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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (15): 2575-2578, 2007
ISSN 1028-8880

Polymorphism of the Bovine POU1F1 Gene: Allele Frequencies and Effects on Milk Production in Three Iranian Native Breeds and Holstein Cattle of Iran

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Abstract: The aim of this study was to estimate the allele frequencies in polymorphic site of exon six of POU1F1 gene in three Iranian native and Holstein cattle. Genomic DNA was extracted from 3 Iranian native cattle breeds, including 97 Mazandarani, 87 Sarabi, 112 Golpaygani and also 110 Holstein cattle. A 451 bp fragment of intron 5 and exon 6 were amplified and digested with *Hinf*I restriction enzyme. Frequencies of allele A were 0.37, 0.27, 0.34 and 0.21 for Mazandarani, Sarabi, Golpaygani and Holstein cattle, respectively. Significant differences in genotype frequencies were found between Mazandarani or Golpaygani and Holstein cattle. No significant differences in genotype frequencies were found between Sarabi and Holstein cattle. Transition A to G in nucleotide 1256 is responsible for *Hinf*I(-) allele. No significant association was observed between POU1F1 polymorphism and milk production. Differences in allelic frequency between native *Bos indicus* breeds (Mazandarani, Golpaygani) and Holstein at the present study might be due to differences in origin breeds, low number of samples and/or as the effect of natural selection in native breeds.

Key words: POU1F1, Mazandarani, Sarabi, Golpaygani, Holstein, PCR-RFLP, milk production

INTRODUCTION

Pituitary Specific Transcription Factor 1 (Pit-1) is a 291 amino acid protein with a DNA binding POU domain. Biochemical and ontogenetic studies have shown that Pit-1 is the critical cell-specific transcription factor for activity expression of the prolactin (PRL), growth hormone (GH) genes in the anterior pituitary gland (Bona *et al*., 2004). It activates also other pituitary genes, including POU1F1 gene itself and beta subunit of thyroid-stimulating hormone (TSHb). Mutations in the human POU1F1 are responsible for a Combined Pituitary Hormone Deficiency (CPHD) with deficiencies of growth hormone, PRL and TSH. There is more variability in the degree of hypothyroidism or delay puberty (Bona *et al*., 2004). Results suggest that transcription of the bGH brings at the 8 to 16 cell stage in bovine embryos, possibly under control of the transcription factor, POU1F1 (Joudrey *et al*., 2003). Because PRL and GH are essential for mammary gland development and milk yield, the POU1F1 gene has potential as a marker for genetic variation in yield traits (Renaville *et al*., 1997a). This gene is localized in centromic region of bovine chromosome 1q21-22 (Woolard *et al*., 1999), located midway between TGLA57 and RM95 (Moody *et al*. 1995). Several polymorphisms have been reported in cattle, that POU1F1 can be differentiated by restriction enzymes, including *Hinf*I-RFLP in exon 6 (Renaville *et al*., 1997a and b, Mattos *et al*., 2004). Interval mapping to detect Quantitative Trair Locus (QTL) revealed significant effects on milk and protein yield associated with chromosome 1 in the region of POU1F1 (Georges *et al*., 1995). Renaville *et al*. (1997a) reported that *Hinf*I (-) (allele A) in exon 6 was superior for milk and protein yields, inferior for fat percent and also superior for body depth, angularity and rear leg set in Italian Holstein Friesian cattle. Influence of *Pou1f*1 on angularity is expected because this linear trait is considered to be strongly related to milk production yield. The influence on body depth can be explained by its role as an indirect indicator of body development. They reported a superior effect of allele A for milk (+73.6±44.6 kg) and protein yields (2.93±1.45 kg), inferior for fat percentage (-0.038±0.018%) and superior for body depth (0.301±0.161), angularity (0.235±0.120) and rear leg set (-0.393±0.227). It is reported that the frequency of AA genotype (*Hinf*I -/-) in Canadian

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AI Holstein bulls was higher than in the Italian bulls. The statistical analysis revealed a significant superiority of A allele on milk yield (+222.4±18.5 kg), protein yield (9.17±0.65 kg), but an inferior for fat yield (-2.29±0.51%) (Parmentier *et al*., 1999). The substitution value of a B allele by A was +46.3, +1.9 and +1.5 kg for milk, protein and fat yield, respectively (Parmentier *et al*. 2001). Mattos *et al*. (2004) have reported the heterozygous Gyr bulls were superior for milk fat production in relation to homozygous (+/+). No *Hinf*I -/- homozygous was observed because of low frequency of *Hinf*I (-) allele (0.05). They suggested that opposite results on fat yield in Gyr breed and Holstein might be due to different genomic background, or another linked functional locus, other than POU1F1 can affect milk production. Zhao *et al*. (2004) investigated four region of POU1F1 gene (introns 3, 4, 5 and exon 6) on Angus beef cattle were divergently selected for high or low-blood Serum IGF-1 concentration. They reported two new polymorphisms in intron 3 and one in intron 4, but they had no significant effect on growth and carcass traits. Renaville *et al*. (1997b) reported that the B allele could be associated to early body weight in double-muscle Belgian Blue cattle. No relationships were observed between average daily gain or feed efficiency and POU1F1 genotypes. Di Stasio *et al*. (2002) found no association of the genotypes with meat production traits in Piemontese cattle. The aims of our present study were to identify allelic frequencies in exon six of POU1F1 gene and to evaluate effect of polymorphism on milk production trait in three Iranian native breeds and also in Holstein cattle of Iran.

MATERIALS AND METHODS

DNA samples and data collection: In this study 97 Mazandarani cattle from 14 distance villages, 87 Sarabi and 112 Golpaygani cattle breeds from 4 research institutes and 110 Holstein from 9 Industrial dairy farms in 5 cities were selected randomly. Mazandarani and, Golpaygani breeds are considered as *Bos indicus* and Sarabi breed *as Bos taurus*. DNA was extracted from whole blood (Miller *et al*. 1988) in Iran.

Genotyping and sequence analysis: A specific Primer pairs were used to amplify a 451 bp fragment of intron 5 and exon 6 (Woolard *et al*., 1994). DNA was amplified in a total volume of 25 μL containing 100 ng genomic DNA, 0.2 μM of each primer, 200 μM or 0.2 mM dNTP, 2.5 mM $MgCl_2$, 1 X PCR buffer and 1 unit Taq DNA polymerase. PCR conditions were 94°C for 1 min, 56°C for 30 sec and 72°C for 40 sec with 35 cycles and finally 72°C for 5 min. Ten microliter of PCR product was digested by 10 unit *Hinf*I (Promega, Germany) restriction enzyme. The digested PCR products were electrophoresed and the restricted fragments were determined under UV light. One sample from each genotype was sequenced to control the restriction results. Sequencing PCR was done in a total volume of 10 μL containing, 2 μL Ready Reaction Premix 1 μL Big Dye Sequencing Buffer, 0.5 μL sequencing primer, DNA (2 ng per 100 bp PCR fragment length) and ddH_2O amount to 70μL total volume. The sequencing PCR conditions were 96°C for 10 sec, 50°C for 5 sec and 60°C for 4 min for 25 cycles. The ABI Sequencer 310 (Applied Biosystems, USA) were used for DNA sequencing. Also 1 sample from each genotype was sequenced by ABI Sequencer. All laboratory experiments were conducted in Humboldt University of Berlin, Germany.

Statistical analysis: Frequencies for POU1F1-*Hinf*I polymorphism in all cows were determined by direct counting for each first or second calving year. Milk records were available only for Golpaygani and Holstein breeds. Not all genotypes in the dataset were included in this analysis (Golpaygani, n = 40; Holstein n = 87). Variance analysis was performed using statistical package JMP (version 4.0.4). Milk production records were adjusted for breed, year and season of calving, lactation number and days-in-milk. Statistical linear model included;

$$y_{jklmn} = M + B_i + Y_j + S_k + L_l + P_m + \alpha(x_{ijklmn} - X) + e_{ijklmn}$$

where y_{jklmn} is the milk production measured on each of ijklmnth animal, M is the overall population mean, B_i is the effect of ith breed, Y_j is the effect of jth calving year, S_k is the effect of kth, season L_l is the effect of lth lactation number, P_m is the fixed effect associated with the mth POU1F1 polymorphism, α is the lineal regression coefficient of days-in-milk on milk production and e_{ijklmn} is the random error.

RESULTS

Polymorphism and sequence analysis: Based on Pit-1 sequence (GenBank, BTY15995), there is one restriction site for *Hinf*I enzyme (GAnTC) in 451 bp PCR amplified fragment. In presence of *Hinf*I recognition site, PCR product will be cleaved to 207 and 244 bp fragments. Therefore, three genotypes are recognizable according to *Hinf*I (+) and *Hinf*I (-) alleles on 2 percent agarose gel (Fig. 1). Allele A frequency for Mazandarani, Sarabi, Golpaygani and Holstein breeds were 0.37, 0.27, 0.34 and 0.21, respectively (Table 1). Chi-Square tests showed significant differences between genotype frequencies of *Bos indicus* (Mazandarani and Golpaygani) with Holstein, but there was no difference between Sarabi (*Bos taurus*)

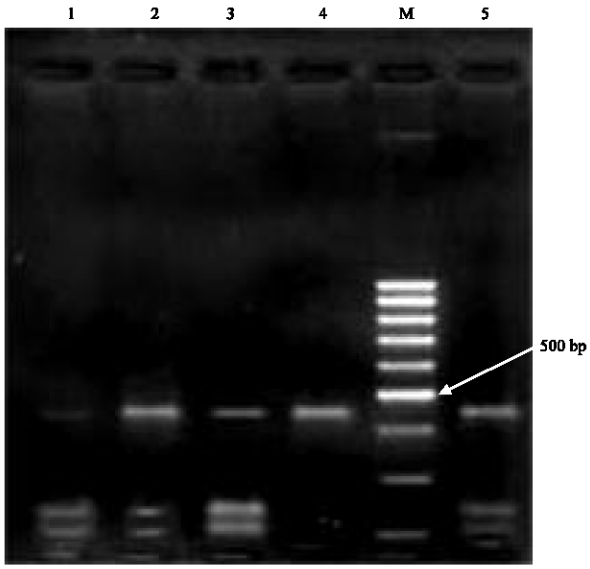

Fig. 1: Determination of genotypes on 2% Agarose gel. 1, 3 lanes are *Hinf*I (+/+); 2, 5 lanes are *Hinf*I (+/-); lane 4 is *Hinf*I (-/-); M = 100 bp Ladder

Table 1: Total number of different POU1F1 *Hinf* I genotypes and allele frequencies for each breed

	POU1F1 *Hinf* I genotypes			Allelic frequencies	
Breed	*HinfI* ++	*HinfI* +-	*HinfI* --	*f*(*HinfI*(+))	*f*(*HinfI*(-))
Mazandarani	41	39	16	0.63	0.37
Sarabi	45	32	7	0.73	0.27
Golpaygani	48	50	12	0.66	0.34
Holstein	65	30	6	0.79	0.21

Table 2: Chi-Square tests of genotype frequencies between different breeds

Breed	Holstein	Golpaygani	Sarabi
Mazandarani	11.03[a]	1.54 ns	3.61 ns
Sarabi	2.23 ns	1.91 ns	---
Golpaygani	9.19[a]	---	

A = $p<0.05$, ns = non-significant

Table 3: Variance analysis of effects on milk production

Source	df	Sum of squares	F ratio	Prob > F
Breed	1	166201569	86.3588	<0.0001
Year	9	17574646	1.0146	0.4330
Season	3	18005194	3.1185	0.0291
Lac	1	2723139	1.4150	0.2368
Days-in-milk	1	23905888	12.4216	0.0006
POU1F1 polymorphism	2	1548227	0.4022	0.6698

and Holstein cattle (Table 2). The sequencing analysis has shown that A to G transition in nucleotide 1256 leads to *Hinf*I (-) allele, because this enzyme could not recognize this sequence.

Statistical analysis: Analyses of variance showed highly significant effect of the breed ($p<0.0001$) (Table 3). Golpaygani breed produces much less milk than Holstein (LSM = 1884 kg vs. LSM = 7559 in Holstein). No significant association was found between POU1F1 polymorphism and milk production in both of two breeds.

DISCUSSION

In the present study the allele frequency of *Hinf I* (-) or the A allele was 0.27 to 0.37 in native breeds. It has been reported *Hinf I* (-) frequency in Holstein, 0.15 (Woolard *et al*., 1994), 0.18 (Renaville *et al*., 1997a), 0.26 (Moody *et al*., 1995), 0.32 (Dierkes *et al*., 1998, Parmentier *et al*., 1999), 0.05 in *Bos Indicus* Gyr cattle (Mattos *et al*., 2004), 0.53 in Belgian Blue (Renaville *et al*., 1997b), 0.21 in Herford (Moody *et al*., 1995), 0.33 and 0.45 in Angus (Zhao *et al*., 2004, Moody *et al*., 1995) and in Sarabi cattle 0.27 (Tavakolian *et al*., 2004). Gene and genotype frequencies between Mazandarani or Golpaygani (both as *Bos Indicus* breeds) and Holstein cattle were significantly different ($p<0.05$), but there was no difference between Sarabi (*Bos taurus*) and Holstein cattle. There was also no significant difference in genotype frequencies between three Iranian native cattle. Differences in allelic frequency between native *Bos indicus* breeds and Holstein at the present study might be due to differences in origin breeds, low number of samples and/or as the effect of natural selection in native breeds. Sequence analysis showed transition A to G at nucleotide 1256 (based on BTY15995) in Sarabi breed. Parmentier *et al*. (2001) reported the same result in Holstein. There was no significant association between polymorphisms of exon 6 in POU1F1 and milk production trait, although some contradictory results have been reported (Renaville *et al*., 1997a; Parmentier *et al*. 1999, 2001; Mattos *et al*., 2004). Our opposite result might be due to of low number of animals in dataset which had milk record data simultaneously.

ACKNOWLEDGMENT

This study was financially supported by Animal Science Research Institute of Iran, No 2-020-210000-04-0000-83014. Laboratory analysis was done in Molekularbiologisches Zentrum der Landwirtschaftlich-Gärtnerischen Fakultät, Humboldt-Universität zu Berlin. The authors acknowledge the helpful cooperation of Mrs Peschel in laboratory.

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