

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, it 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. χ^2 -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.

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 with 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 demonstrated that an enhancer element, located more than 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* autoregulatory 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 μ L blood (after thawing), 400 μ L of lysis buffer (Guanidin

Thiocyanate, 20 mM; EDTA, 20 mM; Tris-HCl, 10 mM; Triton x100, 40 g L⁻¹; DTT, 10 g L⁻¹) was added, the 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×g. The pellet was resuspended in 200 µL of lysis buffer again. The suspended white blood cell suspension was then 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×g. The DNA was precipitated with 45-55 µL of extra gene solution (Ion Exchange Resin): 10%, Orange G color: 0.02%, Triton x100: 0.01%) and was incubated in 65°C for 3-5 min. Then protein was precipitated by centrifugation (3 min at 1000×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 MgCl₂, 200 µM of each dNTPs, 15 pmol of each primers, 2 µL 10X PCR buffer, 1 U *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.

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

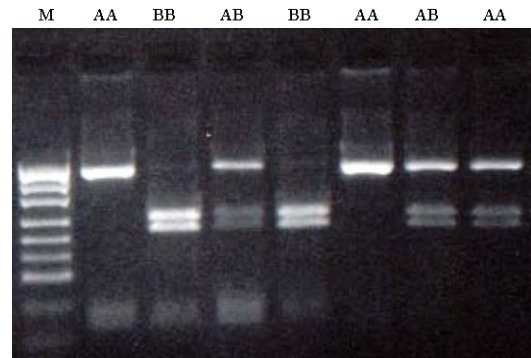


Fig. 1: Representative results of *PIT1-HinfI* analysis detected by agarose gel electrophoresis (M-DNA marker)

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

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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