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Study of *DGAT1* Gene Polymorphisms with Carcass Traits in Iranian Zel and Lori-Bakhtiari Sheep Breeds

Research Article

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ABSTRACT

Diacylglycerol acyltransferase1 (DGAT1) plays an important role in the metabolism of triglycerides which catalyze the final step of triglyceride synthesis in animals. The objective of this study was to investigate the single-nucleotide polymorphisms (SNPs) in 5'UTR, exon-1, and exon-2 of *DGAT1* in two Iranian indigenous sheep breeds. A total of 309 animals including fat-tailed Lori-Bakhtiari (n=152) and thin-tailed Zel (n=157) were used in this study. The genotypic patterns were detected by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). Five SSCP patterns were detected for 5'UTR and exon-1 fragment by PCR-SSCP and subsequently confirmed by sequencing PCR products. The sequencing results revealed that there are three novel polymorphisms in 5'UTR and exon-1fragment of *DGAT1* at the studied breeds. Out of the detected polymorphisms only A277G substitution in exon-1 of *DGAT1* leads to the changes in amino acids (p.Arg26Gly). There was significant correlation ($P<0.05$) between fat-tail weight (FTW) and back-fat thickness (BFT) and the observed genotypes in Lori-Bakhtiari breed; therefore, animals with G5 pattern had higher FTW and BFT compared to G1 pattern. The G1 and G5 genotypic patterns or haplotypes were different at their position 101 of 5'UTR region. No significant relationship ($P<0.05$) was found between the detected genotypes of 5'UTR and exon-1 fragment of *DGAT1* in Zel breed and carcass traits. These results revealed that detected *DGAT1* novel SNPs had significant effects on carcass traits and they can be used as a marker for these traits.

KEY WORDS *DGAT1* gene, Iran, Lori-Bakhtiari, PCR-SSCP, sheep, Zel.

INTRODUCTION

The main goal of sheep breeding programs is to improve meat production and carcass traits (Kosgey *et al.* 2006). During the last forty years, the genetic improvement of livestock has been achieved by selection based on phenotypic information, but getting fast genetic improvement is difficult by this method. However marker-assisted selection (MAS) can increase the genetic improvement of polygenic traits which results from the presence of major genes and their effect on the performance of these traits. Therefore, this method has provided opportunities to increase response to selection, especially for traits with small heritability (Dekkers, 2004). Lori-Bakhtiari breed, with more than 1.7 million heads, is one of the most common indigenous sheep breeds in Iran. This breed adapted to the hilly and mountainous regions in the west of Iran and has been raised mostly in villages under semi-intensive systems. The Lori-Bakhtiari breed has high body weight and the largest fat tail compared to other Iranian Indigenous breeds (Kianzad *et al.*

2003; Vatankhah and Talebi, 2008). Zel breed, with about 1.5 million heads, is the only thin-tailed Iranian breed. Zelis raised in the north part of Iran located between the northern slopes of the Alburz mountain and the Caspian Sea (Kamalzadeh *et al.* 2008).

Diacylglycerol acyltransferase1 (*DGAT1*) gene is involved in the metabolism of triglycerides and catalyzes the last step of the triacylglycerol synthesis (Hatzopoulos *et al.* 2011; Li *et al.* 2013). *DGAT1* is extensively expressed in almost all tissues including the mammary glands, small intestine, adipose tissue, and skeletal muscle, with the highest expression level in adipose tissue and small intestine (Xu *et al.* 2008).

The *DGAT1* gene spans approximately 8.5 kbp and comprises 17 exons 16 introns, located on chromosome 14 of bovine (Grisart *et al.* 2002). The homologous sequence was found on chromosome 9 of sheep (*Ovis aries*). This gene contains about 1470 nucleotides that code a polypeptide with 489 amino acids (Xu *et al.* 2009). Specific polymorphisms in *DGAT1* were detected and their association with some production traits were confirmed in farm animals, especially cattle. Then, *DGAT1* was introduced as a candidate gene for milk production traits in dairy (Banos *et al.* 2008; Li *et al.* 2009; Signorelli *et al.* 2009; Cerit *et al.* 2014) and carcass traits (Anton *et al.* 2010; Souza *et al.* 2010; Pannier *et al.* 2010; Li *et al.* 2013; Tait *et al.* 2014; Ardicli *et al.* 2017) in cattle.Moreover, DGAT1 due to its key role in fat metabolism (Scata *et al.* 2009) is a candidate gene for milk fat content (Scata *et al.* 2009; Xu *et al.* 2009; Yang *et al.* 2011; An *et al.* 2013; Tabaran *et al.* 2014; Martin *et al.* 2017) and carcass traits (Xu *et al.* 2009; Mohammadi *et al.* 2013) in dairy sheep and goat.

However, there are only a few studies on the association between *DGAT1* polymorphisms and carcass traits in sheep (Xu *et al.* 2009; Mohammadi *et al.* 2013; Noshahr and Rafat, 2014; Nanekarani *et al.* 2016). Xu *et al.* (2009) sequenced the whole coding region of sheep DGAT1 and surveyed the associations between polymorphism of *DGAT1* and 11 meat production traits. They reported the positive effect of the T allele in exon-17 of *DGAT1* on meat quality and quantity in three Chinese sheep breeds. Mohammadi *et al.* (2013) reported that there is a significant correlation betweenexon-17 of *DGAT1* polymorphisms (T487C) and carcass weight and dressing percentage, and concluded that the CC genotypes had higher carcass weight and dressing percentage.

Few studies have been carried out on the association of *DGAT1* polymorphisms with carcass traits in sheep breeds. All of these studies suggested that further investigations are necessary to make a definitive statement about the association of this gene polymorphisms with carcass traits in sheep (Xu *et al.* 2009; Noshahr and Rafat, 2014; Mohammadi *et al.* 2013). The aim of this study is to investigate 5'UTR, exon-1 and exon-2 regions of *DGAT1* polymorphisms and their association with carcass traits in Lori-Bakhtiari and Zel Iranian indigenous breeds.

MATERIALS AND METHODS

Animals and data collection

A total of 309 blood samples were collected from Lori-Bakhtiari (n=152) and Zel (n=157) sheep breeds. Lori-Bakhtiari and Zel samples were taken from two industrial slaughterhouses located in the Qom and Mazandaran provinces of Iran, respectively. Before slaughter, body weight (SBW) of lambs was measured. After slaughter, carcass weight (CW), fat-tail weight (FTW), carcass weight without fat-tail (CWFT), and back fat thickness (BFT) immediately were measured and fat-tail percentage (FTP), dressing percentage (DP) and dressing percentage without fat-tail (DPWFT) were calculated. Genomic DNA was extracted by the Salting-out procedure (Miller *et al.* 1998). The quality and concentration of extracted DNA were evaluated by visualizing on 0.8% agarose gel under UV light and spectrophotometer (Thermo, NanoDrop 1000).

DNA amplification with PCR

Two primer pairs DGAT1 (5'UTR and exon-1) F:5'-GGAACTACGCTTCCCAGGAC-3'; R:5'-ACGTCTCCGTCCTTGTCTGT-3'; and (exon-2) F:5'-GTCTTGCATCACCAGCTCCT-3'; R:5'-CAGGCATCTACTGGGATTCAG-3' were designed based on the published sheep sequences (GenBank accession no.EU178818) to amplify the determined fragment by Primer-BLAST (Ye *et al.* 2012). The length of the amplified fragments were 360 bp (5'UTR and partial exon-1) and 445 bp (exon-2) in both breeds. PCR was carried out on 25 µL volume of the following ingredients: 1 × PCR buffer (16 mM $(NH4)_2SO_4$, 67 mM Tris-HCl pH 8.8, 0.1% Tween-20), 2 mM $MgCl_2$, 200 µM dNTP, 6 pmol of each primer, 1.5 unit Taq polymerase (GenetBio, Korea) and 50-100 ng of genomic DNA. PCR reactions were performed with a Genecycler (Bio-Rad, My Cycler, USA) with the amplification program consisted of an initial denaturation at 96 ˚C for 2 mins, then 32 cycles of 96 ˚C for 1 min, 57 ˚C for 45 seconds and 72 ˚C for 1 min, and a final extension of 72 ˚C was maintained for 10 mins for the exon-1 of *DGAT1* gene. For exon-2 fragment, the thermal cycling conditions consisted of initial denaturation at 96 ˚C for 5 mins, followed by 30 cycles of denaturation at 96 ˚C for 1 min, annealing at 60 ˚C for 45 seconds, extension at 72 ˚C for 1 min, with a final extension at 72 ˚C for 10 mins. The PCR products were identified by 2% agarose gel electrophorese and visualized using UV light.

Single-strand conformation polymorphism

SSCP method was applied to detect SNPs in 5'UTR, partial exon-1 and exon-2 of the DGAT1 gene. For SSCP, 5µl PCR products were diluted in 5µl denaturing solution; the mixture was centrifuged and denatured at 98 ˚C for 10 mins, then rapidly chilled on ice for 5 mins and detected on 12% polyacrylamide gel. The electrophoresis was performed in a vertical unit (Bio-rad), in 1× TBE buffer at 4 ˚C. The gel running conditions for the 360 bp fragment of DGAT1 was 350 V for 18hours and 445 bp fragment of DGAT1 was 380 V for 21 hours, respectively. Then, DNA fragments were stained with 0.1% silver nitrate and visualized in 3% NaOH solution (Zhang *et al.* 2007). Two samples from each SSCP patterns were sequenced in both directions (Bioneer, Seoul, South Korea) and were determined by Bio Edit (Version 7.2.0) and Vector NT1 software. Different SSCP pattern sequences were aligned according to Genbank (EU178818.1) by BLAST algorithm to find homologous sequences in NCBI databases (http://www.ncbi.nlm.hih.gov/).

Statistical analysis

Genotypes patterns or haplotypes frequencies were calculated using GENALEX6.4 software (Peakall and Smouse, 2006). Two fixed models were used to make an association between 360 bp fragment of DGAT1 variants and carcass traits using a generalized linear model (GLM) procedure of SAS software (SAS, 2007). Exon-2 of DGAT1 was eliminated from association analysis due to monomorphic state. Tukey–Kramer's multiple range tests were used for least-squares means (LSM) comparison. The statistical model used for determining CW, DP, BFT and DPWFT traits in two breeds was as follows:

$$Y_{ijklm} = \mu + A_i + B_k + G_l + \beta(W_{ijklm} - \overline{W}) + S_j(B_k) + (AG)_{il} + (SG)_{jl} + (BG)_{kl} + e_{ijklm}$$

Another model used for FTW, FTP and DP traits in Lori-Bakhtiari sheep breed was as follows:

$$Y_{ijlm} = \mu + A_i + S_j + G_l + \beta(W_{ijlm} - \overline{W}) + (AG)_{il} + (SG)_{jl} + e_{ijklm}$$

Where:

y_{ijklm}: phenotypic value of carcass traits.

μ: overall population mean for each trait.

A_i: fixed effect of the i^{th} age.

S_j: fixed effect of the j^{th} sex.

B_k: fixed effect of the k^{th} breed.

G_l: fixed effect of the l^{th} genotype.

$\beta(W_{ijklm} - \overline{W})$: covariate fixed effect of weight at slaughter.

$S_j(B_k)$: effect of the j^{th} sex in k^{th} breed.

$(AG)_{il}$: interaction between the i^{th} age and the l^{th} genotype.

$(SG)_{jl}$: interaction between the j^{th} sex and the l^{th} genotype.

$(BG)_{kl}$: interaction between the k^{th} breed and the l^{th} genotype.

e_{ijklm}: residual random effect.

RESULTS AND DISCUSSION

Single strand confirmation polymorphism analysis

The results showed that both PCR products including 360 bp and 445 bp fragments, successfully amplified by the used primers. Five SSCP patterns were detected for exon-1 and its 5′UTR (Figure 1).

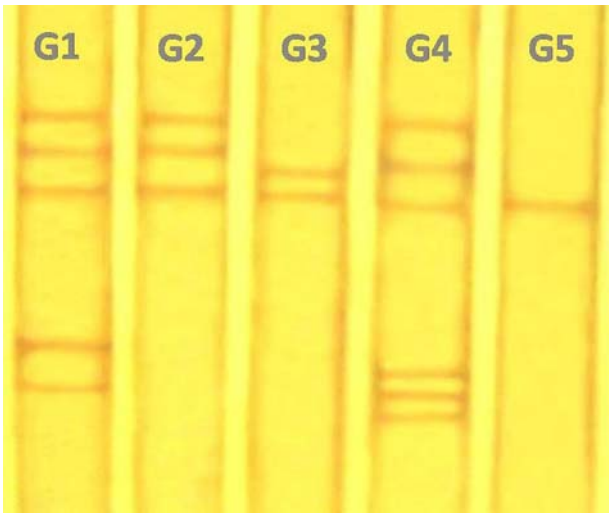

Figure 1 Five PCR-SSCP genotypes of exon-1 and its 5'UTR fragment (360 bp) of *DGAT1* gene for Lori-Bakhtiari and Zel breeds

Furthermore, one SSCP pattern was observed for exon-2 and this segment was monomorphic in both breeds (Figure 2).

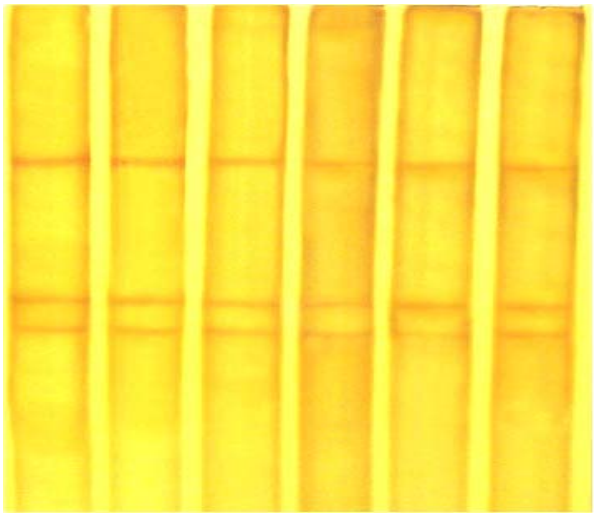

Figure 2 PCR-SSCP genotype of exon-2 fragment (445 bp) of *DGAT1* gene for Lori-Bakhtiari and Zel breeds

The frequency of the observed genotypic patterns of 360 bp fragment for each breed is shown in Table 1. The G5 and G2 had higher frequency in Lori-Bakhtiari and Zel breeds, respectively.

The G3 had the lowest frequency in both breeds. Also, the frequency of G2, G4, and G5 was different between Lori-Bakhtiari and Zelbreeds.

DNA sequence analysis

In this study, sequences of PCR amplicons from five different SSCP genotypic patterns of 360 bp fragment variants were analyzed and compared with the NCBI reference sequences (Table 2). For 360 bp fragment (including 5′UTR and exon-1), three novel single nucleotide polymorphisms (SNPs) were detected in the studied breeds. One of the discovered SNPs is located in the coding region (gA277G) and two of them located in the 5′UTR region (g.T101- and C129A) of DGAT1 (Table 2). Moreover, the protein sequences of exon-1 region were aligned to NCBI reference sequence (Figure 3). The only substitution in exon-1 of G3 pattern (A 277G) leads to the changes in amino acids (p.Arg54Gly) (Figure 3).

Correlation between DGAT1 polymorphisms and carcass traits in sheep

In the present study, exon-2 of DGAT1 was monomorphic and omitted from association studies. Association of the 360bp fragment of DGAT1 polymorphisms with carcass traits was analyzed (Table 3). Significant relationship (P<0.05) was found between FTW and BFT traits in the Lori-Bakhtiari breed, and animals with G5 had higher FTW and BFT compared to G1 pattern.

Although no significant differences (P>0.05) were observed for DP and FTP among five identified genotypic patterns in Lori-Bakhtiari, animals with G3 pattern for DP and G5 pattern for FTP had the highest performance compared to other genotypes. No significant association (P<0.05) was found between observed genotypes of exon-1 of DGAT1 in Zel breed and the studied carcass traits.

DGAT1 was selected as a candidate gene due to its effects on carcass traits that had been confirmed in previous studies on cattle (Pannier *et al.* 2010; Souza *et al.* 2010; Li *et al.* 2013; Tait *et al.* 2014) and sheep (Xu *et al.* 2009; Mohammadi *et al.* 2013; Noshahr and Rafat, 2014). The detection of SNPs in 360 bp fragment was consistent with results of Scata *et al.* (2009) who amplified and sequenced whole of the *DGAT1* gene using 18 primer pairs and reported novel SNPs at 5'UTR (g. C127A) in Sarda, Altamurana and Gentile di Puglia sheep breeds. But their result was not consistent with present study for exon-1. The detection of SNPs in 360 bp fragment was consistent with the results of Scata *et al.* (2009) who identified novel SNP in Sarda, Altamurana and Gentile di Puglia sheep breeds. Furthermore, results indicated that *DGAT1* had significant genetic effects on fat content in Lori-Bakhtiari breed are consistent with the findings of Xu *et al.* (2009), Mohammadi *et al.* (2013) and Noshahr and Rafat (2014). Based on the sequence analysis, two differences, including one deletion and one substitution, were found in 5'UTR region between detected genotypes (Table 2). Regulation of *DGAT1* expression in adipocytes mostly happens at the transcriptional and post-transcriptional steps (Yu *et al.* 2002), therefore mutations in 5'UTR region may influence DGAT1 expression and consequently fat content.

Table 1 Genotypic patterns frequencies of single strand conformation polymorphism (SSCP) variants of 5′ UTR and exon-1 of *DGAT1* gene in Zel and Lori-Bakhtiari breeds

Lori-Bakhtiari sheep breed (n=152)		
Genotypic patterns	**No.**	**Frequencies**
1	30	0.195
2	36	0.239
3	16	0.108
4	21	0.141
5	48	0.315
Zel sheep breed (n=157)		
1	30	0.189
2	81	0.515
3	13	0.084
4	15	0.094
5	18	0.115

Table 2 Nucleotide substitutions and types of single-nucleotide polymorphisms (SNPs) for exon-1 of *DGAT1* gene in Zel and Lori-Bakhtiari breeds

Accession no. EU178818				**Genotypic patterns of haplotypes**				
Allele	**Location**	**Nucleotide position**	**Codon No.**	**1**	**2**	**3**	**4**	**5**
T	5' UTR	(101)	-	-	-	T	T	T
C	5' UTR	(129)	-	C	A	C	A	C
A	Exon-1	(277)	26	A	A	G	A	A

Table 3 Association of *DGAT1* genotypes with carcass traits in Zel and Lori-Bakhtiari breeds

Lori-Bakhtiari sheep breed	***DGAT1* genotypes (LSE±SE)**				
Traits	1 (30)	2 (36)	3 (16)	4 (21)	5 (48)
Carcass weight (kg)	18.25±0.67	18.03±0.64	17.70±081	17.07±0.79	16.95±0.57
Fat-tail weight (kg)	2.63±0.38^{a}	3.22±0.36^{ab}	3.66±0.45^{ab}	3.23±0.44^{ab}	3.81±0.32^{b}
Fat-tail percentage (%)	14.93±2.41	15.80±2.29	15.38±2.88	17.34±2.82	20.68±2.03
Dressing percentage (%)	47.46±0.89	48.66±0.85	49.28±1.07	47.29±1.04	47.80±0.75
Dressing percentage without fat-tail (%)	41.15±1.48	41.30±1.41	41.16±1.78	39.96±1.74	38.88±1.25
Back fat thickness (mm)	3.37±0.31^{a}	3.83±0.29^{ab}	3.98±0.37^{ab}	3.83±0.36^{ab}	4.27±0.26^{b}
Zel sheep breed	***DGAT1* genotypes (LSE±SE)**				
Traits	1 (30)	2 (81)	3 (13)	4 (15)	5 (18)
Carcass weight (kg)	11.13±0.19	11.24±0.13	11.68±0.29	11.50±0.28	11.38±0.25
Dressing percentage (%)	45.87±0.86	46.23±0.58	48.02±1.28	47.53±1.22	46.11±1.09
Back fat thickness (mm)	4.34±0.41	4.27±0.28	3.39±0.62	4.16±0.28	4.37±0.53
Zel and Lori-Bakhtiari breeds	***DGAT1* genotypes (LSE±SE)**				
Traits (Pooled data)	1 (60)	2 (117)	3 (29)	4 (36)	5 (66)
Carcass weight (kg)	15.86±0.21	16.07±0.17	16.58±0.28	15.87±0.27	15.85±0.21
Dressing percentage (%)	46.82±0.60	47.53±0.47	49.17±0.79	47.80±0.75	47.00±0.58
Back fat thickness (mm)	3.90±0.25	4.11±0.20	3.74±0.34	4.05±0.32	4.44±0.25

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SE: standard errors.

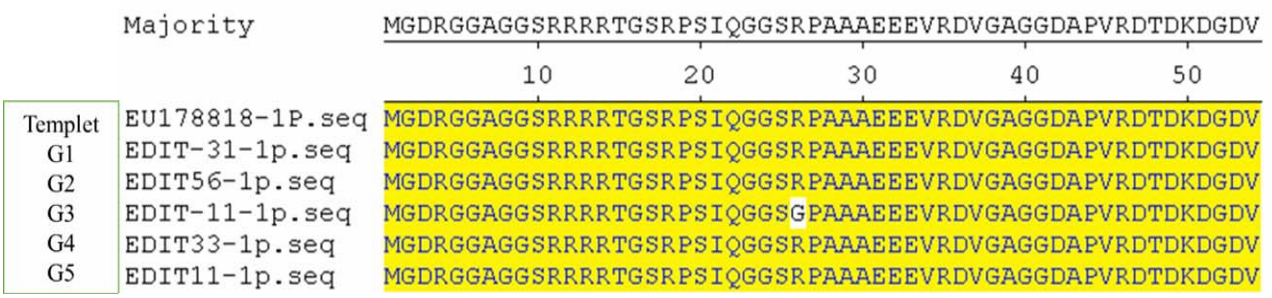

Figure 3 The amino acid sequence of exon-1 of *DGAT1* gene for Lori-Bakhtiari and Zel breeds

Therefore, identified mutations in the 5'UTR region of DGAT1 could affect the function of DGAT1 enzyme in lipid metabolism. For instance, DGAT1 has considerable effect on fat deposition and fatty acid composition in sheep meat and milk (Scata *et al.* 2009).

In the beginning cod on (ATG) has started from position 202, mutations have occurred before it was not able to change the protein sequence and only the mutation occurred in the exon-1 region of the *DGAT1* gene which was able to change the amino acid sequence of translated protein derived from this gene. Therefore, mutation only occurred in position 277 of genotypic pattern G3 and led to the transformation of amino acid arginine to glycine (AGG to GGG). However, there was no significant difference (P>0.05) between this genotypic pattern and other genotypes. This result indicated that despite the change in the sequence of the enzyme, performance did not change significantly. Therefore, this SNP could not be introduced as a marker for the studied traits.

By the way, future investigations can be useful to detect possible effects of this SNP on the production traits of sheep.

Only CW, DP and BFT traits were analyzed in Zel breed because this breed does not have any fat-tail for measuring other traits. In Zel breed, no significant difference (P> 0.05) was found among the observed five genotypes in carcass traits, but G3 pattern showed higher DP and lower BFT compared to others. The lack of significant differences between most of the identified genotypic patterns may be due to the high standard errors found for some genotypic patterns, resulting from the low number of animals for these genotypes. Therefore, increasing the number of animals will probably change the results. The effects of different genotypes on common carcass traits in Zel and Lori-Bakhtiari breeds were similar, when analyzed separately or with each other. Possible contradictory results can be due to the influences of different genomic background and different genotypic frequencies.

CONCLUSION

In this study, five different PCR-SSCP genotypic patterns were detected for 360 bp fragment (including 5′UTR and exon-1) and three novel SNPs were detected for this region. There was a significant correlation (P<0.05) between FTW and BFT traits and observed genotypes in Lori-Bakhtiari breed, but there was no significant correlation (P<0.05) between detected genotypes of exon-1 of DGAT1 in Zel breed and carcass traits. These results indicated that DGAT1 is a potential candidate gene for carcass traits in MAS and breeding programs to improve FTW and BFT traits by selection in Lori-Bakhtiari breed. Although significant correlation (P<0.05) was found between carcass fat content and *DGAT1* gene, but it can be due to the effect of other mutations in regions that were not investigated in present study (Xu *et al.* 2008) and also another QTLs close to the DGAT1 (Coppieters *et al.* 1998). Further investigation is necessary to make a conclusive decision about using the detected polymorphisms of this gene in breeding programs for carcass traits in sheep.

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