

## Genetic Variation of *POUIF1* gene and Its Effect on Milk yields and Some Traits in Cyprus Goats

Muqdad Ali Ahmed  · Dhiaa Sabah Ibrahim 

Department of Student Affairs, University Presidency, University of Samarra, Iraq

Department of Biology, College of Education for Pure Sciences, University of Samarra, Iraq.

E-mail: [dya.sabah@uosamarra.edu.iq](mailto:dya.sabah@uosamarra.edu.iq)

### Abstract

main goal of this study is to diagnose the polymorphisms of *POUIF1* in Cyprus goats and its influence on some of their economic traits. In the current study goats belonging to the ruminant research station and living under the same condition. 75 goats were tested in this experiment. polymorphism was diagnosed using the polymerase chain reaction- single strand conformation polymorphism technique and Sanger sequencing to identify the variations in the animals. genotype and allele frequencies were done by POPGENE32. In this study, two alleles A and G with the frequencies 0.57 and 0.43 respectively, three genotypes GG, insA, and GA. The Frequencies of these genotypes were 0.21, 0.36, and 0.43 respectively. insA was significantly superior in the milk yield at a significance level of 0.05 in average daily milk, insA genotype showed a high value in this trait, 989, whereas GG and GA showed 875 and 859 g/day, respectively. On the other hand, some of the characteristics in milk yield demonstrated no differences between animals. From results, it is concluded that the *POUIF1* influences milk yield in goats. Therefore, this gene could be considered a candidate to enhance the programs of farm animals and increase the income for these projects.

**Keywords:** Goats, PCR-SSCP, Polymorphism, Transcription factor.

### I. Introduction

Given the widening gap between the supply of animal products and increasing global demand driven by population growth, researchers have sought ways to enhance the productivity of farm animals. This need has been highlighted in numerous studies focused on improving goat productivity across various geographical regions (Canon *et al.*, 2006; Colli *et al.*, 2018; Oget *et al.*, 2019). Despite changes in agriculture and technological advancements worldwide, goat populations have continued to grow in recent years (İşik and Bilgen, 2019).

using of molecular genetics techniques in recent decades has led to the discovery of genes that significantly influence key economic traits in farm animals. By identifying the locations of quantitative trait loci (QTL), researchers can better understand the impact of these genes. One such important gene, *POUIF1* is directly related to many economic traits in farm animals, particularly goats. Located on the first chromosome, *POUIF1* consists of 6 exons, 5 introns, and spans approximately 21,000 base pairs (Saleh *et al.*, 2021).

*POUIF1* is considered one of the most important candidate genes influencing the growth of various livestock herds. It is also known by other names, including *PIT-1* and *GHF1*. This gene plays a crucial role in regulating the expression of *PRL* (prolactin), *GH* (growth hormone) and TSH-beta (thyroid stimulating hormone-beta), and is essential for the differentiation, reproduction, and survival of somatotropin, prolactin, and thyrotropin cells (Pawar *et al.*, 2021). It exerts a positive effect by promoting the maturation of the anterior lobe of the pituitary gland and influencing gene expression regulation (Sobrier *et al.*, 2016). Polymorphisms in this gene have been highly correlated with birth weight, body weight, milk yield, milk protein and fat content, and body size, particularly in cattle and goats (Ma *et al.*, 2017)." So, this study aims to investigate the main role of this gene in productive performance.

## II. Material and methods

### sample and milk yielded

In this study, 75 goats from the Agricultural Research Office of the Ministry of Agriculture, located in the Abu Ghraib region of Baghdad province, were examined. Milk yield for each goat was recorded every day in morning and evening throughout the lactation period (2022-2023). For each goat, 3 mL of blood was drawn from the jugular vein and collected in an EDTA K3 tube (anticoagulant, AFCO/Jordan). Genomic DNA was extracted (Fig. 1) using a Geneaid mini kit, following the manufacturer's protocol.

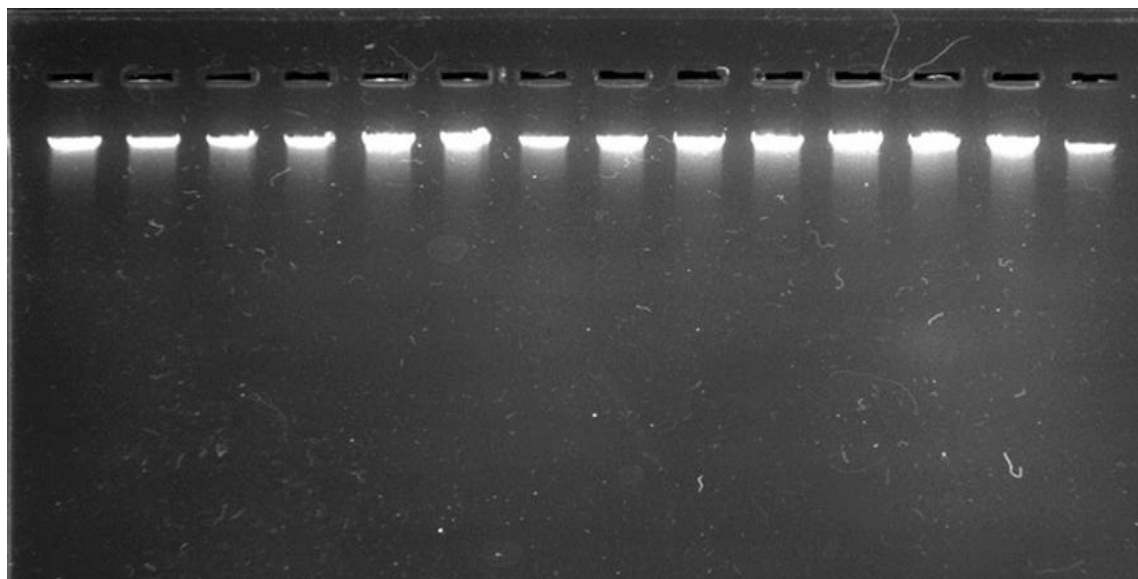


Fig (1): Extraction of gDNA (1% Agarose Stained with Red Safe).

### Amplification of DNA

Pairs of primers were used in this study to amplify the *POUIF1* gene, following the method described by Gündüz and Biçer (2023). Table (1) provides details about the primers used. The amplification targeted exon 6 and the flanking 3' region of the gene.

Table (1): Primer Details of *POUIF1* Goats.

| Primers | Sequence 5' - 3'    | Length | GC%   | Product |
|---------|---------------------|--------|-------|---------|
| F       | CCATCATCTCCCTTCTT   | 17     | 47.06 | 450 bp  |
| R       | AATGTACAATGTGCCTTCT | 19     | 36.84 |         |

### Polymerase Chain Reaction (PCR) and Genotyping

The PCR reaction was optimized using primer optimization, The optimal annealing temperature, determined by running the PCR products through electrophoresis, was found to be 58°C. After optimizing the conditions, PCR reaction was performed under the following parameters (Table 2): an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds. A final extension was carried out at 72°C for 10 minutes with holding 4 °C infinity, using a thermal cycler (Applied Biosystems, Taiwan). The total PCR reaction volume was 25µl

Table (2): PCR Conditions of *POUIF1*.

| Steps                   | Time      | Temp °C | No of Cycle |
|-------------------------|-----------|---------|-------------|
| Initiation Denaturation | 5.00 min  | 94      | 1           |
| Denaturation            | 0.30 sec  | 94      | 35          |
| Annealing               | 0.30 sec  | 58      |             |
| Extension               | 0.30 sec  | 72      |             |
| Final Extension         | 10.00 min | 72      | 1           |
| Hold                    | ∞         | 4       |             |

After completing the PCR protocol, 10 µl of the product was taken and subjected to electrophoresis (Fig. 2) for verification.

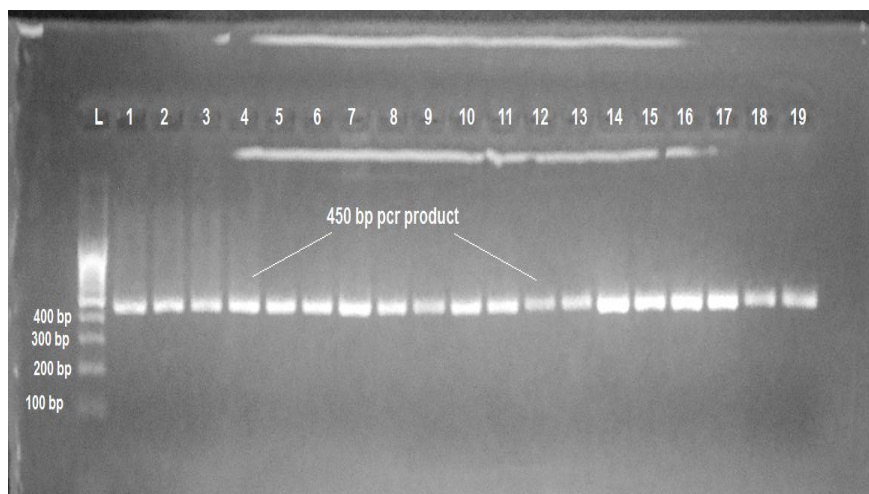


Fig (2): Amplicon of *POUIF1* with Ladder 100 bp (2% agarose).

### PCR-SSCP

To identify genotypes, SSCP analysis revealed three distinct genotypes of *POUIF1* (Fig. 3), designated as pattern A, pattern B, and pattern C.

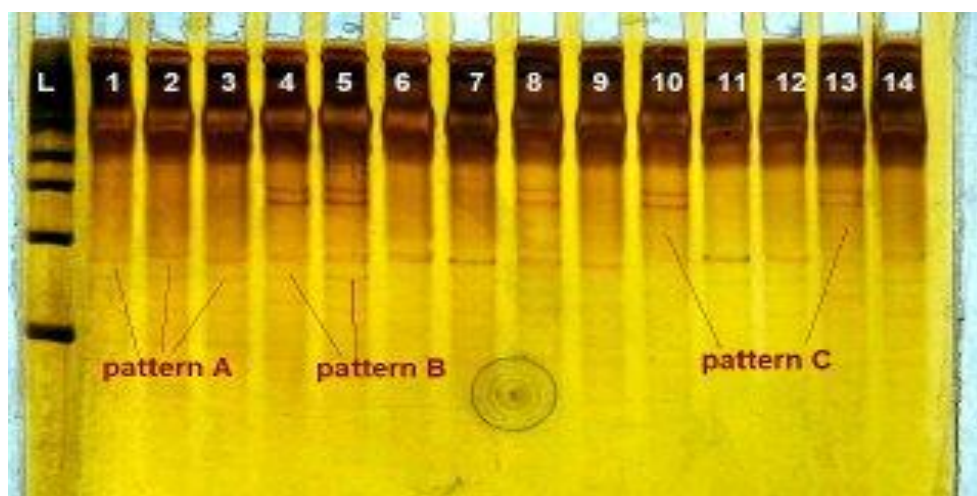


Fig (3): Patterns of *POUIF1* gene by SSCP Technique.

**Statistical analysis**

The current study uses (SAS, 2012) for testing the difference level between genotypes in the population. Pop GENE (Yeh et al., 1997) is used for calculating allelic and genotype frequencies with Hardy-Weinberg equilibrium. For checking the relation between traits and polymorphism F-test is used at a significance level of (P < 0.05).

$$\text{Chi-square value by } \chi^2 = \sum \frac{(\sigma - e)^2}{e}$$

$$\text{Statistical model: } Y_{ijklm} = \mu + G_i + A_j + P_k + W_l + e_{ijkl}$$

$\mu$ : mean,  $G_i$ : effect of genotype,  $A_j$ : effect of age,  $P_k$ : effect of parity,  $W_l$ : effect of weight,  $e_{ijklm}$ : Random error that is normally distributed with a mean equal to zero and a variance of  $\sigma^2e$ .

**Results and Discussion**

**Allelic and Genotypic Frequency**

Table (3) shows the allelic and genotypic frequency for the studied samples and the number of animals per genotype using PopGene32 software packages for the population. It also shows the value of Chi  $\chi^2$  and the level of significance at P=0.05. The frequencies of the genotypes GG, insA, and GA, are 0.21, 0.36, and 0.43 respectively. No significant differences are seen within the samples.

**Table (3): Allele, Genotypic Frequency and Chi-square.**

| Genotype                 | No  | Genotype frequencies |
|--------------------------|-----|----------------------|
| GG                       | 16  | 0.21                 |
| insA                     | 27  | 0.36                 |
| GA                       | 32  | 0.43                 |
| Total                    | 75  | 100 %                |
| Chi $\chi^2$             | --- | 1.63 ns              |
| Allele frequency         |     |                      |
| A                        |     | 0.57                 |
| G                        |     | 0.43                 |
| NS: Non-significant 0.05 |     |                      |

**Sequencing**

In this study, three polymorphic loci were identified in the investigated genotypes of the *POU1F1* (fig.4). In the first, homozygous guanine (G/G) was detected in the 152<sup>th</sup> position of the B1 genotype without any insertion in the upstream region. In the second genotype, the same homozygous guanine (G/G) existed but one adenine insertion was detected between 152<sup>th</sup> and 153<sup>th</sup> loci (insA152-153). In the third genotype, no insertion was detected between the 152<sup>th</sup> and 153<sup>th</sup> loci, but it was found Substitution, that guanine was represented in a heterozygous status either guanine or adenine (G/A) status.





This chromatogram fig (5) shows the variation between genotypes in the population and the differences between homozygotes and heterozygotes.

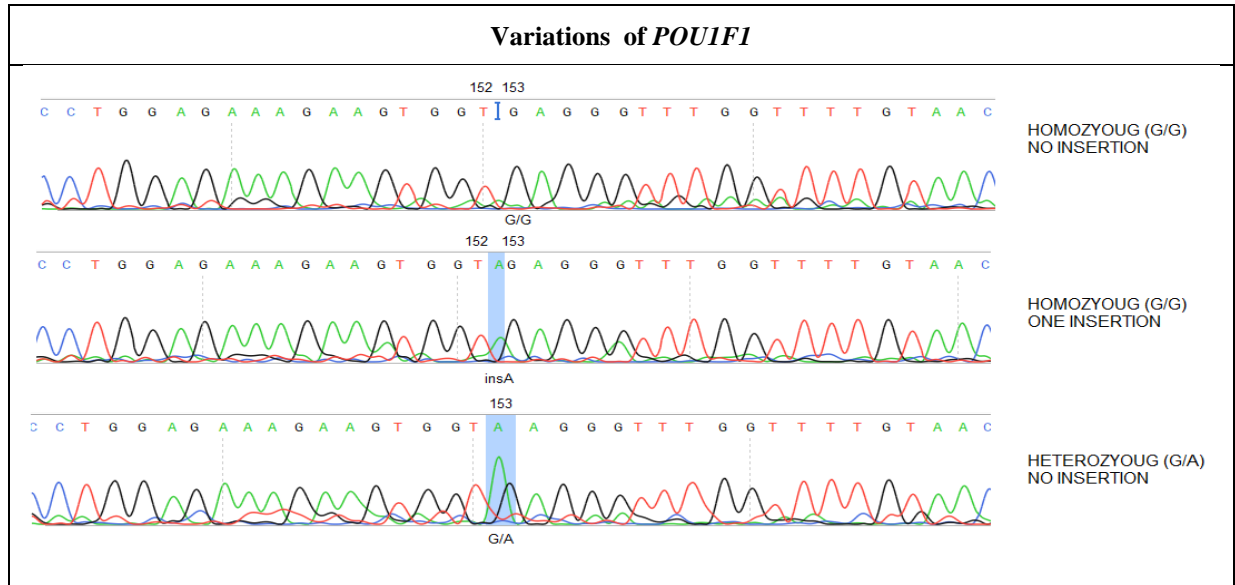


Fig (5): The Pattern of *POU1F1*, the Identified SNPs were highlighted according to their positions.

**Polymorphism of *POU1F1* and Effect of some milk traits in goats.**

Table (4) shows no significant differences in Total Milk Yield (TMY), Lactation Period (LP), and the Peak of production. Average Daily Milk (ADM) shows a significant difference according to the genotype, animals that have insA mutation recorded a high milk production per day. Homozygous and heterozygous animals do not record any differences between the same population, so ADY was 989, 875, and 859 g/day.

**Table (4): Effect of *POU1F1* Polymorphism on TMY, LP, ADY, and Peak.**

| Genotype    | TMY/kg      | LP/day      | ADY/gm                 | Peak/day   |
|-------------|-------------|-------------|------------------------|------------|
| GG          | 120.60±3.53 | 137.81±2.11 | 875 <sup>b</sup> ±0.02 | 38.12±1.70 |
| insA        | 133.59±6.61 | 135.77±1.53 | 989 <sup>a</sup> ±0.05 | 39.18±1.32 |
| GA          | 119.25±1.87 | 138.71±1.10 | 859 <sup>b</sup> ±0.01 | 40.09±1.33 |
| Significant | NS          | NS          | *                      | NS         |

NS: non-significant; (\*) meaning significant at level 0.05.

**Polymorphism of *POU1F1* and Effect of chemical characteristics.**

Results of table (5) shows no significant differences in all the characteristics of chemical composition for milk between the individuals for protein, fat, solid nonfat, lactose.



Table (5): Effect of *POU1F1* Polymorphism on chemical characteristics

| Genotype    | Protein % | Fat %     | Solid nonfat % | Lactose % |
|-------------|-----------|-----------|----------------|-----------|
| GG          | 2.82±0.04 | 2.02±0.04 | 8.28±0.07      | 4.61±0.04 |
| insA        | 2.82±0.03 | 2.07±0.03 | 8.18±0.06      | 4.62±0.03 |
| GA          | 0.02±2.81 | 2.03±0.03 | 8.24±0.05      | 4.59±0.03 |
| Significant | NS        | NS        | NS             | NS        |

### III. Conclusions

This study demonstrates the significant effect of *POU1F1* polymorphism on certain milk traits. insA animals exhibited a significant difference ( $p < 0.05$ ) in (ADY). No significant differences were observed in other genotypes. Variations between studies on this topic may stem from differences in the animals studied or environmental conditions across regions. This study suggests that *POU1F1*, particularly exon 6 and the flanking 3' untranslated region (3' UTR), could serve as a molecular marker for selection in goat breeding programs. These findings can be applied to breeding programs across different types of animal farms and various breeds.

#### Acknowledgments

The authors would like to thank for Ruminant Research Station/Ministry of Agriculture and Biotechnology Department at Samarra University.

#### Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication

#### Ethical approval:

All recommended instructions regarding scientific research ethics in dealing with animals were followed, by the document of Tikrit university – scientific research ethics in 23/9/2024.

### IV. References

- Canon, J., Garcia, D., Garcia-Atance, M.A., Obexer-Ruff, G., Lenstra, J. A., Ajmone- Marsan, P., Danner, S. and The Econogene Cnsortium. Geographical partitioning of goat diversity in Europe and the Middle East. *Anim Genet.* 2006; 37(4):327–334.
- Colli, L., Milanese, M., Talenti, A., Bertolini, F., Chen, M., Crisà, A., and AdaptMap Consortium. Genome-wide SNP profiling of worldwide goat populations reveals strong partitioning of diversity and highlights post- domestication migration routes. *Genet Sel Evol.*2018; 50:1-20.
- Gündüz, Z., and Biçer, O. Milk-y Way: the impact of single-nucleotide polymorphisms on milk production traits in Kilis dairy goats. *Arch Anim Breed.* 2023; 66(4):369-378.
- Işık, R. and Bilgen,G. Associations between genetic variants of the *POU1F1* gene and production traits in Saanen goats. *Arch Anim Breed.* 2019; 62(1):249-255.
- Ma, L., Qin, Q., Yang, Q., Zhang, M., Zhao, H., Pan, C., and Lan, X. Associations of six SNPs of *POU1F1-PROP1-PITX1-SIX3* pathway genes with growth traits in two Chinese indigenous goat breeds. *Annals of Anim Sci.* 2017;17(2):399-411.
- Oget, C., Servin, B. and Palhière, I. Genetic diversity analysis of French goat populations reveals selective sweeps involved in their differentiation. *Anim Genet.* 2019; 50(1):54–63.



- Pawar, V., Sawane, M., Zunjarrao, K., and Doiphode, A.** Characterization of third exonic region of POU1F1 gene in the Osmanabadi breed of goat. *Indian J Anim Sci.* 2021; 91(2):151-154.
- Saleh, A.A., Rashad, A.M.A., Hassanine, N.N.A.M., Sharaby, M.A., Zhao, Y.** Evaluation of morphological traits and physiological variables of several Chinese goat breeds and their crosses. *Trop. Anim. Health Prod.* 2021; 53:1-15.
- Sobrier, M.L., Tsai, Y.C., Pérez, C., Leheup, B., Bouceba, T., Duquesnoy, P., Copin, B., Sizova, D., Penzo, A., Stanger, B.Z., Cooke, N.E., Liebhaber, S.A., and Amselem, S.** Functional characterization of a human POU1F1 mutation associated with isolated growth hormone deficiency: A novel etiology for IGHD. *Hum. Mol. Genet.* 2016;25(3):472–483.
- Yeh, F. C., Yang, R. C., Boyle, T., Ye, Z. H., and Mao, J. X.** (1997). POPGENE, the user-friendly shareware for population genetic analysis. Version 1.32. Edmonton, Alberta: Molecular Biology and Biotechnology Centre, University of Alberta.