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# Correlation analysis between polymorphism of leptin and IGFI genes and body measurements in Barki and Farafra sheep

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## Abstract

**Background** Growth performance, which can be evaluated using various body measurements, is crucial for providing red meat for human consumption. This study aims to improve the growth performance of sheep by identifying the different genotypes of leptin and IGFI genes associated with higher body measurements.

**Methods** In this study, we collected blood samples from Barki ( $n=30$ ) and Farafra ( $n=30$ ) sheep, and measured their body height, width, length, and weight, and tail width and length to assess their growth performance. We used single strand conformation polymorphism polymerase chain reaction (SSCP-PCR) and sequencing analysis to identify single nucleotide polymorphisms (SNPs) and different genotypes of the leptin and insulin-like growth factor receptor (IGFI) genes associated with sheep body measurements.

**Results** The leptin gene (209 bp) and IGFR gene (345 bp) were found to have three (TT, TG, and GG) and (TT, TC, and CC) genotypes, respectively, by SSCP-PCR. Sequence analysis revealed a SNP in the leptin gene (200G>T) with two alleles (G allele: OR058867 and T allele: OR058868). This SNP resulted in the substitution of the amino acid valine with leucine, which increased the width, length, and weight of the body and tail width in the heterozygous form (TG). The IGFI gene had three SNPs: 305 G>T in TT genotype (OR260670), 184C>T, 265T>C, and 305G>T in TC genotype (OR260669), and 265T>C in CC genotype (OR260671). The TC genotype of the IGFR gene was correlated with higher values of body measurements. Barki sheep were found to have the highest values for body weight, width, length, and tail width compared to Farafra sheep.

**Conclusion** We recommend the use of entire Barki sheep in inbreeding programs to improve growth performance in Egyptian sheep.

**Keywords** Polymorphism, Leptin, IGFI, Body measurements, Sheep

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## 1 Background

Sheep are a vital source of meat and its by-products in dry areas. They provide a significant income for farmers and contribute 6.4% to the total red meat production in the Egyptian economy [1]. The most prevalent domestic breeds in Egypt include Al-Osaimi, Farafra, Al-Barky, and Al-Rahmani [2]. Barki sheep, raised under a transhumant system, graze on vegetation available from August to June and are well-suited to the harsh desert environment, including feed scarcity and high temperatures. Despite these conditions, they produce substantial amounts of meat, wool, and milk [3]. Farafra sheep, a native fat-tailed breed, are prevalent in the El-Farafra Oasis in the Egyptian western desert and have been kept under intensive management at the Mallawi Animal Production Research Station since 1992 [4]. Body weight and size can serve as indicators for future breeding and reproductive success in animals [5]. Several factors contribute to the significant variation in these measurements among sheep herds, including genetics, sex, birth type, and environmental conditions [6]. Recently, researchers have focused on specific body measurements that indicate an animal is well-fed and has a high meat yield, due to their economic importance [7]. Researchers have used molecular markers to study the association between candidate genes and performance traits in various sheep breeds, including milk traits in Sfakia sheep [8], carcass traits in Iranian Mehraban sheep [9], and tail types in Hu, Tibetan, and Dorper-Hu hybrid sheep [10]. Key candidate genes associated with milk and growth traits include PRL, LEP, IGF1, DGAT1, STAT5, CSN1S2, GHR, and GHRHR [11, 12].

The LEP gene, discovered in 1994 by Zhang [13], produces a protein composing 167 amino acids. This protein plays a role in growth, energy reserves, metabolism, immunity, reproduction, and regulating feed intake and body weight. It is a strong candidate gene for economically important traits in livestock [14]. Leptin plays a crucial role in regulating appetite and can affect factors related to eating, such as body weight and fat storage. Variations in the LEP gene have been linked to growth, body measurements, and carcass characteristics in various sheep breeds [15]. Polymorphisms in the LEP gene have also been shown to have additive effects on the weights and yields of primal cuts and carcasses [16].

IGF1 mediates many biological effects, including glucose absorption, myogenesis stimulation, apoptosis inhibition, cell cycle gene activation, lipid synthesis, progesterone production in granular cells, and DNA, protein, RNA synthesis and cell proliferation [17]. IGF1, a member of the IGF family (GHRH, GH, GHRHR, GHR, IGF-IR and IGF-IIR), is involved in processes such as reproduction, embryonic development, metabolism, cell

differentiation, protein synthesis, skeletal growth, and fetal growth [18], also it was considered as a functional gene for a number of production traits such as meat and carcass quality traits, and growth in livestock. Variations in the IGF1 gene have been linked to growth traits in sheep [19]. Specific variants of the IGF1 gene have been associated with internal carcass length, rib yield, and neck weight in Santa Inês sheep. Additionally, a variant in the IGF1 gene has been linked to the weights and yields of primal cuts such as rib, loin, leg, and neck [20]. It has been shown that IGF1 gene polymorphisms had significant effects on many growth traits in Makooei and Balushi sheep [21, 22]. Hajhousseinlou et al. [23] studied the relationship between IGF1 gene SNPs and several body traits in sheep. To our knowledge, this is the first study focusing on genotypes of leptin and IGF1 genes associated with body measurements in Barki and Farafra sheep. This study examines mutations of the leptin and IGF1 genes and their association with body measurements in Barki and Farafra sheep using SSCP-PCR, sequencing analysis, and appropriate statistical analysis.

## 2 Methods

### 2.1 Animal and blood samples

For the association analysis, 60 sheep were used as the experimental population, including 30 Barki and 30 Farafra sheep from the same farm. Blood samples, approximately 6 ml from vein of each animal, were taken by sterilized syringe and collected in tube containing EDTA. All samples were transferred into lab using ice-box and stored at  $-20^{\circ}\text{C}$  until DNA extraction.

### 2.2 Body measurements

A calibrated electronic scale was used to measure the body of Barki and Farafra sheep. The same person used a clearly graduated leather ruler to measure the height and length of body as well as the length and width of tail. The all measurements were recorded and statistically analysed.

### 2.3 DNA extraction and PCR conditions

We used the Genomic DNA Kit (Geneaid, cat. No. GB 100) to extract DNA from whole blood samples, following the manufacturer's instructions. We then measured the quantity and quality of the DNA using a NanoDrop 2000 spectrophotometer (Thermo, Waltham, MA, USA).

For the PCR reaction, we mixed 12.5  $\mu\text{L}$  Master Mix, 1  $\mu\text{L}$  of Primer-F, 1  $\mu\text{L}$  of Primer-R, 4  $\mu\text{L}$  of DNA, and 6.5  $\mu\text{L}$  of ddH<sub>2</sub>O in a 25  $\mu\text{L}$  reaction mixture. The primer sequence and PCR reaction were summarized in Table 1. We examined the PCR products on a 1% agarose gel.

**Table 1** Primer sequence of leptin and IGFI genes

Name	sequences	Accession number	Annea	Size	PCR condition
Leptin	5'-GCTCCACCCTCT CCTGAGTTTGTC-3' 5'-TGTCCTGTAGAG ACCCCTGTAGCCG-3'	KJ918739.1	65/30 s	290bp	1 cycle at 94 °C/3 min., 35 cycles at 95 °C/30 s., 72 °C/ 30 s., and final extension at 72 °C/ 10 min
IGFI	5'-ACTGACCTCATG CGCATGTGCTGG-3' 5'-CTCGTTCTTGCG GCCCCGTTTCAT-3'	XM_027957015.2	52/30 sec	345bp	

## 2.4 Genotyping of leptin and IGFI genes and SNPs detection

We used the SSCP-PCR technique to study the genetic polymorphism of leptin and IGFI genes based on previous study for Darwish et al. [24]. A 5 µL of each PCR product was mixed with 45 µL of loading dye (98% Formamide, 10 mM EDTA, 0.025% bromophenol blue, and 0.025% xylene-cyanol). After denaturation at 95 °C for 10 min, the samples were cooled on ice and loaded on 29% acrylamide:bisacrylamide gels. The gels were stained in a solution of 10% ethanol, 0.5% acetic acid, and 0.2% ethidium bromide for 10 min. Finally, the gels were washed with distilled water, and then photographed and analysed by gel documentation. MacroGen Co., Ltd. (Soul, Korea) sequenced the PCR products. We then compared the sequences using DNAMAN 6.0 software (<https://www.lynnon.com>) and Chromas 2 software (<http://technelysium.com.au/wp/chromas>) to identify any potential SNPs. These SNPs had been recorded at GenBank.

## 2.5 Statistical analysis

We calculated allele frequencies, polymorphic information content (PIC), heterozygosity (He), and the effective number of alleles (Ne) using Gene Calc, Copyright © 2023 according to Szymon and Jan [25]. We tested for Hardy–Weinberg equilibrium at each site using a chi-square test. We also used SPSS software version 18 to analyze the association between SNP genotypes and/or haplotypes and body measurements, using the following animal model:

$$y_i = \mu + G_i + b_i + e_i.$$

In this model:

$y_i$  represents the trait measured in an individual.  $\mu$  is the overall mean.  $G_i$  is a fixed effect corresponding to the genotype of polymorphisms.  $b_i$  is the breed effect.  $e_i$  is a random residual effect.

## 3 Results

### 3.1 Detection of leptin and IGFI genes and genotypes

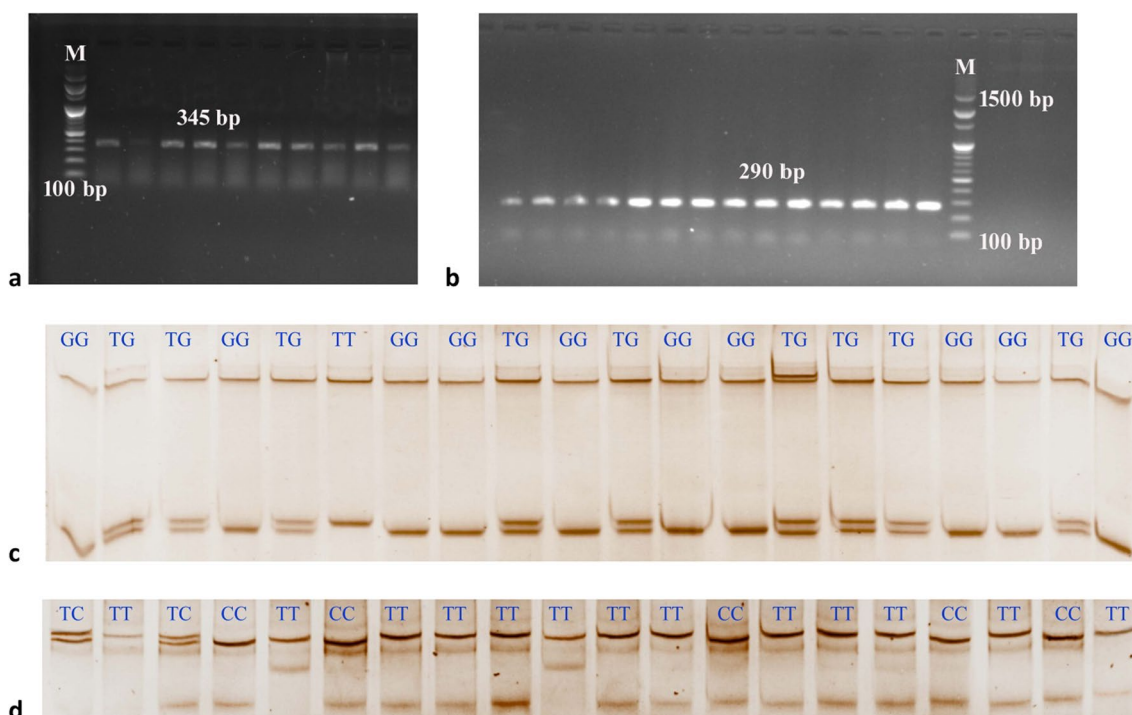
PCR detected the Leptin and Insulin-like Growth Factor 1 (IGFI) genes at 290 bp and 345 bp, respectively (Fig. 1a and b). SSCP-PCR revealed three genotypes for each gene. In Barki sheep, the leptin gene had TT (frequency 6), TG (frequency 17), and GG (frequency 7) genotypes. In Farafra sheep, the frequencies were 3, 11, and 16, respectively (Fig. 1c). The IGFI gene had TT (frequency 19), TC (frequency 2), and CC (frequency 9) genotypes in Barki sheep. In Farafra sheep, the frequencies were 19, 4, and 7, respectively (Fig. 1d).

### 3.2 SNP Detection

Sequencing results revealed one SNP (200G > T) in the leptin gene with two alleles (T and G) as shown in Fig. 2. This SNP was genotyped and classified into TT, TG, and GG genotypes in an experimental population of 30 Barki sheep and 30 Farafra sheep (Fig. 3). The IGFI gene had three SNPs (184 C > T, 265 T > C, and 305 G > T) which were genotyped and classified into three genotypes in the same experimental population. These SNPs were recorded at GenBank under accession numbers: OR058867 for G allele and OR058868 for T allele of leptin gene. While IGFI gene had the following accession numbers: OR260670 for TT genotype, OR260669 for TC genotype, and OR260671 for CC genotype (Fig. 3a, b, and c).

### 3.3 Amino acids changes

In the leptin gene, a point mutation of the substitution type occurred at base 198G > T, resulting in a change of the amino acid valine to leucine, as shown in Fig. 4. All mutations (186 C > T, 266 T > C, and 306 G > T) in the IGFI gene occurred in the intron region.



**Fig. 1** a show detection of leptin gene at 290 by PCR, b show detection IGFI gene 345bp by PCR, c different genotypes of leptin gene in Barki and Farafra sheep by SSCP-PCR, and d different genotypes of IGFI gene in Barki and Farafra sheep by SSCP-PCR

### 3.4 Genetic parameters calculation

Table 2 displays the results of the genetic parameters calculation. All leptin gene sites were in Hardy–Weinberg equilibrium ( $p > 0.05$ ). The OR058868 G > T site had moderate PIC status ( $0.25 < PIC < 0.5$ ) in both Barki and Farafra sheep, with He and Ne values of 0.42–0.48 and 1.72–1.96, respectively. This site was highly variable and its alleles were evenly distributed. Similarly, all IGFI gene sites were in Hardy–Weinberg equilibrium ( $p < 0.05$ ). The OR260670 C > T site had moderate PIC status ( $0.25 < PIC < 0.5$ ) in both Barki and Farafra sheep, with He values of 0.42–0.46 and Ne values of 1.81 in Barki sheep and 1.96 in Farafra sheep. This site was also highly variable and evenly distributed, as shown in Table 3.

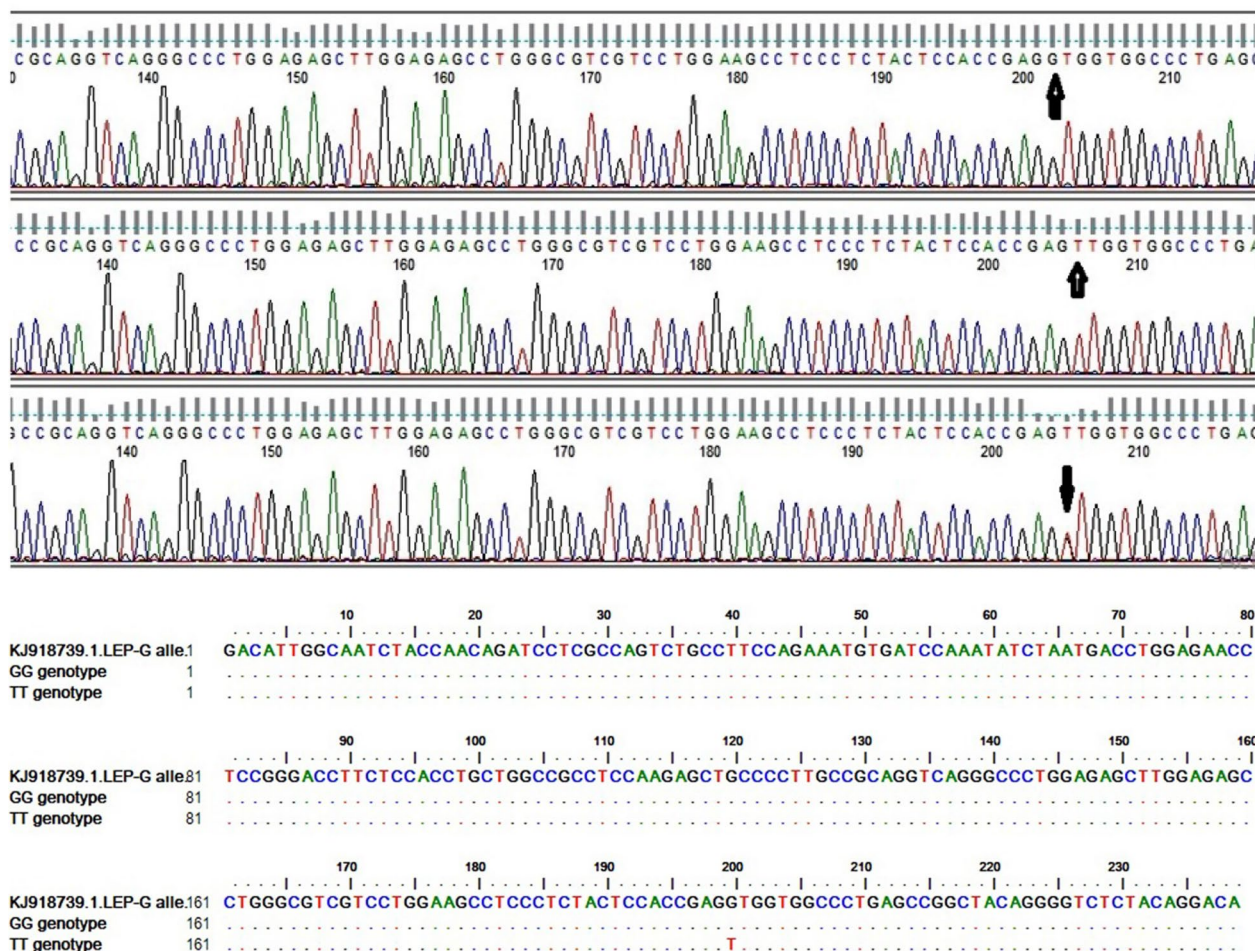
### 3.5 Effect of leptin genotypes, breed type, and interaction between breed and genotypes on body measurements

Barki sheep had better body measurements than Farafra sheep, with significantly higher body width ( $18.2 \pm 0.6$ ), tail width ( $15.1 \pm 0.5$ ), and tail length ( $29.5 \pm 0.5$ ) compared to Farafra sheep ( $8.9 \pm 0.7$ ,  $8.7 \pm 0.6$ , and  $25.6 \pm 0.6$ ), respectively. However, animal height was significantly higher in Farafra sheep compared to Barki sheep (Table 4). Regarding the interaction between leptin genotypes and breed type, the TG genotype in Barki sheep

had the highest significant value for animal height and body length traits, while no significant differences were observed in other traits between Barki and Farafra sheep (Table 4). The animal height trait was correlated with the GG genotype while body width, body length, tail width, and body weight traits were correlated with the TG genotype (Table 6).

### 3.6 Effect of IGFR genotypes, breed type, and interaction between breed and genotypes on body measurements

Body width, body length, and tail width traits were significantly higher in Barki sheep compared to Farafra sheep. Tail length and body weight traits were also better in Barki sheep than Farafra sheep. However, animal height was highest in Farafra sheep compared to Barki sheep (Table 5). Regarding the interaction between IGFI genotypes and breed type, the TC genotype in Barki sheep had the highest significant value for body width, body length, and tail width traits while the animal height trait was correlated with the TT genotype. No significant differences were observed in other traits between Barki and Farafra sheep (Table 5). Sheep with the TC genotype had significantly taller body length than TT and CC genotype carriers. The highest values of animal height, body width, tail width, and body weight were also correlated with the TC genotype (Table 6).



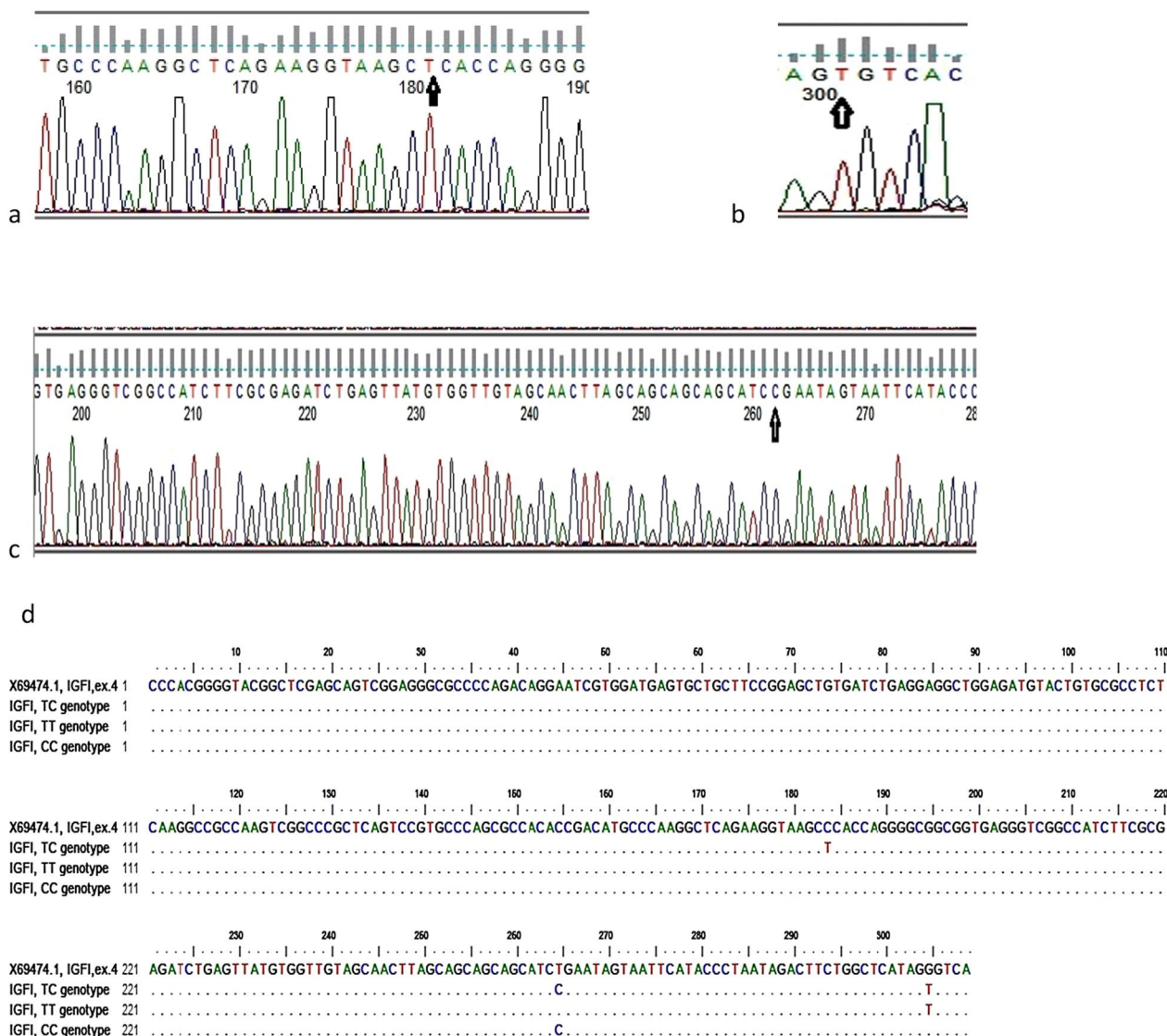
**Fig. 2** Alignment of leptin gene fragment with sequence of leptin gene deposited in gene bank under accession number KJ918739.1 by Bioedit software, also it shows SNP of leptin gene (198G>T) by sequence analysis

### 4 Discussion

The effectiveness of using markers to select desirable traits in animals for sale relies on several factors. These include finding markers that are closely linked to the genes responsible for the traits, verifying that variations in these genes are present in the population, and understanding how these genes interact with other genes or environmental factors that affect economically important traits. This must be done in a cost-effective and efficient manner for the technology to be adopted by livestock industries [26]. These genes can be closely linked to genetic markers and can be inherited together. A good genetic marker should have multiple forms, be inherited in a simple manner, and be easy to detect [27]. There are two ways to study the genes responsible for variations in phenotypic characteristics. The first is to start with the phenotypic characteristic and work toward the genome, using association mapping based on linkage disequilibrium or by targeting specific candidate genes based on

their similarity to known genes. The second is to start with the genome and work toward the phenotypic characteristic, using statistical analysis of genomic data to identify likely targets of past selection through selective sweep analysis [28, 29].

Fat tail breeds make up approximately 25% of the world’s sheep population and are grazed in many countries, particularly in Asia and northern Africa. Sheep breeding plays a vital role as a source of protein through increasing body weight of sheep, thus improving the local economy in these countries. The variants of genes associated with fat tail trait affecting the phenotypic expression are of both theoretical and economic interest. The fat tail is an important trait for coping with environmental hazards and serves as a valuable energy reserve for animals during migration and winter. However, the desire of producers and consumers for fat-tailed sheep decreased in the intensive and non-intensive systems. As a result in the industrialized country, fat tail size reduction could be



**Fig. 3** **a** show SNPs of IGFI gene at position 184 C>T in TT genotype, **b** show SNP of IGFI gene at position 305C>T in TC, and **c** show SNP of IGFI at position 265T>C in CC genotype by sequence analysis, and **d** show alignment of IGFI gene fragment with sequence of IGFI deposited in gene bank under accession number X69474.1 by Bioedit software

a goal in sheep. In sheep breeding, tail type traits have a significant impact on performance [11]. Due to the presence of fat in the tail, it has additional value to humans because it provides high-energy food during times of drought and famine. Nowadays, the requirements for high-quality meat consumption have increased, and the basic points for determining meat quality are the distribution and fat content of the animal carcass [30]. Behind, the fats found in sheep tails are considered healthy for humans as they are unsaturated fats and they also reduce cooking time [31], and the current study showed that there is a positive relationship between fat tail and high body weight, which may change this tendency for

producers and consumers. In many sheep studies, including those on growth, inheritance, and nutrition, measurements of size and body shape are important [11]. For species raised for meat, body shape and growth rate are important selection criteria. The average weight of the fat tail was 1.03 kg, and the ratio of tail fat to carcass varied widely [32]. This could be an indicator of biological diversity in this breed and could be used to select lambs with smaller fat tails. Compared to Turkish Awassi sheep, where the average tail fat weight was 3 kg and accounted for 15% of the carcass weight [33], as well as compared to the Lori, Mehraban, and Ghezeli breeds of Iran [34], the fat tail of the Makoei breed was relatively light.

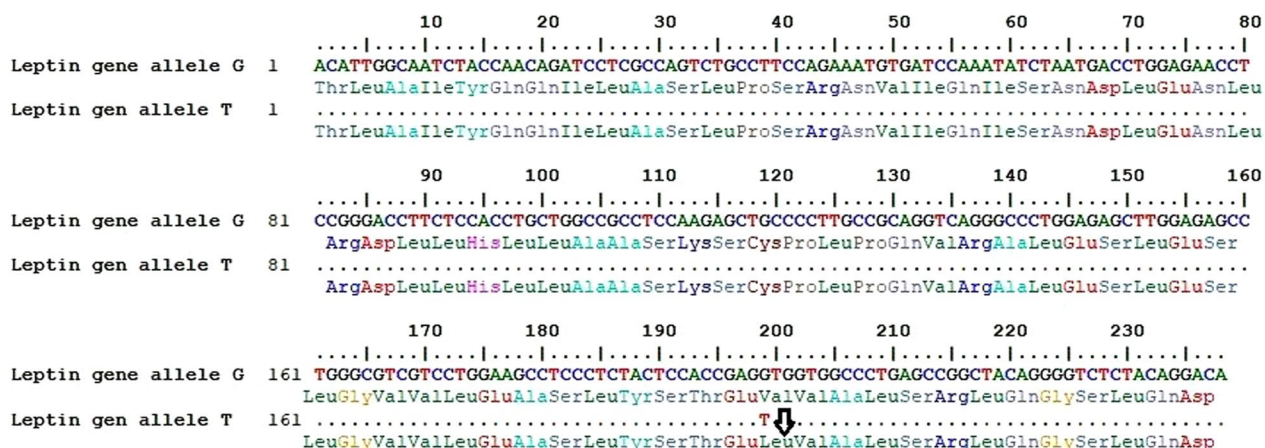


Fig. 4 Show amino acids changes (valine to leucine) in leptin gene

Table 2 Genetic parameters calculation of G > T of leptin gene

	Breed	Genotype	Geno. freq	Allele	Allele freq	He	Ho	Ne	PIC	Hardy	Chi. Sq						
Leptin	Barki	TT(6)	0.2	T	0.48	0.49	0.51	1.96	0.37	0.76	0.54347						
		TG(17)	0.57	G	0.52												
		GG(7)	0.23														
	Farafra	TT(3)	0.1	T	0.3							0.42	0.58	1.72	0.33	0.86	0.28
		TG(11)	0.37	G	0.7												
		GG(16)	0.53														

Geno. Freq.: genotype frequency, Allele Freq.: allele frequency, He: heterozygosity, Ho: homozygosity, Ne: effective number of allele, PIC: polymorphic information content

Table 3 Genetic parameters calculation of C > T of IGFI gene

Gene	Breed	Geno	Geno. freq	Allele	Allele freq	He	Ho	Ne	PIC	Hardy	Chi. Sq						
IGFI	Barki	TT(19)	0.3	T	0.67	0.46	0.54	1.81	0.36	0.0002	21.67						
		TC(2)	0.06	C	0.33												
		CC(9)	0.63														
	Farafra	TT(19)	0.4	T	0.7							0.42	0.58	1.96	0.33	0.001	13.9
		TC(4)	0.13	C	0.3												
		CC(7)	0.47														

Geno. freq.: genotype frequency, Allele freq.: allele frequency, He: heterozygosity, Ho: homozygosity, Ne: effective number of allele, PIC: polymorphic information content

Indeed, information on the relationship between genetic polymorphisms and tail fat measurements in sheep is scarce. Most investigations have focused on the correlation of carcass traits with genetic polymorphisms of related genes. In fact, there are few reports were done on association of fat tail traits with presence of SNPs in the IGF-1 and PIT-1 genes of sheep. Recently, few polymorphisms of the IGF-1 and PIT-1 genes have been detected in sheep compared to other small ruminants. Little information is available to compare the

different Iranian sheep breeds. This is the first study that attempts to reveal the relationship between allele variation in sheep IGF-1 and leptin genes and body measurements associated with tail traits in Egyptian sheep. The current study found that the TC genotype of the IGFI gene in Barki sheep had the highest significant value for body width, body length, and tail width traits while the animal height trait was correlated with the TT genotype. Sheep with the TC genotype of the IGFI gene had significantly taller body length than TT and CC genotype

**Table 4** Effect of breed type and interaction between breed type and genotypes of leptin gene on body measurements

Breed		Geno	Animal height	Body width	Body length	Tail width	Tail length	Body weight
Barki	Br.*Ge. effect	TT	33.16 ± 1.8 <sup>b</sup>	17.3 ± 1.3	43.8 ± 2.5 <sup>b</sup>	14 ± 0.9	29.7 ± 1.1	32 ± 0.9
		TG	39.5 ± 1.1 <sup>a</sup>	18.9 ± 0.7	49.6 ± 1.5 <sup>a</sup>	16.1 ± 0.6	28.8 ± 0.7	33.1 ± 0.6
		GG	38.4 ± 1.7 <sup>a</sup>	18.2 ± 1.1	46.7 ± 2.3 <sup>ab</sup>	15.3 ± 0.9	30 ± 1	33.4 ± 0.9
	Br. effect	Barki	37 ± 0.9	18.2 ± 0.6*	46.7 ± 1.3	15.1 ± 0.5*	29.5 ± 0.5*	32.8 ± 0.5
		Farafra	51 ± 1.1*	8.9 ± 0.7	47.9 ± 1.4	8.7 ± 0.6	25.6 ± 0.6	32.6 ± 0.5
Farafra	Br.*Ge. effect	TT	50.6 ± 2.6	8.6 ± 1.8	47.7 ± 3.6	8.7 ± 1.4	26.7 ± 1.6	32 ± 1.3
		TG	50.4 ± 1.3	8.7 ± 0.9	47.4 ± 1.8	8.5 ± 0.7	24.3 ± 0.8	31 ± 0.7
		GG	51.8 ± 1.1	9.4 ± 0.8	48.7 ± 1.6	8.8 ± 0.6	25.9 ± 0.7	32.8 ± 0.6

Br.\*Ge. Effect: breed and genotype interaction effect, Geno.: genotype, all value within table were expressed as mean ± S.E., <sup>a,b,c</sup> alphabetic indicates to significant between means of three leptin genotypes at  $p < 0.05$ , and \* indicates to significant between means of two breeds at  $p < 0.05$

**Table 5** Effect of breed type and interaction between breed type and genotypes of IGFI gene on body measurements

Breed		Geno	Animal height	Body width	Body length	Tail width	Tail length	Body weight
Barki	Br.*Ge effect	TT	39.1 ± 1.1 <sup>a</sup>	18.5 ± 0.7 <sup>b</sup>	48.3 ± 1.3 <sup>b</sup>	15.7 ± 0.5 <sup>ab</sup>	29.3 ± 0.6	32.8 ± 0.5
		TC	37.5 ± 3.4 <sup>ab</sup>	21.5 ± 2.2 <sup>a</sup>	59.5 ± 4.1 <sup>a</sup>	18 ± 1.6 <sup>a</sup>	28.5 ± 1.9	34 ± 1.6
		CC	35.9 ± 1.6 <sup>b</sup>	17.5 ± 1 <sup>b</sup>	44.1 ± 1.9 <sup>c</sup>	14.3 ± 0.7 <sup>b</sup>	29.4 ± 0.9	32.9 ± 0.7
Farafra	Br. effect	Barki	37.5 ± 1.3	19.2 ± 0.8*	50.6 ± 1.6	16 ± 0.6*	29 ± 0.7*	33.3 ± 0.6*
		Farafra	51.5 ± 1.1*	9.3 ± 0.7	48.5 ± 1.3	8.9 ± 0.5	25.3 ± 0.6	31 ± 0.8
	Br.*Ge effect	TT	50.7 ± 1.1	8.8 ± 0.7	48.1 ± 1.4	8.4 ± 0.6	25.7 ± 0.7	32 ± 1.3
		TC	53.7 ± 2.4	9.5 ± 1.5	49.5 ± 2.9	9.5 ± 1.2	26.3 ± 1.4	31.7 ± 0.5
		CC	50.1 ± 1.8	9.6 ± 1.2	48.1 ± 2.2	8.9 ± 0.9	23.8 ± 1	32.8 ± 1.1

Br.\*Ge. Effect: breed and genotype interaction effect, Geno.: genotype, all value within table were expressed as mean ± S.E., <sup>a,b,c</sup> alphabetic indicates to significant between means of three IGFI genotypes at  $p < 0.05$ , and \* indicates to significant between means of two breeds at  $p < 0.05$

**Table 6** effect of leptin and IGFRI genotypes on body measurements in sheep

	Animal height	Body width	Body length	Tail width	Tail length	Body weight
<i>Genotypes (Leptin)</i>						
TT	39 ± 1.6 <sup>c</sup>	14.4 ± 1.1 <sup>ab</sup>	45.1 ± 2.2 <sup>b</sup>	12.2 ± 0.8 <sup>ab</sup>	28.6 ± 0.9	32 ± 0.8
TG	43.8 ± 0.9 <sup>b</sup>	14.9 ± 0.6 <sup>a</sup>	48.7 ± 1.2 <sup>a</sup>	13.1 ± 0.5 <sup>a</sup>	27 ± 0.5	32.3 ± 0.4
GG	47.7 ± 1 <sup>a</sup>	12.1 ± 0.7 <sup>b</sup>	48.1 ± 1.4 <sup>a</sup>	10.7 ± 0.5 <sup>b</sup>	27.1 ± 0.6	32.2 ± 0.5
<i>IGFI</i>						
TT	44.9 ± 0.8	13.7 ± 0.5	48.2 ± 0.9 <sup>b</sup>	12.1 ± 0.4	27.5 ± 0.5	32.3 ± 0.4
TC	45.6 ± 2.1	15.5 ± 1.3	54.5 ± 2.5 <sup>a</sup>	13.8 ± 1	27.4 ± 1.2	33.4 ± 0.9
CC	43 ± 1.2	13.5 ± 0.8	46.1 ± 1.5 <sup>b</sup>	11.6 ± 0.6	26.7 ± 0.7	31.9 ± 0.6

All value within table were expressed as mean ± S.E., <sup>a,b,c</sup> alphabetic indicates to significant between means at  $p < 0.05$

carriers. The highest values of animal height, body width, tail width, and body weight were also correlated with the TC genotype. There significant correlations were found for all fat tail measurement traits with genotypes of the IGF-1 and PIT-1 genes in the Makooei sheep. Sheep with

longer, wider tails have the P3 genotype of the PIT-1 gene while sheep with thicker tails have the P4 genotype [35]. In Lori Bakhtiari and Zil breeds, the exon 17 region of the DGAT1 gene was associated with carcass weight and dressing percentage in sheep [36]. A significant



association was also found in Makooei sheep between various transferrin proteins genotypes and fat tail weight, hot carcass weight, and hot carcass weight free of fat tail [37]. A nearly significant relationship was reported between band patterns and tail length and tail down circumference in the fourth exon of the growth hormone gene in Kermanian sheep [12]. Yakar et al. [38] showed that IGF-1 plays an important role in skeletal development, while Sun et al. [39] found a connection between IGF1 gene expression and body weight in Hu sheep. The IGF1 gene is crucial for animal growth, including cell proliferation, maintenance of skeletal muscle satellite cells for regeneration, and muscle hypertrophy [40]. An association between BL, TW and body weight traits in Balami sheep and WH traits in Yankasa sheep with IGF1 gene was detected by PCR-RFLP [41]. A PCR-SSCP in exon 1 was linked to ADG and BL in Makui sheep [42], and ADG in both Baluchi [43] and Makooei [44] sheep breeds. The IGF-I AA genotype was found with a frequency of 83.3% in Colored Polish Merino sheep, had significant effects on meat color, outer carcass fat, and drip loss in the study by Grochowska et al. [45], and were associated with longissimus dorsi, width and flavor of muscle. Leptin helps regulate the balance between food intake and energy expenditure by sending signals to the hypothalamus [46]. Due to its ability to break down fat and regulate fat stores, genetic variations in LEP may be important in mobilizing lipids for milk production, with potential implications for the body weight of offspring in sheep. In line with this, genetic variations in leptin have been shown to affect milk performance in cattle [47]. The LEP gene is considered a candidate gene that affects body fat content [48]. A LEP polymorphism was also found to be significantly associated with milk yield in Najdi ewes from Saudi Arabia [49]. Several studies have highlighted the role of LEP in growth traits in sheep [50]. Statistical analysis results of recent study showed a significant relationship between Leptin gene (exon 3) and tail, chest, abdomen, and neck circumference as well as backfat thickness. This study revealed new associations where the C allele had a positive effect on fat tail weight and back fat thickness in fat-tailed sheep [51]. The SNP of leptin gene in position A113G was associated with an increase in fat-tail percent, carcass weight, and total body fat weight in the Shal sheep breed while it was associated with reduction in carcass weight, lean meat weight, and slaughter weight, and an increase in fat-tail percent [52]. The polymorphism of Leptin gene at position 332G>A had significant effect on body length. This non-synonymous mutation caused change in amino acid from Arg to Gln [15]. The present study found that the TG genotype of the Leptin gene in Barki sheep had the highest significant value for animal height and body length traits, while

no significant differences were observed in other traits between Barki and Farafra sheep. The animal height trait was correlated with the GG genotype while body width, body length, tail width, and body weight traits were correlated with the TG genotype. There are haplotypes in IGF1 and LEP genes associated with body traits in Santa Inês sheep [53]. Therefore, this study concluded that SNPs in the leptin and IGFI genes could be useful for improving growth performance in sheep.

## 5 Conclusion

The present study found that the leptin and IGFI genes had these genotypes: TT, TG, and GG for leptin, and TT, TC, and CC for IGFI. Sequence analysis of the leptin gene revealed two alleles with one SNP, resulting in the substitution of valine with leucine. This increased body and tail width, length, and weight in the TG heterozygous form. The IGFI gene had three SNPs in intron 1 region, with the TC genotype correlating with higher body measurements. Barki sheep had higher values for body weight, width, length, and tail width compared to Farafra sheep. These results are important in breeding programs to improve body measurements in Egyptian sheep.

### Abbreviations

SSCP-PCR	Single strand conformation polymorphism polymerase chain reaction
SNP	Single nucleotide polymorphisms

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### Authors' contribution

This study was done in collaboration with all authors. AD designed this study. AD, MAA, and ZA participated in the conduct of the study. AD, AA and SO analyzed the data. AD drafted the manuscript. AD and ZA critically revised the manuscript. All authors read and approved the final manuscript.

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### Availability of data and materials

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

### Declarations

#### Ethics approval and consent to participate

The experimental procedure used in this investigation was approved by the Animal Care and Use Committee of National Research Centre in Egypt

#### Consent for publication

Not applicable.

#### Competing interest

The authors declare that they have no conflict of interest.

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