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Short Communication

Genetic polymorphisms and protein structures in growth hormone, growth hormone receptor, ghrelin, insulin-like growth factor 1 and leptin in Mehraban sheep

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ABSTRACT

The somatotropic axis, the control system for growth hormone (GH) secretion and its endogenous factors involved in the regulation of metabolism and energy partitioning, has promising potentials for producing economically valuable traits in farm animals. Here we investigated single nucleotide polymorphisms (SNPs) of the genes of factors involved in the somatotropic axis for growth hormone (GH1), growth hormone receptor (GHR), ghrelin (GHRL), insulin-like growth factor 1 (IGF-I) and leptin (LEP), using polymerase chain reaction–single-strand conformation polymorphism (PCR–SSCP) and DNA sequencing methods in 452 individual Mehraban sheep. A nonradioactive method to allow SSCP detection was used for genomic DNA and PCR amplification of six fragments: exons 4 and 5 of *GH1*; exon 10 of *GH receptor* (*GHR*); exon 1 of *ghrelin* (*GHRL*); exon 1 of *insulin-like growth factor-I* (*IGF-I*), and exon 3 of *leptin* (*LEP*). Polymorphisms were detected in five of the six PCR products. Two electrophoretic patterns were detected for *GH1* exon 4. Five conformational patterns were detected for *GH1* exon 5 and *LEP* exon 3, and three for *IGF-I* exon 1. Only *GHR* and *GHRL* were monomorphic. Changes in protein structures due to variable SNPs were also analyzed. The results suggest that Mehraban sheep, a major breed that is important for the animal industry in Middle East countries, has high genetic variability, opening interesting prospects for future selection programs and preservation strategies.

1. Introduction

The conservation of animal genetic resources has been a topic of discussion since the 1950s (Simon, 1984) from biological, economic, cultural and emotional standpoints. It is essential to avoid the loss of genetic variability, in part because these resources may be valuable for future breeding requirements (Hodges, 1984). Genetic variability in indigenous breeds is a major concern considering the necessity of preserving what may be a precious and irreplaceable richness, particularly in light of increasing demands for animal products around the world. Conservation should be based on a deep knowledge of the genetic resources of each specific breed. It is therefore important to make efforts to characterize genetically indigenous breeds. The Mehraban breed of sheep is a major genetically indigenous breed in the animal industry in Middle East countries.

Growth hormone (GH) is one of the major regulators of postnatal growth and metabolism in animals, and thus GH affects growth rate, body compositions, health, milk production and aging by direct actions and indirect effects including the secretion of insulin-like growth factor I (IGF-I) (Ho and Hoffman, 1993; Lincoln et al., 1995; Sumantran et al., 1992). The control mechanism for GH secretion is known as "the somatotropic axis," which includes growth hormone-releasing hormone (GHRH), somatostatin (SRIF), ghrelin (or GH secretagogue, GHS), IGF-I and leptin (Frohman et al., 2000). Bovine GH gene is a single-copy gene located in the band region of q26-qter in cattle chromosome 19. It is composed of four introns and five exons with approx. 1800 bp of length (Vukasinovic et al., 1999), encoding 191 (or 190) amino acid residues with a four-helix structure (Secchi and Borromeo, 1997). The ovine GH and bovine GH genes are 97.5% homologous in the coding regions (Jacqueline et al., 1988).

It is also well known that the binding of GH to GH receptor (GHR) causes receptor dimerization, and thus initiates signaling cascades through the cytoplasmic domain (Frank, 2001), causing biological effects of GH on the target cells by the regulation of the transcription of other genes, including insulin-like growth factor-I (IGF-I), metabolic

Abbreviations: GH, growth hormone gene; GHR, growth hormone receptor gene; GHRL, ghrelin gene; IGF-I, insulin-like growth factor 1 gene; LEP, *leptin* gene; SNP, single nucleotide polymorphism; PCR–SSCP, polymerase chain reaction–single-stranded conformation polymorphism; QTL, quantitative trait loci; SAS, statistical analysis system; Tyr, tyrosine acid; Gln, glutamine acid; Glu, glutamic acid; MAS, marker-assisted selection.

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Table 1
Oligonucleotide primer sets used for the study.

Set	Gene	Primers sequence (5′ → 3′)	Annealing temperature (°C)	Product size (bp)
1	*GH1* (Exon 4)	F: 5′-CCACCAACCACCCATCTGCC-3′ R: 5′-GAAGGGACCCAAGAACGCC-3′	60	214
2	*GH1* (Exon 5)	F:5′-GAAACCTCCTTCCTCGCCC-3′ R:5′-CCAGGGTCTAGGAAGGCACA-3′	62	365
3	*GHR*	F: 5′-GCCAAAACAATAAGACTGGGAACC-3′ R: 5′-GGCTGTAGTGGTAAGGCTTTCTGTG-3′	62	218
4	*GHRL*	F: 5′-CCCTGCTCTGGATGGACTTGGC-3′ R:5′-GGCTTTGGGCATTTAGGACGC-3′	59	114
5	*IGF-I*	F: 5′-ATTACAGCTGCCTGCCCCTT-3′ R: 5′-CACATCTGCTTACACCTTACCCG-3′	60	265
6	*LEP*	F: 5′-AGGAAGCACCTCTACGCTC-3′ R:5′-CTTCAAGGCTTCAGCACC-3′	60	471

enzymes and transcription factors (Rotwein et al., 1994). Mutations in the GHR gene have been associated with Laron-type dwarfism in humans (Godowski et al., 1989), sex-linked dwarfism in chickens (Burnside et al., 1992), growth traits in beef cattle (Hale et al., 2000) and milk production traits in Holstein cattle (Aggrey et al., 1999).

Ghrelin, the endogenous ligand for the growth hormone secretagogue receptor (GHSR), is secreted mainly by the stomach in response to fasting, but it is also synthesized locally in the hypothalamus and various peripheral tissues. Ghrelin stimulates GH release via a dual action on hypothalamic GHRH cells and on anterior pituitary somatotrophs (Korbonits et al., 2004). In our previous study (Bahrami et al., 2012) we investigated the association between single nucleotide polymorphisms (SNPs) in the growth hormone secretagogue receptor (GHSR) genes and carcass traits, and we found that sheep with genotype TT and B protein structure showed a positive effect on carcass weight and body length, and that other genotypes affected the abdominal fat and some blood parameters.

Another gene is *leptin*; besides being a regulator of excessive fat deposition, leptin seems to play an important role during the adaptation of animals to undernutrition. Indeed, the rapid decrease in plasma leptin concentrations in underfed animals could be an acute signal to stimulate refeeding behavior and glucocorticoid secretion, decrease thyroid activity, energy expenditure and protein synthesis, and block reproduction (Ahima et al., 1996; Friedman and Halaas, 1998; Heiman et al., 1999). Leptin is also reported to suppress GH secretion (Roh et al., 2001).

In farm animals, many polymorphisms have been identified in *GH* and discussed in association with animal production. It is well known that DNA variations in exon 5 of *GH1* ($GH^{Leu127Val}$) that cause amino acid substitutions have been associated with growth and fat traits in cattle (Ardiyanti et al., 2009a,b; Chrenek et al., 1998; Schlee et al., 1994; Sneyers et al., 1994). Several investigations have been carried out on genetic polymorphism in *GHR* of human (Buzi et al., 2007; Hadjiyannakis et al., 2001; Millar et al., 2008), chicken (Feng et al., 1997, 1998), cow (Di Stasio et al., 2002, 2005; Ge et al., 2000; Varvio et al., 2008), and sheep (O'Mahoney et al., 1994). Ghrelin (GHRL) and obestatin are encoded by the same gene and propeptide, but different post-translational processes generate two peptides with opposing functions: obestatin was originally proposed to counteract the effects of ghrelin on food intake (Zhang et al., 2005), and ghrelin's original function was depicted in terms of a strong growth hormone-releasing effect (Kojima et al., 1999; Seoane et al., 2000). Five novel single nucleotide polymorphisms (SNPs) were detected in the bovine *GHSR* (Zhang et al., 2007). Bahrami et al. (2012) found that *GHSR* gene polymorphisms may contribute to carcass weight and body length in Mehraban sheep. The combination of location and biological function makes the *GHRL* gene an excellent candidate gene that may impact genetic breeding. The IGF-I gene is extremely conserved among species, and few polymorphisms have been described. The presence of a microsatellite at the promoter region of this gene in cow, human and horse allows analyses of genetic variations related to this locus (Caetano and Bowling, 1998; Kirkpatrick, 1992; Vaessen et al., 2001). Several SNPs have been reported in *LEP* (Buchanan et al., 2002; Lagonigro et al., 2003; Nkrumah et al., 2005). In recent years, studies have been performed to determine the association between *LEP* polymorphisms and carcass traits in animals. Polymorphisms and an association with food intake, milk production, carcass, and meat quality traits in the bovine and ovine *LEP* have been described (Buchanan et al., 2003; Lagonigro et al., 2003; Liefers et al., 2002; Shojaei et al., 2010).

Table 2
Genotypic and allelic frequencies of SSCP variants of *GH1* exon 4 in Mehraban sheep breed.

GH1 exon 4				
Genotype	No.	Genotype frequency	Allele	Allele frequency
AA	303	0.67	A	0.83
AB	149	0.33	B	0.17
Total	452			

As mentioned above, *GH*, *GHR*, *GHRL*, *IGF-I* and *LEP* encoding major factors controlling the somatotropic axis are important candidate genes for the identification of genetic markers for growth, carcass traits, and milk production in livestock animals. The aim of the present study was the evaluation of the genetic variability of *GH*, *GHR*, *IGF-I*, *GHRL* and *LEP* using a nonradioactive single-strand conformation polymorphism (SSCP) protocol. This will be a first-step study for the Mehraban sheep breed toward establishing a breeding program based on marker-assisted selection.

2. Materials and methods

2.1. DNA extraction

Genomic DNA samples were obtained from 452 Mehraban sheep bred at an industrial slaughterhouse in Hamedan Province, Iran.

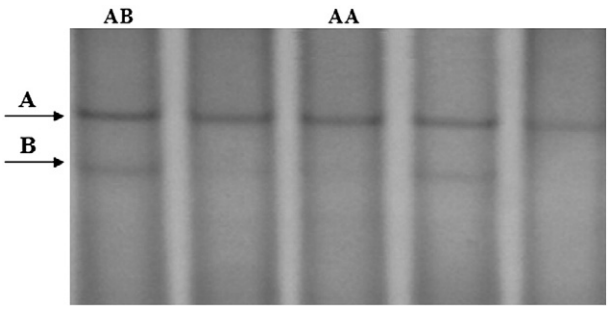

Fig. 1. PCR–SSCP electrophoresis patterns for *GH1* exon 4 gene in Mehraban sheep breed.

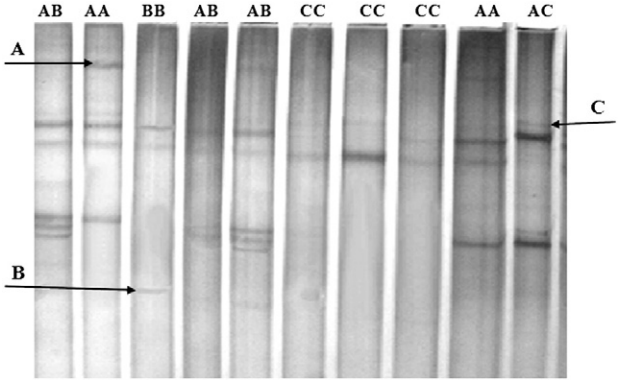

Fig. 2. PCR–SSCP electrophoresis patterns for *GH1* exon 5 gene in Mehraban sheep breed.