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Molecular Study of Some Candidate Genes Affecting Milk Production Traits in Holstein Cattle

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ABSTRACT:

Most of the economically important traits in dairy cattle are quantitative in nature, which means that they are affected by environmental factors and by large number of gene. Selection of superior animals has been made more effective through studies of major milk protein genes that are known to affect both milk yield and composition. The present study was carried out to detect polymorphism in *kappa casein (CSN3)* and *beta-lactoglobulin (LGB*) genes in Holstein cattle under Egyptian condition through DNA sequencing and polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP). Fifty Animals were divided into high and low milk producing according to their breeding value. PCR amplification of exon III o *CSN3* and *LGB* was performed followed by restriction fragment length polymorphism (PCR-RFLP) using *Hind-III* and *HinfI* for CSN3 and *HaeIII* restriction endonuclease for *LGB*. Nucleotide polymorphisms between high and low producing cows were detected by DNA sequencing. The restriction enzymes digestion failed to produce restriction patterns and revealed no polymorphism in all studied animals. Comparison of nucleotide sequences between high and low producing cows revealed lack of polymorphism in *CSN3* and four nucleotide changes in *LGB* gene; C179 T, C225T, T246C, and C294G. The further study using other specific restriction endonuclease was required to detect polymorphisms of *CSN3* and *LGB* in Egyptian Holstein cattle. SNPs discovered in this study can be used as molecular genetic markers for marker assisted selection (MAS) to increase and accelerate the rate of genetic improvement of milk production traits.

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1. INTRODUCTION

Milk is an important source of essential nutrients for lactating calves and a key raw material for human food preparations. All over the world people fulfill approximately 13% of their protein requirement from milk and milk products (Reinhardt et al., 2012). Milk proteins polymorphism has been of great interest in animal breeding and dairy industry due to its relationships with production traits, milk composition and quality and its potential use in genetic selection of bovine breeds (Hristov et al., 2013).

Milk protein comprises casein and whey protein. Caseins that make up about 80% of the milk proteins, consisting of four proteins Alpha S1 (CSN1S1, 39-46% of total caseins), alpha S2 (CSN1S2, 8-11%), beta (CSN2, 25-35%), and kappa (CSN3, 8- 15%). Whey proteins have two major fractions alpha-lactalbumin and beta-lactoglobulin (Meignanalakshmi et al., 2013).

Kappa casein *(CSN3)* has been mapped on bovine chromosome (BTA6), spans 13.1 Kb that is arranged in five exons and four introns and the most coding sequences for the mature CSN3 protein are contained in the fourth exon. It is considerably different from other caseins in structure and other properties, serving as a stabilizing factor during the formation of micelle structure in curdling milk and had a great influence on the manufacturing properties of milk (Dinc et al., 2013).

Beta lactoglobulin gene *(LGB*) has been located on bovine chromosome (BTA11), spans 4.7 Kb which is arranged in seven small exon, six introns and encodes 162 amino acid residues, synthesis in the mammary glands during pregnancy and the lactation stages. It could have a role in metabolism of phosphate in the mammary gland and the transport of retinol and fatty acids in the gut. *LGB* affect the milk production parameters, milk fat and quality of milk protein. Their polymorphisms partly explain the genetic variance and improve the estimation of breeding value (Karimi et al., 2009; Miluchova et al., 2011).

Molecular genetic markers represent one of the most powerful tools for the analysis of genomes and enable the association of heritable traits with underlying genomic variation. Additionally, studies of candidate genes and their effect on the

phenotypic traits are the basis for marker-assisted selection (MAS) (Kulibaba and PodStreshnyi, 2012).

The aim of the present work was to detect polymorphisms in kappa casein (*CSN3*) and beta lactoglobulin *(LGB)* genes in Holstein Frisian cattle reared under Egyptian condition through PCR-RFLP and DNA Sequencing.

2. MATERIALS AND METHODS

This study was carried out at the Genetics and Genetic Engineering Lab, Animal Wealth Development Department, Faculty of Veterinary Medicine, Zagazig University.

2.1. Animals and sample collection

Fifty Holstein Frisian cattle were selected from private farm at Algharbia government. The data on animal number, date of birth, date of calving, lactation, milk yield, and lactation length were collected from daily farm records. Animals were divided into high (n = 25) and low (n = 25) milk yield depending on the breeding value of each animal which were calculated according to ***Falconer and Mackay (1996)***. Blood samples (5 mL) were collected from *jugular vein* into sterilized vacutainer tubes containing EDTA as an anticoagulant and then brought to the laboratory in ice box and stored at -20° C till the isolation of DNA.

2.2. Genomic DNA extraction

Total genomic DNA was extracted using DNeasy Blood & Tissue Kit (QIAGEN, Germany) according to the manufacturer's protocol. The quality of the extracted DNA was evaluated by 0.7 % agarose gel electrophoresis and the quantity was measured by UV spectrophotometer taking optical density (OD) at 260 and 280 nm. The 260/280 nm absorbance ratio ranged from 1.7 to 1.9 indicating high quality.

2.3. PCR amplification

Amplification of 438 bp fragment spaning exon III of *CSN3* and 402 bp fragment covering exon I of *LGB* genes were performed using specific primers with the following sequence: F: 5′ GCC ATT GCC TTC TCT GTC AC 3', R: 5′TGG CCT GTA AAG TGA TAC CAA G3' and F: 5′ AGG CCT CCT ATT GTC CTC GT 3′, R: 5′ ATT TGT CAG GCG GCT CTA GC 3′, respectively. The primers were designed with primer3 software using accession no. AY380228.1 and X14710.1 for *CSN3* and *LGB*, respectively. Polymerase chain reaction (PCR) was carried out in a final volume of 25µl, containing 12.5 µl of the master mix (Thermo Scientific, Fermentas), 2 µl of the DNA template, 1 µl of each primer (10 pmol/µl) and deionized water up to 25 µl. amplification was performed using T-professional thermal cycler (Biometra, Germany) with an initial denaturation at 94ºC for 2 min, followed by 40 cycle of 94ºC for 20 sec, 61ºC for 20 sec,72ºC for 45sec with a final extension of 8 min at 72°C . The PCR products were checked by agarose gel electrophoresis using 1.5% agarose gel in 1×TAE buffer. The amplified product was visualized under UV transilluminator.

2.4. PCR- RFLP

The amplified PCR products were digested with three restriction enzymes *HindIII and HinfI* for *CSN3* fragment and *HaeIII* endonuclease for LGB gene fragment. About 5 µl of PCR product was digested with 2.5 units of restriction enzyme using suitable 10× restriction buffer. *HindIII and HinfI* digestion was carried out at 37°C for 20 min and *HaeIII* digestion at 37°C for 5 min. The RE digested PCR products were electrophoresed in agarose gel containing ethidium bromide as staining agent in 1×TAE buffer. The digested products were visualized under UV transilluminator and scored using gel documentation system.

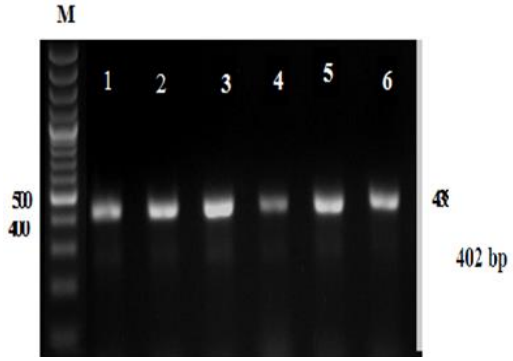

Fig 1. Restriction pattern of exon III of CSN3 gene in low (L) and high (H) milk producing cows lanes (1-5), amplification product lane (6), M: 100 bp ladder.

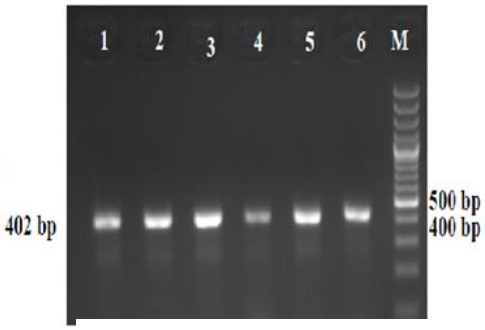

Fig 2. Restriction pattern of exon I of LGB gene in low (L) and high (H) milk producing cow's lanes (1-5), amplification product lane (6), M: 100 bp ladder.

2.5. DNA sequencing

PCR products were purified with Gene JET PCR purification kit (Fermentas) according to the manufacturer's instructions and were directly sequenced using both the forward and reverse primers of PCR amplification. The sequencing process was performed by European Custom Sequencing Centre (GATC Biotech AG, Germany). The obtained sequences were edited manually using Chromas Lite Ver. 2.01, (http://www.technelysium.com.au/chromas.ht) and aligned with CLC Main Workbench 7 and Clustawl Omega software.

2.6. Statistical Analysis

Non genetic factors were corrected or adjusted as described by Schmidt and Vanvleck (1974). The frequencies of gene and genotypes were estimated for each locus by method described by Falconer and Mackay (1996). Association between milk production traits and the genotype of *CSN3* and *LGB* genes could not be achieved due to monomorphism at *CSN3 gene and few non-informative SNPs at LGB gene*.

3. RESULTS AND DISCUSSION

The breeding value (BV) for fifty cows was calculated and animals are ranked according to their corrected milk and BV. Results indicated that the higher milk producing animal, the higher breeding value possess which ranged from 4861 to 8050.

The amplified product of the *CSN3 exonIII* was 438 bp in length. The restriction digestion of this product was conducted with endonucleases *HindIII and HinfI*. These enzymes failed to produce restriction patterns capable of discriminating between the different *CSN3III* genotypes, and produced similar band (uncut 438 bp) revealing no polymorphism in all studied animals (Fig 1).

Monomorphism of the genotype of *CSN3* gene was confirmed by nucleotide sequencing. PCR product of multiple individuals was sequenced and the obtained sequences were analyzed by chromas software (lit 2.01). Sequence comparison of the *CSN3* coding region between high and low milk production cows showed that there was no nucleotide difference in this region. These results are in agreement with the study of Robitaille et al. (2005) and Bartonova et al. (2012).

Several studies were done in the bovine CSN3 gene and mutations in another region were detected, for example, Nari et al. (2011) identified two base substitutions in exon IV resulted in amino acid changes therionine (ACC) and aspartic acid (GAT) at position 136 and 148 respectively given variant A, while in variant B, Isoleucin (ATC) substitutes Threonine and Aspartic acid is substituted by Alanine (GCT). Choobini et al. (2014) identify four SNPs in exon IV in Iranian Holstein cows at positions C10828T and A10863C created the B variant, G10711A created a C variant and A10884G created the E variant of this gene.

On screening the *LGB*/*HaeIII in* 50 Holstein cattle, all the samples showed an identical restriction pattern with the absence of restriction site producing 402 bp fragment only. All the animals revealed only one genotype (Fig 2).

Sequence based marker was informative and able to discriminate and confirm polymorphisms in *LGB* gene (exon I) between different studied animals. PCR product of multiple individuals was sequenced separately and the obtained sequences were analyzed by chromas software. Sequence comparison of the LGB coding region between high and low milk producing animals revealed that, there were four SNPs arise from three base substitutions in coding region (C179 T, and C225Tand T246C) and one in non coding region (C294G) (Fig 3) resulting in one amino acids change at position 11 $^{\text{Thr (ACC) / Ile (ATC)}}$ and three silent base substitutions corresponding to amino acid residues 12 $^{\text{pro (CC}\mathbf{A}\text{_CC}\mathbf{G}\text{)}}$, 26 $^{\text{Thr (AC}\mathbf{C}\text{_ AC}\mathbf{T}\text{)}}$ and 33 $^{\text{Ile (AT}\mathbf{T}\text{_AT}\mathbf{C}\text{)}}$.

Sequencing of these loci revealed variation between the studied animals which was not detected using a conventional PCR-RFLP method. These results are in agreement with the study of Braunschweig and Leep (2006) and Mercadante et al. (2012). The nucleotides sequence in the highest milk production animals' (C179T, C225Tand T246C) can be used as a molecular genetic markers for marker assisted selection (MAS) to increase and accelerate the rate of genetic improvement on milk production trait. Caroli et al. (2009) identified 2 nucleotide differences in exonIV resulted in amino acid change from (GAT) Aspartic acid to Glycine (GGT) at 46 and (G**T**C) Valine to Alanine (G**C**C) at 118. These SNPs were found to be significantly associated milk production traits.

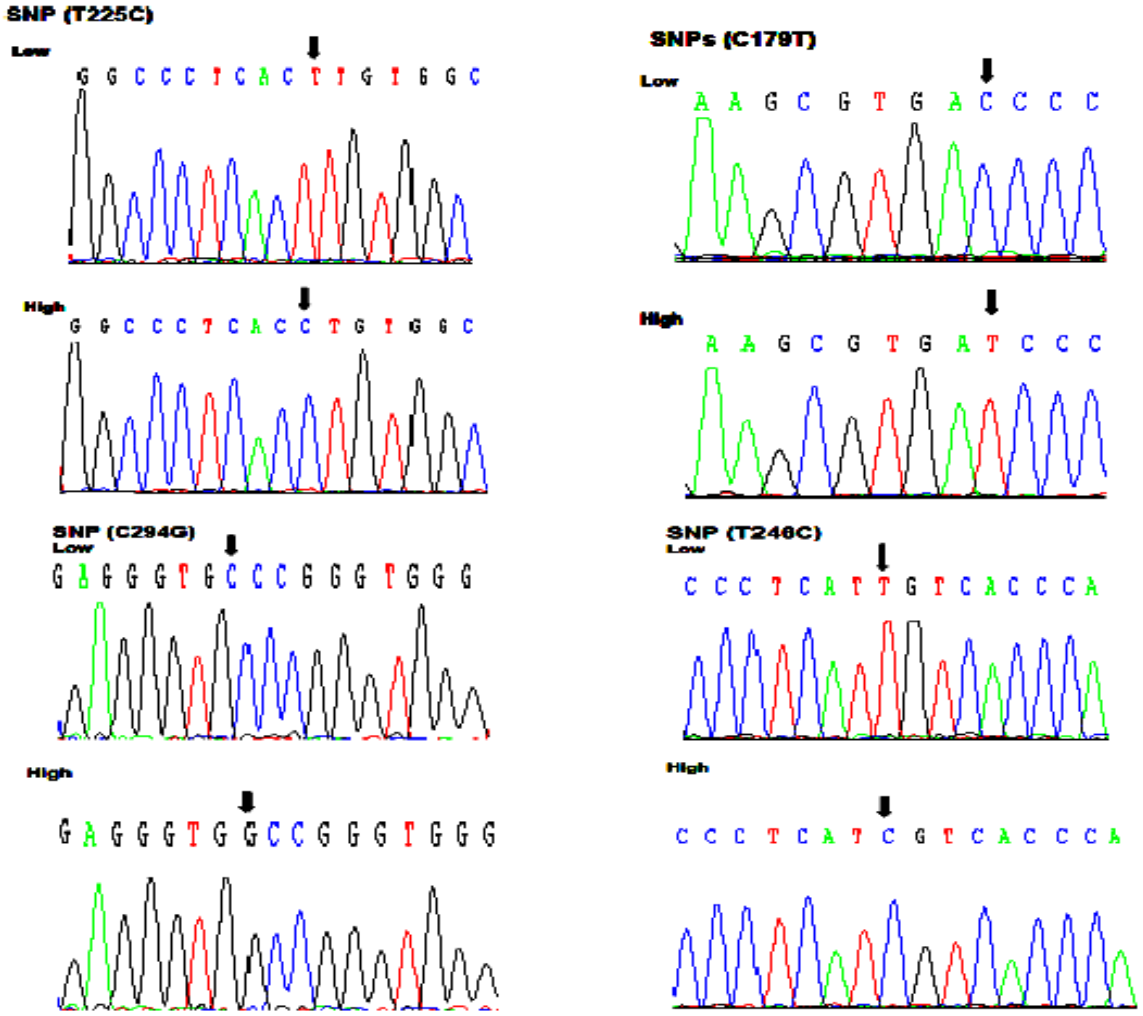

Fig 3. Relative sequenced peaks in exon I of LGB gene in high and low milk producing cattle. Arrows refers to sites of bases change.

4. CONCLUSION

PCR-RFLP failed to produce restriction patterns capable of discriminating between the different *CSN3* and *LGB* genotypes revealing no polymorphism in all studied animals. Further study using other specific endonuclease was required to detect different genotypes of these genes. Four single nucleotide polymorphisms were detected in *LGB*, but no difference in *CSN3* sequences. SNPs discovered in *LGB* in this study provided suitable markers for association studies of candidate genes with milk production traits.

5. ACKNOWLEDGEMENT:

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