minshan black fur goats, Jining grey goats and Matou goats. The genetic variations in exon 1,6, and 7 of *LPL* gene were detected by PCR-SSCP. Genetic effect of *LPL* gene on intramuscular fat content was analyzed by Anhui White and Boar populations. The results exhibited as follows: one polymorphism site was found in the exon1 of *LPL* and five genotypes were defined. Besides that, one polymorphism site was found in exon 6 and 7 of *LPL* gene, respectively, andthree genotypes AA, AB and BB were defined in both of exon 6 and 7. *LPL* gene increased intramuscular in the order of P1 (exon 1 of *LPL*): BB>AB>AA, P6 (exon 6 of *LPL*): AA>AB>BB, and P7 (exon 7 of *LPL*): BB>AB>AA. These results indicated that *LPL* gene could be considered as a candidate gene affecting intramuscular fat content in special goat populations.

Key words: Goat; Lipoprotein lipase gene; Intramuscular fat content; PCR-SSCP.

INTRODUCTION

Lipoprotein lipase (*LPL*) is a kind of proteolytic enzyme which is composed of 448 amino acid residues (human) (Spakrs *et al*., 2001), and is mainly composed of some parenchymal cells like adipose, skeletal muscle, breast, macrophages cell, and so on. *LPL* was involved in the metabolism and transport of lipids, regulated energy balance, fat deposition and growth traits. The *LPL* gene is located at 8p22. In human, the *LPL* gene encodes 448 amino acid residues, and comprises 10 exons spanning about 30kb. *LPL* gene is present in different tissues, especially in adipose tissue and skeletal muscle and provides energy in the form of fatty acids to many tissues. It regulates fat deposition, energy balance, body weight and growth traits. Fat deposits contribute significantly to meat quality attributes such as juiciness, flavor, taste and other organoleptic properties. The genes, such as *ACSBG2*, *FASN*, *ELOVL6* and *DDT*, have been found were significantly associated with fat deposits, the differences in fat deposition were reflected with differential gene expression in fast and slow growing chickens (Hirwa *et al*., 2014). In human, exon 1 of *LPL* gene encodes 5'-UTR and initiation site of signal peptide. The other eight exons encode 446 amino acids, of which the exon 2 is N-binding sites of glycosylation, exon 4 is a binding site of heparin, activation and hydrolysis of apolipoprotein (ApoC Ⅱ),exon 6 is a binding site of phospholipid hydrolysis and exon 8 is the binding sites of another N-glycosylation (Deeb *et al*., 1989).A correlation exists between genetic variation, expression of *LPL* gene and intramuscular fat (IMF)content in animals, which is a very important genetic marker to improve IMF content (Wang *et al*., 2013; Jeong *et al*., 2012; Zhang *et al*., 2014; Gao *et al*., 2004). At present, the association between *LPL* gene polymorphism and IMF has been found in human, mouse, cattle and pigs (Radha *et al*., 2006; Zhang, 2005; Guo *et al*., 2007), however, the relationship between *LPL* gene and IMF content of goats was not reported yet.

In the present study, Polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP), which is a rapid, precise technique that allows the identification of single-nucleotide polymorphisms (SNPs), was used to detect Anhui white goats (AB), Boer goats (BO), Linqu dairy goats (LQ), Minshan black fur goats (MS), Jining grey goats (JN) and Matou goats (MT), including337 individuals totally. Firstly, we analyzed the mutant allelein exon 1, 6, 7 of *LPL* gene and the distribution ofgenotype frequencies in 6 groups, and studied the relationship between *LPL* gene genetic polymorphism and the IMF content in Anhui white goats and Boer goats populations. The research implied that *LPL* gene could be used as a candidate gene for improving the IMF content of goats.

MATERIALS AND METHODS

Animals: In total, 337 goats were collected from different areas of P.R. China. Among them,100 ABs and 64 BOs were obtained from Boda animal husbandry industry ofbreeding sheep (Hefei, Anhui,), 48 MSs were obtained from Tao River, Minxian, Gansu, 27 MTs were obtained from Enshi, Hubei, 44 LQs were obtained from Linqu, Shandong, and 54 JNs were obtained from Jining, southwest of Shandong. The blood samples, 8-10ml of each blood sample, were collected from 337 goats, ACD was used as anticoagulant. All samples were stored under -20 ℃at laboratory. About 100g of *carcass longissimus dorsi* of 12-13 rib in the slaughter site was collected for extraction of crude fat, and weak kept frozen at -20 ℃.

Genomic DNA extraction: Genomic DNA from blood samples was extracted by conventional phenol chloroform, and was dissolved in TE buffer. The quality and purity of the isolated DNA were determined by agarose gel electrophoresis and spectrophotometry, respectively. The DNA samples that showed a single band on agarose gel and had an A_{260nm}/A_{280nm} ratio of ~1.8 were used for further analysis. DNA was quantified by comparison with lambda DNA of known concentration in 0.8% agarose gels.

Polymorphism analysis: According to the DNA sequences of *LPL*, gene of goats (GenBank No. NC_022300.1), the primers was designed by Primer 5.0 (Table 1).The primers were synthesized by BGI Company.

Table 1. The primers used for polymorphism analysis.

Exon	Direction	Sequence
First exon (P1)	Forward	5’-AGCCGTGGAGTGGAAACA-3’
	Reverse	5’-GCGGACAGAGCGGTAGAA-3’
Sixth exon (P6)	Forward	5’-ATGTGGACCAGCTAGTGA-3’
	Reverse	5’-GTAAGGCATCTGAGAACG-3’
Seventhexon (P7)	Forward	5’-AGATGCGGTCTGCTGTTG-3’
	Reverse	5’-AAATCCTGCGGCTACTCA-3’

Actual results are different from expectation, thus we can use gradient –PCR to find the most suitable annealing temperature, and improve each influence factor in turn. The polymerase chain reaction was performed in a 25ul of total reaction volume composed of 2.5ul of 10×LA PCR Buffer, 0.25ul of Taka Ra LA Tap HS, 4ul of dNTP Mixture, 50~100ng of genomic DNA as template and added ddH_2O to 25ul. The PCR reaction was performed as follows: 5 min at 95℃ for initial denaturation; followed by 30~35 cycles of amplification (30s at 94℃ for denaturation, 30s at 49~58℃ for annealing, 30s at 72℃ for extension) and final extension for 5min at 72℃; stored at 4℃. The PCR products were analyzed by electrophoresis in 1.5% agarose gel to detect whether there were specific bands. Silver staining and photos were used for Single-Strand Conformation Polymorphism (SSCP) analysis. After genotyping by PCR-SSCP, the PCR products were send to BGI company for purification and sequencing.

Data statistics and analysis: Count upgenotype and allele frequencies. According to the fixed effect model, the effect of genotype on IMF was analyzed in the general linear models (GLM) of the SPSS (Ver. 13.0). The statistical analysis model is $Y_{ijn}=u+g_i+q_j+e_{ijn}$ (Y_{ij}=the value of observations of analytic characters; u=the population means; g_i=genetic effect; q_j=mass effect; e_{ijn}=the random error).

RESULTS

PCR-SSCP detection and sequencing: 3 primer pairs were selected and the length of P1、P6、and P7 were 178bp, 239bp, and 232bp respectively. However, 3 pairs of primers showed polymorphism in different degree.

There was polymorphism in the P1 locus by SSCP (Figure 1), in which five genotypes, AA, AB, BB, AC and BC , were defined. Comparing with the *LPL* gene(GenBank accession No. NC_022300.1) of goats, the synonymous of mutation, G→A, occurred at the 287 bp locus of P1 was found (Figure 2).

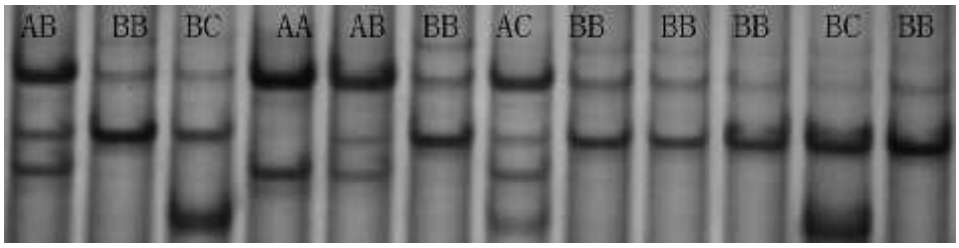

Figure1 The amplification of fragments ofpolymorphism site P1 in*LPL* gene through SSCP electrophoresis.

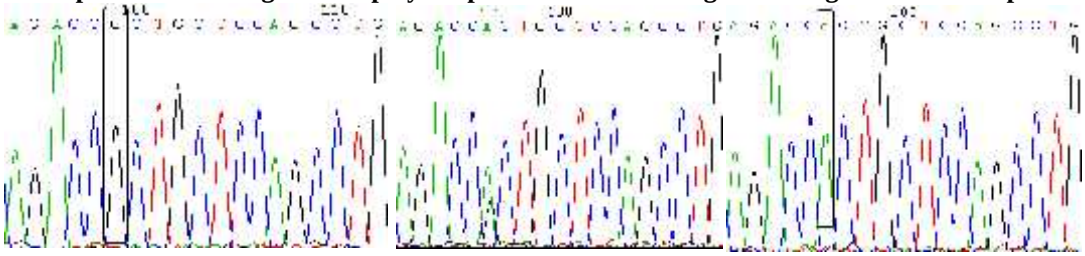

Figure2 The sequencing results of different genotypes ofP1 polymorphism sitein *LPL* gene

The polymorphism was found in the P6 locus (Figure. 3), and three kinds of genotypes, AA, AB, and BB, were found. Comparing the results of sequencing with the *LPL* gene (GenBank accession No. NC_022300.1) of goats, the G→A occurred at the 16997 bp locus of P6 (Figure 4), which is due to the mutation in glutamine (Gln) to serine (Ser).

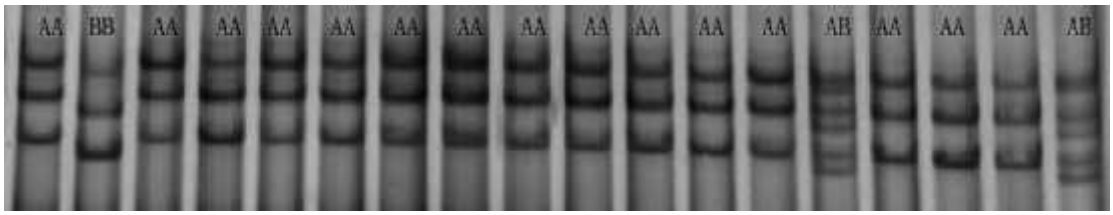

Figure 3 The amplification of fragments of polymorphism site P6 in *LPL* gene through SSCP electrophoresis.

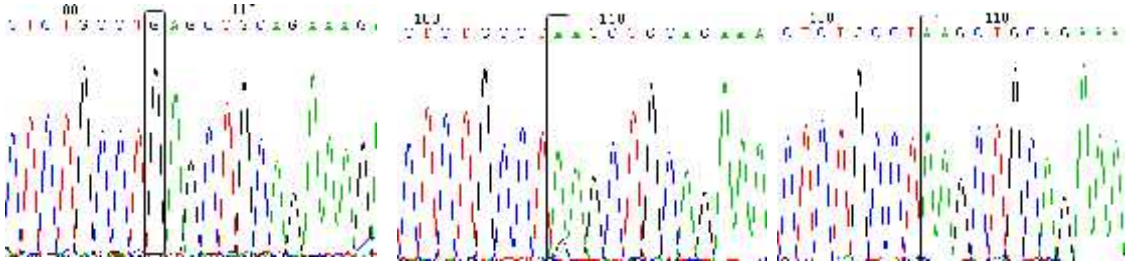

Figure4. The sequencing results of different genotypes ofP6 polymorphism sitein *LPL* gene.

The polymorphism has also been found in the P7 locus (Figure 5), and three kinds of genotypes were defined as AA, AB, and BB. Comparing the sequencing results with *LPL* gene(GenBank accession No. NC_022300.1) of goats, the mutation G→A occurred at the 18313 bp locus of P7 was found (Figure. 6), which is due to the mutation, Aspartic acid (Asp) to Alanine acid (Ala).

Figure5. The amplification of fragments ofpolymorphism site P7 in*LPL* gene through SSCP electrophoresis.

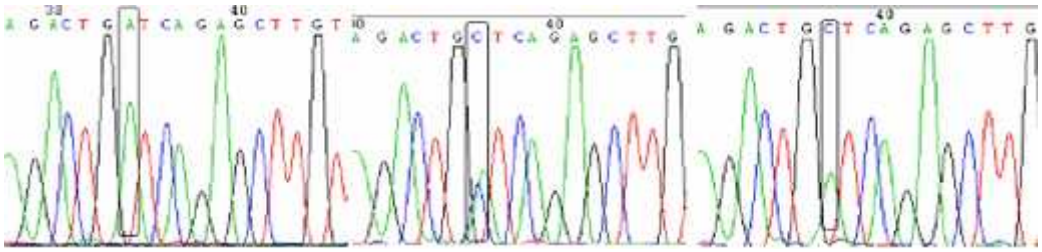

Figure6. The sequencing results of different genotypes ofP7 polymorphism sitein *LPL* gene.

Population genetics statistics of polymorphism sites in *LPL* gene from different goats: Population genetics analysis showed that five genotypes were detected in 6 goat populations in the P1 site, in which BB genotype is homozygous dominant, allele B is the dominant allele (Table 2). Except for the P1 site, three genotypes, AA, AB, and BB, were observed in P6 and P7 site in the 6 goat populations. Among them, AA was a dominant genotype in P6 and BB was a dominant genotype in P7.

The results of X^2 fitness test indicated that genotype of each population in three loci did not reach significant levels, which showed that the 6 goat populations were in the Hardy-Weinberg equilibrium in the three loci of the *LPL* gene (P>0.05). The frequency of allele B in P6 and P7 in 6 populations was quite stable, which was in the range of 0.6 - 0.7, whereas the distribution of alleles A of P6 in different populations showed the specificity, distribution in AB was the most significant (0.9750), followed by BO (0.8828), MT (0.8333), LQ (0.7500), JN (0.7037) and MS (0.6979).

Table 2. Population genetic analysis in polymorphic sites of *LPL* gene

Breed	Site	Genotype frequency					Allele frequency			PIC	X^2
		AA	AB	BB	AC	BC	A	B	C		
AB	P1	0.1000	0.1800	0.6300	0.0100	0.0800	0.1950	0.7600	0.0450	0.0283	8.2530
BO		0.0312	0.2187	0.5937	0.0468	0.1093	0.1639	0.7577	0.0784	0.2997	0.7202
MS		0.1041	0.3541	0.4583	0.0208	0.0627	0.2916	0.6667	0.0417	0.3457	0.1875
MT		0.1481	0.2592	0.4074	0.0372	0.1481	0.2963	0.6110	0.0927	0.3623	0.5510
LQ		0.0454	0.2727	0.5909	0.0000	0.0910	0.1817	0.7727	0.0456	0.2896	0.0548
JN		0.0555	0.1667	0.6481	0.0185	0.1112	0.1481	0.7870	0.0649	0.2790	1.5855
AB	P6	0.9600	0.0300	0.0100			0.975	0.0250		0.0476	14.7929
BO		0.7969	0.1719	0.0313			0.8828	0.1172		0.1855	1.8349
MS		0.5833	0.2292	0.1875			0.6979	0.3021		0.3328	10.0033
MT		0.7073	0.2593	0.0370			0.8333	0.1667		0.2392	0.1200
LQ		0.6136	0.2727	0.1136			0.7500	0.2500		0.3047	3.2727
JN		0.5741	0.2593	0.1667			0.7037	0.2963		0.3301	7.7276
AB	P7	0.2500	0.2500	0.5000			0.3750	0.6250		0.3589	21.7778
BO		0.1875	0.2969	0.5156			0.3359	0.6641		0.3466	7.1657
MS		0.1875	0.2917	0.5208			0.3333	0.6667		0.3457	5.6719
MT		0.1852	0.3704	0.4444			0.3704	0.6296		0.3576	1.1445
LQ		0.2045	0.3636	0.4318			0.3864	0.6136		0.3618	2.3911
JN		0.1667	0.4630	0.3704			0.3981	0.6019		0.3644	0.0624

Correlation analysis between the content of IMF and genotype effect: The least squares method was used to examine the relativity between *LPL* genotype and content of IMF (Table 3), which showed that the least squares means of IMF contents of BO were slightly higher than AB, the effect of AA genotype in P6 site on IMF was that BO was significantly higher than AB (P<0.05). The least squares means of IMF content of genotypes BB and AA in P1 site of BO were 4.573and 3.150, respectively (genotype number of AC and BC were few, they would be negligible) (P< 0.05), the effect of genotype BB on IMF content was large. The least squares mean of IMF content on P6 sites of genotypes AA, AB and BB in the BO were 4.750, 3.850, and 3.457, the difference between genotype AA, BB, and AB were significantly (P<0.05) and the effect of genotype AA on the content of IMF was maximum.

Table 2. The correlation of the *LPL* gene polymorphic site with IMF in 2 goat populations

Polymorphic site	Genotypes	AB	BO
P1	AA	3.012[a]	3.150[a]
	AB	3.500[a]	4.225[a]
	BB	3.985[a*]	4.573[a*]
P6	AA	3.872[a*]	4.750[b*]
	AB	3.223[a]	3.850[a]
	BB	2.900[a]	3.457[a]
P7	AA	3.132[a]	3.433[a]
	AB	3.750[a]	3.988[a]
	BB	3.957[a*]	4.625[a*]

Notes: The values with different small and capital superscripts in the same row indicated the significant difference (P<0.05), * meant the significant difference (P<0.05).

The least squares mean of IMF content on P7 sites of genotypes AA, AB， and BB in the BO were 3.433, 3.988, and 4.625, the difference between genotype BB, AA, and AB were significantly (P<0.05) and effect of genotype BB on the content of IMF was highest.

DISCUSSION

Intramuscular fat (IMF) content is an important factor that affects meat quality, there are many factors affecting the content of IMF. One of the most important factors is the genetic (Badaoui *et al*., 2007; Li *et al*., 2008). By integrating data from cattle and sheep, Guo *et al*. have identified 30 genes with robust correlation with IMF% in both cattle and sheep LM., based on this gene set, they identified *CIDEA* as the gene, whose expression was most correlated with IMF% in both cattle and sheep (Guo *et al*., 2014). *LPL* gene is one of the candidate genes that influence the meat quality of animals, and plays an important role in deposition of intramuscular fat. The present research on genetic relationship between polymorphism of *LPL* gene and IMF content most focused on pig, chicken, and cattle(Pan *et al*., 2004; Hu *et al*., 1994; Guo, 2007), while the reports on the relationship between the *LPL* gene polymorphism and fat deposition in goat were few.

Using 5 varieties of yak as the object, gene mutation (C→T) was found d in exon7 of the *LPL* gene, moreover, the body weight, body height and chest circumference of BB genotype were significantly higher than those of AA and AB genotype (P<0.05) (Xing, 2009). PCR-SSCP and DNA sequencing were applied to analysis a total of 398 samples from 5 yak breeds, and the SNP, AA, AB and BB, was found in the exon 7 of *LPL* gene, and C→T occurred in 19913bp site. The polymorphism of *LPL* gene was related to the lipid deposition (Ding *et al*., 2012). Some other people took Xiangxi yellow cattle as a test object, they detected by polymerase chain reaction and DNA sequencing method to determine the association of SNPs in *LPL* gene with growth traits, and found that SNPs of C-355157T, 355186G, T355348A,T355420C, A355427Thad abundant genetic diversity, while the variability of T355169C and TA355210Gwas low. Linkage disequilibrium analysis showed that there are 18 different haplotypes in Xiangxi cattle. In addition, H1H1 haplotype combinations has a higher body width. At the same time, the animals with CC genotype maintain higher mean values for BW than those with the CT and TT genotypes (P < 0.05) at T355420C locus. The animals with the AA genotype have lower mean values for WH, BL, HG and BW than those with the AT and TT genotypes at A355427T locus (P < 0.05).The results suggested that the SNPs of the *LPL* gene might be useful genetic markers for growth traits in the bovine reproduction and breeding (Wang *et al*., 2012).In order to study the effect of *LPL* gene on CAD and blood lipid levels in the group, PCR-SSCP was used to detect the polymorphisms of *LPL* gene, and three polymorphism loci (Hind Ⅲ locus, Pvu II and Ser-447Ter) was found in CAD infected patient (Daoud *et al*., 2013).

In present study, PCR-SSCP was used to analysis the polymorphism of *LPL* gene, and found the polymorphism of *LPL* gene in AB, BO, MS, MT, JN, and LQ. Polymorphism information content (PIC)in the exon 1 of *LPL* gene in 6 goat populations was between 0.25~0.5, which belongs to the moderate polymorphism and have a significant genetic variation. According the exon 6, AB, BO and MT belong to the low polymorphism, and the other three populations were moderately polymorphic and genetic coefficient of variation was medium. PIC of exon 7 was more than 0.34 in6 goat populations, which was belonged to moderate polymorphism and had a significant genetic variation. The genotype of polymorphic loci in *LPL* gene and genetic effects of IMF content, AA genotype in the P6 site of BO was significantly higher than AB (P< 0.05). The SNPs distribution of exon 1, 6, and 7 in*LPL* gene was detected between AB and BO. However, the Chi-squares independence test showed that there was no significant correlation between three mutation sites and species distribution (P>0.05). The correlation between SNP in exon 1, 6, 7 of *LPL* gene and fat deposition showed that there were significant differences between P1-287GG, P6-16997GG, P7-18313AA in BO and AB and other genotypes (P<0.05). P6-16997GG in BO and AB also had a significant difference (P<0.05). There was no significant correlation between G-287Ain exon 1 and G-16997A in exon 6 with IMF content(P>0.05) and had a negative correlation between G-16997A mutation and IMF(r=-0.799, P=0.161), andA-18313C mutation site in exon 7 and IMF havea significant relationship (P<0.05). The relationship between each genotype of least squares

means about IMF content was that P1: BB>AB>AA, the effect of P1-BB on IMF content was larger; P6: AA>AB>BB, influence of P6-AA on IMF content was larger; P7: BB>AB>AA; P7: BB>AB>AA, P7-BB have a larger effect on IMF content. The analyzing the genotype and allele frequency in 3 polymorphic loci of *LPL* gene (P1, P6 and P7), distribution of different degrees have happened in AB, BO, MS, MT, JN, and LQ. At some time, the effects of 3 polymorphic loci on IMF content in AB and BO was significant. According to the biological function of *LPL* gene and the existing results, it implied that *LPL* gene could be used as a candidate gene for influencing the intramuscular fat content in particular groups.

Conclusions: In the present study, 6 goats breeds, including mutton sheep, milk sheep and sheep milk skin, were selected to study the genetic relationship between polymorphism of lipoprotein lipase (*LPL*) gene and intramuscular fat content,, using PCR-SSCP and GLM method of the SPSS software. The results showed that this gene polymorphism in goat was poor, only found mutations in exon 1, exon 6 and exon 7of *LPL* gene. This information may be helpful in laying the solid theoretical foundation for the molecular breeding of Chinese goat breeds.

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