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# Studying of Cystein and Glycin Rich Proteins Gene and Relationship with Body Dimensions in Awassi Sheep

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**Abstract: This study investigated the association between single-nucleotide polymorphisms (SNPs) in the cysteine- and glycine-rich protein 2 gene (CSRP2) and body dimensions in Awassi sheep. Blood samples and measurements of body length, front height, and back height were collected from 50 animals raised at a private station in Babylon Province, Iraq, during 2022–2023. Genomic DNA was extracted, and exon 7, together with the adjacent terminal region, was amplified and sequenced. Allele and genotype frequencies were assessed using the chi-square test, while genotype effects were analyzed with a general linear model followed by Duncan's multiple range test. Two C>T substitutions were identified at positions 20315 and 20094, producing the CC, CT, and TT genotypes. The T allele was the most frequent at both positions, with frequencies of 0.67 at position 20315 and 0.57 at position 20094. Genotype distributions differed significantly at both loci (P≤0.01). At position 20315, sheep with the CC genotype had significantly greater body length (64.09±2.22 cm), front height (59.51±0.45 cm), and back height (60.10±1.20 cm) than animals with the CT or TT genotypes. At position 20094, the CC genotype was associated with the greatest body length (61.17±2.22 cm) and back height (61.05±1.20 cm), while front height also varied significantly among the genotypes. These results suggest that CSRP2 polymorphisms are associated with growth-related body dimensions in Awassi sheep and that the CC genotype may be favorable for these traits. However, the findings should be validated in larger, independent populations before these variants are used in marker-assisted selection programs.**

***Keywords:*** *Awassi Sheep; CSRP2 Gene; Single Nucleotide Polymorphism; Body Dimensions; Growth Traits; Marker-Assisted Selection.*

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## I. INTRODUCTION

Awassi sheep can be classified as the most famous and important sheep breed in middle east counties as well as in another regions around the world. The breed characterized as multipurpose but many of countries improved it to be a good dairy sheep breed in addition to its mutton from lambs fattening (AL-Barazanji and Othman ,2013).

Many studies in molecular biology and genetics proved that the sheep traits can improved by markers assisted selection (MAS) and determination the quantitative traits loci (QTL) to increase the economic yield from animals products such as meat , milk and secondary products ( ).

The genes coded with high cystein and glycin are some of the candidate genes under this group and the corresponding previous research refers to for employability of this genes as genetic markers for the purpose of creating a genetic variance of utility in genetic selection and decision for choice of best individuals as sires or dams for the future generations. In sheep, it is located at 3rd chromosome and has eight expression area (exons) and seven interruption area (introns) (NCBI, 2023) which are coding many functional proteins to play crucial function in cells growth or differentiation, immunological properties, histones modification (Brants et.al., 2012; Liu et.al., 2016 ; Zhang et.al., 2021).

The major aim of the current study is to determine the relationship of single nucleotide polymorphism of cystein-Glycine rich protein encoding gene (CSRP2) with the body dimension of Awassi sheep under farming conditions and used the results as a guidelines or indicator to make a selecting base for Iraqi sheep improving.

## II. MATERIALS AND METHODS

The current research was conducted at private station at Babylon province (Middle of Iraq) with 50 blood samples and genetic analysis at available Middle east laboratories at Baghdad (AL-harithya) during 2022 – 2023 with the aim to purify DNA and detect the genotypes of Cystein – Glycin rich protein gene (CSRP2) at 7th exon position along with the terminator region.

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Blood was collected from jugular vein and DNA was isolated according to the procedure of (Samprook and Russel, 2001), kit was from Promega company. The primer target region amplified and detected was: 5-GGG TGA CTT GCT CTA ACA CTC ATC- 3 (Forward) and 5'-AGG CCT CAC TTC CCT ACA TCC CTA-3 (Reverse).

The body dimensions ( body length, front height and – height) were recorded for each ewes.

Statistical inference: SAS (2016) computer software was used to test the data through general linear model procedure (GLM) as follows:

$$Yijk = \mu + Ti + eijk$$

Where: μ: grand mean

Ti: CSRP2 genotypic effect

eijk : is a random error.

Chi- square test was used to compare the significance differences between phenotypes:

$$X^2 = \sum \frac{(Observed\ No. - Expected\ No.)^2}{Expected\ No.}$$

General linear model (GLM) revealed significant group differences and Duncan's multiple range test (Duncan,1955) was used for comparing differences between means.

## III. RESULTS AND DISCUSSION

Results of genetic analyses (Fig.1) indicated that a single nucleotide polymorphism (SNP) was noticed in the site 20315 (exone 7) , nitrogen base changed from cytosine to Thiamin (C< T) that lead to three genotypes : the wild type (CC), the hetero type (CT) and the mutant type (TT).

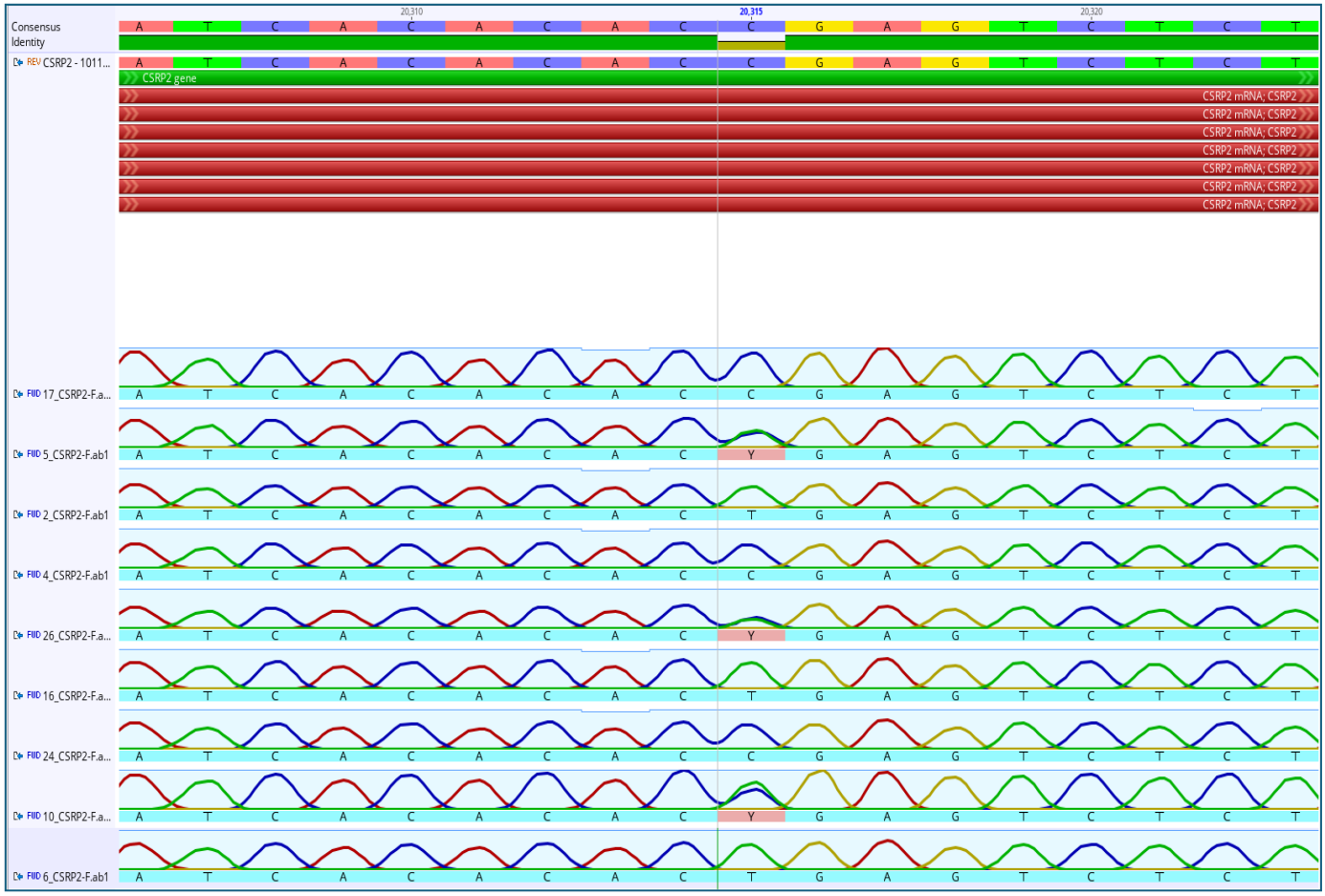

Fig 1 Single Nucleotide Polymorphism in the Site 20315 of CSRP2 Gene

Results of genetic analyses indicated that a single nucleotide polymorphism (SNP) was noticed in the site 20094 (exone 7) (Fig.2) and lead to similar changes in the site 20315 above where the nitrogen base cytosine was altered to Thiamin (C< T) that lead to three genotypes : the wild type (CC), the hetero type (CT) and the mutant type (TT).

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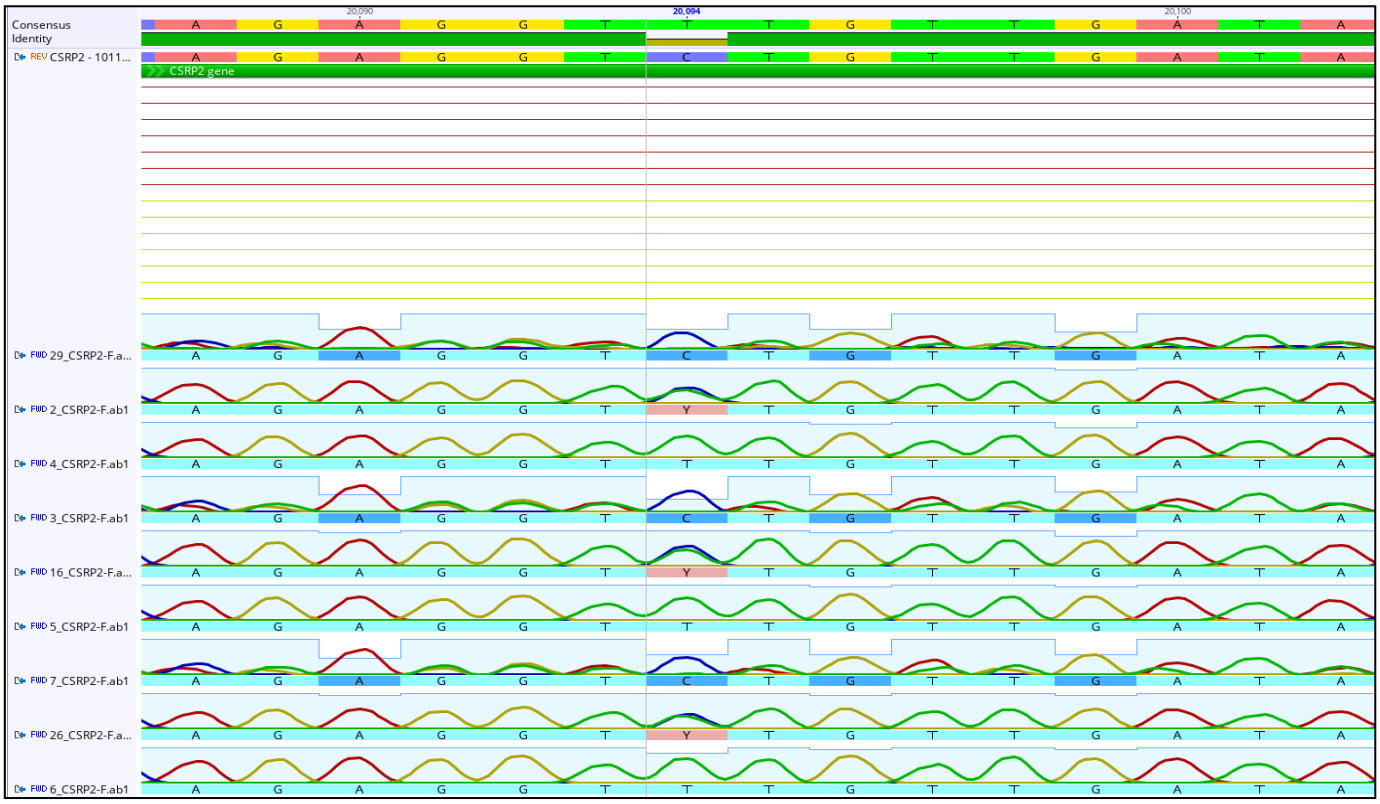

Fig 2 Single Nucleotide Polymorphism in the Site 20094 of CSRP2 Gene

Results revealed a very significant difference (P≤ 0.01) of sample distribution according to alleles due to single nucleotide polymorphism in position (Table – 1).

Two co-dominant alleles and three genotypes was indicated in 1st position (site 20315), frequencies of alleles was 0.33 and 0.67 for C and T alleles respectively whereas frequency of genotypes were 0.26, 0.14 and 0.60 for CC, TC and TT respectively.

Distribution of samples within 2nd region (location 20094) was remarkably different (P≤0.01), 0.43 and 0.57 represented the frequency of C and T alleles respectively, and the genotype frequency was 0.38, 0.10 and 0.52 for genotypes CC, TC and TT respectively.

Table 1 Distribution of Genotypes and Allele Frequency of CSRP2 Gene

| **Genotype (site 20315)** | **No.** | **%** | **Allele frequency** | |
|---|---|---|---|---|
| CC | 13 | 0.26 | C | T |
| TC | 7 | 0.14 | 0.33 | 0.67 |
| TT | 30 | 0.60 | | |
| $X^2$ | 17.2576** | | | |
| **Genotype (site 20094)** | **No.** | **%** | **Allele frequency** | |
| CC | 19 | 0.38 | C | T |
| TC | 5 | 0.10 | 0.43 | 0.57 |
| TT | 26 | 0.52 | | |
| $X^2$ | 13.8636** | | | |

**(P≤0.01)

Results showed that the SNP in site 20315 of CSRP2 gene lead to a significant difference among animals according to the base change (Table-2), sheep with wild genotype (CC) were the longest body length about 64.09 cm while the body length of hetero and mutant groups were 53.88 and 52.91 respectively (P≤0.01).

A significant difference also found among groups in front height (P≤0.05), greatest body was found in wild group i.e., 59.51 cm vs. lowest body height which found in hetero group i.e., 54.22 cm.

The back height of the body ranged widely (P≤0.05) among the genetic groups, and the wild type sheep were 60.10 cm compared to hetero and mutant types, which had recorded approximately 55.33 and 56.42 cm respectively.

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Table 2 Effect of CSRP2 Gene Polymorphism on Awassi Sheep Body Dimensions at Weaning Age

| Genotype (site 20315) | S.E ±Means | | |
|---|---|---|---|
| | B.L (Cm) | F.H (Cm) | B.H (Cm) |
| CC wild | 64.09±2.22 a | 59.51± 0.45 a | 60.10±1.2 a |
| TC Hetero | 53.88±1.05 b | 54.22± 0.67 c | 55.33± 1.61 b |
| TT Mutant | 52.91±1.13 b | 57.04± 0.39 b | 56.42± 1.04 b |
| Significance | ** | * | * |

B.L: Body length, F.H: Front height, B.H: Body height. **: P≤0.01 , * :P≤0.05

Table-3 results reported a considerable variation between genetic groups in body measures (P≤0.05), body length at weaning was variable and increased remarkably in wild type ewes to an extent of 61.17 cm while in hetero and mutant groups were 55.82 and 53.89 cm respectively.

Table 3 Effect of CSRP2 Gene Polymorphism on Awassi Sheep Body Dimensions at Weaning Age

| Genotype (site 20094) | S.E ±Means | | |
|---|---|---|---|
| | B.L (Cm) | F.H (Cm) | B.H (Cm) |
| CC wild | 61.17±2.22 a | 57.51± 0.45 a | 61.05±1.2 a |
| TC Hetero | 55.82±1.05 b | 55.72± 0.67 c | 54.31± 1.61 b |
| TT Mutant | 53.89±1.13 b | 57.54± 0.39 b | 56.50± 1.04 b |
| Significance | * | * | * |

B.L: Body length, F.H: Front height, B.H: Body height. * :P≤0.05

Front height and back height increased significantly in wild type group namely, 57.51 and 61.05 cm respectively while the measurements in hetero type group were 55.72 and 54.31 cm respectively and the mutant group were 57.54 and 56.50 cm respectively.

Current findings revealed that cystein rich protein genes and its gene expression are play significant role in sheep growth and this findings are agreed with previous research such as Levtchenko et. al. (2014) who stated the cystein importance for various organs in mammalian body such as kidney functions, lysosomal activity, Cerebral and Neuro-cognitive, male fertility and liver function.

The results also support an earlier result by Abayomi et.al. (2017) who reported that sulfur amino acids like cystein are vital for life and metabolism in the majority of the mammalian species and catabolism of cystein is an essential process for health and its first step is catalyzed by a cystein deoxygenate enzyme which is accountable for iron metabolism in the body.

Most of the research cited that CSRP genes family are contribute many genes to enhance growth and metabolism and its considered as a candidate genes for muscles development in sheep therefore, it very crucial factors to increase the economic income of domestic animals (Yi et.al., 2017 ; Robka et.al., 2018 ; Mohammadabadi et.al., 2021 ). The present study is comparable with findings of Cao et.al. (2023) who showed that the growth genes cystein amino acids that are coded are epigenetically can regulate postnatal muscle growth by transcription and translation efficiency without the sequence of DNA being altered.

Collectively, these findings indicate that genes encoding or regulating sulfur-rich amino acid proteins contribute to growth and muscle development. The observed single nucleotide polymorphisms in CSRP2 were associated with measurable differences in sheep body dimensions, supporting the potential use of this genetic variation in selection programs designed to improve the performance of domestic animals.

## IV. CONCLUSION

This study identified two C>T single-nucleotide polymorphisms in the examined region of the CSRP2 gene at positions 20315 and 20094. Both variants were significantly associated with body measurements in Awassi sheep. Although the T allele and TT genotype were the most common at both loci, sheep with the CC genotype generally exhibited more favorable growth-related traits. At position 20315, the CC genotype was associated with significantly greater body length, front height, and back height. Similarly, at position 20094, animals carrying the CC genotype had the greatest body length and back height, while front height also varied significantly among the genotypes. These findings suggest that CSRP2 may play an important role in postnatal growth and muscle development. The identified polymorphisms may therefore have potential as preliminary molecular markers in Awassi sheep breeding programs. However, the study was based on a relatively small sample from a single production environment. Further research involving larger, independent flocks is needed to confirm these associations. Factors such as age, sex, management conditions, pedigree, and additional productive traits should also be considered before the favorable genotype is incorporated into routine marker-assisted selection programs.

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