Polymorphisms in hormone-sensitive lipase and leptin receptor genes and their association with growth traits in Barki lambs

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Abstract

Background and Aim: Marker-assisted selection has many advantages over conventional selection in animal breeding. The candidate gene approach has been applied to identify genetic markers associated with economically important traits in livestock. This study was established to investigate variation in the hormone-sensitive lipase (HSL) and leptin receptor (LEPR) genes, and their association with growth traits in Barki lambs.

Materials and Methods: Records for birth weight (BW), pre-weaning average daily gain (ADG1), weaning weight (WW), post-weaning average daily gain (ADG2), and marketing weight (MW) were obtained from 247 Barki lambs. Polymerase chain reaction–single-stranded conformational polymorphism analyses were used to detect variation in exon 9 of HSL and exon 19 of LEPR. General linear models were used to test for associations between the variation in ovine HSL and LEPR, and growth traits.

Results: The SSCP banding patterns for HSL showed three variants (H1, H2, and H3), which contained two nucleotide-sequence differences (c.1865C>T and c.2038T>C). Two SSCP banding patterns (L1 and L2) were observed for LEPR and these contained two nucleotide-sequence differences (c.2800G>A and c.2978C>G). The HSL genotype showed no effect on the studied traits. The LEPR genotype was proven to have significant effects (p<0.05) on ADG2 and MW. The presence of the L1 variant was associated (p<0.01) with decreased ADG2 and MW.

Conclusion: The finding of an association between LEPR gene variation and growth rate after weaning in Barki lambs warrants efforts to improve this trait.

Keywords: Barki lambs, growth traits, hormone-sensitive lipase, leptin receptor.

Introduction

In Egypt, there are major problems associated with supplying sufficient food to the population, especially meat products. This has directed attention toward improving the productivity of livestock adapted to the conditions of arid and semi-arid areas, which constitute about 94% of Egypt’s land. In these areas, Barki sheep have advantages over large ruminants, in that they can utilize a wider diversity of plants and have a higher reproductive rate, allowing populations to recover more quickly than large ruminants. Developing the productivity of sheep from meat is very important to overcome the shortfall of meat products. The growth traits of lambs are important factors influencing the meat productivity of sheep. Rapid growth during the early stage of lamb life could compensate for some of the rearing costs and result in a higher net profit for sheep producers. Understanding the genetic mechanisms that control growth traits is crucial in improving meat productivity in sheep [1]. The genetic factors that regulate appetite, lipolysis, and energy homeostasis are important factors in controlling growth and weight gain [2]. Mutations in these genetic factors could disrupt energy homeostasis. Two of the most important factors in regulating energy intake and consumption are hormone-sensitive lipase (HSL) and leptin receptor (LEPR), which are responsible for transferring information related to controlling food intake, metabolism, lipolysis, and energy expenditure [3,4].

HSL is an intracellular enzyme predominantly found in white and brown adipose tissues, along with skeletal muscles, and intestinal mucosa, steroidogenic tissues including adrenals, ovaries, and testis, and pancreatic β cells. It plays a role in many metabolic processes, energy homeostasis, and steroidogenesis through its unique ability to hydrolyze a wide variety of substrates, such as all forms of acylglycerols (triglycerol, diacylglycerol, and monoacylglycerol), cholestereryl esters, steroid esters, para-nitrophenol esters, and retinyl esters [5]. HSL is encoded by the HSL gene, which is located on chromosome 14 and contains 10 exons separated by 9 introns (GenBank Gene ID: 100169699).

Leptin is an adipocyte-secreted hormone that regulates food intake, energy expenditure, and body weight in mammals. It acts through the LEPR, a
member of the cytokine receptor superfamily, and has been found on the surface of cells in many organs and tissues, including the hypothalamus, liver, heart, kidneys, lungs, small intestines, pituitary cells, testes, ovaries, spleen, pancreas, adrenal glands, and adipose tissue [6]. Leptin and its receptor are known to play a role in regulating a multitude of physiological processes, including appetite, lipid metabolism, energy expenditure, growth, reproduction, and immune function [7]. LEPR is encoded by the LEPR gene, located on chromosome 1, and contains 20 exons and 19 introns. Numerous studies have focused on variation in the LEPR gene and its association with performance traits in livestock.

Given the crucial roles played by HSL and LEPR in glucose and fat metabolism as well as energy expenditure, variation within these genes might cause variation in metabolic function and lipase function and result in variation in growth traits in Barki lambs. Against this background, the main objective of this study was to determine the variation in a region covering a portion of exon 9 of the HSL gene and that in a region covering a portion of exon 19 of the LEPR gene, as detected using polymerase chain reaction–single-stranded conformational polymorphism (PCR-SSCP) analysis. Another objective is to reveal associations between these genetic variations and variation in growth traits in Egyptian Barki lambs.

Materials and Methods

Ethical approval

The study was carried out under permission and the guidelines of Desert Research Center and the Ministry of Agriculture and Land Reclamation, Egypt.

Study period and location

A total of 247 Barki lambs, born in four successive years (2014-2017) and reared at Mariout Research Station (belonging to the Desert Research Center and located in the northwest of Egypt), were genotyped. The genotyping was conducted at Molecular Genetics Laboratory of Animal Breeding Department, Desert Research Center, in 2018-2019.

Phenotypes and blood collection

At birth, the lambs were ear-tagged and weighed. Lambs suckled their ewes until weaning (80-95 days). Live weights at weaning weight (WW) and marketing weight (MW; 8-9 months) were recorded. Pre- and post-weaning average daily gains (ADG1 and ADG2) were estimated from these weights.

Blood samples were collected from the jugular vein of the phenotyped lambs using vacuum tubes treated with 0.25% EDTA, and stored at −20°C on DNA extraction. DNA was extracted using a genomic DNA extraction kit (Qiagen, Hilden, Germany).

Polymerase chain reaction–single-stranded conformational polymorphism

Regions of HSL2 and LEPR were amplified using pairs of primers (Table-1). The PCR mixture for each gene contained 50 ng of genomic DNA, 0.25 mM of each primer, 160 mM dNTPs (GenElute; Merck KGaA, Darmstadt, Germany), 1.5 µL of 10× polymerase buffer (including 1.5 mM MgCl$_2$), 0.6 U Taq DNA polymerase (GenElute; Merck KGaA, Darmstadt, Germany), and deionized water up to a final volume of 15 µL. Thermal cycling was conducted using a Bio-Rad C 1000 touch thermal cycler (Bio-Rad, Hercules, CA, USA), and the thermal cycle parameters for both genes were 95°C for 3 min, followed by 35 cycles of 96°C for 30 s, 60°C (for HSL) or 61°C (for LEPR) for 30 s, and 72°C for 30 s. This was followed by final elongation for 5 min at 72°C.

A total of 15 µL of each PCR amplicon was denatured at 105°C for 7 min, rapidly chilled on ice, and then loaded onto the electrophoresis unit [Protein II xi cells (Bio-Rad, USA)]. The amplicons from the HSL gene were screened using 14% acrylamide:bisacrylamide (37.5:1) gels at 250 V and 25°C for 16 h, and the amplicons from the LEPR gene were screened using 14% acrylamide:bisacrylamide gels at 280 V and 15°C for 18 h. The method of Byun et al. [8] was used to stain gels.

Sequencing and analysis of variant polymorphisms

Two amplicons from lambs with homozygous SSCP patterns were purified using a PCR clean-up kit (GenElute; Merck KGaA, Darmstadt, Germany). The purified amplicons were delivered to the Macrogen sequencing company (Seoul, South Korea), to be sequenced in both directions using the BigDye terminator tool. DNA sequences, alignments, translations, analysis, and comparisons were achieved using DNAMAN and DNASTAR software.

Statistical analysis

Using the general linear mixed model in SAS (version 9.1), two sets of analyses were run to test the effect of HSL/LEPR gene variation on the studied traits. In the first, genotype was modeled as a class variable. In the second, absence/presence of the detected variants was collapsed into two categories. Variation in the HSL/LEPR gene, year of lambing, parity of ewe, and gender of lamb were included as fixed effects. Sire was included as a random effect. Age at weaning was fitted as a covariate in the model testing the effect of variation in the HSL/LEPR gene on ADG1 and WW; additionally, age at marketing was fitted as a covariate in the model testing the effect of variation in the HSL/LEPR gene on ADG2 and MW. The significant differences were further explored using a Duncan test at p<0.05.

The generalized statistical model was as follows:

\[ Y_{ijklmn} = u + R_i + T_j + B_k + P_l + T_{jkl} + \epsilon_{ijklmn} \]

\[ Y_{2ijklmno} = u + R_i + P_l + B_k + T_{jklm} + bAW_m + \epsilon_{ijklmno} \]

\[ Y_{3ijklmn} = u + R_i + P_l + B_k + T_{jklm} + bAM_n + \epsilon_{ijklmn} \]

where,

- \( Y_1 \) = the evaluated ADG2 or MW;
- \( Y_2 \) = the evaluated ADG1 or WW;
- \( Y_3 \) = the evaluated ADG2 or MW;
Sequence variation

Five genotypes were observed for HSL in the Barki lambs (Table-2 and Figure-1). These genotypes were derived from three sequence variants (Figure-2), which were named H1, H2, and H3. A total of two single-nucleotide polymorphisms (SNPs) (c.1865C>T and c.2038T>C) that were previously reported in the NBCI were detected by performing DNA sequencing of the three detected variants in exon 9 of the HSL gene. Notably, the c.1865C>T substitution results in the amino acid change threonine>methionine (p.Thr.622Met); however, the c.2038T>C substitution results in the amino acid change cysteine>arginine (p.Cys680Arg). As shown in Table-2 and Figure-3, two different SSCP banding patterns were observed for amplicons from the amplified region of LEPR in the Barki lambs and three combinations of SSCP patterns corresponding to three different genotypes were detected. These genotypes comprised two variant sequences (Figure-4), which were named L1 and L2. The results of sequencing the two detected variants revealed two SNPs (c.2800G>A and c.2978C>G), which were previously reported in exon 19 of the LEPR gene. These are missense SNPs and do result in amino acid changes, namely, valine>methionine (p.Val934Met) and serine>cysteine (p.Ser993Cys), respectively.

Effect of sire

The sire exhibited a significant effect on WW (p<0.005) and MW (p<0.01).

Effect of gender

The gender of lamb showed a highly significant effect (p<0.001) on birth weight (BW), ADG1, WW, and MW. Male lambs were heavier than female lambs in BW, WW, and MW.

Effect of non-genetic factors

Ewe parity significantly affected (p<0.001) BW, whereas the year of lambing did not affect any of the studied traits. Age at weaning showed a significant effect (p<0.01) on WW, whereas age at marketing had no significant effects on the studied traits.

Effect of HSL gene variation

The results presented in Tables-3 and 4 show no significant association of ovine HSL exon 9 variants or genotypes with growth traits.

Effect of LEPR gene variation

The SSCP patterns at exon 19 of LEPR of 247 individuals were analyzed for correlations with growth traits (Table-5). Statistically significant results (p<0.05) were found for associations of ADG2 and MW values with LEPR genotypes; however, no significant associations of LEPR genotypes with BW, ADG1, and WW values were detected (p>0.05). Individuals with genotype L2L2 had superior ADG2 and MW when compared with those with L1L1 and L1L2.
As shown in Table-6, the presence of the L1 variant in the lamb genotype was associated (p<0.01) with decreases in ADG2 and MW.

The obtained results of the additive/dominance effects of LEPR variants on the growth traits are shown in Table-7. These results revealed a significant additive (p<0.01) effect for the LEPR genotype on ADG2 (5.878 g/day±2.076) and MW (1.889 kg±0.688).

**Discussion**

**Effect of HSL genotype on growth traits**

This is the first report regarding the effect of HSL gene variation in Barki sheep. The SNPs detected in this study were not the same SNPs as were detected in Suffolk sheep [9], which were three single-nucleotide substitutions in intron 5 and one non-synonymous substitution in exon 9 of HSL. None of those substitutions was associated with post-weaning growth, whereas the detected substitutions in intron 5 were associated with eye muscle depth, eye muscle width, and fat depth above the eye muscle.
Various SNPs have been reported in other livestock, but only a few of them have been further analyzed. In cattle, two SNPs (c.276C>T and c.51C>T) have been found to be associated with carcass and meat quality traits in Chinese Simmental-cross steers [10]. Furthermore, three SNPs (rs109759779, rs109598915, and rs41887406), selected on the basis of evolutionary conservation, were studied and the obtained results suggested a possible association of SNP1 with the levels of oleic acid and total monounsaturated fatty acids (SFA) (p<0.01), and SNP2 and SNP3 with the level of heneicosylic acid (p<0.01) [11].

In goats, two synonymous polymorphisms were identified at exons 2 (c.327C>A>T) and 3 (c.558C>T).

**Table-4:** Association of the absence/presence of hormone-sensitive lipase variants with growth traits in Barki lambs.

| Trait   | Variant being assessed | N   | Absent variant | Present variant | Significance |
|---------|------------------------|-----|----------------|-----------------|-------------|
| BW      | H1                     | 44  | 3.59±0.09      | 3.50±0.04      | 0.446       |
|         | H2                     | 136 | 3.56±0.05      | 3.54±0.06      | 0.226       |
|         | H3                     | 217 | 3.52±0.04      | 3.45±0.08      | 0.612       |
| ADG1    | H1                     | 44  | 174.32±4.90    | 176.33±2.46    | 0.580       |
|         | H2                     | 136 | 174.79±3.06    | 177.43±3.14    | 0.495       |
|         | H3                     | 217 | 175.95±2.41    | 176.13±4.93    | 0.974       |
| WW      | H1                     | 44  | 19.77±0.50     | 19.84±0.25     | 0.668       |
|         | H2                     | 136 | 19.71±0.31     | 19.98±0.33     | 0.473       |
|         | H3                     | 217 | 19.81±0.25     | 19.98±0.52     | 0.948       |
| ADG2    | H1                     | 44  | 89.68±3.70     | 85.53±1.62     | 0.290       |
|         | H2                     | 136 | 86.59±1.91     | 85.89±2.36     | 0.829       |
|         | H3                     | 217 | 86.21±1.60     | 86.72±4.24     | 0.826       |
| MW      | H1                     | 44  | 44.22±1.25     | 43.17±0.53     | 0.491       |
|         | H2                     | 136 | 43.32±0.64     | 43.40±0.77     | 0.880       |
|         | H3                     | 217 | 43.31±0.53     | 43.71±1.29     | 0.874       |

BW=Birth weight, ADG1=Pre-weaning daily gain, WW=Weaning weight, ADG2=Post-weaning daily gain, MW=Marketing weight.

**Table-5:** Least square means and their standard errors for growth traits in Barki lambs according to the leptin receptor genotypes.

| Trait   | Genotype | Significance |
|---------|----------|--------------|
|         | L1L1 (134) | L1L2 (81) | L2L2 (32) |          |
| BW      | 3.49±0.05 | 3.54±0.07 | 3.59±0.10 | 0.347 |
| ADG1    | 174.64±3.11 | 176.69±3.85 | 179.75±5.04 | 0.754 |
| WW      | 19.67±0.32 | 19.93±0.39 | 20.25±0.49 | 0.690 |
| ADG2    | 84.14±2.02 | 94.36±2.66 | 100.08±2.95 | 0.012* |
| MW      | 42.61±0.68 | 45.93±0.83 | 47.56±1.11 | 0.017* |

BW=Birth weight, ADG1=Pre-weaning daily gain, WW=Weaning weight, ADG2=Post-weaning daily gain, MW=Marketing weight. *Refers to significance at (p<0.05)
of the HSL gene [12]. The same study revealed a miss-
se sense polymorphism at exon 6 (c.1162G>T), which
involves an alanine to serine substitution at position 388.

In pigs, the influence of HSL gene polymorphism
(c.442G>A) on carcass traits was investigated [13], and
two alleles A and G were detected with frequencies of
0.738 and 0.262, respectively. Moreover, three gen-
otypes AA, AG, and GG were found with frequen-
cies of 0.700, 0.075, and 0.225, respectively. In the
observed population, allele A was associated with bet-
ter animal muscularity, while allele G was associated
with greater fat content. In addition, the HSL variation
in two local Chinese pig breeds (Nuogu bei Luobo
and backfat thickness.

Duroc crossbreeds, which was associated (p<0.05)
with increased rib eye area and decreased intramuscu-
lar fat and backfat thickness.

In general, variation in exon 9 of HSL is not
associated with growth traits. This might be due to the
variation in the studied region not directly affecting the
weight gain of animals, instead altering the subcutane-
ous, and intramuscular fat composition of the body [8].

Effect of LEPR genotype on growth traits

In this study, analysis of exon 19 in LEPR
(Figure-4) revealed two missense mutations, the first
of which (c.2800G>A) resulted in the amino acid
substitution Val934Met, and the second of which
(c.2978C>G) resulted in the amino acid substitution
Ser993Cys. Tables-5 and 6 show significant associa-
tions of the variation in LEPR gene with growth speed
after weaning (ADG2 and MW). There is a lack of
reports on studies concerning the effect of the LEPR
gene on growth traits in sheep in the literature; how-
ever, many previous reports have demonstrated associa-
tions between genetic variation in LEPR and other
economically important traits. A previous study [15]
sequenced a region covering exon 2 to exon 16 of the
ovine LEPR gene using complementary DNA and
identified two SNPs in exon 2 (T240C and T279C),
one SNP (A16830G) in exon 10, and one SNP
(C2373T) in exon 14. The SNP in exons 2 tended to
associate with feed intake during gestation (p=0.087),
whereas the SNP in exon 2 significantly (p=0.0229)
associated with residual feed intake during lactation.

Another study [16] detected three different muta-
tions in the coding region of the LEPR gene: SNP A
(chr1: 40778726C>T), which resulted in the amino
acid change Arg62Cys; SNP B (chr1: 40857869C>T),
which resulted in the amino acid change Pro1019Ser;
and SNP C (chr1: 40858019A>G), and which resulted
in the amino acid change Lys1069Gln, in Davisdal
ewe. In Indonesian breeds of sheep (fat-tailed sheep,
thin-tailed sheep, and Garut composite sheep), a novel
SNP in the genomic region (g.40854778A>C) of the
LEPR gene was also identified and found to be signifi-
cantly (p<0.05) associated with SFA (including trico-
sanoic acid [C23:0] and tetracosanoic acid [C24:0])
and polyunsaturated fatty acid (docosahexaenoic acid
(C22:6n3)] [17].

Moreover, in cattle, variation in the LEPR gene
was confirmed to have associations with body weight
and ADG at 6 and 12 months of age in Nanyang
calves [18]. In humans, several studies found associ-
ations of the polymorphisms of Lys109Arg in exon 4,
Gln223Arg in exon 6, and Lys656Asn in exon 14 of
the LEPR gene with body weight and body gain. The
LEPR Arg109Lys and Arg223Gln polymorphisms
were shown to be positively associated with weight

**Table-6**: Association of the absence/presence of leptin receptor variants with growth traits in Barki lambs.

| Trait       | Variant being assessed | N     | Absent variant | LSM±SE | Present variant | N     | LSM±SE | Significance |
|-------------|------------------------|-------|----------------|-------|----------------|-------|-------|--------------|
| BW          | L1                     | 32    | 3.59±0.10      | 215   | 3.51±0.04      | 0.209 |
|             | L2                     | 134   | 3.49±0.05      | 113   | 3.55±0.06      | 0.230 |
| ADG1        | L1                     | 32    | 179.75±5.03    | 215   | 175.42±2.42   | 0.507 |
|             | L2                     | 134   | 174.65±3.11    | 113   | 177.56±3.10   | 0.547 |
| WW          | L1                     | 32    | 20.25±0.50     | 215   | 19.77±0.25    | 0.454 |
|             | L2                     | 134   | 19.67±0.32     | 113   | 20.02±0.32    | 0.479 |
| ADG2        | L1                     | 32    | 100.08±2.96    | 215   | 84.22±1.61    | 0.003 **|
|             | L2                     | 134   | 84.14±2.03     | 113   | 88.81±2.18    | 0.156 |
| MW          | L1                     | 32    | 47.56±1.11     | 215   | 42.73±0.53    | 0.005 **|
|             | L2                     | 134   | 42.61±0.69     | 113   | 44.24±0.70    | 0.134 |

BW=Birth weight, ADG1=Pre-weaning daily gain, WW=Weaning weight, ADG2=Post-weaning daily gain, MW=Marketing weight. **Refers to significance at (p<0.01)

**Table-7**: Genetic effects of the ovine leptin receptor gene on growth traits in Barki lambs.

| Trait       | Genetic effect | Additive p-value | Dominance p-value |
|-------------|----------------|------------------|-------------------|
| BW          |                | 0.051±0.052      | 0.324             |
|             |                | 0.028±0.079      | 0.722             |
| ADG1        | 2.415±3.110    | 0.438            | 1.063±4.697       | 0.821 |
| WW          | 0.281±0.317    | 0.376            | 1.49±0.480        | 0.756 |
| ADG2        | 5.878±2.076    | 0.005**          | -2.854±3.177      | 0.370 |
| MW          | 1.889±0.688    | 0.006**          | -0.637±1.053      | 0.546 |

BW=Birth weight, ADG1=Pre-weaning daily gain, WW=Weaning weight, ADG2=Post-weaning daily gain, MW=Marketing weight. **Refers to significance at (p<0.01)
gain in Dutch adults [19]. The weight-gainers with the Arg109 or Arg223 allele had higher leptin levels than those not carrying these alleles.

The effect of the LEPR genotype on growth traits after weaning might be due to the critical roles played by LEPR in the control of glucose metabolism and energy balance [20]. Energy balance has associations with whole-body mass and skeletal muscle mass through affecting the metabolism of whole-body protein as well as skeletal muscle protein. A negative energy balance causes decreases in skeletal muscle mass as a result of imbalanced rates of muscle protein synthesis and degradation. The previous work showed that the effect of energy deficits resulted in a 5-10% loss in initial body mass and a 19% decrease in the synthesis of skeletal muscle protein [21].

LEPRs are present on β cells as well as on muscle and fat cells, enabling leptin to modulate the secretion and action of insulin [22]. Insulin causes weight gain when the cells absorb too much glucose and the body converts this into fat [23]. Despite sheep being more tolerant of insulin than non-ruminants, insulin treatments have been reported to increase weight gain and fat deposition in sheep [24].

The effect of LEPR variation on growth traits after weaning might also be explained by other phenomena. Markedly, the L2 variant, which is associated with higher ADG2 and heavier MW, carries the nucleotide substitutions c.2800G>A and c.2978C>G, which would lead to putative amino acid substitutions of valine to methionine and serine to cysteine, respectively. Furthermore, the L2 variant has a higher degree of additive effects because the L2L2 homozygote was found to have a higher value of additive effect than the heterozygote L1L2, which, in turn, had a higher value than the L1L1 homozygote. These findings might be due to the amino acid substitutions. The amino acid point mutations might change the structure of LEPR and thus its function, such as the affinity of binding to LEPR, the expression level of the protein, or the protein’s durability [25]. These effects could lead to variation in growth traits after weaning. Against this background, further studies are needed to verify the binding affinity of the mutants to LEPR as well as to determine the expression level of the LEPR.

Conclusion

This study revealed associations of variation in the LEPR gene with ADG2 and MW. The detected variant might be used in breeding programs to produce animals with superior growth traits after weaning in Barki sheep in Egypt and elsewhere.

Authors' Contributions

AHMI conceived and designed the research, conducted the sample collection, processed samples in the Molecular Laboratory, carried out the data analysis and writing of the manuscript, edited the manuscript and approved the submitted version of the manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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