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The Study of PIT1 Gene Polymorphism in the Najdi Cattle Using PCR-RFLP Method

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Abstract: The part of the bovine genome which shows a superior action and explains the major part of variation of the economical production traits were known as QTL. PIT1 gene which is also termed hormone factor-1 is a pituitary-specific transcription factor which has responsible for pituitary development and hormone expression in mammals. The main function of PIT1 is for binding and trans-activity promoters of both Growth Hormone (GH) and Prolactin (PRL) gene. It was subjected to different molecular studies as a key role for understanding genetic variation in dairy cattle. This study was aimed to detect the polymorphism in a part of PIT1 gene, exon 6, in Najdi cattle. DNA was extracted from blood sample collected from 84 Najdi cattle and submitted for Polymerase Chain Reaction (PCR) followed by digestion with HinfI restriction enzyme. Base on PCR-RFLP method, the researchers found that the frequency of the A and B alleles of this gene for Najdi cattle were 18.45 and 81.55%, respectively. The genotypic frequencies of AA, AB and BB were 3.57, 29.76 and 66.66%, respectively.

Key words: PIT1 gene, polymorphism, Najdi cattle, allele, growth hormone, prolactin

INTRODUCTION

The growth hormone factor-1/pituitary-specific transcription factor, PIT1, a member of the POU family of homeo-domain transcription factors, activates gene expression for thyrotropin and Prolactin (PRL) and Growth Hormone (GH) but also has a role in pituitary cell differentiation and proliferation (Renaville *et al*., 1997). The inhibition of PIT1 synthesis leads to a marked decrease in expression of PRL and GH to a dramatic decrease in proliferation of cell lines producing PRL and GH. In humans, different mutations of the PIT1 gene also have been reported in patient swith familial pituitary hypoplasia or with sporadic combined pituitary hormone deficiency (Scully *et al*., 2000). Finally, mutations in the PIT1 gene are responsible for the dwarf phenotypes of the Snell and Jackson mice and lead to anterior pituitary hypoplasia. During development, PIT1 gene expression precedes GH and PRL gene expression in somatotrophic and lactotrophic cells, respectively and in the major cell specific activator of hormone expression from this cell types. The PIT1 gene is controlled by several factors that interact with its 5' regulatory the PIT1 gene itself also occurs as there are two PIT1 binding sites in the 5' flanking region. Zwierzchowski *et al*. (2002) explored the molecular mechanism responsible for activation of the PIT1 gene. They described that an enhancer element located >10 kb upstream of the transcriptional start site was essential for pituitary specific expression of the PIT1 gene in transgenic mice. Rajas *et al*. (1998) characterized 12 kb of genomic DNA upstream of the PIT1 promoter. They identified a distal region that decreased the basal transcriptional activity of the PIT1 minimal promoter, indicating that this region behaves as a silencer. This distal regulatory region contains 3 PIT1 auto regulatory elements (Oprzadek *et al*., 2003).

MATERIALS AND METHODS

Samples and bleeding locations: A total of 84 individuals were used in this study. Whole blood samples were collected from the following populations: Shoushtar (n = 32), Shadegan (n = 39), Mahshahr (n = 13). DNA extraction was carried out by the method of Boom *et al*. (1990) as follows: briefly, to an aliquot of 100 μL blood (after thawing), 400 μL of lysis buffer (Guanidin Thiocyanate, 20 mM; EDTA, 20 mM; Tris-HCl, 10 mM; Triton X 100, 40 g L^{-1}; DTT, 10 g L^{-1}) was added, the

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mixture was vortexed and incubated at 65°C for 5 min. The cells were resuspended in 20 μL of nuclease solution (Silica gel: 4 g, Guanidine solution: 100 mL) and spun for 10 sec at 12,000 x g. The pellet was resuspended in 200 μL of lysis buffer again. The suspended white blood cell suspension wasthen added to 400 μL of saline buffer (NaCl, 1M; Tris-HCL, 10 mM; Kcl, 1M and EDTA, 20 mM), the mixture was vortexed and then spun for 10 sec at 5,000 x g.The DNA was precipitated with 45-55 μL of extra gene solution (Ion exchange resin): 10%, orange G color: 0.02%, Triton X 100: 0.01% and was incubated in 65°C for 3-5 min. Then protein was precipitated by centrifugation (3 min at 1000 x g) and the upper layer containing the DNA was transferred to another tube. The relative purity of DNA was determined using a spectrophotometer based on absorbances at 260 and 280 nm, respectively.

PCR-RFLP analysis: The sequences of the forward and reverse primers for the amplification of the PIT1 gene were:

PIT1F: 5'-AAACCAT-CATCTCCCTTCTT-3'
PIT1R: 5'-AATGTACAATGTGCC-TTCTGAG-3'

The polymerase chain reaction for the PIT1 gene was performed in a 25 μL reaction mixture containing 1.5 mM MgC_{12}, 200 μM of each dNTPs, 15 pmol of each primers, 2 μL 10X PCR buffer, 1U Taq polymerase and 100 ng of genomic DNA template. The reaction mixture was placed in a DNA thermal cycler. Thermal cycling conditions included: an initial denaturation step at 94.5°C for 5 min, followed by 30 cycles of 94°C for 40 sec, primer annealing at 56°C for 40 sec, PCR products synthesis at 72°C for 40 sec and final synthesis at 72°C for 4 min using a DNA thermal cycler. Amplified DNA was digested with HinfI enzyme. The digestion product was separated by horizontal electrophoresis through 2% agarose gels in 1X TBE and 1 μM ethidium bromide.

RESULTS AND DISCUSSION

The following DNA restriction fragments were obtained for the PIT1-HinfI polymorphism: 244 and 207 bp for the BB genotype, 451, 244 and 207 for the AB and 451 for the AA (Fig. 1). The BB genotype was the most frequent in all the studied herds 0.6666 followed by the heterozygotic AB 0.2976 whereas the AA was the least frequent 0.0357. The frequency of the $PIT1^A$ ranged was 0.1845 and $PIT1^B$ 0.8155. The genetic equilibrium in the studied population was not detected. Genotype and gene frequencies of three regions investigated separately and the results shown in Table 1. Each population follows Hardy-Weinberg equilibrium. Average heterozygosity for

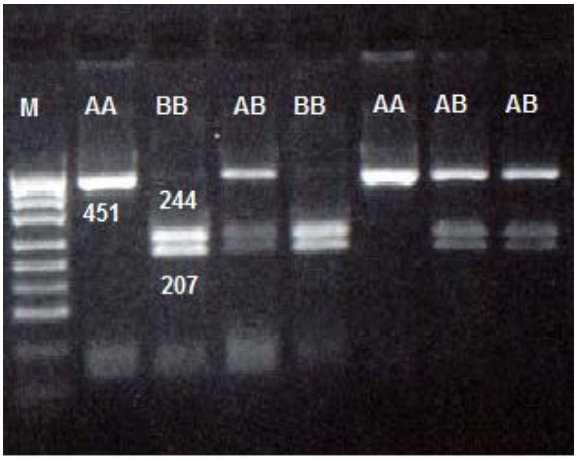

Fig. 1: Representative results of PIT1-HinfI analysis detected by agarose gel electrophoresis (M-DNA marker)

Table 1: The frequency of PIT1 gene and genotypes

	Genotypes (%)			Alleles (%)	
Region	AA	AB	BB	B	A
Shoushtar	6.25	31.25	62.50	78.12	21.87
Shadegan	2.56	25.64	71.79	84.62	15.38
Mahshahr	0.00	38.47	61.53	80.77	19.23

Table 2: The rate of hetrozygosity of najd cattle PIT1 gene in different region

Region	Observed heterozygosity	Expected heterozygosity
Shoushtar	0.31	0.32
Shadegan	0.25	0.25
Mahshahr	0.38	0.30
Average	0.31	0.29

three regions estimated (Table 2). The results showed that the heterozygosity of Mahshahr is the high in compared with others city.

A main goal of the animal breeder is to select superior animals for breeding. Screening favorable alleles for selection at the DNA level provides an ideal tool for marker-assisted selection. RFLP polymorphism within the bovine PIT1 gene was first detected with HinfI nuclease by Woollard *et al.* (1994), Sabour *et al.* (1996) showed that allele A in PIT1 locus positively affected milk production traits in Friesian cattle. This allele (frequency of 0.18) showed a significant superiority over allele B for milk and milk protein yields and body conformation traits within Italian Holstein Friesian cattle.

The allele and genotype frequencies are variable among different studied populations and also the favorable allele and genotype frequencies in Iranian populations (Bos indicus) were comparable to published results, especially on Bos taurus.

The PIT1 A allele frequencies have been estimated to be 0.45 in Angus; 0.26 in Holstein; 0.21 in Herford; 0.28 in Gelbvieh; 0.1 in Brahman; 0.25 in Polish and 0.95 in Gry cattle (Zwierzchowski *et al*., 2002).

A heterozygosity of <0.5 indicated low variation for these genes in studied populations. It is suggested that the strategies such as migration, introduction of new diversity and crossbreeding for increasing gene diversity and its conservation besides exploration of this potential genetic diversity should be adapted.

Although, the allele frequency of B is high for some Iranian populations, the AB genotype (favorable genotype) frequency is not too high.

Therefore, it is suggested that crossbreeding should be done between these populations and/or with exotic breeds to increase the frequency of the favorable genotype.

CONCLUSION

Chi-square test indicated that these two populations were at Hardy-Weinberg equilibrium. Thus, the selection base on these loci in the population is possible to be done.

The results of this study also indicated that there was no any significant effect of the obtained genotypes on milk traits.

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