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Association of polymorphisms in *kappa casein* gene with milk traits in *Holstein Friesian* cattle

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Abstract

The genetic polymorphism of milk proteins can be a useful guide for selection and an informative marker in breeding research. *Kappa casein* (*CSN3*) is a standout amongst the most vital milk proteins in the mammals that assumes a crucial part in the milk quality and coagulation. Considering polymorphism of *CSN3* and its relationship with milk characteristics in Holstein Friesian cattle was the target of the current study. The PCR-RFLP utilizing *Hind III* endonuclease enzyme and DNA sequencing were performed on DNA samples extracted from fifty animals. Restriction digestion analysis of 633bp PCR product indicated two genotypes AA (uncut 633 bp), and AB (633, 416, and 217 bp) with higher frequency of A allele (0.80) than B allele (0.20). Animals with AB genotypes had a significantly higher milk yield and SNF % (10724 and 9.26%, respectively), whereas animals with AA genotype had a superior estimate effect on fat (3.36%) and proteins (3.14%). Comparison of the nucleotide sequences between different genotypes revealed only one nucleotide changes (A405G), corresponding to the same amino acid residue alanine. Molecular selection for animals carrying the B allele could impact breeding programs for dairy production in Egypt.

Key words: Holstein Friesian cattle, Milk traits, *Kappa casein* gene, PCR-RFLP

Introduction

Milk is a complex biological fluid made out of mainly water, lactose, fat, proteins, organic acids and minerals. It represents a fundamental source of energy, proteins, minerals, and vitamins for young mammals during the first period of their life¹⁰⁾. The major milk proteins of all mammals comprise of the four caseins; α s1-casein, α s2-casein, β -casein and k-casein which represents 80% of total milk proteins as well as the two major whey protein, α -lactoalbumin and β -lactoglobulin which represent 20% of the total proteins. These previous six proteins represent 95% of total proteins in bovine milk¹⁾.

The candidate gene methodology is a standout amongst the most imperative technique to investigate genetic markers Linked to production traits and to determine the polymorphisms in the protein coding regions. Recently, genetic marker research applied to animal breeding and production is concentrated mainly on analyzing mutations located within candidate genes and their association with economically important traits¹²⁾.

CSN3 gene has been located on bovine chromosome 6 and comprised of five exons and four introns⁷⁾. It encoded protein affecting the amount, composition and technical properties of milk. Eleven genetic variants of *CSN3* had been distinguished¹⁴⁾, the most widely recognized alleles

are A and B which differ in two amino acids at positions 136 (Thr → Ile) and 148 (Asp → Ala).

The target of the current study was to analyze *kappa* casein gene using PCR-RFLP followed by DNA sequencing and to explore conceivable relationship with milk production characteristics in Holstein Friesian cattle.

Material and Methods

The current study was conducted at the Biotechnology unit, Animal Wealth Development Department, Faculty of Veterinary Medicine, Zagazig University, Egypt.

Phenotypic data and sample collection:

Fifty Holstein Friesian cattle were randomly selected from private farms and the information including, date of birth, date of calving, lactation, milk yield, and lactation length was gathered from daily farm records. Blood samples (5 ml) were gathered from the jugular vein into cleaned, sterilized vacutainer tubes containing EDTA as an anticoagulant and after that got to the lab refrigerator and put away at - 20° C until DNA extraction. Fifty milk samples were gathered during morning milking in order to represent the whole milking of each animal for fat, total protein, solid not fat and lactose analysis by ultrasonic portable milk analyzer (Milko Tester Model- Master Mini).

DNA Extraction:

DNeasy Blood & Tissue Kit (QIAGEN, Germany) was used for genomic DNA extraction following the manufacturer's protocol. The quality and amount DNA was evaluated by 0.7 % agarose gel electrophoresis and by UV spectrophotometer, respectively.

PCR amplification:

A 633 bp fragment covering intron 3 exon 4, intron 4 in CSN3 gene were amplified with following sequences: F:5'CAGCGCTGTGAGAAAGATGA3' and R:5'CCCATTTGCCTTCTCTGTA3'.

The primers were outlined using Primer 3.0 software and the published nucleotide sequence of the Bos taurus CSN3 gene (GenBank accession AY380228.1). PCR reactions were done in a

total volume of 25 µL, consisting of 12.5 µl master mix (Thermo Scientific, Fermentas), 2 µl DNA template, 1 µl of each primer (10 pmol/µl) and deionized water up to 25 µl. Amplification was carried out in a thermal cycler (Biometa, Germany) with the accompanying conditions: initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 20 sec, 61°C for 20 sec, 72°C for 45sec with a final extension of 8 min at 72°C. The amplified fragments were separated on 1.5% agarose gel electrophoresis, imaged under UV transilluminator. The size of the amplified product was compared with the 100-bp ladder DNA marker.

PCR -RFLP method:

About 5 µl of PCR product was digested with 2.5 units of *HindIII* endonuclease enzymes using suitable 10 × restriction buffers at 37°C for 20 min. The digested products were separated in 1.5% agarose gel containing ethidium bromide as the staining agent in 1×TAE buffer, imaged under UV transilluminator and scored utilizing gel documentation system.

DNA sequencing:

PCR products were purified with Gene JET PCR purification kit (Fermentas) following the manufacturer's guidelines and were straightforwardly sequenced utilizing both the forward and reverse primers of PCR amplification. The sequencing procedure was done by European Custom Sequencing Centre (GATC Biotech AG, Germany). The obtained sequences were edited manually utilizing Chromas Lite Ver. 2.01, (<http://www.techneleysium.com.au/chromas.ht>) and aligned with CLC Main Workbench 7 and Clustal Omega software.

Statistical analysis:

The alleles and genotypes frequencies were defined by method depicted by⁵⁾. Genotype deviation from Hardy-Weinberg equilibrium was evaluated by the exact chi-square test. Associations between CSN3 genotypes and milk production traits (milk yield, fat %, protein % lactose % and solid not fat %) was conducted by least square method of the general linear model (GLM) procedure of the statistical

packages for social science¹³⁾ using the following linear model.

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where, Y_{ij} = Observation of the target trait,
 μ = Overall mean, G_i = Fixed effect of i^{th} genotype,
 e_{ij} = Random error. Additive effects (allele substitution effect) were estimated through adding an additional regression covariate with value 0, 1 and 2 to account for number of different genotypes.

Results and Discussion

Usage of the PCR-RFLP method for distinguishing proof the genetic polymorphism in *CSN3* permitted both fast and effective determination of the genetic variation in this gene, regardless the age and sex of animals, promoting the foundation and elevation the frequency of desired alleles among our Egyptian animals.

Genomic DNA was amplified using specific primers for *CSN3* gene, the amplified product was 633bp. The Restriction digestion of this fragment with *Hind III* indicate the presence of two restriction patterns which are designated as AA (uncut 633 bp), and AB (633, 416, and 217 bp) (Fig.1).

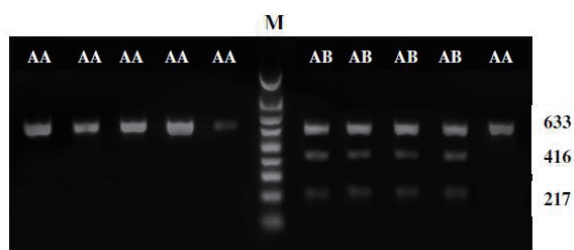


Fig.1. Restriction fragment patterns of Exon 4 of *CSN3* after digestion with *Hind III*. M: 100 bp ladder.

The genotypes denoted in the present study were similar to those revealed by^{4,6)} in Egyptian, Iranian Holstein and Sahiwal cattle breeds, respectively while²⁾ observed three different genotypes AA, AB and BB in Busa cattle, Chinese Holstein cattle and Mexican Jersey cattle, respectively.

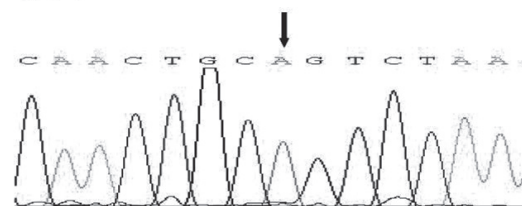
The AA genotype frequency (0.60) was higher than that of AB genotype (0.40) and frequency of A allele (0.80) was higher than that of the B allele (0.20). The distribution of *CSN3* genotypes was fitted with Hardy-Weinberg equilibrium ($P < 0.05$) for the studied population

Our results agree with that of^{14, 9)} they observed a higher frequency of allele A (0.69, 0.76, 0.63 and 0.81 respectively) comparing with allele B (0.31, 0.24, 0.37 and 0.19 respectively) in Holstein cows. Contrary to these findings⁴⁾ reported predominance of the B allele (0.71) in Iranian Holstein cattle³⁾ in Mexican Jersey cattle.

PCR products of multiple individuals were sequenced and the two sequences revealing A and B alleles were deposited in Genbank database under accession numbers (KP894162 and KP894163, respectively). The nucleotide sequence analyses uncovered SNP at the nucleotide position of 405bp (A to G transition) in exon 4 which translated to the same amino acid residue alanine (Fig. 2). This result is on the same line with¹¹⁾ they revealed two synonymous SNPs in an exon IV of *CSN3* gene in Holstein cattle. Also⁴⁾ detected four SNPs; C10828T, A10863C, G10711A and A10884G in *CSN3* gene in Iranian Holstein cows and identified B, C and E variants of this gene.

SNP (A405G)

Genotype (AA)



Genotype (AB)

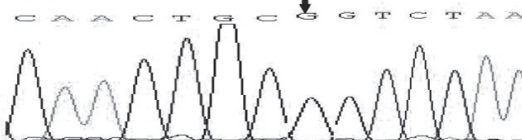


Fig.2. Relative sequenced peaks of *CSN3* genotypes AA and AB.; arrow refers to site of base change.

The associations between *CSN3* genotypes and milk production traits were studied. Animals with genotype AB had a significantly higher milk yield and SNF % (10724 and 9.26%, respectively) compared with Animals with genotype AA (3328 and 8.66%, respectively). However, Animals with genotype AA had a superior estimate effect on fat (3.36%) and proteins (3.14%);

Furthermore, no significant difference between *CSN3* genotypes for lactose % (**Table 1**). The similar effect of *CSN3* genotypes had been noticed by⁸⁾ they showed that animals with *CSN3* AB genotype were better milk producers in Bulgarian Rhodopean cattle breeds, Holstein, and Sahiwal cattle breed, respectively As opposed to these outcomes^{3, 14)} set up that, the *CSN3*

genotype has no statistical effect on daily milk, fat and protein yield. These negating reports can be pointed mostly to some elements, e.g. population size, breed frequency of occurrence of specific variants under study, ways of treating and demonstrating performance traits and the statistical method applied.

Substituting the A allele with the B allele increased the milk yield with 7396.22 kg; this exhibit the predominance of the B allele regarding quantitative milk traits. A similar inclination was noticed for solid % where the substitution of the B allele for the A allele elevate the percentage of milk solids not fat by 0.60% but decreased fat and protein % by 0.38 and 0.48, respectively.

Table 1 Least square means $\pm$ SE for milk production traits in Holstein cattle with different *CSN3* genotypes and Allel substitution effect.

Trait	Genotype(mean $\pm$ SE)			Allel substitution effect (allele B)
	AA	AB	t-value	
Milk yield	3328 $\pm$ 493	10724 $\pm$ 491	-14.97 *	7396.22 *
Fat%	3.36 $\pm$ 0.071	2.98 $\pm$ 0.076	5.38 *	-0.38 *
Protein%	3.14 $\pm$ 0.083	2.66 $\pm$ 0.098	4.86 *	-0.48 *
Lactose%	4.17 $\pm$ 0.050	4.10 $\pm$ 0.052	1.27 ^{NS}	-0.07 ^{NS}
SNF%	8.66 $\pm$ 0.093	9.26 $\pm$ 0.096	-6.38 *	0.60 *

* = Significant difference ($P \leq 0.05$).

NS= Non-significant.

Conclusion

The nucleotide sequence variation in the *Kappa casein* gene and positive association with milk trait can be also a useful information resource for cattle genetic improvement, conservation, and breeding decisions. Also, this study can help in maintaining a high frequency of 'B' allele as the favorable one for increasing milk quality and quantity in our commercial cattle breeds.

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