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# ASSOCIATION OF THE ACETYL-COENZYME-A CARBOXYLASE α EXON 1 GENETIC PATTERNS WITH MILK TRAITS IN MAHABADI GOAT

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## ABSTRACT

The current study suggests the presence of an association among milk production and genetic polymorphism in the exon I region of Acetyl Coenzyme A Carboxylase α (ACACA) in Iranian indigenous Mahabadi goat. The DNA form 150 does were analysed by PCR-SSCP technique. The results revealed two polymorphic patterns at the exon I region of the ACACA gene on this breed. Each conformation pattern tended to affect milk production, differently ($p<0.05$). While milk fat and protein percentage and somatic cell score were not statistically associated with these patterns. The result indicated the importance of polymorphism in the exon I region of the ACACA gene as an indicator for marker assisted selection in dairy goat breeds.

**Key words:** PCR-SSCP, goat, Acetyl coenzyme A carboxylase α, milk traits

## INTRODUCTION

Among enzymes involved in lipogenesis, acetyl-coenzyme A carboxylase α (ACACA) has been considered for its special regulatory role in fatty acid biosynthesis by catalyzing the ATP-dependent formation of malonyl-coenzyme A (Cronan and Waldrop, 2002; Badaoui *et al*., 2007; Garcia -fernandez *et al*, 2010). ACACA is highly expressed in adipose tissue, lactating mammary gland and liver (Ponce -Castaneda *et al*., 1991; Abu -Elheiga *et al*., 1995). The complete sequence of 7041 bp of bovine ACACA, located in chromosome 19, was first reported by Mao *et al*. (2001). Barber *et al*. (2005) described the regulation of bovine ACACA in a tissue-specific fashion which is transcribed applying 4 different promoters. Shin *et al*. (2011) proposed ACACA gene as a candidate marker for meat quality traits in Hanwoo beef steers. A significant association has also been identified among polymorphic conformations of promoter I of ACACA gene and back fat thickness, triacylglycerol content and fatty acid composition of longissimus dorsi muscle, in various beef breeds (Zhang *et al*., 2010).

Similar sequence to bovine ACACA has been reported for sheep (Barber and Travers, 1995) and goat (Badaoui *et al*., 2007). On these species, ACACA is located in chromosome 11 and encodes a protein including 2346 amino acids (Barber and Travers, 1995). Badaoui *et al*. (2007) sequenced 5.5 kb of ACACA of goat and found about 99% amino acid identity with its ovine and bovine orthologous. They also identified a silent SNP in exon 45 that was associated with milk fat, lactose and somatic cell count, during lactation. By sequencing a fragment of promoter III of ACACA gene in various goat breeds, Signorelli *et al*. (2009) discovered three SNPs that were associated with fat yield in Sannan and the Local Grey breeds. Additional SNP associated with milk yield has also been identified in the exon 45 region of Alpine goats (Crepalidi *et al*., 2013).

The goat genetic resource in Iran is consisted of more than 20 breeds (Valizadeh, 2011) with an estimated population of 20.5 million goats in 2013 (Deputy of Livestock Affairs of Iran, 2014). The Mahabadi goat population, as a dual purpose (milk and meat) breed, is estimated to be approximately 1 million head. This breed is mainly distributed in the East and West Azerbaijan and Kurdistan regions of Iran and contributes to the economy of the rural population of these regions (personal communication, Animal Breeding Center of Iran). Currently, Mahabadi goat is the only local goat breed in Iran that has been gone under regular milk recording program, since early 2011. In the current study we applied PCR-SSCP analysis to discover conformational patterns of the exon I region of ACACA gene in Mahabadi goats. Further, we aimed to report the association of the polymorphic patterns of this gene with milk traits in Mahabadi goats.

## MATERIALS AND METHODS

**DNA extraction and genotyping:** The trials were carried out on the experimental farm of Mahabadi goat in Tehran University, Alborz, Iran. All animal were fed by similar diet. Blood samples were collected from a total of 150

Mahabadi does (ranged in age from 1 to 9 years). Genomic DNA was extracted from each animal whole blood using modified salting-out protocol on whole blood (Miller *et al*., 1988). ACACA gene primer pairs were designed utilizing Vector NTI and based on the DNA sequence of the ovine ACACA gene GeneBank accession no. NC_019468.1. A 388 bp fragment of the exon 1 region of ACACA gene was amplified using PCR primers 5'-GTG GCA AAC GTT GTC TTT CT-3' and 5'-CGT ATG GGC TTC ACT GAC TG-3' (synthesized by Metabion company). The PCR reaction was carried out in a 25µl final volume containing 1X reaction buffer, 0.5 ppm of each primer, 0.2 mM of each dNTP, 2mM $MgCl_2$ , 1 unit of *Taq* DNA polymerase and 150 ng of goat genomic DNA as template. Thermal cycling condition was set at 95°C for 5 min for denaturation, followed by 33 cycles of 95 °C for1 min, 60 °C for 45 sec. for annealing, DNA extension at 72 °C for 45 sec. and final elongation of 10 min at 72 °C. About 10µl of the PCR product was mixed with 8 µl of gel loading solution containing 95% formamide, 20 mM EDTA, 0.05% bromophenolblue and 0.05% xylene cyanol. The volume of the solution was adjusted using distilled $H_2O$. The mixture was denatured at 95 °C for 5 min, cooled on ice for 10 min and loaded on nondenaturing 12% polyacrylamide gels (37.5:1 acrylamide to bis-acrylamide). Electerophoresis was performed in 1x TBE buffer at 250 V for 15 hours at -4 °C. After electrophoresis, the DNA fragments in the gel were detected by silver staining.

**Statistical analysis:** Association analyses of PCR-SSCP conformational patterns with milk traits were performed using SAS 9.2. The following mixed model was used to explore the effect of variation in ACACA gene on milk yield:

$$y_{ijkl} = \mu + Temp_i + Age_j + S_k + b_w(W_{ijkl} - \overline{W}) + b_{int}(Int_{ijkl} - \overline{Int}) + e_{ijkl}$$

Where $y_{ijkl}$=milk yield (g/d), « = overall mean, $Temp_i$=fixed effect of $i^{th}$ genetic pattern (2 levels), $Age_j$ : = fixed effect of $j^{th}$ goat age at parturition (9 levels), $S_k$= fixed effect of kidding season (2 levels : winter and spring), $b_w$=regression coefficient of *W* on *Y* (*W*= the weight of goats at parturition), $\overline{W}$ =average weight of goats at parturition, $b_{int}$= regression coefficient of *Int* on *Y* ($Int_{ijkl}$ = interval from parturition to recording /day), $\overline{Int}$=average interval from parturition to recording, and $e_{ijkl}$ =random residual. Similar model was applied for milk fat and milk protein percentage and somatic cell score (SCS) (log transform of SCC: $SCS1 = log_2\left(\frac{SCC}{10^5}\right) + 3$ (Reents *et al*., 1995)). However, the fixed effect of kidding season was removed from the model because all observations on the latter traits had been collected in winter. Moreover, no record was available for the age of 7 to 9, for these composition traits.

## RESULTS

PCR amplification of exon 1 of ACACA locus was carried out in 150 samples of Mahabadi does. During PCR reaction a fragment of 388 bp of ACACA gene was amplified (fig1). PCR-SSCP method resulted in 2 different genetic patterns (fig 2). The frequency of these patterns was 86% and 14% respectively.

Association analysis of SSCP genetic patterns with production traits is presented in table 1. Results indicated that various genetic patterns of exon I of ACACA gene do not have significant effect on production traits, except for milk production. Milk production was also affected by the age and weight of goats at parturition. Fat and protein percentage and somatic cell score were not affected by goat age and weight at parturition, except for fat percentage that was influenced by goat weight at parturition. Kidding season did not declared any significant effect on milk production.

Least square means of the most frequent genetic pattern (pattern 1) was greater than that of the second one (table 2). Least square means of fat and protein percentage was greater (but non-significant) for genetic pattern 2 compared with genetic pattern 1. While for somatic cell score, a greater least square mean was obtained for the first genetic pattern.

The age of doe at parturition significantly affected milk production. Least square means of production traits for different doe ages are presented in table 3. The greatest and the lowest least square means of milk yield were related to the age 6 and 2, respectively. No significant difference did exist among least square means of fat and protein percentage and somatic cell scores at different doe ages and various ACACA genetic patterns.

**Table 1. Results of analysis of variance (F value) for production traits considering the effect of genetic patterns of the exon I region of ACACA gene**

| Source of Variation | Milk Yield¥ | Fat% | Pro% | SCS |
|---|---|---|---|---|
| Genetic pattern | 3.87* | 0.4 | 0.8 | 0.07 |
| goat age | 6.90*** | 0.87 | 1.89 | 1.53 |
| goat weight at parturition | 8.61** | 7.71** | 0.34 | 1.66 |
| interval from parturition to recording | 14.96*** | 2.26 | 0.13 | 5.43* |
| kidding season | 2.54 | - | - | - |

*:p<0.05 ; **:p<0.01 ; ***: p<0.001

**Table 2. Least square means (standard errors) of production traits at different conformation patterns**

| Pattern | Milk (g) Yield(gr) | Fat% | Pro% | SCS |
|---|---|---|---|---|
| 1 | 635.95(56.76)[a] | 1.82(0.33) | 3.96(0.11) | 4.58(0.41) |
| 2 | 481.19(78.38)[b] | 2.19(0.44) | 4.13(0.15) | 4.38(0.54) |

Lsmeans with different letters are significantly different.

**Table 3. Least square means (standard errors) of production traits at different doe ages**

| Age | Milk Yield(g) | Fat% | Pro% | SCS |
|---|---|---|---|---|
| **1** | 717.43(130.66) | 1.55(1.00) | 3.88(0.34) | 4.82(1.24) |
| **2** | 230.19(104.93)[a] | 2.61(0.60) | 3.98(0.21) | 3.60(0.75) |
| **3** | 491.09(141.18) | 1.77(0.68) | 4.27(0.23) | 4.96(0.84) |
| **4** | 511.67(68.40) | 2.20(0.52) | 3.63(0.18) | 5.93(0.64) |
| **5** | 373.26(67.15)[a] | 2.55(0.56) | 4.46(0.19) | 4.00(0.70) |
| **6** | 839.82(67.10)[b] | 1.32(0.73) | 4.05(0.25) | 3.56(0.90) |
| **7** | 447.56(84.57)[a] | - | - | - |
| **8** | 427.91(109.99)[a] | - | - | - |
| **9** | 988.19(383.55) | - | - | - |

Lsmeans with different letters are significantly different.

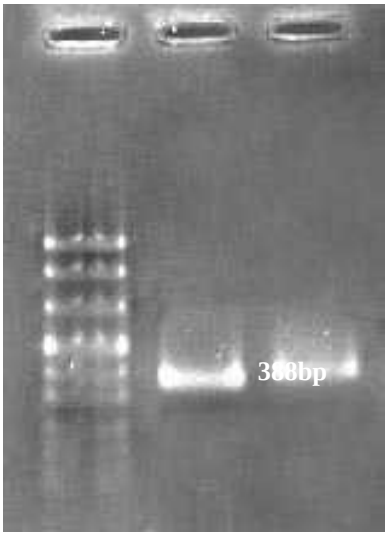


**Fig 1. PCR product of the exon I of the ACACA gene**

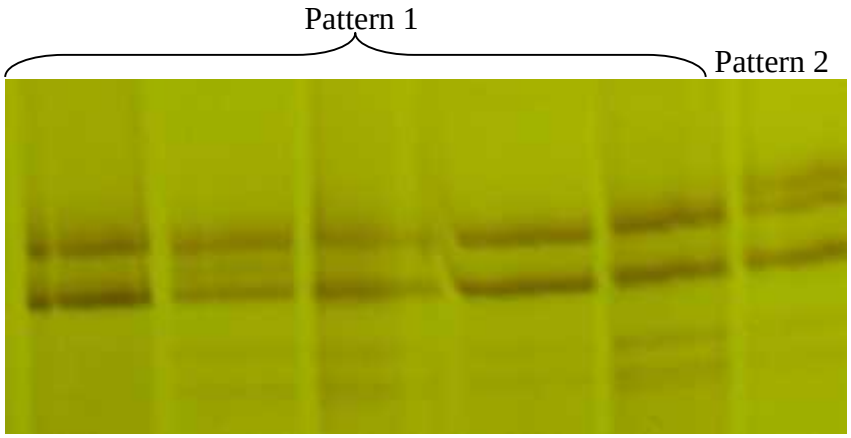


**Fig 2. PCR-SSCP electrophoretic separation of the exon I of the ACACA gene**

## DISCUSSION

Milk quantity and its composition are of economic importance in domestic animals and are under the control of either multi-genes or environmental factors. Scientific researches on genetics and genomic technologies have helped in identification of genes or SNPs associated with genes that affect milk production and its composition (Moioli *et al*., 2005; Badaoui *et al*., 2007; Ibeagha-Awemu *et al*., 2008; Macciotta *et al*., 2008; Deng *et al*., 2010; Zidi *et al*., 2010) in goat. So far, several studies have reported the role of ACACA gene polymorphism on economic important traits in goat (Badaoui *et al*., 2007; Signorelli *et al*., 2009; Crepaldi *et al*., 2013) and other domestic animals (Moioli *et al*., 2005; Zhang *et al*., 2010). However, none of these studies have involved the association of the exon I region of ACACA gene with these traits. According to the results we identified two different genetic patterns in exon I of the ACACA gene. Association of these patterns with various production traits including milk production, milk fat, milk protein and milk somatic cell score were identified. The genetic patterns had a significant effect on milk production. Hence the possible SNP on the sequence of exon I of ACACA could be considered as a potential candidate DNA marker for milk quantity of Mahabadi goats in Iran. Eight SNPs in promoter I region of the ACACA gene of cross bred cattle have been reported to be associated with adjusted back fat thickness, fatty acid composition of *longissimus dorsi* muscle and triacylglycerol content by Zhang *et al* (2010). Studies about the association of ACACA gene with milk production traits in sheep (Moioli *et al*., 2005) and goat (Badaoui *et al*., 2007) have showed that the SNP markers is associated with fat yield, lactose content and somatic cell count. Considering that ACACA gene influences the biosynthesis of fatty acids, we expected that the potential SNPs on this region affect the milk quality, as well. In contrary to our expectation, detected genetic patterns did not influence the milk fat, significantly. However, milk fat and milk protein percentage were greater in the second pattern. It could be due to the role of exon I in biosynthesis of fatty acids in liver and adipose tissue. ACACA gene is expressed in a tissue specific fashion. Transcription of ACACA gene could be initiated from three different promoters. Promoter I which includes exon I, in addition to exon 5 and /or 4, is regulated by nutritional state and its expression is related to the fatty acid synthesis and storage capacity of the adipocyte and no transcript of this promoter is expressed in mammary tissue (Travers and Barber, 2001). While promoter 2 and 3 transcripts increases during lactation. It could be the reason for weak association of milk fat and milk protein percentage with polymorphism in the exon I region of this gene. It is necessary to sequence the amplified fragment to identify potential SNPs on this area. The results of the current study need to be followed in other goat breeds in order to achieve a more reliable understanding on the diversity of this locus and to confirm SNP markers effects of the exon I region of ACACA gene on milk traits in goat.

**Conclusion:** In this preliminary study we found that genetic patterns of the exon I of the ACACA gene is significantly associated with milk quantity. The SSCP polymorphic variation makes it a potential candidate for the establishment of associations with quantitative traits (Malveiro *et al*., 2001). Hence the ACACA gene could be a useful DNA marker for the study of milk yield in Mahabadi goat of Iran. Subsequent sequencing of the obtained patterns is necessary to find out the potential SNPs in this area. However, before choosing a specific pattern as a candidate gene, we need to expand the current study with more animals and more breeds to establish a strict and a more reliable hypothesis about the significant association of the observed polymorphism and milk production.

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