

***PROPI* Gene polymorphisms associated with growth traits in local Iraqi sheep**

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Abstract

The *PROPI* gene is considered an important candidate gene due to its role in growth, reproduction, metabolism, and selection traits. This study aimed to detect mutations in exon 1 and intron 1 of the *PROPI* gene and assess their association with growth traits in 20 local Iraqi Awassi ewes. Sequencing analysis revealed a single nucleotide polymorphism (SNP) at position 78 (G>A), which resulted in three genotypes: GG, GA, and AA. The SNP was located at position 26 of the partial polypeptide sequence and at position 63 of the complete protein sequence (accession number NP_001009767.1). The codon changed from AGG to AGA; however, no amino acid substitution occurred because both codons encode arginine, indicating a synonymous mutation. No significant associations were found between the GG, GA, and AA genotypes and the studied growth traits, including birth weight and body measurements. All sequences obtained in this study were submitted to NCBI GenBank under accession numbers LC833880 to LC833899.

Keywords: *PROPI*; Polymorphism; Growth traits; Association; Sheep

Introduction

Growth performance in sheep is affected by multiple genetic and physiological factors, motivating the search for genes linked to key production traits. Among these, *PROPI* is a crucial transcription factor required for anterior pituitary development and hormone-producing cell differentiation. Its regulatory role extends to pathways controlling growth hormone and related endocrine functions, making it an important candidate in growth-trait studies [1,2]. This has encouraged researchers to investigate new genetic tools to enhance growth performance while maintaining the adaptive traits that are vital for survival in local environments, one gene of particular interest is the *PROPI* (Prophet of Pit-1) gene, which plays a crucial role in the development of the pituitary gland. The pituitary gland regulates several essential hormones, including growth hormone (GH), thyroid-

stimulating hormone (TSH), and adrenocorticotrophic hormone (ACTH) [3]. Mutations or polymorphisms in the *PROPI* gene can alter the function or expression of these hormones, leading to variations in body weight, growth rate, and other production traits throughout the animal's life. Previous studies in sheep and cattle have demonstrated significant associations between *PROPI* gene polymorphisms and growth-related characteristics such as birth weight, weaning weight, and post-weaning gain [4]. Similarly, [5] reported that variations in this gene could serve as valuable genetic markers for selecting animals with superior growth potential. Investigating the genetic diversity and potential polymorphisms of this gene in local breeds could provide valuable insights into the genetic mechanisms controlling growth. Such information could support breeding

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strategies aimed at improving growth performance while preserving the unique adaptive characteristics that make these

sheep well-suited to Iraq's challenging environmental conditions

Material and Methods

The research was conducted on a private farm north of Nasiriyah. Thirty Awassi ewes, with an average weight of 45 kg and ages ranging from approximately 4 to 6 years, were randomly selected. The ewes were kept in semi-open pens and provided with green fodder and hay, and concentrated feed was given to all the ewes. The lambs were weighed at birth, and their body dimensions (body length, front height, back height, chest circumference, and abdominal circumference) were estimated. 20 blood samples were drawn from the ewes' jugular vein, and DNA was extracted using a kit produced by the Taiwanese company Genaid, the same steps were followed in the company's protocol. The *PROPI* gene was used to identify polymorphisms resulting from mutations along the sequence of the studied segment of the *PROPI* gene, calculate the frequencies of genotypes & alleles, and investigate the relationship between polymorphisms and growth traits. The forward primers 5'-ATG AAC ACT CAG CCC TAC CG-3' and the reverse primers 5'-TAT GCC CCG TAG TTC CCA AG-3' were used to amplify a 307bp fragment of the *PROPI* gene, encompassing the first exon and part of the first intron. These primers were selected based on Accession number (AY533708) for [6], the polymerase chain reaction (PCR) protocol designed to amplify the specific fragment of the *PROPI* gene included 95°C for 5 minutes for initial denaturation and 35 cycles. Each cycle

Results and Discussion

The results of agarose gel electrophoresis showed successful amplification of the

included 95°C for 30 seconds for denaturation, followed by 56°C for 1 minute for annealing and 72°C for 30 seconds for extension. After the 35th cycle, the protocol concluded with 72°C for 7 minutes for final extension. The PCR mixture was 25 µL, containing 12 µL of 2x Amplicon master mix and 1 µL of forward and reverse primers), 3 µL of medium-concentration DNA template (approximately 50 ng/L), and 8 µL of free nuclease water (FNW). After completing the PCR amplification technique The *PROPI* gene was selected using electrophoresis on a 1.5% agar gel with TBE solution, used DNA standard for 100-3000 bp, and a safe green dye (safeview dye ID: G108 from Canadian AMP company). The PCR products for all samples were sent to Macrogen, Korea, for sequencing analysis. Immediately after receiving the results, multiple sequence alignment analysis was performed using BioEdit v7.2.5 software [7]. Gene and allelic frequencies and Hardy-Weinberg equilibrium were estimated using SPSS v23 software. For analysis relationship between *PROPI* gene polymorphisms and growth traits, the following model was used:

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

Where, Y_{ijk} is the phenotypic value of the trait, μ is overall population mean, G_i is the effect of *PROPI* genotype, and e_{ijk} is the random error effect.

Prope 1 gene fragment at a size of 307 bp (Figure 1).

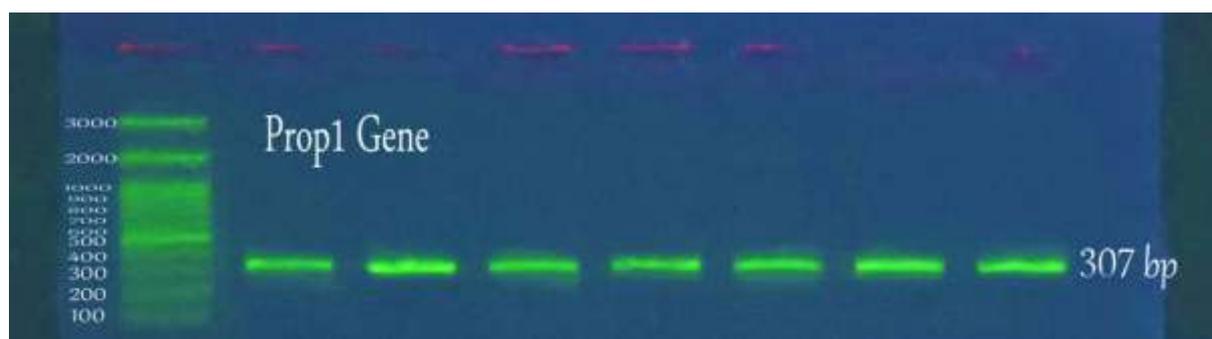


Figure (1) Electrophoresis of the *Prope 1* gene fragment for local sheep on 1.5% agarose gel

The multiple alignment analysis of the studied Probe 1 gene fragment revealed a genetic variation at position 78 (G>A). This mutation produced three distinct genotypes, as shown in Table (1) and Figure (3). The nucleotide change occurred at position 26 of the polypeptide chain within the studied region, and at position 63 of the complete polypeptide chain of the gene, according to the probe1 protein accession number NP_001009767.1. The mutation was located in the AGG codon, which encodes the amino acid arginine. With the AA genetic change, the codon was converted to AGA. This alteration did not lead to an amino acid substitution,

since both AGG and AGA encode arginine.

The statistical analysis of the genetic variation in the 78 G>A mutation showed that the frequency of the GG genotype was the highest at 0.55, compared to 0.25 for the heterozygous GA genotype and 0.20 for the homozygous recessive AA genotype. In addition, the Chi-square test for genotype distribution yielded a value of 6.28, which was statistically significant at the 0.05 level. This result may be attributed to the relatively small sample size and the differences observed between the expected and actual genotype distributions.

Table (1) Frequencies of genotypes and alleles for the *PROPI* gene of local sheep

Genotype	(%)	count	Chi-square
GG	0.55	11	* 6.28
GA	0.20	4	
AA	0.25	5	
All	%100	20	
Alelle	(%)		
C	0.65		
G	0.35		
Signification		** P≤0.01	

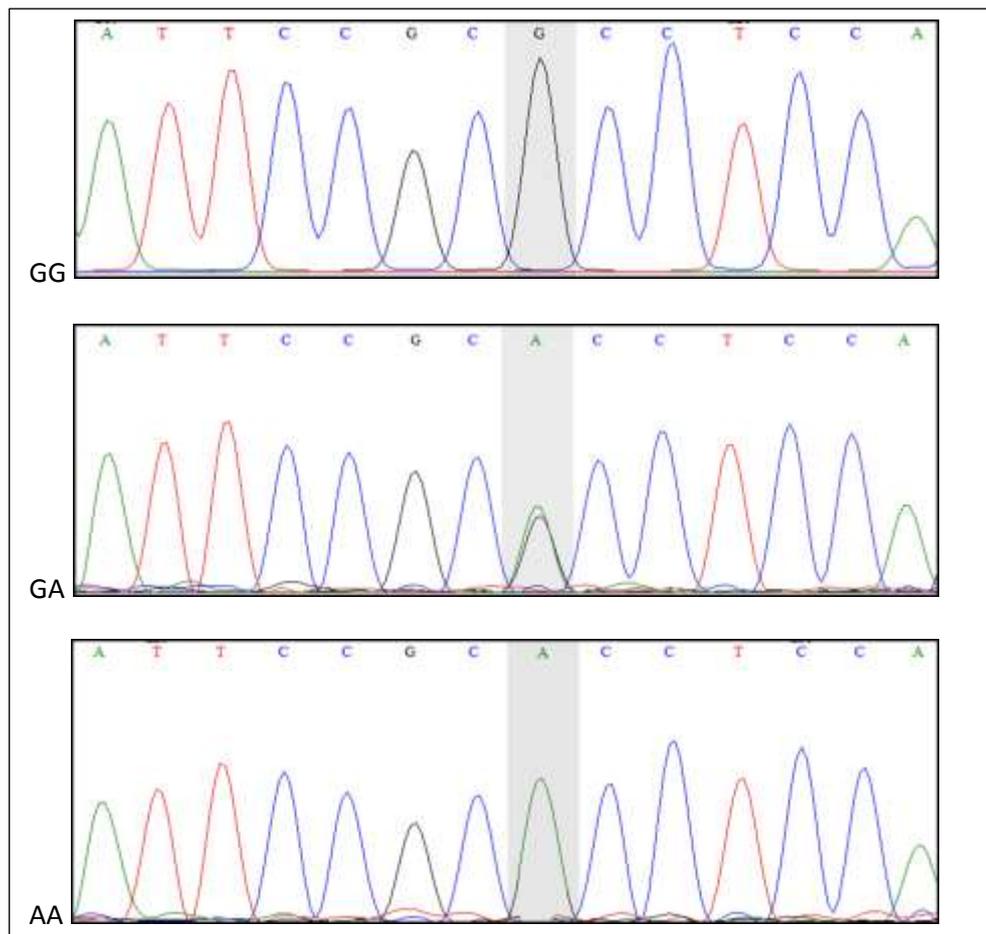


Figure (2) shows the 78.G>A mutation in the *PROP1* gene of local sheep.

The statistical analysis using the Least Significant Difference (LSD) test in SPSS Table (3) revealed no significant differences among the GG, GA, and AA genotypes of the *PROP1* gene for the measured productive traits—including body length, front height, background height, chest circumference, and abdominal circumference—of local Awassi lambs (Table 3). However, numerical differences were observed in birth weight. Lambs carrying the AA genotype recorded an average birth weight of 3.71 kg, compared with 3.64 kg for the GG genotype and 3.66 kg for the GA genotype. In addition, abdominal circumference was slightly higher in lambs with the AA genotype (37.3 cm) than in those carrying the GG (36.63 cm) and GA (36.50 cm) genotypes.

The results of the present study differ from those reported by [8], who found significant associations between the c.109+40T>C and c.109+207C>T polymorphisms of the *PROP1* gene and several growth traits in New Zealand Romney lambs, including birth weight, weaning weight and average daily gain. Their findings highlight a more prominent influence of *PROP1* variants on early growth performance than observed in the Awassi population examined in the current study. Further evidence from other species supports the variability of *PROP1* gene effects across breeds and environments. For instance, [9,10] reported significant associations between *PROP1* polymorphisms and milk production, milk chemical composition, weaning weight, and average daily gain in Mahabadi goats.

Table (2) Effect of the Probe 1 gene Polymorphism on production traits \pm standard error

P value	Mean	AA	GA	GG	Polymorphism 78.G>A PROB1 Gene
	20	5	4	11	count
0.58 N.S	3.6 \pm 50.03	3.71 \pm 0.08	3.6 \pm 60.4	3.64 \pm 0.03	Birth Weight (Kg)
0.26 N.S	37.07 \pm 0.2	37.7 \pm 0.54	36.87 \pm 0.42	36.86 \pm 0.26	Body length (cm)
0.51 N.S	38.40 \pm 0.19	38.80 \pm 0.46	38.25 \pm 0.32	38.27 \pm 0.27	Front Height (cm)
0.23 N.S	38.52 \pm 0.17	38.64 \pm 0.40	38.60 \pm 0.32	38.60 \pm 0.23	Background Height (cm)
0.42 N.S	36.60 \pm 0.16	37.51 \pm 0.30	36.05 \pm 0.32	37.20 \pm 0.24	Chest circumference (cm)
0.20 N.S	36.78 \pm 0.17	37.30 \pm 0.37	36.50 \pm 0.36	36.63 \pm 0.20	Abdominal circumference (cm)

However, similar to the present findings, their study did not detect significant differences among genotypes for birth weight, suggesting that the influence of *PROPI* on early growth may be limited or masked by environmental factors in some breeds. Comparable trends have also been observed in the study of [4], who investigated a missense mutation (A79V) in the *PROPI* gene of goats and found variable effects on growth traits, with limited influence on birth-related characteristics. Moreover, the work of [10] on Turkish sheep breeds demonstrated notable differences in *PROPI* allele frequencies among populations, indicating that genetic background may play a key

role in determining the phenotypic impact of this gene. Finally, broader genomic studies—such as those summarized by [12,13] emphasize that genes regulating pituitary function, including *PROPI* and related transcription factors like *POUIF1*, may influence growth and productivity through complex regulatory pathways. Overall, the collective evidence indicates that the phenotypic influence of *PROPI* polymorphisms is not universal but rather depends on breed-specific genetics, environmental conditions, and the interaction of multiple regulatory pathways.

The fig.3 presents a multiple sequence alignment of the Prop-1 gene for the three genotypes GG, GA, and AA. The alignment is used to compare the nucleotide sequences in order to identify similarities and differences among them. The asterisk (*) symbol indicates positions where the nucleotide bases are identical in all three genotypes, showing regions of high sequence conservation within the gene. The differences between the sequences represent single nucleotide polymorphisms (SNPs). In these variable positions, the genotype GG contains the nucleotide G, while the genotype AA

contains the nucleotide A, whereas the heterozygous genotype GA shows both forms. These polymorphic sites are considered important because they may influence gene expression or protein function, and therefore can be associated with phenotypic traits such as growth performance and reproductive characteristics in sheep. Overall, this alignment visually demonstrates the location of the SNP within the Prop-1 gene and highlights the genetic variation among individuals carrying different polymorphisms.



Figure (3) Multiple sequence alignment of GG, GA, and AA polymorphisms of the Prop-1 gene

The Fig.4 displays the amino acid sequence alignment of the *PROPI* gene for the GG, GA, and AA genotypes. The

sequences show full similarity, and the highlighted position corresponds to the amino acid arginine (R), which is

conserved in all genotypes. This indicates that the nucleotide variation observed at the DNA level does not result in a change

in the amino acid sequence, suggesting that the polymorphism is synonymous and does not alter the protein structure.

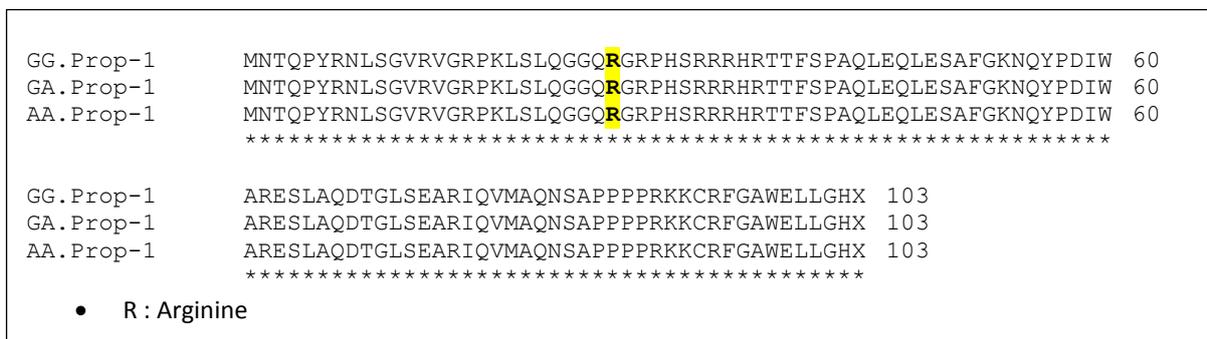


Figure (4) ultiple sequence alignment of amino acid sequences for GG, GA, and AA polymorphisms of the PROPI gene

All sequences of the 20 samples in the current study have been registered in the NCBI GenBank and accession numbers

have been obtained, as shown in Table (4), to be made available as a database for researchers.

Table (4) Accession numbers for the local sheep gene prop 1, which were registered in the NCBI gene bank.

Sequence size	Accession Numbers	Gene
307bp	LC833880, LC833881, LC833882, LC833883, LC833884, LC833885, LC833886, LC833887, LC833888, LC833889, LC833890, LC833891, LC833892, LC833893, LC833894, LC833895, LC833896, LC833897, LC833898&LC833899	<i>PROPI</i>

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