IJAS Iranian Journal of Applied Animal Science

Genetic Polymorphism Detection of the Exon 1 Region of Acetyl-CoA Carboxylase Alpha Gene in Iranian Mahabadi Goat Breed

Short Communication

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Received on: 25 Feb 2015
Revised on: 10 Apr 2015
Accepted on: 30 Apr 2015
Online Published on: Dec 2015

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Online version is available on: www.ijas.ir

ABSTRACT

Acetyl-coenzyme A carboxylase α (ACC-alpha) is considered as the key regulatory enzyme in fatty acid biosynthesis. ACC-alpha gene is located on Caprine chromosome 11 and is polymorphic in many goat breeds. In the current study, we aimed to find possible single nucleotide polymorphisms (SNPs) in the exon 1 region of the ACC-alpha gene in Iranian Mahabadi goat breed. Genomic DNA was extracted from blood samples of 150 Mahabadi does. The exon 1 region of the ACC-alpha gene was amplified to produce a 390 bp fragment. The PCR products were analyzed by both polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP) techniques. RFLP was performed utilizing *HinfI* endonuclease enzyme. No polymorphism was observed after digestion of the PCR products using *HinfI*. However, SSCP of the PCR products revealed two conformation patterns at the exon 1 region of goat ACC-alpha gene with frequencies of 86% and 14%, respectively. Sequencing the observed conformation patterns presented more than 10 SNPs compared with its reported squences in the exon 1 region of ovine ACC-alpha gene. Most observed polymorphic sites did not follow the Hardy-Weinberg equilibrium. Sequence results indicated more similarity in the exon 1 of the ACC-alpha gene among indigenous sheep and goat breeds.

KEY WORDS acetyl-CoA carboxylase alpha gene, Mahabadi goat, polymorphism.

INTRODUCTION

Acetyl-CoA carboxylase α (ACC-alpha) is the key regulatory enzyme in fatty acid biosynthesis. This biotin-dependent enzyme is regulated by the Acetyl- CoA carboxylase α gene (ACC-alpha) and can catalyze irreversible carboxylation of Acetyl-coA for producing malonyl-CoA (Badaoui *et al.* 2007; García-Fernández *et al.* 2010a). Malonyl-CoA is considered as substrate for the synthesis of long chain fatty acids (García-Fernández *et al.* 2010b). Lipogenic tissues including adipose tissues, liver and lactating mammary gland are the target tissues where the ACC-alpha is predominantly expressed (Ponce-Castañeda *et al.* 1991; Zhang *et al.* 2010). The complete sequence of the bovine ACC-alpha, located in chromosome 19, was first reported by Mao *et al.* (2001). Bovine ACC-alpha is regulated in a tissue-specific pattern depending on four various promoters (Barber *et al.* 2005). The ACC-alpha gene in sheep is under the control of a single gene located in chromosome 11 (Barber and Travers, 1995). This gene involves a number of 58 exons which encodes a protein including 2346 amino acids (Barber and Travers, 1995). By sequencing a large

fragment of caprine ACC-alpha gene, Badaoui *et al.* (2007) reported the existence of a great amino acid identity among goat and sheep. Genetic polymorphisms of the ACC-alpha gene have been reported in various species including cattle, sheep, goat and fish (Badaoui *et al.* 2007; Signorelli *et al.* 2009; Zhang *et al.* 2010; Cheng *et al.* 2011; Crepaldi *et al.* 2013).

Several studies have also reported related association effects between ACC-alpha gene polymorphisms and milk and meat traits in farm animals (Crepaldi *et al.* 2012; Crepaldi *et al.* 2013; Badaoui *et al.* 2007; Milanesi *et al.* 2010). ACC-alpha gene has been proposed as a candidate marker for meat quality traits in Hanwoo beef steers (Shin *et al.* 2011). Significant association has also been reported among polymorphic conformations of promoter I of the ACC-alpha gene and back fat thickness, triacylglycerol content and fatty acid composition of longissimus dorsi muscle (Zhang *et al.* 2010). Badaoui *et al.* (2007) identified the association among a silent SNP existing in the exon 45 region of the ACC-alpha gene and milk fat, lactose and somatic cell count in lactating goats. Signorelli *et al.* (2009) reported three SNPs in promoter III of the ACC-alpha gene that was associated with milk fat yield in goat. Possible existing polymorphism in the exon 1 region of the ACC-alpha gene has not been reported in any goat breeds, so far. In this study we aimed to find polymorphism in this region, in Mahabadi goats of Iran using two different methods (including PCR-RFLP and PCR-SSCP).

MATERIALS AND METHODS

Animals

Random blood samples were collected from a total of 150 Mahabadi goats belonging to the experimental farm of the Tehran University, Alborz, Iran. Samples were collected from does at different ages. For each individual approximately 5 mL of vein blood was gathered in EDTA tubes and was transferred to -20 ˚C freezer.

DNA extraction and polymerase chain reaction (PCR)

Genomic DNA was extracted from blood samples using modified salting-out protocol on whole blood (Miller *et al.* 1988). A 390 bp fragment of the exon 1 region of ACC-alpha gene was amplified through PCR technique. Primer pairs were designed utilizing Vector NTI (Vector NTI®, Infor Max Inc, North Bethesda, MD) and based on the DNA sequence of the ovine ACC-alpha gene (Gene Bank access number AJ292285.1). Arrangement of forward and reverse primers (synthesized by the Metabion company) is presented in Table 1. The PCR reaction was carried out in a 25 µL final volume containing 1X reaction buffer, 0.5 ppm of each primer, 0.2 m*M* of each dNTP, 2 m*M* $MgCl_2$, 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 a final elongation of 10 min at 72 ˚C. About 10 µL of the PCR product was mixed with 8 µL of gel loading solution containing 95% formamide, 20 m*M* EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol. The volume of the solution was adjusted using distilled H_2O.

Table 1 The sequence of forward and reverse primers for amplifying the exon 1 region of the acetyl-CoA carboxylase α gene

	Sequence of primers
Forward primer	5'-GTG GCA AAC GTT GTC TTT CT-3
Reverse primer	5'-CGT ATG GGC TTC ACT GAC TG-3'

Restriction fragment length polymorphism (RFLP) technique

Genotyping of each animal was done using RFLP technique. Aliquots of 10 µL of the amplified DNA were digested with 5 U of *Hinf1* endonuclease at 37 ˚C for 10 hours following the supplier's guidelines for buffer condition. The digested products were then visualized by loading on 14% PAGE in 0.5x Tris-Borate-EDTA (TBE) buffer and constant voltage of 100 V for 5 h.

SNP genotyping using PCR-SSCP

Two µL PCR product was admixed with 18 µL SSCP DYE (1960 µL formamid, 60 µL 0.5 M EDTA, 1 µL zinol sianid and 1 µL bromophenol blue). The mixture was denatured at 96 ˚C for 10 min, cooled on ice for 10 min and was loaded on nondenaturing 12% polyacrylamide gels (37.5:1 acrylamide to bis-acrylamide). Electerophoresis was performed in 0.5x TBE buffer at 250 V for 15 hours at -4 ˚C. After electrophoresis, the DNA fragments in the gel were detected by silver staining.

Sequence analysis

The PCR product of each SSCP pattern of the exon 1 region of ACC-alpha gene was purified and sequenced by Biotech Company in both directions (Seoul, south Korea) in order to identify the existing SNPs in different genotypic patterns.

RESULTS AND DISCUSSION

ACC-alpha gene has been reported as a polymorphic marker for selection in milk or meat fat in farm animals (Badaoui *et al.* 2007; Crisà *et al.* 2010; Signorelli *et al.* 2009; Crepaldi *et al.* 2013). Therefore, the effect of the ACC-alpha genetic polymorphism is important due to its potential impact on milk and meat quality traits. As expected, the amplified goat ACC-alpha gene resulted in a

390 bp DNA fragment covering the exon 1 region. *HinfI* recognizes 5'G▼ANTC3' cutting site. No polymorphism was observed after digestion of the PCR products using *Hinf1* restriction enzyme. Under PCR-SCCP two different conformations were detected after electrophoresis on gel (Figure 1).

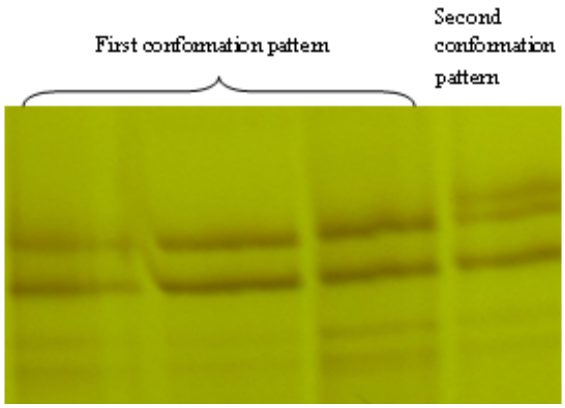

Figure 1 Observed SSCP conformation patterns of the exon 1 of the acetyl-coenzyme A carboxylase α gene in Mahabadi goat

The frequency of these conformational patterns were about 86% and 14%, respectively. Sequence result of these SSCP conformational patterns indicated three monomorphic and 11 polymorphic sites (Table 2) compared with its reported sequence in ovian ACC-alpha gene (AJ292285.1, 98% identity). Monomorphic loci in the population contained 2 insertions (AJ292285.1-4578-4579:->A and AJ292285.1-4599-4600:->G). Allele frequencies and the result of chi-square test for Hardy-Weinberg equilibrium at different loci are presented in Table 2. Also sequence alignment of the studied region in Mahabadi goat declared a greater level of identity (99%) compared with its related sequence reported in Iranian sheep strains (KM460818.1) (Table 2).

Comparing the sequence of the exon 1 region of the ACC-alpha gene in Mahabadi goat breed with registered sequence in gene bank (accession number KM460818.1) represented 11 polymorphic and 1 monomorphic sites in Mahabadi goat breed.

This could be due to higher degree of genetic similarity among sheep and goat strains in Iran compared with the breeds in other countries. The new mutations (insertion type) in Iranian sheep and goat strains observed in this study could be a result of geographical adaptation and it needs to be studied in depth in order to find out its possible effect on resulted protein structure and its biologic performance.

Sequence heterogeneity among strains and species of different geographic areas has also been reported in previous studies (Bozkaya *et al.* 2007).

CONCLUSION

In this study the sequence analysis of the exon 1 region of the ACC-alpha gene was reported for the first time. The results of the current study revealed more than ten polymorphic sites in the exon 1 region of the Caprine ACC-alpha gene compared with its previous reported Ovine sequences after genotyping the observed conformational patterns using PCR-SSCP. This refers to a great level of variability in the exon 1 region of this gene among goat and sheep. On the other hand alignment of the studied region indicated more sequence identity among Iranian sheep and goat strains. Considering the results of this study, it would be more reliable if this study expands for other native goat breeds in order to prove our hypothesis about the observed variability in the studied region. Also further studies have to be designed to find out possible association of the observed conformation patterns with productive traits in goat.

Table 2 Alignment results of the exon 1 region of the acetyl-CoA carboxylase α gene of Mahabadi goat with previous reported sequence in sheep breeds

AJ292285.1	KM460818.1	Pattern 1	Pattern 2	Condition	Observed Allele frequency		χ^2 test result[b]
4412(G>C)	150(G>C)	CC	GG	Polymorph	f(C)=0.86	f(G)=0.14	***
4485(C>G)	-	GG	GG	Monomorph	f(G)=1	-	***
4528(G>C)	266(G>C)	GC	GG	Polymorph	f(C)=0.43	f(G)=0.57	***
4565(A>G)	303(A>G)	AA	AG	Polymorph	f(A)=0.93	f(G)=0.07	NS
4567(C>T)	305(C>T)	CT	CT	Polymorph	f(C)=0.5	f(T)=0.5	***
4569(G>A)	307(G>A)	GA	GG	Polymorph	f(G)=0.57	f(A)=0.43	***
4572(G>C)	310(G>C)	CC	GG	Polymorph	f(C)=0.86	f(G)=0.14	***
4576(C>G)	314(G>C)	GC	GG	Polymorph	f(C)=0.43	f(G)=0.57	***
4578-4579(->A)	-	AA	AA	Mono morph	f(A)=1	-	***
4580(G>C)	319 (G>C)	GC	GG	Polymorph	f(C)=0.43	f(G)=0.57	***
4583(G>A)	322(G>A)	GG	GA	Polymorph	f(G)=0.93	f(A)=0.07	NS
4586(A>T,C)	325(A>T,C)	TT	TC	Polymorph	f(T)=0.93	f(C)=0.07	NS
4599-4600(->G)	-	GG	GG	Mono morph	f(G)=1	-	***
4603(T>A)	343(T>A)	TA	TT	Polymorph	f(T)=0.57	f(A)=0.43	***
-	359(R>A)[a]	AA	AA	Mono morph	f(A)=1	-	***

[a] According to IUPAC nucleotide codes, R refers to A or G.
*** (P<0.001).
NS: non significant.

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