Short Communication

Genetic polymorphism at the growth hormone locus in Iranian Talli goats by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP)

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Abstract
The growth hormone gene could be an attractive candidate gene for milk production in goats. Single-strand conformation polymorphism was used to identify polymorphism at the goat growth hormone (*gGH*) gene. For this purpose, genotyping of 90 Talli goat breeds was performed. Nine conformational patterns were observed in exon 4 of the *gGH* gene, with frequencies of 27.7% for the homozygous pattern (AA) and 72.2% for all of other heterozygous patterns (A/B, A/C, A/B/C, A/B/D/E, A/B/C/F, A/C/F, A/B/E, A/B/F). The results showed that exon 4 of the *GH* gene in Talli goats is highly polymorphic.
Keywords: Growth hormone gene; Polymorphism; goat-PCR-SSCP

The applications of molecular genetics have many important advantages. One such significant advantage is the genotyping of individuals for specific genetic loci (Dekkers, 2004). The genes that affect a polygenic trait such as milk production are not exactly known, however a number of candidate genes with major effects have been recognized. In candidate gene approach to identify genes responsible for variation in a polygenic trait, the process is selection of candidate genes based on the relationship between physiological or biochemical processes involved in the expression of the phenotype then testing the selected genes as putative quantitative trait loci (QTL) (Yao *et al*., 1996). Growth hormone (GH) affects a wide variety of physiological parameters such as lactation (Baldi, 1999), reproduction (Scaramuzzi *et al*., 1999), growth (Breier, 1999), and metabolism (Bauman, 1999). There is evidence of an association between plasma levels of the GH and its genetic variants (Schlee *et al*., 1994). A substitution of a cytosine (C) for a guanine (G) at position 2141 in the bovine growth hormone (bGH) gene causes an amino acid change from Leu (L, codon CTG) to Val (V, codon GTG) at residue 127 (Zhang *et al*., 1992). In homozygous animals either a unique band (211 bp, VV variants) or two bands (159 and 52 bp, LL variants) patterns were observed. Heterozygous animals give a three-band (211, 159 and52 bp) pattern. The *Alu*I (+/-) polymorphism is believed to be related to plasma levels of GH as suggested by Schlee (1994). This author reported that the genotype LL was usually associated with higher circulating concentrations of GH when compared to genotype LV. The Holstein breed has a higher frequency of the Leu allele than the Jersey breed (Lucy *et al*., 1993). Falaki *et al*. (1997) have reported a GH-*Taq*I polymorphism in Simmental and Holstein bulls associated with milk production. Single-strand conformation polymorphism (SSCP) is a powerful method for identifying sequence variation in amplified DNA. Lagziel *et al*. (1996) have found 14 different haplotypes for the

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entire *bGH* gene using the SSCP technique and have reported favorable milk protein percentages with a specific haplotype. Malveiro *et al*., (2001) have analyzed exons 1-5 of the goat growth hormone (*gGH*) gene by the PCR-SSCP method in Algarvia goats and have identified conformational patterns. Their results showed that patterns F/F of exon 4 and A/A of exon 5 are positively associated with milk production ($p<0.05$). Marques *et al*. (2003) have also studied exons 1-5 of gGH gene and have found an association between patterns of exons 2 and 4 with milk yield in two ecotypes of Serrana goats. As pointed out by Malveiro *et al*. (2001) and Marques *et al*. (2003) it has been suggested that the exon 4 is more polymorphic than other exons of *gGH* gene.

In the present study, exon 4 of the *GH* gene was analysed in Talli goat which is an endogenous Iranian breed, with high production potential and good reproduction traits. Talli goats are mainly reared in the south of Iran and Oman, where they are very well adapted to warm and humid areas and play an important role as an economic resource to the rural populations. In the present study, SSCP assay was used to investigate polymorphisms at the *GH* gene in the Talli goats. For this study 90 blood samples (34 female and 56 male animals varying from 6 months to 2 years of age) were randomly obtained from 120 Talli goats reared at the breed conservation station in Bandar Abbas, Hormozgan, Iran. Genomic DNA was extracted from 100 µl of blood by the guanidinium iso thiocyanate-silica gel method (Boom *et al*., 1990). Position 1416-1615 of exon 4 belonging to *gGH* was amplified by PCR culminating in a 200 bp fragment. For this purpose the following forward (5′CTGCCAGCAGGACTTGGAGC 3′) and reverse primers (5′GAAGGGACCCAACAACGCCA3′) were used (Marques *et al*., 2003).

PCR reactions were performed in a 25 µl reaction mixture containing 3 µl of DNA (50 ng), 2.5 µl of 10X PCR buffer, 2.5 mM $MgCl_2$, 2 mM of each dNTPs, 3 µl of a primer mix (5 pmol of each primer), 1 Unit of *Taq* DNA polymerase and 6 µl of ddH_2O. The thermal cycling conditions (Biometra T-Personal Ver: 1.11 thermocycler (Biometra, Germany) included an initial denaturation at 95ºC for 5 min, 30 cycles of denaturation at 95ºC for 30 s; annealing at 60ºC for 30 s and extension at 72ºC for 30 s followed by a final extension at 72ºC for 5 min. Each amplification product was analyzed by electrophoresis on a 2% agarose gel and stained with ethidium bromide (Fig. 1).

For SSCP analysis, 3 µl of PCR products were added to 7 µl of running buffer. The running buffer

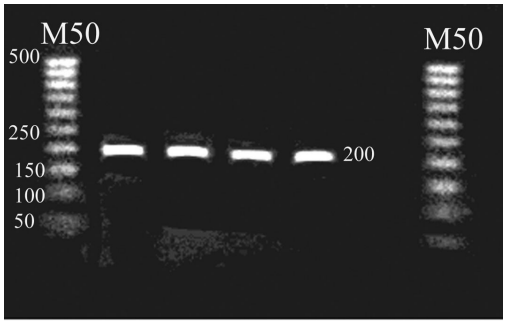

Figure 1. Amplification of a 200 bp fragment from exon 4 of the *gGH* gene in Talli goat. Molecular marker is represented by M50 (IsoGene, Russia) (500, 450, 400, 350, 300, 250, 200, 150, 100, and 50bp).

included 80% formamide, 20% glycerol, 0.05% xylene cyanol, 0.05% bromophenol blue and 0.2% EDTA (0.5 M). After heating at 95ºC for 5 min, the tubes were immediately placed on ice and the SSCP was performed at 7ºC and 200 V on a 8% polyacrylamide/TBE gel for 3-4 h. A constant temperature is essential for band sharpness and reproducibility of strand separation (Hongyo *et al*., 1993). For this purpose the electrophoresis unit was coupled to a thermostatic bath. DNA fragments were visualized by the silver staining method. The observed band patterns were genotyped by comparing it with proposed patterns for exon 4 of *gGH* (Table 1) (Marques *et al*., 2003). In this study nine conformational patterns were observed and only one of them was homozygous, with a frequency of 27.7%. Genotypic frequencies were 11.1%, 25.5%, 13.3%, 3.3%, 1.1%, 2.2%, 14.4%, 27.7% and 1.1% for patterns of A/B, A/C, A/B/C, A/B/D/E, A/B/C/F, A/C/F, A/B/E, A/A, and A/B/F, respectively and allele frequencies were 0.577, 0.162, 0.183, 0.008, 0.056 and 0.014 for A, B, C, D, E and F, respectively (Fig. 2). The equation below was used for the calculation of the genotype frequency of the A/B genotype:

$$F(A/B) = \frac{n(A/B)}{N}$$

Where $F(A/B)$ is the genotype frequency for the A/B genotype, is number of genotypes for the A/B genotype and N is the total number of genotypes in the population.

SSCP polymorphisms can be rapidly and inexpensively detected in a population of animals. Marques *et al*. (2003) have studied exons (1-5) of the *GH* gene in Serrana goats and have reported ten genotypes with a frequency of 96% for heterozygote goats. They have thus concluded an association between these polymor-

Table 1. Proposed band patterns for exon 4 of the *gGH* gene. A-J are SSCP patterns. A, B, C, D, E, F, G, I and J are SSCP patterns and A/B, A/C, A/BC, A/B/D/E, A/B/C/F, A/C/F, A/B/F, A/A and A/B/F are genotypes.

SSCP patterns	A	B	C	D	E	F	G	I	J
Genotype	A/B	A/C	A/BC	A/B/D/E	A/B/C/F	A/C/F	A/B/F	A/A	A/B/F
f									
d									
c									
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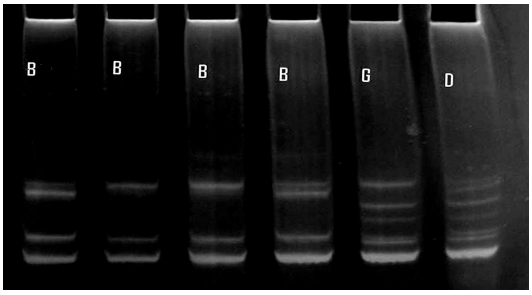

Figure 2. Some genotypic patterns at exon 4 of the *gGH*. B, D and G are SSCP patterns for studied goats.

phisms and milk traits, in which animals with pattern A/B in exon 4 have a superior milk yield. Malveiro *et al*. (2001) have identified 6 homozygote genotypes in exon 4 of the *GH* gene in Algarvia goats using PCR and have shown that animals with pattern FF produce more milk. The present study shows that exon 4 of the *gGH* gene is highly polymorphic. The *gGH* gene could be exploited as a candidate gene for marker-assisted selection (MAS) in dairy goat breeds.

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