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REGULAR ARTICLES

Co-inherited novel SNPs of the *LIPE* gene associated with increased carcass dressing and decreased fat-tail weight in Awassi breed

Tahreer M. Al-Thuwaini[1] · Mohammed Baqur S. Al-Shuhaib[1] · Frederic Lepretre[2] · Zainab A. Mahdi[1]

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Abstract

The lipase E hormone-sensitive (LIPE) enzyme is one of the lipolytic enzymes, and it plays a key role in the regulation of adipose tissue deposition. This study was conducted to investigate the possible association between the *LIPE* gene variations and the main body weight measurements in Awassi sheep. A total of 160 of sexually mature Awassi rams (*Ovis aries*) that aged between 2 and 3 years were included in the present study. Genomic DNA was extracted and two specific PCR amplicons were designed to amplify two coding regions within the *LIPE* gene. Genotyping experiments were performed using polymerase chain reaction-single-strand conformational polymorphism (PCR-SSCP). Two different SSCP banding patterns were identified, CC and CD in exon 2, and AA and AT in exon 9. Five novel single-nucleotide polymorphisms (SNPs) were detected by sequencing, namely g.151C > A and g.198C > T in exon 2, and g.213G > C, g.226G > T, and g.232A > C in exon 9. Haplotype block analysis showed strong linkage disequilibrium values between the two SNPs in exon 2 and the three SNPs in exon 9. Association analysis of haplotypes with carcass traits demonstrated a significantly higher dressing percentage ($P < 0.05$) and lower fat tail weight (FTW) in CACT and GCGTAC haplotypes made these haplotypes more favorable for human consumption. The current research is the first one to report a tight association between the *LIPE* genetic polymorphism and the dressing percentage and FTW traits, suggesting a pivotal role played by these co-inherited SNPs in the metabolism of carcass traits in sheep.

Keywords Awassi · Carcass dressing · *HSL* gene · Haplotype

Introduction

Lipolysis in adipose tissue plays a major role in energy homeostasis and body weight and is regulated, at least in part, by the multiple lipolytic genes (Nishizawa and Shimomura 2019; Kulyte et al. 2020). Lipolytic genes are shown to be related to several body weight measurements in a variety of animal species, and thus, they have the potential to provide an insight into growth traits in sheep (Carlsson et al. 2006). Lipolytic genes have recently demanded more attention because of their inevitable role in obesity and diseases (Luglio et al. 2015). One of these genes is the hormone-sensitive lipase (*HSL*), or lipase E hormone-sensitive (*LIPE*) gene. The ovine *LIPE* gene is a highly conserved gene, which consists of 10 exons and located on chromosome 14 (Jiang et al. 2014). Due to the versatile roles of this gene, its expression is not only confined in adipose tissues. Instead, *LIPE* gene is also highly expressed in other tissues, such as ovary, testis, heart, adrenal glands, skeletal, and muscle tissues (Qiao et al. 2007). The *LIPE* gene encodes HSL, a multifunctional enzyme that is involved in the control of hormone-stimulated lipolysis, free fatty acid biosynthesis, and mobilization (Zechner et al. 2012; Fang et al. 2017). It acts as a rate-limiting enzyme in the conversion of stored intracellular triacylglycerol to diacylglycerol and releases non-esterified fatty acids (Xue et al. 2015; Jocken et al. 2018). Furthermore, HSL enzymes play a key role in

✉ Tahreer M. Al-Thuwaini
tahrearmohammed@agre.uoqasim.edu.iq;
tahreermohammed@ymail.com

Mohammed Baqur S. Al-Shuhaib
mohammed79@agre.uoqasim.edu.iq; baquralhilly_79@yahoo.com

Frederic Lepretre
frederic.lepretre@univ-lille.fr

Zainab A. Mahdi
zanibali861@gmail.com

[1] Department of Animal Production, College of Agriculture, Al-Qasim Green University, Al-Qasim, Babil 51001, Iraq

[2] Plateau de genomique fonctionnelle et structurale, CHU Lille, University of Lille, Lille, France

the determination of insulin resistance degree and tissue content of diacylglycerol (Hsiao et al. 2013). Two different forms of LIPE enzymes have been identified in sheep. Both forms exhibit considerable homology with LIPE amino acid sequences with several other mammalian species. This homology suggests a remarkable functional constraint over evolutionary time (Lampidonis et al. 2011). In dairy goats, *LIPE* gene variation may exert an essential role in altering the mobilization of medium-chain and unsaturated fatty acids and body composition traits (Zidi et al. 2010). It is well established that the genetic polymorphism of the *LIPE* gene has been associated with multiple carcass traits in several species. Goszczynski et al. (2014) have reported high polymorphism in the *LIPE* gene and confirmed a significant association of this gene variation with the fatty acid composition in cattle. Similarly, it has been found that single-nucleotide polymorphisms (SNPs) of the *LIPE* gene have been associated with the carcass and meat quality traits in cattle (Fang et al. 2014). Significant associations between the variation in the *LIPE* gene and subcutaneous fat deposition, including an increased body mass index, waist circumference, and obesity in humans, have been observed (Carlsson et al. 2006). Given the essential role the *LIPE* gene plays in fat metabolism, it is suggested that the nucleic acid substitution within this gene may cause alterations in the function of its encoded LIPE enzyme and may result in varied phenotypic traits in the studied population. Unfortunately, only limited numbers of studies have investigated the association of this gene with the primary productive traits of sheep (Yang et al. 2014). Keeping in mind the above aspects, the purpose of this study was aimed to investigate the possibility of the association between polymorphisms of the *LIPE* gene with several production traits in the Iraqi native Awassi sheep. To the best of our knowledge, the present manuscript is the first one to describe the association of the *LIPE* gene polymorphisms with several fat depositions and composition traits in sheep.

Materials and methods

Sheep population and carcass growth trait measurements

The study was conducted according to the regulations of the international recommendations for the care and use of animals (Federation of Animal Science Societies 2010) and animal experimentation was approved by Al-Qasim Green University (Approval No. 12.10.15). A total of 160 of sexually mature Awassi rams (*Ovis aries*) aged between 2 and 3 years were included in the present study. The referred animals were the progeny of the different males and females and they were randomly selected from three flocks in the middle Euphrates regions of Iraq. Any relationship between the parents of the selected animals was not recorded. In each studied flock, 10–12 rams were randomly allocated to mate with about 20–25 ewes per ram, with male identification recorded. All the selected animals were confirmed to be healthy in terms of the absence of any noticeable clinical symptoms. Animals were kept on natural pasture during spring and autumn, while in winter, animals were kept indoors and fed about 2.5% of their live body weight daily, comprising a mixture of barely (59%), bran (40%), and salt (1%) concentrates. From the period of October 2017 to June 2018, several phenotypic measurements were taken from the Awassi sheep from the main abattoirs in the referred regions, such as live body weight (BW), back fat thickness (BFT), abdominal fat (AF), fat tail weight (FTW), and carcass weight, which were recorded at the specified slaughterhouses as described by Rehman et al. (2013). Dressing percentage of each animal was calculated according to the following formula (Warriss 2000):

$$Dressing\ percentage = Carcass\ weight / Live\ body\ weight \times 100.$$

Genomic DNA extraction

Genomic DNA was extracted from the whole blood by a universal salting-out technique (Al-Shuhaib 2017). The integrity of the extracted DNA was evaluated by direct electrophoresis on 0.8% agarose gel, while the purity of the extracted DNA was assessed by a nanodrop spectrophotometric method (BioDrop μLITE, BioDrop, UK).

Polymerase chain reaction

Two pairs of specific primers were designed by NCBI primer BLAST software to amplify the biggest two coding exons within the *LIPE* gene, including the *LIPE*-exon-2 and *LIPE*-exon-9 fragments, of 342 bp and 278 bp, respectively (Fig. 1a). Further details of the designed primers are described in Table 1. The PCR reaction was performed using the *Accu*Power PCR PreMix (Bioneer, South Korea). Each 20 μl of PCR premix contained 250 μM of dNTPs, 10 mM of Tris-HCl (pH 9.0), 30 mM of KCl, 1 U of *Top* DNA polymerase, and 1.5 mM of $MgCl_2$. The PCR reaction mixture was completed with 10 pmol of each primer and 10–30 ng of genomic DNA. The PCR program was initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation (94 °C for 30 s), annealing (61.8 °C for *LIPE*-exon-2 and 60.4 °C for *LIPE*-exon-9 for 30 s), elongation (72 °C for 30 s), and a final extension (72 °C for 5 min). The PCR products were verified by electrophoresis on a 1.5% (w/v) agarose gel, and photos were taken using a photodocumentation unit (ChemiDoc, Bio-Rad, USA). It was

Table 1 Oligonucleotide primer sets designed for the amplification of the ovine *LIPE* gene

Primer label	Sequence (5′-3′)	Primer binding coordinates*	Amplicon length	Region	Annealing temperature
LIPE -exo2-F	ACATGGCCTTCTTC TCCAGC	49839123–49839142	342 bp	Exon 2	61.8 °C
LIPE-exo2-R	CCTC AAAGAAGAGC CTCCCG	49839445–49839464			
LIPE-exo9-F	CCAA CTCCCTCAAG AGCCTG	49847442–49847461	278 bp	Exon 9	60.4 °C
LIPE-exo9-R	TGAG TAGAGGGGCA TCCACA	49847700–49847719			

LIPE, lipase E hormone-sensitive; exo2, exon 2; the symbols "F" and "R" refer to forward and reverse primers, respectively

*Primer binding coordinates refer to the *Ovis aries* NCBI Reference Sequence NC_019471.2

confirmed that all electrophoretic PCR bands were specific before being submitted to the next genotyping step.

Single-strand conformation polymorphism

The initial denaturation of the PCR amplicons, as well as SSCP protocol, was performed according to Al-Shuhaib et al. protocol (Al-Shuhaib et al. 2018). The samples were loaded into 8% SSCP gels (acrylamide:bis-acrylamide = 37.5:1). The running conditions were set at 200 V, 100 mA for 4 h at a constant temperature of 20 °C using mini-wide gels (216 × 110) mm with gel thickness 1.0 mm (JY-CZ-B, Junyi-Dongfang Electrophoresis Equipment). The DNA fragments in the gels were visualized by silver nitrate as described by Byun et al. (2009).

Sequencing of PCR amplicons

Each observed PCR-SSCP electrophoretic pattern was commercially sequenced from both directions according to the instruction manuals recommended by sequencing laboratories (Macrogen Inc. Geumcheon, Seoul, South Korea). The reference databases of the two *LIPE* fragments were retrieved from the NCBI website (https://www.ncbi.nlm.nih.gov). Subsequently, the sequences of the observed genotypes were edited, aligned, and analyzed by BioEdit Sequence Alignment Editor Software Version 7.1 (DNASTAR, Madison, WI, USA). The electropherogram variations were then visualized by SnapGene Viewer Ver. 4.0.4, (http://www.snapgene.com). The novelty of the observed SNPs was confirmed by the dbSNP database (https://www.ncbi.nlm.nih.gov/projects/SNP/). Further confirmations of the observed SNPs were obtained from the ensemble genome browser 95 (https://asia.ensembl.org/index.html).

Statistical analysis

The allele and genotype frequencies of both studied loci of *LIPE* gene were estimated by direct counting, and the differences of the observed and expected frequencies of exon 2 and exon 9 genotypes were estimated using chi-square test to verify if the targeted ovine population was in Hardy-Weinberg equilibrium. Genetic indices of Awassi sheep, including observed heterozygosity (H_o) and expected heterozygosity (*He*), were performed by PopGen32 software, v. 1.31 (Yeh et al. 1999). Pairwise linkage disequilibrium (LD) between SNPs was calculated using SHEsis software (She and He 2006). All statistical analyses were performed using SPSS v23.0 (IBM, NY, USA). General linear mixed-effects models (GLMMs) were used to evaluate the effect of genotype on phenotypic traits. The model used to test the SNP genotype effect was fitted as follows:

$$Y_{ijk} = \mu + G_i + \alpha_j + e_{ijk}$$

where Y_{ijk} is the phenotypic traits observed, μ is the overall mean for each trait, G_i is the fixed effect associated with the *i*th SNP genotype or haplotype, α_j is the random effect of *j*th sire, and e_{ijk} is the random error for $_{ijk}$. Preliminary statistical analyses indicated that age, season, and nutrition were not found to affect carcass characteristics and thus they were not included in the model.

Results

Polymorphism of the *LIPE* gene

Both amplified loci of the *LIPE* gene were observed alongside with two SSCP banding patterns. Exon 2 showed two genotypes, one with two single-strand (ss) DNA bands, CC genotype, and the other one with three ssDNA bands, CD genotype. Similarly, exon 9 showed two genotypes, one with three

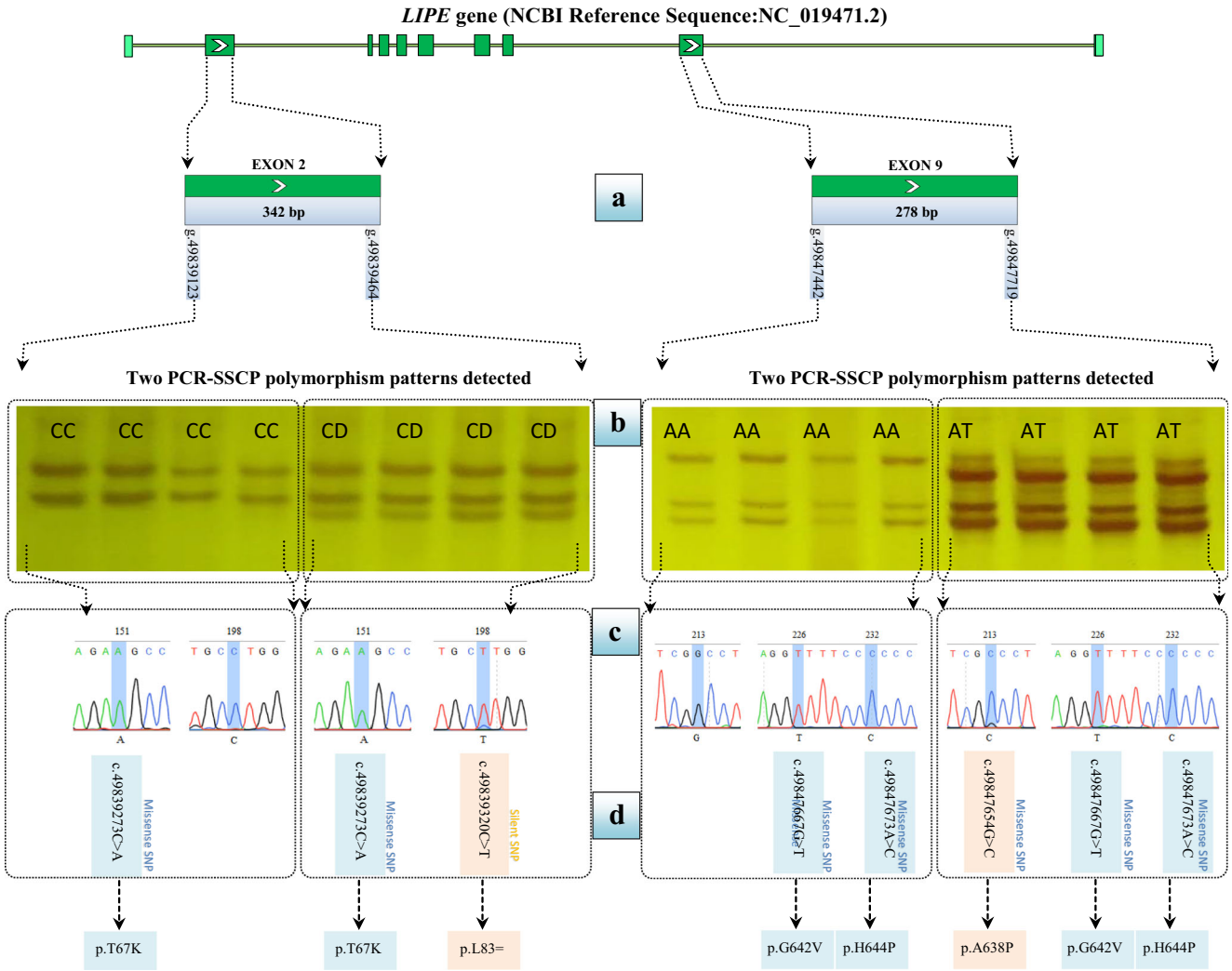

Fig. 1 Genomic organization of the *LIPE* gene and its PCR-SSCP-sequencing strategy. (A) PCR design of two PCR specific primer pairs for the amplification of 342 bp and 278 bp in exon 2 and exon 9, respectively. (B) Post-PCR genotyping using SSCP technique, in which both exons showed two different genotypes. (C) Sequencing reaction interpretation of the detected genotypes, in which two SNPs were detected in the exon 2 and three SNPs were identified in exon 9. (D) Characterization of the observed SNPs, in which one silent p.L83= and one missense p.T67K SNPs were observed in the exon 2, and three missense p.A638P, p.G642V, and p.H644P SNPs were observed in the exon 9. The cyan color refers to the presence of a particular SNP in both genotypes, while the pink color refers to the detection of a particular SNP in one specific genotype. The positions of primers and the nomenclature of the observed SNPs are listed according to NCBI Reference Sequence: NC_019471.2 following the nomenclature described in varnomen.hgvs.org/

ssDNA bands, AA genotype, and the other one with four ssDNA bands, AT genotype, within the same gel conditions (Fig. 1b). The nucleic acid substitutions of the detected genotypes were analyzed by comparing DNA sequencing electropherograms of both observed genotypes in both sequences of exon 2 and exon 9, respectively (Fig. 1c).

LIPE gene sequence interpretation

No previously published data concerning the *LIPE* gene were deposited under Awassi breed in the NCBI GenBank database. Therefore, the exon 2 and exon 9 DNA sequences obtained in this study, and those previously reported for other highly related ovine sequences (DQ647326.1 and NM_001128154.1 for exon 2, and DQ647326.1, NM_001128154.1, KC585035.1, KC585036.1 for exon 9), were compared to identify polymorphic sites. Based on alignments and annotations conducted by Blastn, dbSNP, ensemble, and DNA STAR tools, the novelty of all the five detected SNPs was confirmed in the investigated Awassi population. Two transition SNPs were detected in exon 2: c.49839273C > A, with a missense effect of p.T67K that was observed in both genotypes, and c.49839320C > T, with a silent effect of p.L83= that was observed only in the CD genotype. However, both transition/transversion forms were detected in exon 9, in which three SNPs, c.49847654G > C, c.49847667G > T, and c.49847673A > C, with three respective missense effects of p.A638P, p.G642V, and p.H644P

were observed (Table 2). While p.G642 and p.H644P were detected in both AA and AT genotypes, p.A638P was detected only in the AT genotype (Fig. 1d).

The genetic diversity of the observed variations and association of polymorphisms with carcass traits in Awassi breed

Frequencies of individual alleles and genotypes in the analyzed populations of Awassi sheep are presented in Table 3. The χ^2 test showed that the polymorphism of the *LIPE* gene at both loci did not deviate from the Hardy-Weinberg equilibrium ($P < 0.05$). The observed heterozygosity was lower than its expected value. This entailed a low level of genetic diversity. Pairwise linkage disequilibrium (LD) between five SNPs was calculated in the Awassi breed. Two SNPs (g.151C > A and g.198C > T) in exon 2 and three SNPs (g.213G > C, g.226G > T, and g.232A > C) in exon 9 were in high LD ($r^2 > 0.9$), signifying a closely and strongly linked between pairs of SNPs. Concerning exon 2 variation, differences between combined genotypes were statistically significant just for FTW and dressing percentage ($P < 0.05$), in which individuals with CACT haplotype showed lower FTW and higher dressing percentage than those with CCCC haplotype. Similarly, variation in the exon 9 appeared to exhibit significant effects on FTW and dressing percentage. The individuals with GCGTAC haplotype showed a significant association with lower FTW and higher dressing percentage ($P < 0.05$) than those with GGGGAA haplotypes (Table 4). Thus, sheep with CACT and GCGTCA haplotypes had lower FTW and higher dressing percentages and could be used in the improvement of meat quality in Awassi breed. Haplotype analysis of five SNPs revealed a statistically significant association with carcass traits, indicating that these variants of the *LIPE* loci could be helpful genetic markers in sheep breeding.

Discussion

LIPE gene encodes the HSL enzyme, an intracellular neutral lipase, which hydrolyzes a variety of esters and participated in the mobilization of free fatty acids to the body. Since the *LIPE* gene is directly involved in regulating lipolysis, alterations in its sequences are expected to modify the fatty acid composition of tissues. For this reason, mutations in *LIPE* gene may affect the chemical composition of meat and alter meat quality traits (Goszczynski et al. 2014). This study describes a tight association between the *LIPE* gene variation and carcass measurements in sheep. This association was carried out based on the PCR-SSCP genotyping of the *LIPE* gene in 160 Awassi rams. The polymorphisms of exon 2 and exon 9 were associated with dressing percentage and FTW measurements in the investigated Awassi breed. Regarding the exon 2, only one SNP was detected with a silent effect, p.L83=. Although a silent SNP may change the protein conformation and translation kinetics (Komar 2007; Hunt et al. 2009), the currently observed silent SNP had obvious association with the studied carcass measurements. Concerning exon 9, three SNPs with three different missense effects were observed, p.A638P, p.G642V, and p.H644P. However, the observed association in the sheep between the exon 2 and exon 9 genetic variations of the *LIPE* gene and the carcass traits is quite remarkable since no previous study has demonstrated such association (Yang et al. 2014). However, our results are in line with several reports that demonstrated the linkage of *LIPE* variation with several carcass traits in cattle and pigs (Lei et al. 2005;

Table 2 Nucleotide substitutions and types of genotypes among the *LIPE* gene-based SSCP genotypes of the studied Awassi sheep. The positions of SNPs are listed according to the NCBI Reference Sequence: NC_019471.2

No.	Gene region	Nucleotide position in the PCR amplicon	Nucleotide position in the NCBI reference sequence	Amino acid change	Type of SNP	SNP summary	Genotype
1	Exon 2	151	49839273	P67K	Missense	exon2; c.49839273C > A (p.T67K)	CC and CD
2		198	49839320	L83=	Silent	exon2; c.49839320C > T (p.L83=)	CD
3	Exon 9	213	49847654	A638P	Missense	exon9; c.49847654G > C (p.A638P)	AT
4		226	49847667	G642V	Missense	exon9; c.49847667G > T (p.G642V)	AA and AT
5		232	49847673	H644P	Missense	exon9; c.49847673A > C (p.H644P)	AA and AT

SSCP, single-strand conformation polymorphism; *NCBI*, National Center for Biotechnology Information; *PCR*, polymerase chain reaction; *SNP*, single-nucleotide polymorphism

Table 3 Effects of *LIPE* gene polymorphism on the main body traits of Awassi sheep

Gene region	Combined genotypes (*n*)	LSM ± SE					
		Live body weight (kg)	Fat tail weight (kg)	Abdominal fat weight (kg)	Back fat weight (kg)	Dressing (%)	Carcass weight (kg)
Exon-2	CCCC (137)	48.795 ± 0.451	1.993 ± 0.076^{b}	2.092 ± 0.064	2.963 ± 0.069	53.820 ± 0.615^{b}	26.259 ± 0.309
	CACT (23)	46.400 ± 1.392	1.450 ± 0.023^{a}	1.900 ± 0.026	2.100 ± 0.024	57.389 ± 1.777^{a}	25.600 ± 1.318
	P value	0.340	0.05*	0.426	0.662	0.04*	0.571
Exon-9	GGGGAA (129)	49.000 ± 0.484	2.045 ± 0.084^{b}	2.088 ± 0.075	3.049 ± 0.060	53.539 ± 0.659^{b}	26.232 ± 0.354
	GCGTAC (31)	47.470 ± 1.105	1.808 ± 0.015^{a}	2.394 ± 0.075	2.394 ± 0.016	57.249 ± 1.511^{a}	27.176 ± 1.022
	P value	0.254	0.04*	0.299	0.123	0.04*	0.350

Least square means ± standard error. Significant differences in means represent by different letters in the same column. The letter (*n*) refers to the number of individuals

*($P < 0.05$)

Fang et al. 2014; Goszczynski et al. 2014; Xue et al. 2015). While it is likely that the association between the *LIPE* genetic polymorphisms with economic traits is may be informative to improve the quality of meat in sheep (Goszczynski et al. 2014), likewise, involving haplotype analysis can also improve the power of association studies with carcass traits in sheep (An et al. 2017). Based on haplotype analysis, sheep with the CACT and GCGTAC haplotypes had lower FTW and higher dressing percentages and are thus more favored for human consumption than those with the CCCC and GGGGAA haplotypes. However, consumers prefer leaner meat, since the increased FTW burdens breeders in terms of reproduction, growth, and carcass marketing conditions. Moreover, the presence of more tail fat in the body lowers the quality of meat and requires more energy than the production of lean tissue. Besides, consumers are not usually preferring fat dispersed throughout the tail over the meat fibers (Gokdal et al. 2003). Consequently, increased FTW reduces the commercial value of the carcass (Bingol et al. 2006). Therefore, it can be stated that the higher proportion of the fat in the carcass lowers its desirability to the consumers (Bingol et al. 2006; Skapetas et al. 2006). In agreement with our findings, a tendency for genotypes with lower FTW was found to have a higher percentage of fat in carcass cuts (Ünal et al. 2006). Though lipolysis is controlled by the expression of many lipolytic genes that may exhibit multifactorial regulation of this critical metabolic activity (Fan et al. 2019), this study detected a tight correlation between the polymorphisms of *LIPE* gene with FTW and dressing percentage. Accordingly, meat quality seems to be under the detected genetic polymorphism of the *LIPE* gene. Therefore, it is maybe rational to consider the present haplotype analysis as a valuable tool for assessing the degree of *LIPE* gene association with carcass traits.

Conclusion

Haplotype analysis of five novel SNPs observed in the *LIPE* gene demonstrated a tight linkage to some carcass and lipid traits in sheep. Since it exhibited significant association ($P < 0.05$) with higher dressing and lower FTW values in the

Table 4 Genetic diversity of the *LIPE* gene for two different exons in the Awassi sheep

Gene region	Combined genotypes		Genotype frequencies		Allele frequencies		*Ho*	*He*	*Ne*	χ^2
Exon 2	CCCC	CACT	CCCC	CACT	CC	CT				
	n = 137	*n* = 23	0.86	0.14	0.93	0.07	0.143	0.133	1.540	0.915
Exon 9	GGGGAA	GCGTAC	GGGGAA	GCGTAA	GGA	CTC				
	n = 129	*n* = 31	0.81	0.19	0.90	0.10	0.193	0.175	1.212	1.776

χ^2, chi-square; *Ho*, observed heterozygosity; *He*, expected heterozygosity; *Ne*, effective allele number; *n*, number of individuals

*All chi-square tests have one degree of freedom and within the significance level $P < 0.05$

Awassi breed. Therefore, the *LIPE* gene variation is an interesting marker for the assessment of carcass traits, and it can be recommended for future marker-assisted selection programs.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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