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Association between lactoferrin gene polymorphisms and mastitis susceptibility in Baladi goat

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ORIGINAL ARTICLE

Association Between Lactoferrin Gene Polymorphisms and Clinical Mastitis Susceptibility in Baladi Goat

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Abstract

OBJECTIVE: To explore the association between lactoferrin (*LTF*) gene polymorphisms and mastitis resistance and susceptibility in Baladi goats.
DESIGN: Crossover study.
ANIMALS: Thirty female Baladi goats.
PROCEDURES: Blood samples were collected from each animal into tubes containing disodium EDTA as an anticoagulant for DNA extraction. Polymerase chain reaction was performed to amplify the 430-bp LTF gene.
RESULTS: Polymerase chain reaction-DNA sequencing genetic assessment revealed nucleotide sequence variants in the *LTF* gene associated with mastitis susceptibility among the enrolled Baladi goats (GenBank accession numbers gb|PP037928| and gb|PP037929| for healthy and mastitic does, respectively). One nonsynonymous single nucleotide polymorphism G299A was discovered by DNA sequencing of *LTF* gene, which caused the amino acid C100Y to be substituted.
CONCLUSION: *LTF* gene could be used as a candidate gene for mastitis resistance/susceptibility in goats, enabling marker-assisted selection in mastitis-resistant animals.

Keywords: Baladi goat, Gene polymorphism, *LTF* gene, Mastitis

1. Introduction

It is well known that domestic goat (*Capra hircus*) is among the first domesticated livestock species and therefore integral to animal husbandry. Goats are reared extensively on a global scale, particularly in the developing world, and serve as a vital resource for meat, milk, and fiber [1]. Goats are the species of choice in marginal environments because of their ability to convert fibrous fodder resources into food products that are edible to humans [2]. Five native goat breeds exist in Egypt: Baladi, which is common in the Nile Delta, Barki and Sahrawi, which are common in the desert; Sinaoy, also called Bedouin goat, which is primarily found in the Sinai Peninsula; Saidi, which is widely distributed in Upper Egypt, and Zaraibi, also called Egyptian Nubian, which is common in the Nile Valley and Delta [3]. The Anglonubian breed, one of the five goat breeds officially recognized in Egypt, was created by crossing the Egyptian Nubian breed with various British breeds [4]. Goat genetic development programs in Egypt have also included several crossbreeding experiments with other breeds, such as the Syrian-bred Damascus goats [5].

Mastitis is a complex disease that results from interactions between agents, animals, and the environment. It is associated with the presence of microorganisms in most cases and is considered an important problem of animal health and public

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health, with great economic repercussions in practically every country in the world [6].

Mastitis is the most important health problem in dairy small ruminants and represents the main cause of economic losses in this industry, owing to a decrease in milk yield and quality [7]. Mastitis can be classified as clinical or subclinical. Animals with clinical mastitis may present with edema, increased temperature, hardening, and pain in the mammary gland. Meanwhile, macroscopic alterations do not occur in the subclinical form, but alterations in milk composition do not show signs of inflammation in the mammary halves [8].

Lactoferrin (*LTF*) is an iron-binding protein found in most bodily fluids [9]. It is found on goat's chromosome 22 [10] and is the most important iron-binding protein in milk [11]. Neutrophils and inflamed tissues release LTF, are considered to have direct antimicrobial properties, and play a role in innate immunity [12]. In addition, LTF is found in various biological fluids, is strongly related to transferrin, and possesses multiple antimicrobial and physiological properties [13].

Previous studies have investigated *LTF* gene polymorphisms and their connections with mastitis susceptibility using restriction fragment length polymorphisms (RFLP). The aim of this study was to identify a possible link between *LTF* gene and the incidence of mastitis in Baladi goats using a polymerase chain reaction (PCR)-DNA sequencing approach.

2. Materials and methods

2.1. Ethics statement

The collection of samples and care of animals used in this study followed the guidelines for experimental animals established by the Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University (code M/82).

2.2. Animals

The present study was conducted on a total number of 30 female Baladi goats (*C. hircus*) obtained from a private farm located in Dakhlia governorate, Egypt. All environmental and hygienic conditions of the farm were inspected, especially housing, health, vaccination problems, production patterns, and feeding (concentrate feed mixture was offered at 3% of body weight while Berssem was fed ad libitum). The investigated doses were put through a thorough clinical examination in accordance with pre-established standard protocols, and the results were documented at the same time. Clinical mastitis was identified based on physical examination of the glands through inspection and palpation and evaluation of milk produced for aberrant color and consistency. Physical examination was also performed to determine body temperature, pulse, and respiratory rates. Based on the aforementioned criteria, the investigated animals were assigned to two equal groups: 15 healthy and 15 mastitic. Blood samples were collected from each animal into tubes containing disodium EDTA as an anticoagulant for DNA extraction.

2.3. DNA extraction and polymerase chain reaction (PCR) amplification

Genomic DNA was extracted from whole blood using a Gene JET whole blood genomic DNA extraction kit (Thermo Scientific, Lithuania). The concentration and purity of the extracted DNA were examined using a Nanodrop spectrophotometer for further analysis.

PCR was performed to amplify a fragment (430-bp) of *LTF* gene using the primers described previously [14].

F: 5′- TGTCCCTGGGCTCTTTAG -3′.

R: 5′- CCGAAGTGGCTTGTGAA -3′.

The polymerase chain reaction was carried out in a final volume of 100 μL containing 6 μL DNA, 1.5 μL of each primer, 50 μL PCR master mix (Jena Bioscience, Germany), and 41 μL H_2O (distilled water). The final reaction mixture was placed in T-professional thermal cycler (Biometra, Germany) and exposed to initial denaturation 95 for 5 min, followed by 30 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 45 s, extension at 72 °C for 1 min, and final extension at 72 °C for 10 min. PCR products were held at 4 °C and detected by agarose gel (2%) electrophoresis then the fragment patterns were visualized under U.V using gel documentation system (Gel Doc. Alpha-chem. Imager, USA).

2.4. DNA sequencing

Primer dimers, nonspecific bands, and other contaminants were eliminated before DNA sequencing. According to [15], purification of PCR products was done using a PCR purification kit (Jena Bioscience # pp-201s/Germany). To ensure adequate concentrations and purity of the PCR products, quantification of the PCR products was performed using Nanodrop (Uv−Vis spectrophotometer Q5000/USA) [16]. According to the enzymatic chain terminator technique created by [17],

PCR products with target bands in all investigated does (15 healthy and 15 mastitic) were sent for DNA sequencing in forward and reverse directions using an ABI 3730XL DNA sequencer (Applied Biosystem, USA).

Blast (2.0) and chromas 1.45 (http://www.technelysium.com.au) were used to analyze DNA sequencing data [18]. Single nucleotide polymorphisms (SNPs) were identified as differences between the PCR products of the *LTF* gene in all investigated animals and the reference sequences found in GenBank. The MEGA6 tool was used to identify differences in the amino acid sequence of the *LTF* gene between the enrolled does, based on sequence alignment. In addition, a neighbor-joining phylogenetic tree was constructed to elucidate clustering among the investigated DNA sequences and GenBank sourced reference [19].

3. Results

3.1. Clinical findings

Clinically, healthy Baladi do produce typical, regular milk and do not have any mammary anomalies. Patients with mastitis have swollen, heated, hard, and extremely painful udders. Milk production was also drastically reduced and the milk was dense and yellow. The respiratory and rectal temperatures in this group increased. The mean rectal temperature in this group was 41.5 °C.

3.2. Association between nucleotide sequence variation in LTF gene and mastitis susceptibility

SNP variations in amplified DNA nucleotides associated with mastitis incidence were found in the results of PCR-DNA sequencing on both normal and affected does for the *LTF* (430-bp) gene (submitted to GenBank with accession numbers gb|PP037928| and gb|PP037929| for healthy and mastitic does). Of the 30 investigated does (15 healthy and 15 mastitic), five healthy does exhibited the G299A SNP. Representative DNA sequence differences between the GenBank-sourced nucleotide sequence and the *LTF* gene examined in this study were used to verify the discovered SNP (Fig. 1).

Fisher's exact test revealed considerably different occurrences of the *LTF* gene in the normal and mastitis-affected does ($P < 0.01$). *LTF* gene had exonic region mutations, which resulted in different coding DNA sequences in normal does versus mastitis-affected does. One nonsynonymous SNP

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LC639818.1      TGTCCCTGGGCTCTTTAGGCCGGTGACCGACCCACTGGGGAAGCAGAGGAGGCTCTGGGC 60
H               TGTCCCTGGGCTCTTTAGGCCGGTGACCGACCCACTGGGGAAGCAGAGGAGGCTCTGGGC 60
M               TGTCCCTGGGCTCTTTAGGCCGGTGACCGACCCACTGGGGAAGCAGAGGAGGCTCTGGGC 60
                ************************************************************
LC639818.1      TGCAGTGACCAGATGGAATTCCCCTTCCCACGAGGGGCTCGTTCAGGGACGGCTGTTTCT 120
H               TGCAGTGACCAGATGGAATTCCCCTTCCCACGAGGGGCTCGTTCAGGGACGGCTGTTTCT 120
M               TGCAGTGACCAGATGGAATTCCCCTTCCCACGAGGGGCTCGTTCAGGGACGGCTGTTTCT 120
                ************************************************************
LC639818.1      CTCTCTCTCTGGTCCAGGCTAAGAGCTCTCGATTCTCTCTACCTTTTCTGCAGCTCCCCA 180
H               CTCTCTCTCTGGTCCAGGCTAAGAGCTCTCGATTCTCTCTACCTTTTCTGCAGCTCCCCA 180
M               CTCTCTCTCTGGTCCAGGCTAAGAGCTCTCGATTCTCTCTACCTTTTCTGCAGCTCCCCA 180
                ************************************************************
LC639818.1      AACCCACTATTATGCTGTGGCCGTCGTGAAGAAGGGCAGCAACTTTCAGCTGGACCAGCT 240
H               AACCCACTATTATGCTGTGGCCGTCGTGAAGAAGGGCAGCAACTTTCAGCTGGACCAGCT 240
M               AACCCACTATTATGCTGTGGCCGTCGTGAAGAAGGGCAGCAACTTTCAGCTGGACCAGCT 240
                ************************************************************
LC639818.1      GCAAGGCCAGAAGTCCTGCCACATGGGCCTTGGCAGGTCCGCTGGGTGGAACATCCCTGT 300
H               GCAAGGCCAGAAGTCCTGCCACATGGGCCTTGGCAGGTCCGCTGGGTGGAACATCCCTAT 300
M               GCAAGGCCAGAAGTCCTGCCACATGGGCCTTGGCAGGTCCGCTGGGTGGAACATCCCTGT 300
                ************************************************************ *
LC639818.1      GGGAATCCTTCGCCCGTTCTTGAGCTGGACAGAGTCGGCCGAGCCCCTCCAGGGAGGTAA 360
H               GGGAATCCTTCGCCCGTTCTTGAGCTGGACAGAGTCGGCCGAGCCCCTCCAGGGAGGTAA 360
M               GGGAATCCTTCGCCCGTTCTTGAGCTGGACAGAGTCGGCCGAGCCCCTCCAGGGAGGTAA 360
                ************************************************************
LC639818.1      GATGTGTGGGGGCTCTGGTGATCTCAGGCTGGGGTCCCTACTCCAGTGGCACCTTCACAA 420
H               GATGTGTGGGGGCTCTGGTGATCTCAGGCTGGGGTCCCTACTCCAGTGGCACCTTCACAA 420
M               GATGTGTGGGGGCTCTGGTGATCTCAGGCTGGGGTCCCTACTCCAGTGGCACCTTCACAA 420
                ************************************************************
LC639818.1      GCCACTTCGG         430
H               GCCACTTCGG         430
M               GCCACTTCGG         430
                **********
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Fig. 1. Representative DNA sequence alignment of lactoferrin gene (430-bp) between Healthy (H) and mastitic (M) does and GenBank gb|LC639818.1|. Asterisks represent similarity.

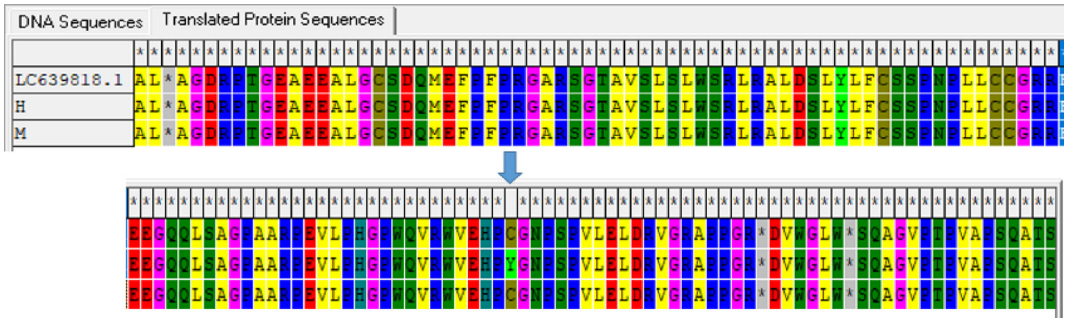

Fig. 2. Representative amino acids sequence alignment of lactoferrin gene (430-bp) between Healthy (H) and mastitic (M) does and GenBank gb|LC639818.1|. Asterisks represent similarity.

(G299A) was discovered by DNA sequencing of *LTF* gene in five healthy does, which caused the amino acid C100Y to be substituted. The representative amino acid sequence alignment of *LTF* gene (430-bp) between healthy and mastitic does and the reference sequence available in GenBank are shown in Fig. 2.

The neighbor-joining constructed phylogenetic tree of the representative sequenced samples compared with accession number gb|LC639818.1|available in GenBank showed that healthy animals were found in one cluster and mastitic animals were found in different clusters, as shown in Fig. 3.

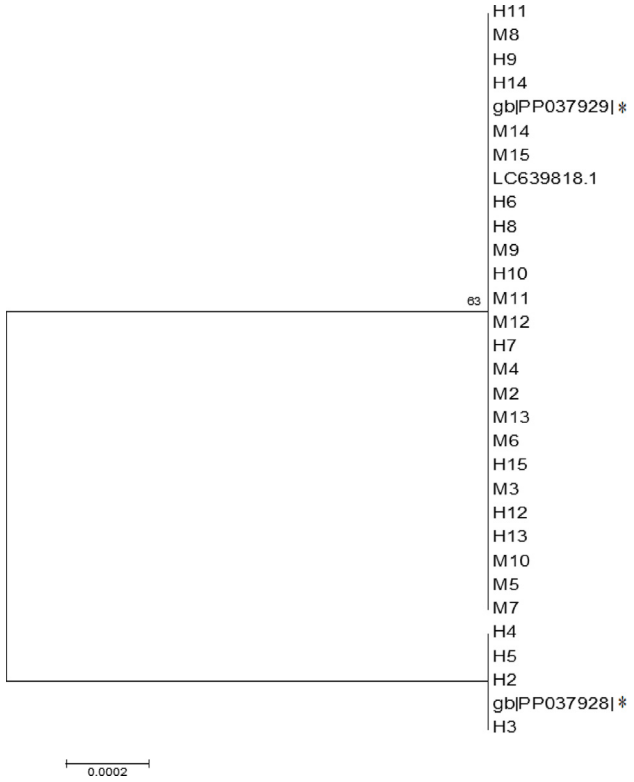

*Fig. 3. Neighbor joining phylogenetic tree of lactoferrin gene between the healthy (H) and mastitic (M) goats compared with GenBank gb|LC639818.1|. * is our submitted GenBank sourced sequences.*

4. Discussion

Knowing the genes, fundamental mutations, and communication with other variables that confer resistance to animals is crucial for the efficient exploitation of disease-resistant sheep or the complete elimination of diseased animals [20]. In this context, PCR-DNA sequencing was carried out for the molecular characterization of a 430-bp fragment of *LTF* gene in Baladi goats exposed to environmental conditions in Egypt. Our results revealed nucleotide sequence variations among the enrolled goats associated with mastitis susceptibility (submitted to GenBank with accession numbers gb|PP037928| and gb|PP037929| for healthy and mastitic does). Interestingly, the denoted SNP was considered novel when compared with the GenBank sequence.

Our findings demonstrate the presence of one nonsynonymous SNP, G299A, in the *LTF* gene (430-bp). According to the results of a basic local alignment search tool (BLAST), the altered base of the sheep database was conserved (GenBank accession number FJ541507.1). Additionally, the buffalo database contained the G299A SNP (GenBank accession number EU581859.1). The conservation behavior in the altered bases may be attributed to close relatedness among ruminant species, where genetic resource conservation programs contribute to an increase in the number and preservation of valuable gene reservoirs [21]. Another cause is PCR-DNA sequencing of the conserved part (CDS) of the *LTF* gene [22].

Little information is available on polymorphisms in *LTF* gene and mastitis in goats. The initial evidence for this connection can be found in the gene sequences from *C. hircus* that were used in our work and were published in PubMed. In contrast to

earlier studies, this study investigated polymorphisms using SNP genetic markers to compare the prevalence of mastitis in healthy individuals and those with mastitis.

Genetic characterization of breeds, biodiversity evaluation, and conservation decisions have been transformed using SNP genetic markers [23]. SNP research may offer a more accurate understanding of the evolution of European cattle than other markers [24,25]. SNPs are thought to be particularly important in the search for connections between a marker at an unidentified gene locus and a known site in the genome. It is possible to assess a phenotypic effect by understanding its genetic basis, making the search for such relationships essential [26,27].

In terms of the relationship between *LTF* gene polymorphisms in livestock, an association between *LTF* and subclinical mastitis in goats has been studied [28]. The authors reported that the different genotypes found in *LTF* were not significantly associated with the occurrence of mastitis. Polymorphism and association studies of *LTF* gene with milk yield, milk composition, and somatic cell count have been conducted in beetal goats [29]. In addition, *LTF* has been shown to be associated with milk composition traits in dairy goats [20,30]. *LTF* is linked to mastitis resistance in Holstein cows [10] and buffaloes [31].

However, the candidate gene method has been used to assess the veracity of mastitis in livestock. [32] reported a correlation between susceptibility to or resistance to mastitis and variations in the *TLR2* gene. Sequence analysis variations in the *TLR2* and *TLR4* genes have been linked to mastitis in river buffalo [33]. In addition, PCR-DNA sequencing of immunological (*SELL*, *ABCG2, FEZL*, and *SLC11A1*) markers from the investigated Holstein and Brown Swiss dairy cows revealed nucleotide sequence alterations in the form of SNPs related to mastitis tolerance/susceptibility [34,35]. *TLR4* gene polymorphisms and mastitis susceptibility have been found to be significantly correlated in sheep [36] and cattle [37]. *TLR4* SNPs were found to be significantly correlated in mastitic Barki sheep [38].

The limitations of this study are as follows: first, a higher number of animals was needed. Second, other breeds of goats should be considered. Third, PCR-DNA sequencing should be conducted for different candidate genes associated with mastitis resistance and susceptibility.

5. Conclusion

In 30 Baladi goat breeds (15 healthy and 15 mastitic), PCR-DNA sequencing of *LTF* gene revealed a nucleotide sequence variation in the form of SNPs between the investigated healthy and mastitic breeds. By employing genetic markers along with normal goat selection, a promising opportunity exists to reduce the occurrence of mastitis due to these functional variations. The gene domain identified in this study may facilitate future methods for treating mastitis in goats.

Authors' contributions

Ahmed Ateya conceived and designed the experiment, collected blood samples, and performed the molecular analysis; Osama Muhammad contributed to the molecular analysis; Mohamed Fouda and Basma Hendam analyzed the data. All authors contributed to the writing of the manuscript.

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Data availability

The Data that support the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Ethical approval the authors confirm the ethical policies of the journal, as noted on the journal's author guidelines page, with approval number (code M/82) obtained from the Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University, Egypt.

Informed consent all the authors declare their consent to participate in this study. All the authors declare their consent to participate in this study.

Conflicts of interest

The authors declare no conflicts of interest.

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