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Individual genomic loci in the caprine β-lactoglobulin gene linked to mastitis incidence in Baladi goats

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ORIGINAL ARTICLE

Individual Genomic Loci in the Caprine *β-lactoglobulin* Gene Linked to Mastitis Incidence in Baladi Goats

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Abstract

BACKGROUND: This study aimed to investigate genetic differences in the *β-lactoglobulin* (*β-LG*) gene and its effect on mastitis tolerance/susceptibility in Baladi goats.
METHODS: Blood samples were collected from 30 female Baladi goats to extract DNA and loaded into tubes containing the anticoagulant, disodium EDTA. PCR was performed to amplify the *β-LG* gene's 427 base pairs of the *β-LG* gene.
RESULTS: PCR-DNA sequencing analysis identified *β-LG* gene differences taking the form of C390A single-nucleotide polymorphism correlated with mastitis tolerance in Baladi goats (GenBank with accession numbers gb|PP265530| and gb|PP265531| for healthy and mastitis, respectively).
CONCLUSION: The *β-LG* gene may be an intriguing indicator of mastitis susceptibility or resistance in goats, allowing for marker-assisted selection of tolerant individuals.

Keywords: Baladi does, Individual genomic loci, Mastitis incidence, *β-Lactoglobulin* gene

1. Introduction

Globally, developing nations produce the vast majority of goat milk; thus, goat milk plays an important role in nourishing millions of people and serves as an important source of meat, milk, and fiber [1]. It was recently concluded that goats will continue to play an important role in challenging circumstances in the subtropical tropics, deserts, and Mediterranean regions [2]. Nevertheless, goats are recognized for their ability to adapt to harsh environments [3].

There are four main Egyptian goat breeds, three of which are extensively distributed throughout the country: Egyptian Baladi goats in the Delta, Saidi goats in Upper Egypt, and Barki goats in the Northern Coastal Zone of the Western Desert. The fourth most significant breed is Zaraibi goats (Egyptian Nubian), which have a great reputation as a prospective milk-productive breed and are raised in the Northeast Delta. Other minor indigenous breeds include Wahati goats in the New Valley, Black Sinai goats located in the Sinai Peninsula, and Abouramada-Halaieb-Shalateen goats in the Halaieb-Shalateen triangle [4].

Mastitis minimizes milk production and quality because of the interaction between various factors, including animals, the environment, and microorganisms. Infection of the udder frequently occurs straight up through the teat canal [5]. Thus, numerous microorganisms can influence mammary epithelial cells and alveolar function, impairing milk quality and quantity and posing a severe public health risk [6,7].

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Mastitis may be localized within a single gland or diffuse inflammation in one or more mammary glands. Mastitis affects the parenchymal tissue. It causes the release of toxic compounds, resulting in lesions varying from increasing the infiltration of leukocytes without visible alterations in milk to hyperemia due to high vascular permeability, which can lead to fibrosis, toxemia, and milk loss [8].

β-Lactoglobulin (β-LG) is an important protein in mammalian milk. It has a substantial effect on milk quality. *β-LG* is a highly acid-resistant protein with a molecular weight of 36,000 Da. The complete amino acid arrangement of *β-LG* has been defined, and genetic variations in its sequence have been identified [9]. Previous studies have analyzed *β-LG* gene polymorphisms using restriction fragment length polymorphisms (RFLP). This study investigated the potential association between the *β-LG* gene and the incidence of mastitis in Baladi goats using PCR-DNA sequencing.

2. Materials and methods

2.1. Ethics statement

Sample collection and handling of the animals used in this investigation conformed to the experimental animal protocols published by the Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University (code M139).

2.2. Animals

The current study included 30 female Baladi goats (*Capra hircus*) obtained from a private farm in MitGhamr city, Dakahlia, Egypt. The does were housed in shaded, semi-open pens and nourished with 650 g of concentrate feed mixture and 650 g of alfalfa hay per head each day, with unlimited access to water. A thorough clinical examination was performed on the goats by previously described standard protocols [10]. Clinical mastitis was identified based on physical examination of the mammary glands through inspection, palpation, and evaluation of milk produced for aberrant color and consistency. Physical examination also determined 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 in vacuum tubes containing anticoagulants (sodium fluoride or EDTA) for DNA extraction.

2.3. DNA extraction and polymerase chain reaction

DNA was extracted from whole blood using a Biospin Blood/Cell/Tissue Genomic DNA Extraction Kit (Cat. No. BSC47S1; Bioer Technology, Munich, Hamburg, Germany). Before further analysis, the concentration and purity of the extracted DNA were examined using a Nanodrop (Uv-Vis spectrophotometer Q5000/USA, Waltham, MA, USA).

PCR was carried out to amplify a noncoding fragment (427 bp) of *β-LG* gene using previously described primers [11].

F: 5′- CGGGAGCCTTGGCCCCTCTGG -3′
R: 5′- CCTTTGTCGAGTTTGGGTGT -3′.

The reaction mixture of PCR was performed with a final volume of 50 μl containing 3 μl DNA, 0.5 μl of each primer, 25 μl PCR master mix (GENEDIREX, Waltham, MA, USA), and 21 μl H_2O (d. d. water). The final reaction mixture was placed in Benchmark TC 9639 Gradient Thermal Cycler (Sayreville, New Jersey, USA) and exposed to initial denaturation 94 for 4 min, followed by 34 cycles of denaturation at 94 °C for 40 s, annealing at 60 °C for 1 min, extension at 72 °C for 2 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 ultraviolet using a gel documentation system (Analytik Jena, Munich, Hamburg, Germany).

2.4. DNA sequencing

Before DNA sequencing, primer dimers, nonspecific bands, and other impurities were removed [12]. A PCR purification kit (Jena Bioscience # pp-201s/ Munich, Hamburg, Germany) was used to purify PCR products. To guarantee acceptable quantities and purity of the PCR products, they were quantified using Nanodrop (NanoDrop One/OneC UV-Vis; Thermo Fisher Scientific, Munich, Hamburg, Germany) [13]. The results of PCR-containing target bands in all studied does (15 healthy and 15 mastitic) were submitted for forward and reverse DNA sequencing using an ABI 3730XL DNA sequencer (Applied Biosystems, Munich, Hamburg, Germany).

For analysis of DNA sequencing data, Blast 2.0 and chromas 1.45 (http://www.technelysium.com.au) were utilized [14]. Single-nucleotide polymorphisms (SNPs) have been detected in PCR products of the *β-LG* gene in all animals analyzed and GenBank-based reference sequences. Furthermore, a neighbor-

joining phylogenetic tree was constructed to clarify the grouping between the DNA sequence under investigation and the reference sequence obtained from GenBank [15].

3. Results

3.1. Clinical findings

Clinically, healthy Baladi produce typical, regular milk and do not have any mammary anomalies. Patients with mastitis have swollen, heated, hard, and extremely painful udder. Milk production was drastically reduced and the milk was dense and yellow. 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 β-lactoglobulin gene and mastitis susceptibility

PCR-DNA sequencing revealed SNP differences in amplified nucleotide sequences linked to mastitis incidence in healthy and affected animals for the *β-LG* (427 bp) gene (submitted to GenBank with accession numbers gb|PP265530| and gb|PP265531| for healthy and mastitis does). Among the 30 analyzed does (15 healthy and 15 mastitic), nine healthy ones had the A390C SNP (Fig. 1). The identified SNP was validated using representative DNA sequence variations between the *β-LG* gene analyzed in the investigated does and the reference gene nucleotide sequence obtained from GenBank (Fig. 2).

According to the SNP Fisher's exact test, the *β-LG* gene was shown to be significantly different in both normal and mastitis-affected does ($P < 0.01$). As illustrated in Fig. 3, the neighbor-joining phylogenetic tree of the representative sequenced samples, when compared to the GenBank reference accession number gb|KM817769.1|, revealed that healthy animals exhibiting the discovered SNP were located in one cluster, and the remaining animals were in a different cluster.

4. Discussion

Genetics plays a vital role in milk production, quality, and disease resistance. Genetic polymorphisms could be used in marker-assisted selection for various target traits [16], especially mastitis resistance and milk production traits in small ruminants [17]. Current methods for controlling mastitis rely on prophylactic and antibiotic treatments. Identifying a genetic marker that allows the inclusion of mastitis resistance in selection

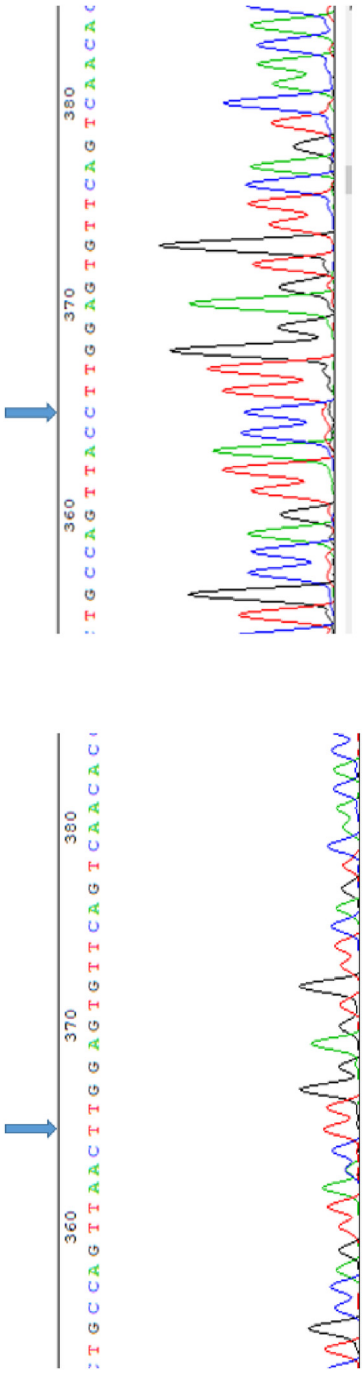

Fig. 1. Partial chromatograph of β-LG gene (427 bp) between healthy (H) and mastitis (M) does indicating T/C SNP. β-LG, β-lactoglobulin; SNP, single-nucleotide polymorphism.

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KM817769.1      CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
PP265530        CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
M2              CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
M3              CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
M4              CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
M5              CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
M6              CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
M7              CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
M8              CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
M9              CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
M10             CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
M11             CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
M12             CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
M13             CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
M14             CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
M15             CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
H10             CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
H11             CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
H12             CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
H13             CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
H14             CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
H115            CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
PP265531        CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
H2              CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
H3              CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
H4              CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
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H8              CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
H9              CGGGAGCCTTGGCCCCTCTGGGGACAGACGACGTCACCCCCGCCTCCCCCATCAGGGGGA    60
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KM817769.1      CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
PP265530        CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
M2              CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
M3              CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
M4              CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
M5              CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
M6              CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
M7              CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
M8              CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
M9              CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
M10             CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
M11             CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
M12             CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
M13             CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
M14             CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
M15             CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
H10             CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
H11             CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
H12             CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
H13             CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
H14             CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
H115            CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
PP265531        CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
H2              CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
H3              CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
H4              CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
H5              CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
H6              CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
H7              CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
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H9              CCAGGAGGGACCGGGACCGCGGTCACCTCTCCTGGGACCCAGGCCCCTCCAGGCCCCTCC    120
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Fig. 2 Representative DNA sequence alignment of β-LG *gene (427-bp) between healthy (H) and mastitis (M) does and reference sequence available in GenBank gb|KM817769.1|. Asterisks represent similarity.* β-LG, β-lactoglobulin.

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KM817769.1      TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
PP265530        TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
M2              TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
M3              TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
M4              TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
M5              TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
M6              TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
M7              TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
M8              TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
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M11             TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
M12             TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
M13             TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
M14             TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
M15             TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
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H11             TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
H12             TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
H13             TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
H14             TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
H115            TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
PP265531        TGTGGCCTCCTGCTCGGGGCCGCTCCTCCTTCAGCAATAAAGGCATAAACCTGTGCTCTC    180
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M7              CCTTCTGAGTCTTTCCTGGACAACGGGCAGGGGGTGGAGAAGGCCCGGCACAGGGTGGGG    240
M8              CCTTCTGAGTCTTTCCTGGACAACGGGCAGGGGGTGGAGAAGGCCCGGCACAGGGTGGGG    240
M9              CCTTCTGAGTCTTTCCTGGACAACGGGCAGGGGGTGGAGAAGGCCCGGCACAGGGTGGGG    240
M10             CCTTCTGAGTCTTTCCTGGACAACGGGCAGGGGGTGGAGAAGGCCCGGCACAGGGTGGGG    240
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M12             CCTTCTGAGTCTTTCCTGGACAACGGGCAGGGGGTGGAGAAGGCCCGGCACAGGGTGGGG    240
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H115            CCTTCTGAGTCTTTCCTGGACAACGGGCAGGGGGTGGAGAAGGCCCGGCACAGGGTGGGG    240
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Fig. 2. (continued).

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H115            AGTGGTCTGGCTCAGAGGATGACAGCGGGGCTGGGATCCAGGGCGTCTGCATCACAGTCT    300
PP265531        AGTGGTCTGGCTCAGAGGATGACAGCGGGGCTGGGATCCAGGGCGTCTGCATCACAGTCT    300
H2              AGTGGTCTGGCTCAGAGGATGACAGCGGGGCTGGGATCCAGGGCGTCTGCATCACAGTCT    300
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H9              AGTGGTCTGGCTCAGAGGATGACAGCGGGGCTGGGATCCAGGGCGTCTGCATCACAGTCT    300
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KM817769.1      TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
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M2              TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
M3              TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
M4              TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
M5              TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
M6              TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
M7              TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
M8              TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
M9              TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
M10             TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
M11             TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
M12             TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
M13             TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
M14             TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
M15             TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
H10             TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
H11             TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
H12             TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
H13             TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
H14             TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
H115            TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
PP265531        TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
H2              TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
H3              TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
H4              TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
H5              TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
H6              TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
H7              TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
H8              TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
H9              TGTGACATCTGGGGGCCCACACACATCACTGTGGCTCTTTGAAACTTTCAGGAACCAGGG    360
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Fig. 2 (continued).

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KM817769.1      AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
PP265530        AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
M2              AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
M3              AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
M4              AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
M5              AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
M6              AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
M7              AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
M8              AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
M9              AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
M10             AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
M11             AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
M12             AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
M13             AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
M14             AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
M15             AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
H10             AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
H11             AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
H12             AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
H13             AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
H14             AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
H115            AGGGACTCAGCAGAGATATCTGCCAGTTACCTTGGAGTGTTCAGTCAACACCCAAACTCG    420
PP265531        AGGGACTCAGCAGAGATATCTGCCAGTTAACTTGGAGTGTTCAGTCAACACCCAAACTCG    420
H2              AGGGACTCAGCAGAGATATCTGCCAGTTAACTTGGAGTGTTCAGTCAACACCCAAACTCG    420
H3              AGGGACTCAGCAGAGATATCTGCCAGTTAACTTGGAGTGTTCAGTCAACACCCAAACTCG    420
H4              AGGGACTCAGCAGAGATATCTGCCAGTTAACTTGGAGTGTTCAGTCAACACCCAAACTCG    420
H5              AGGGACTCAGCAGAGATATCTGCCAGTTAACTTGGAGTGTTCAGTCAACACCCAAACTCG    420
H6              AGGGACTCAGCAGAGATATCTGCCAGTTAACTTGGAGTGTTCAGTCAACACCCAAACTCG    420
H7              AGGGACTCAGCAGAGATATCTGCCAGTTAACTTGGAGTGTTCAGTCAACACCCAAACTCG    420
H8              AGGGACTCAGCAGAGATATCTGCCAGTTAACTTGGAGTGTTCAGTCAACACCCAAACTCG    420
H9              AGGGACTCAGCAGAGATATCTGCCAGTTAACTTGGAGTGTTCAGTCAACACCCAAACTCG    420
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KM817769.1      ACAAAGG 427
PP265530        ACAAAGG 427
M2              ACAAAGG 427
M3              ACAAAGG 427
M4              ACAAAGG 427
M5              ACAAAGG 427
M6              ACAAAGG 427
M7              ACAAAGG 427
M8              ACAAAGG 427
M9              ACAAAGG 427
M10             ACAAAGG 427
M11             ACAAAGG 427
M12             ACAAAGG 427
M13             ACAAAGG 427
M14             ACAAAGG 427
M15             ACAAAGG 427
H10             ACAAAGG 427
H11             ACAAAGG 427
H12             ACAAAGG 427
H13             ACAAAGG 427
H14             ACAAAGG 427
H115            ACAAAGG 427
PP265531        ACAAAGG 427
H2              ACAAAGG 427
H3              ACAAAGG 427
H4              ACAAAGG 427
H5              ACAAAGG 427
H6              ACAAAGG 427
H7              ACAAAGG 427
H8              ACAAAGG 427
H9              ACAAAGG 427
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Fig. 2 (continued).

programs would help reduce the economic impact of the disease as well as the use of antibiotics.

Mastitis has low heritability, making it challenging to select mastitis-resistant animals. Therefore, selective breeding can lower mastitis incidence in goats, using suitable candidate genes associated with mastitis resistance as *β-LG* gene [18]. This study aimed to detect nucleotide sequence variations in *β-LG* gene sequence as a possible genetic marker of mastitis resistance in Baladi goats using PCR-DNA sequencing. In this context, PCR-DNA sequencing was carried out for the molecular characterization of a 427-bp fragment of *β-LG* gene in Baladi goats exposed to environmental conditions in Egypt.

Our results revealed nucleotide sequence variations among the enrolled does associated with mastitis susceptibility (submitted to GenBank with accession numbers gb|PP265530| and gb|PP265531| for healthy and mastitic does). Interestingly, the denoted SNP was considered novel compared to the GenBank reference sequence. In contrast to earlier studies, this study investigated polymorphisms using SNP genetic markers, a more accurate method than RFLP, to compare the prevalence of mastitis in healthy and mastitic individuals. SNP genetic markers have revolutionized breed genetic classification, biodiversity assessment, and conservation decision-making [19]. Compared with other markers, SNP studies may provide a more precise picture of the evolution of European cattle [20,21]. In addition, SNPs are believed to be particularly significant when attempting to find links between a

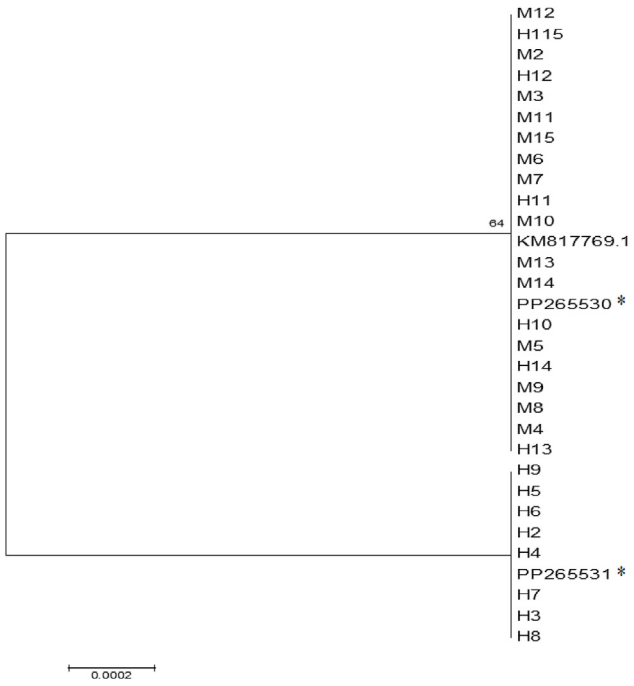

Fig. 3. Representative neighbor-joining phylogenetic tree of β-LG *gene between the healthy (H) (GenBank accession number PP265531) and mastitis (M) (GenBank accession number PP265530). Goats compared with the reference accession number gb|KM817769.1|.* β-LG, β-lactoglobulin.

marker at an unknown gene locus and a known location in the genome. The search for such associations is crucial because it is possible to evaluate phenotypic effects by comprehending their genetic foundation [22,23].

According to the findings of our study, the *β-LG* gene (427-bp) contains a SNP, C390A. The modified mutant base in the buffalo database is preserved based on the results of the basal local alignment search algorithm (BLAST) (GenBank accession number JF274007.1). Furthermore, a database with a modified mutant base for cattle (GenBank accession number DQ489319.1) has been preserved. The close relationship among ruminant species, for whom genetic resource conservation initiatives help boost numbers and preserve important gene reservoirs, may cause the conservation behavior in the modified bases [24].

β-LG gene plays a significant role in mastitis resistance and tolerance in cattle [25]. This gene has bactericidal and bacteriostatic actions against pathogenic agents of mastitis. Previous studies have investigated the relationship between the *β-LG* gene and milk composition via RFLP. *β-LG* gene appears to greatly impact on milk yield and composition (fat and protein) [26,27]. Interestingly, there is previous information on the association between *β-LG* gene and mastitis resistance in ruminants, including cattle [28−31] and sheep [32]; however, there is little information about the relationship between *β-LG* gene and mastitis resistance in goats.

Certain challenges to this study that should be considered in the future. First, a larger number of animals should be considered. Furthermore, several goat breeds should be investigated. Finally, PCR-DNA sequencing should be performed on different potential genes related to mastitis resistance or susceptibility.

5. Conclusion

PCR-DNA sequencing of the *β-LG* gene in 30 animals of the Baladi goat breed (15 healthy and 15 mastitic) demonstrated nucleotide sequence variations (SNPs) between healthy and mastitic goats. These functional differences offer a viable way to reduce the incidence of mastitis using genetic markers alongside regular goat selection. Therefore, the gene investigated in this study provides a useful management strategy for goat mastitis.

Declaration

Ethical approval

The authors confirm the ethical policies of the journal, as noted on the journal's author guidelines page, with approval number (code M139) obtained from the Research Ethics Committee, Faculty of Veterinary Medicine, Mansoura University, Egypt.

Authors' contributions

AA Writing − review & editing, Writing − original draft, Methodology, Data curation, Conceptualization. YA Writing − original draft, Methodology. MF and AK Writing − original draft, Data curation.

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Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of interest

The authors declare no conflicts of interest.

Informed consent

The owner of the private farm has been informed of the nature of the project and agreed to participate in the research project.

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