

## Evaluation of beta-casein locus for detection of A1 and A2 alleles frequency using allele specific PCR in native cattle of Kermanshah, Iran

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**Abstract.** The beta-casein locus with different allelic forms of A1 and A2 has been reported among many breeds of dairy cattle. These allelic forms produce beta-casein variants of A1 and A2. The bioactive peptide of beta-casomorphin-7 is produced from proteolysis of beta-casein A1 variant. Beta-casomorphin-7 has morphine like activity and results in some diseases including type 1 diabetes mellitus, cardiovascular disease, sudden death and madness. The aim of present study was to determine the frequency of beta-casein variants in native cattle of Kermanshah Province. Blood samples were taken from 131 native cattle. DNA was extracted from blood using phenol-chloroform method. A fragment with 854 base pairs including a part of exon 7 and a part of intron 6 was amplified by allele specific-polymerase chain reaction (AS-PCR). The frequency of beta-casein genotypes were in Hardy-Weinberg equilibrium ( $\chi^2=1.17$ ,  $P>0.1$ ). The frequency of A1A1, A1A2, and A2A2 genotypes were 16.8, 42.7, and 40.5%, respectively. The frequency of A1 allele was 38.2 and the frequency of A2 allele was 61.8%. In the present study a relatively high frequency of 38.2% was found for beta-casein A1 allele. Finding this percentage of the beta-casein deleterious allele in the cattle indicates the milk of native cattle from Kermanshah Province might contain a high level of beta-casein A1 variant with subsequent increased level of bioactive peptide of beta-casomorphin-7 and its deleterious effects on health.

**Key words:** Native cattle, beta casein, A1, A2 alleles, milk, Kermanshah.

### Introduction

Milk proteins have high biological value compared to either plant or animal proteins and play significant functional and structural activities in living organisms (Sharma et al. 2013). Two major milk protein groups are caseins and whey proteins ( $\beta$ -lactoglobulin and  $\alpha$ -lactalbumin). Casein, which accounts for around 80% of the total milk protein, is the major component of milk proteins that secreted by cells of the mammary glands (Sulimova et al. 2007). Bovine milk contains four main casein groups, namely,  $\alpha$ s1-,  $\alpha$ s2-,  $\beta$ - and  $\kappa$ -casein accounting for 38, 10, 36, and 13% of total proteins in milk, respectively (Sulimova et al. 2007, Jaiswal et al. 2014). There is also  $\gamma$ -casein, which is produced from degradation of  $\beta$ -casein (Kaminski et al. 2007).

Bovine casein genes locate on chromosome 6q31-33 (Sulimova et al. 2007). Among caseins, the  $\beta$ -casein with 209 amino acids contains 25-30% of total milk proteins (Keating et al. 2008, Haq et al. 2013). There are many genetic variants of  $\beta$ -casein in different breeds of cattle including A1, A2, A3, A4 B, C, D, E, F, H1, H2, I and G. The A1 and A2 variants are the most common forms, the B variant is less common, and the A3 and C variants are rare. Replacement a cytosine nucleotide with an adenine nucleotide in the  $\beta$ -casein gene results in substitution of histidine that is present in A1 variant with proline at position 67<sup>th</sup> of the  $\beta$ -casein chain in A2 variant (Jaiswal et al. 2014, Sharma et al. 2013).

The bioactive peptides are released by enzymatic hydrolysis of proteins with gastrointestinal enzymes, usually pepsin and trypsin (Korhonen & Pihlanto 2006). The A1 variant of  $\beta$ -casein yields the  $\beta$ -casomorphin-7 (BCM-7) as a bioactive peptide with morphine like activity that may play a role in development of some human diseases. The presence of proline instead of histidine in A2 variant prevents the hydrolysis of peptide bond between residues 66<sup>th</sup> and 67<sup>th</sup> in the  $\beta$ -casein A2 and inhibits BCM-7 produc-

tion (Sharma et al. 2013).

In successive gastrointestinal digestion of milk containing the  $\beta$ -casein A1 variant, the BCM-7 level is 4-fold higher than that in milk containing the A2 variant (Kaminski et al. 2007). Published reports suggest the presence of BCM-7 in milk is associated with some diseases including human ischemic heart disease, atherosclerosis, type 1 diabetes mellitus and sudden infant death syndrome (Kaminski et al. 2007). In contrast to A1 allele that could be a risk factor for health, the A2 allele has breeding value to increase the bovine milk and protein production and to decrease the fat level of milk. In order to reduce the frequency of A1 allele, breeding of Poland Holstein bulls has been recommended.

Due to opposite functions of  $\beta$ -casein variants of A1 and A2 on human health and the quality and quantity of milk, the aim of present study was to determine the frequency of  $\beta$ -casein variants of A1 and A2 in native cattle of the Kermanshah Province, Iran.

### Material and methods

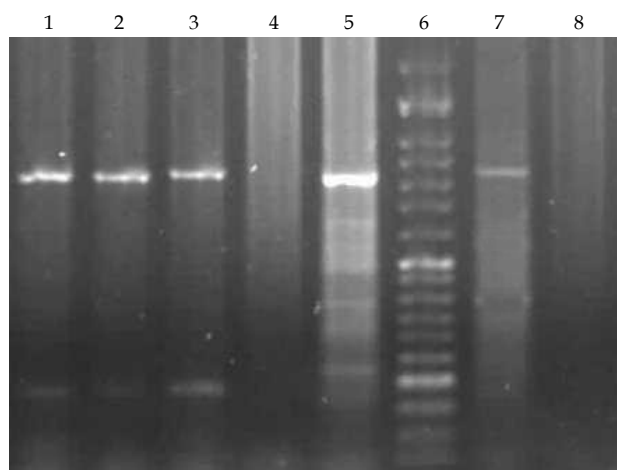
Blood samples were taken from 131 native cattle from the villages of the Kermanshah Province in Western Iran. DNA was extracted from blood using phenol-chloroform method (Rahimi et al. 2006). Extracted DNA was verified by electrophoresis on 1% agarose gel. The concentration and purity of DNA was assessed with a Nanodrop spectrophotometer (Thermo).

A fragment with 854 base pairs including a part of exon 7 and a part of intron 6 was amplified by allele specific-polymerase chain reaction (AS-PCR). The PCR was conducted by Master Cycler Gradient-Eppendorf (Germany). The PCR reagents consisted of 20 pmol of each primer, 300 ng of extracted DNA, 200  $\mu$ M dNTPs, 2.5 mM  $MgCl_2$ , 1 U Taq polymerase and 2.5  $\mu$ l of 10X PCR buffer with final volume of 25  $\mu$ l. The PCR thermal cycling parameters were: 1 cycle at 94 °C for 5 min, 35 cycles by 94 °C for 60s, 58 °C for 60s and 72 °C for 60s followed with final extension for 10 min at 72 °C. The PCR was conducted using the forward primer of 5'-GCC CAG ATG AGA GAA GTG AGG -3', and the reverse primer 1 of 5'-GAT GTT TIG TGG GAG GCT GTT AT -3' for A1 and the reverse primer 2 of 5'-

GAT GTT TTG TGG GAG GCT GTT AG -3' for A2 allele (Keating et al. 2008). The obtained PCR product with 854-bp was electrophoresed on 1% agarose gel. All of the statistical analyses were performed using SPSS statistical software package version 16.0.

## Results

Agarose gel electrophoresis pattern of some PCR products of the beta-casein gene is demonstrated in Fig. 1. The frequency of beta-casein genotypes was in Hardy-Weinberg equilibrium ( $\chi^2=1.17$ ,  $P>0.1$ ). Distribution of beta-casein genotypes in native cattle of Kermanshah Province is depicted in Table 1. The frequency of A1A1, A1A2, and A2A2 genotypes were 16.8, 42.7, and 40.5%, respectively. The frequency of A1 allele was 38.2 and the frequency of A2 allele was 61.8%. In the present study a relatively high frequency of 38.2% was found for beta-casein A1 allele that indicates the milk of native cattle of Kermanshah Province may contain a relatively high percent of the deleterious peptide of beta-casomorphin-7.



**Figure 1.** Agarose gel electrophoresis pattern of AS-PCR products of beta-casein genotypes with 854-bp. Lane 1 shows positive control, lanes 2 and 3 demonstrate A1A2 genotype, lanes 4 and 5 indicate A2A2 genotype, lanes 7 and 8 show A1A1 genotype. The 50-bp DNA molecular weight marker is indicated in lane 6.

**Table 1.** Distribution of beta-casein genotypes and alleles in native cattle of Kermanshah Province.

Parameters	N (%)
Genotype	
A1A1	22 (16.8)
A1A2	56 (42.7)
A2A2	53 (40.5)
Allele	
A1	100 (38.2)
A2	162 (61.8)

## Discussion

Bovine milk and dairy products are the main ingredients of the human diet to fulfill nutritional as well as physiological functions (Keating et al. 2008). Casein and major milk proteins exert various main biological activities. These proteins

are positive regulators of human health with providing critical nutritive elements, and immunological protection (Clare & Swaisgood 2000). Today, increasing attention is being focused on biologically active peptides derived from milk proteins. These peptides are inactive within the sequence of the parent protein molecule and can be released by gastrointestinal digestion of milk, fermentation of milk with proteolytic starter cultures or hydrolysis by proteolytic enzymes (Korhonen & Pihlanto 2006). The A1 and B variants of beta-casein are different from the A2 variant at the position 67 that results in cleavage of the peptide bond upon digestion and releasing of the bioactive peptide of  $\beta$ -casomorphin-7 (Kaminski et al. 2007). Finding a frequency of 38% for beta-casein A1 allele in the cattle of present study indicates that the milk of native cattle of Kermanshah Province may contain a relatively high percent of deleterious variant of beta-casein and bioactive peptide of  $\beta$ -casomorphin-7. However, in cross breed cattle (Karan Fries) of India, a lower frequency of A1 allele of beta-casein (17.5%) was detected (Jaiswal et al. 2014).

BCM-7 may play a functional role in the development of human diseases such as diabetes mellitus and coronary heart disease. Epidemiological reports from New Zealand show that consumption of A1 variant of beta-casein is associated with higher national mortality rates from ischemic heart disease. However, the benefit of milk containing the A2 beta-casein variant for human health compared to milk with the beta-casein variant of A1 has been suggested (McLachlan 2001). It seems that the populations that consume milk with high levels of A2 variant of beta-casein have a lower incidence of cardiovascular disease and type 1 diabetes mellitus (Laugesen & Elliott 2003). However, consumption of milk with a high level of BCM-7 has been suggested as a main cause of sudden infant death syndrome and neurological disorders such as autism and schizophrenia (Ganguly et al. 2013, Kaminski et al. 2007). McLachlan (2001) demonstrated a correlation between consumption of milk containing beta-casein A1 variant and the incidence of heart disease in 30-69 years old males across 16 countries (McLachlan 2001).

It has been reported that consumption of beta-casein A1 milk resulted in higher levels of cholesterol and higher surface area percent of aorta covered by fatty streaks compared to consuming milk with A2 variant of beta-casein (Kaminski et al. 2007). Laugesen & Elliott (2003) reported an association between consumption of milk with beta-casein A1 variant and type 1 diabetes mellitus in children below 14 years old across 19 countries (Laugesen & Elliott 2003, Truswell 2005). Also, it was emphasized that the A1 allele of beta-casein with affecting on immunological processes could lead to type 1 diabetes mellitus (Truswell 2005). The A1 allele is frequent in American with a frequency of 0.01-0.72 and Danish (0.07-0.71) cattle. Also, this allele is the most frequent allele in Holstein-Friesian (0.31-0.66), Ayrshire (0.43-0.72) and Red (0.710) cattle. In contrast, a high frequency of A2 allele has been observed in Guernsey (0.88-0.97) and Jersey (0.49-0.72) cattle (Kaminski et al. 2007).

In the present study a relatively high frequency (38.2%) was found for beta-casein A1 allele that indicates the milk of native cattle of the Kermanshah Province from Western Iran may contain a relatively high percent of deleterious variant of beta-casein A1 and  $\beta$ -casomorphin-7. Our results could be

confirmed and extended in more studies with larger sample size from other parts of the country and also from Asian countries.

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