American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Relationship of Cystein Rich Protein Gene Polymorphism with Sheep Immunity

Majeed Sh. S. Al-Omairi
University of Al-Shatrah, College of Applied Medical Sciences, Department of Pathological Analysis

Sabah Mohammad Al-Haj Nasan
University of Homs- Faculty of Agriculture- Department of Animal Production

Received: 2025, 15, Sep
Accepted: 2025, 21, Oct
Published: 2025, 04, Nov

Annotation: The study was conducted at the Private Station on samples consisting of 60 blood samples taken from 60 Awassi ewes during their 3rd lambing season in the year 2023. The system used for sheep breeding at the station is in open and semi-open pens, with closed facilities for young lambs. Results showed a significant effect of SNP in exone 2 from CSRP-1 gene on all immunity parameters (P<0.05), TNF-α altered significantly and the highest concentration was recorded in sheep with wild genotype (74.99 pg/ml) compared with the sheep with mutant genotype (60.14 pg/ml). IgA, TGF-β and IL-6 differed significantly (P<0.05) between wild and mutant groups, the highest concentration were recorded in wild group namely, 201.07μ / ml, 60.84 pg/ml and 494.55 pg/ml respectively. The TNF-α altered significantly and the highest concentration was recorded in sheep with wild genotype (70.90 pg/ml) compared with the sheep with mutant genotype (64.24 pg/ml). IgA and IL-6 differed significantly between wild and mutant groups and the highest values were

noticed in wild namely, 220.07 and 290.05 pg / ml respectively compared with values in mutant group namely, 161.02 and 393.79 pg / ml respectively. Results showed that the IL-1β and TGF-β did not differed significantly between wild or mutant groups according exon 5 position.

Keywords: CSRP-1, Immunity, local sheep.

Introduction:

Cysteine is a crystalline sulfur amino acid and is considered one of the essential and important components in protein synthesis. Its chemical formula is HO2CCH(NH2) CH2SH, and it is a non-essential amino acid, meaning that the body can produce it (Banjac et al., 2008).

The genetic codes for the production of this amino acid in eukaryotes are UGU and UGC. Cysteine contains a thiol group (SH), which gives it hydrophobic properties (water-repellent). The reason this amino acid is effective in the processes of building several proteins and enzymes is due to its thiol group, which interacts with many other chemical compounds within the body. It is believed that this acid may have played an important role in the origin and evolution of life on Earth (Kitadai and Maruyama, 2018).

Cystine is primarily produced by the breakdown of proteins inside the cell. Under normal metabolic conditions, it passes from lysosomes to the intercellular fluid and then is broken down and reused in the synthesis of proteins. However, in some cases of metabolic disorders, whether due to genetic or environmental reasons, a disruption occurs in the function of the systems responsible for transporting and breaking down cystine across the lysosomal membranes. This leads to the accumulation of cystine within the lysosomes, and since this amino acid is crystalline (difficult to degrade), it results in the formation of crystals within living cells, causing damage to tissues and organs. This condition in humans is specifically known as cystinosis (Levtchenko, 2019).

Dickinson (2002) stated that cysteine has the ability to bind with several compounds responsible for immunity, including immunoglobulin IgG, which leads to its conversion into four different types in the series, depending on the degree of coupling with cysteine. This means an increase in its efficiency as an antibiotic and a diversity in its immune roles.

In another context, Slominski and his colleagues (2004) indicated that cysteine is absorbed by skin cells and interferes in the processes of melanin production, accelerating the conversion of eumelanin to pheomelanin.

Cysteine works in conjunction with other compounds called cathepsins, which are lysosomal proteases specialized in controlling cellular division. In humans, for example, there are eleven types of cathepsins, abbreviated as CCs, which make up about 25% of the total proteolytic enzymes in the body. They work to inhibit abnormal divisions in active tissue cells, provided that the appropriate pH level for their activity is maintained (Javorsek et al., 2019).

CSRP-1 It is one of the genes that encode proteins rich in glycine, cysteine, and methionine, and it is considered one of the important genes in sheep. It is located on chromosome number 12 and consists of seven coding regions (exons) and six non-coding regions (introns), with a total length of 36,417 base pairs (NCBI, 2023).

Recent studies indicate that this gene encodes a large group of similar proteins, all of which share the presence of disulfide bonds. The gene expression of this gene varies according to the tissue type with the strongest expression occurring in the rumen area, while its gene expression decreases in other tissues (NCBI, 2023).

Materials and methods:

The study was conducted at the Private Station on samples consisting of 60 blood samples taken from 60 Awassi ewes during their 3rd lambing season. The system used for sheep breeding at the station is in open and semi-open pens, with closed facilities for young lambs. The herd is managed according to a program that includes feeding, preparation for the breeding season, and arrangements for the stages of pregnancy and lambing, as well as health and veterinary care.

A total of 10 ml of blood was collected from the jugular vein of each ewe into a collection tube containing an anticoagulant of the type EDTAK2 produced by Promega. The samples were transported in a refrigerated container to the laboratory for preservation by freezing at -4°C and immediate DNA extraction.

The primers were selected as shown in Table for the purpose of conducting molecular detection and identifying the phenotypic diversity of the CSRP-1 gene.

After the polymerization reaction was completed, the presence or absence of mutations was detected using sequencing by sending the samples to be read in South Korea and receiving the results via email.

Blood samples were withdrawn from uterine vein through milking and ELISA test was used to determine the immunity parameters concentration from each ewe.

The statistical program SAS (2016) was used to analyze the obtained data according to the Complete Randomized Design (C.R.D.) and according to the mathematical model below:

$Yijk = \mu + Ti + eijk$

where:

Yijk: the effect of each observation in any treatment included in the experiment.

"μ: overall mean

Ti: effect of the genetic composition of gene on the studied traits

eijk: effect of random error.

A Duncan test was conducted to compare the means at a significance level of $P<0.01$ and $P<0.05$. As for the distribution of the studied sample according to the genetic compositions of the two position of CSRP-1 gene.

Results and discussion:

Results showed a significant effect of SNP in exone 2 from CSRP-1 gene on all immunity parameters ($P<0.05$), table (1).

TNF-α altered significantly and the highest concentration was recorded in sheep with wild genotype (74.99 pg/ml) compared with the sheep with mutant genotype (60.14 pg/ml).

IL-1β differed significantly between sheep groups, the higest concentration was recorded in sheep with wild genotype (61.05 pg/ml) compared with the mutant group (37.25 pg/ml).

Results showed that the IgA, TGF-β and IL-6 differed significantly ($P<0.05$) between wild and mutant groups, the highest concentration were recorded in wild group namely, 201.07μ / ml, 60.84 pg/ml and 494.55 pg/ml respectively.

Table-1: effect of single nucleotide polymorphism in exon 2 on immunity parameters concentration.

Genotypes (exone-2)	sd ± mean				
	TNF-α)pg /ml(	IL-1β)pg / ml(	IgA)µ / ml(	TGF-β)pg/ml(	IL-6)pg / ml(
Wild (GG)	74.99± 3.17	61.05± 1.9	201.07± 10.9	60.84± 4.44	494.55± 15.80
Mutant (CC)	60.14 ± 2.66	37.25 ± 4.63	180.02± 17.22	51.11 ± 3.16	389.29 ±8.51
Significance	*	*	*	*	*

***(P<0.05)**

Results showed a significant effect of SNP in exone 5 from CSRP-1 gene on some of immunity parameters, table (2).

The TNF-α altered significantly and the highest concentration was recorded in sheep with wild genotype (70.90 pg/ml) compared with the sheep with mutant genotype (64.24 pg/ml). IgA and IL-6 differed significantly between wild and mutant groups and the highest values were noticed in wild namely, 220.07 and 290.05 pg / ml respectively compared with values in mutant group namely, 161.02 and 393.79 pg / ml respectively.

Results showed that the IL-1β and TGF-β did not differed significantly between wild or mutant groups according exon 5 position.

Table-2: effect of single nucleotide polymorphism in exon 5 on immunity parameters concentration.

Genotypes (exone-2)	sd ± mean				
	TNF-α)pg /ml(	IL-1β)pg / ml(	IgA)µ / ml(	TGF-β)pg/ml(	IL-6)pg / ml(
Wild (GG)	70.90± 1.91	53.23± 3.88	220.07± 13.3	56.43± 4.67	490.05± 10.00
Mutant (CC)	64.24 ± 1.22	55.06 ± 3.68	161.02± 10.6	55.45 ± 5.11	393.79 ±8.77
Significance	*	N.S	**	N.S	**

N.S (no significant), *(P<0.05), **(P<0.01)

There are many studies that indicate that increased concentrations of immunoglobulins, especially IgA, IgG, and IgM, play a crucial role in protecting the fetus and sustaining pregnancy. Furthermore, the increase in their concentrations during pregnancy may be related to elevated levels in colostrum after birth, which positively reflects on the vitality of newborns and supports their immunity (Schuberth et al., 2008). The genetic variation is very important in sheep improving and selection and must interest of the single nucleotide change which lead to increase or decrease the immunity parameters. Trevisi and his colleagues (2016) concluded that the changes occurring within the reproductive system lead to variations in the concentration of most known cytokines to varying degrees, they pointed out the possibility of establishing a relationship between certain cytokines and specific reproductive diseases and issues. They emphasized that elevated levels of interleukins serve as a reliable method for predicting the risk (predisposition) of developing endometritis or retained placenta.

The current results confirmed what Xie and his colleagues (2017) stated, which is that the female reproductive system of mammals is one of the most sensitive systems in the body to changes in cytokine levels. They emphasized the existence of different immune responses in this system compared to other body systems.

These results are consistent with what several previous studies have concluded. Lopez and his colleagues (2010), Young (2016), and Tanaka (2014) emphasized that cytokines play an important role in altering the concentrations of various reproductive hormones according to the requirements of each stage of reproductive activity. This effect manifests in several ways, including that cytokines act as protein messengers between cells or may modulate and regulate the production and metabolism of lipids. In other cases, cytokine concentration may even influence the general behavior of the animal, and all these effects contribute in one way or another to changing the levels of sex hormones. The results of the current study align with previous studies regarding the impact of cytokines on reproductive hormones, showing that some of these compounds, such as interleukins and tumor necrosis factors, can migrate to the brain and regulate body temperature as well as modify metabolic levels during hunger. All these functions contribute to improving the physiological condition of the animal, which positively reflects on the release of reproductive hormones.

In conclusion: the genetic polymorphism of CSRP-1 gene effect significantly on immunity in sheep therefore, we must interest of any single base changes in this gene and determine the positive or negative effect on economic traits and consider it as genetic marker useful in sheep improve ring and selection programs.

Reference:

1. Banjac,A; T. Perisic; H. Sato; A. Seiler; S. Bannai; N. Weiss and P. Kölle.2008. The cystine/cysteine cycle: a redox cycle regulating susceptibility versus resistance to cell death. *Oncogene* volume 27, pages1618–1628 (2008) Cite this article.
2. Dickinson, D. P. 2002. Cystine peptides of mammals theribiol: theribiolgical roles and potential effect in the oral cavity and their tissues in health and disease. Crit Rev Oral Biol Med. 13(3):238-275
3. Duncan, D.B. 1955. Multiple Range and Multiple F Tests. Biometrics, 11 :1- 41.
4. Javorsek, U. et al. 2019. Cysteine cathepsins, and their extracellular roles: shaping the microenvironment. Error! Hyperlink reference not valid..
5. Kitadai, N. and S. Maruyama. 2018. Origins of building blocks of life: A review.. Get rights and content Under a Creative Commons license. https://doi.org/10.1016/j.gsf.2017.07.007
6. Levtchenko, E.2019. Cystinosis: an update.University Hospitals Leuven, Belgium.
7. Lopez-Gatiuz, F. and I. Garcia-Ispierto. 2010.Ultrasound and endocrine findings that help to assess the risk of late embryo/early foetal loss by non-infectious cause in dairy cattle.Reprod. Domest. Anim., 45(3): pp. 15-24.
8. NCBI. 2023.CSRP gene etrieved Mar 19, 2023, from https://www.ncbi. nlm.nih.gov:https:// www. ncbi. nlm. nih. Gov /gene.
9. SAS. 2016. Statistical Analysis System, User's Guide. Statistical. Version 9. 1th ed. SAS. Inst. Inc. Cary. N.C. USA.
10. Slominski A.; D. J. Tobin; S. Shibahara and J. W ortsman.2004. Melanin Pigmentation in Mammalian Skin and Its Hormonal Regulation. 84: 1155–1228, 2004; 10.1152/ physrev.00044.2003.
11. Schuberth, H.J.; Taylor, U.; Zerbe, H.; Waberski, D.; R. Hunter and Rath, D. 2008. Immunological responses to semen in the female genital tract. Theriogenology., 70: 1174–1181.
12. Tanaka, T.; M. Narazaki and Kishimoto, T. 2014. IL-6 in inflammation, immunity, and disease. Cold Spring HarbPerspect. Biol., 6.

13. Trevisi, E; G. Bertoni; A. Ferrari and A. Menuti. 2016. Pro-Inflammatory Cytokine Profile in Dairy Cows: Consequences for New Lactation. https:// doi.org/ 10.4081/ ijas..3862

14. Xie, M.; McCoski, S.R.; Johnson, S.E.; Rhoads, M.L.; Ealy, A.D. 2017.Combinatorial effects of epidermal growth factor, fibroblast growth factor 2 and insulin-like growth factor 1 on trophoblast cell proliferation and embryogenesis in cattle. Reprod. Fertil. Dev. 2017, 29, 419–430. [CrossRef] [PubMed]

15. Weiss, G.; Goldsmith, L.T.; Taylor, R.N.; D. Bellet and Taylor, H.S. 2009. Inflammation in reproductive disorders. Reprod. Sci., 16:216– 229.

16. Young, S.L. 2016. Reproductive immunology: checkered past and bright future. Fertil. Steril., 106(3):497–498.