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Association of PIT1 Gene with Milk Fat Percentage in Holstein Cattle

Research Article

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ABSTRACT

The pituitary-specific transcription factor (PIT1) gene is responsible for pituitary development and hormone secreting gene expression in mammals. PIT-1 is studied as a candidate genetic marker for growth, carcass and also for milk yield traits. In dairy farm animals, the principal goal of the selection is the improvement of milk yield and composition. The genes of milk proteins and hormones are excellent candidate genes for linkage analysis with quantitative trait loci (QTL) because of their biological significance on the quantitative traits of interest. Thus, in this study association between polymorphism of the pituitary transcription factor 1 (PIT1) gene and milk fat percentage of Holstein cattle in Khorasan Razavi province of Iran were analyzed. A total of 100 dairy cows from a herd containing 1000 animals were included in the study. Genomic DNA was extracted from the whole blood. One pair primers were used for amplification of PIT1 gene and PCR products were electrophoresed on 1% agarose gel. Then, PCR products were digested with *HinfI* restriction enzyme. Results were analyzed using PopGene software and allele frequencies A and B were 0.25 and 0.75, respectively. Frequencies of AA, AB and BB genotypes, number of observed alleles, number of effective alleles, expected heterozygosity, observed heterozygosity, mean of heterozygosity, expected hemozygosity, observed hemozygosity, Nei's index and Shanon's index were 6, 40 and 54%, 2, 1.6, 0.37, 0.40, 0.37, 0.62, 0.59, 0.37 and 0.56, respectively. Results of k-square shown that population is in Hardy-Weinberg equilibrium. SAS software with GLM procedure was used for calculation of association between milk fat percentage and observed genotypes and results indicated that the effect of genotype on fat percentage was significant ($P<0.01$) and AB genotype had the highest effect on milk fat percentage. These results imply that the PIT1 genotypes affected milk fat percentage, suggesting that this polymorphism can be used as a molecular marker for this trait.

KEY WORDS Holstein cattle, milk fat, PCR, PIT1 gene, polymorphism.

INTRODUCTION

Iran has a cattle population of 7.9 million of which 45.9, 43.6 and 10.51% are of the indigenous, crossbred and registered (mainly Holstein) genotypes. The contribution of livestock to the national economy is 4% of total GDP. Iranian dairy production has undergone significant and considerable structural changes during the last two decades (FAO, 1993) with creation of larger herds. According to the FAO (1993) report, artificial insemination (AI) coverage in several countries including Iran increased remarkably over the 1980 s decade. In general, four groups of sire in terms of their origin are available to dairy producers through AI in Iran. These are American, Canadian, European and Iranian sires, which regardless of their origin can be further categorized to two groups: summarized or sampling sires. Summarized sires are those that have been progeny tested; thus, an estimate of their daughter's producing ability is available. Sampling sires are those that have been selected to transmit high production qualities or type-related traits

based on their pedigree. However, they also need to be progeny tested to determine more accurately which sires will pass their high production and type-related traits to their daughters. Many Iranian dairy producers are reluctant to use foreign sampling sires because their daughters' performance is considered somewhat unpredictable. However, Iranian sampling sires are very reasonable and hence, some farmers prefer to use them on the repeat breeder cows as well as on the moderate to low prouder cows (Heravi-Moussavi and Danesh-Mesgaran, 2009). Use of genes concerning with economic characteristics of farm animals for marker assist selection (MAS) can aid on the selection of animals with the highest breeding values. To determine the best genotypes carrying alleles by taking into account the phenotypic values of animals in quantitative characters are difficult. In other words, phenotypic values do not always reflect the genotypic values of the animals. The improvement of any trait in population primarily depends on its economic gain. Milk yield and components are a quantitative trait controlled by many genes, each one of them with small effect. Dairy cattle breeders have primarily concentrated in the high milk yield per cow until now. However, the milk components should not be ignored in selection programs. Because component percentages tend to have negative genetic associations with yield traits, however, selection is limited (Othman *et al.* 2011). Although the percentage of milk components, especially the fat and protein level with nutrition may be desirable level, this approach ignores the animal genetic effect, and also not permanent (Soyeurt *et al.* 2006). In short, the planning of a breeding program intended for maintaining the desired levels of milk components as well as increasing milk yield in selection is important. However, focus on the traits and their economic weights in selection would be linked to dairy markets, production systems, feed supply and cost, and the presence of data and its usability with the industry of countries (Shook, 2006). PIT1 (POU1F1) is a member of the POU-domain family of genes that play important regulatory roles in developmental processes (Dybus *et al.* 2004). PIT1, an approximate 31-33-kilodalton protein (291 amino acid), was first associated with a critical role in the transcriptional regulation of growth hormone (GH) and prolactin (PRL) genes (Dybus *et al.* 2003). Molecular basis of this polymorphism was the silent mutation (G→A) located within exon 6 of the PIT1 gene (Dierkes *et al.* 1998). PIT1 gene considered as a candidate marker for milk production due to regulation of expression of bGH and the prolactin genes which are essential for mammary gland development and milk yield (Dybus *et al.* 2004). PIT1 gene has been sublocalized to the centromeric region (1q21-22) of *Bos taurus* chromosome 1 (Moody *et al.* 1995). PIT1 gene consists of six exons and five introns. The PIT1 protein consist 3 domains; POU-homeo, POU-specific and the N-terminal region which plays a role in transactivation (Haugen *et al.* 1993; Kopp and Jameson, 1998). Mutation in the PIT1 gene has been reported to be responsible for the dwarf phenotypes in mice (Camper *et al.* 1990; Li *et al.* 1990). In mammals, some of the mutations in PIT1 gene subvert growth, prolactin and TSH hormones, and even causes abnormalities of pituitary development called hypoplasia (Renaville *et al.* 1997a).

PIT1 is an essential for development of somatotrope, lactotrope, and thyrotrope cells in the anterior pituitary and it transactives expression of the genes encoding GH, PRL, and TSH-b). Mutations in the human PIT1 are responsible for a CPHD (combined pituitary hormone deficiency) with deficiency of GH, PRL or TSH, while the production of ACTH, LH and FSH are preserved and its lead to late to reach puberty and hypothyroidism (Bona *et al.* 2004). PIT1 gene is considered to be a candidate gene for the regulation of growth and development in cattle and other mammals due to PRL and GH are effective in proliferation of somatotrophic cells as well as they are necessary for mammary gland development and milk yield (Zhang *et al.* 2009). In cattle, PIT1 was found to be related to milk yield, protein yield, fat percentage and some conformation traits in Italian Holstein-Friesian bulls (Renaville *et al.* 1997a), body weight in double-muscled Belgian Blue cattle (Renaville *et al.* 1997b), some feeding criteria and carcass dimensions in the fattening performance of Holstein-Friesian bulls (Oprzadek *et al.* 2003), fat milk production in Gyr bulls (De Mattos *et al.* 2004), milk yield in Holstein-Friesian (Vargas *et al.* 2004), growth traits in Nanyang cattle (Xue *et al.* 2006), growth traits of Canchim animals, from two lineages (Carrijo *et al.* 2008) and also birth weight and height at withers of Geman Yellow × Qinchuan beef cattle (Zhang *et al.* 2009). There are no reports on the study of PIT1 gene in Holstein cattle in Khorasan Razavi province of Iran by now. Thus, the aim of this study was to determine the allelic frequencies at the bovine PIT1-*HinfI* locus and to investigate the relationship of the polymorphisms and milk fat percentage of Holstein cattle in Khorasan Razavi province of Iran.

MATERIALS AND METHODS

A total of 100 dairy cows from a commercial herd contains 1000 animals, in Khorasan Razavi province of Iran were included in the study. Whole blood samples (approximately 5 mL per animals) were collected from the Jugular vein of each dairy cattle into tubes with EDTA and stored at -20 °C needed for DNA extraction. DNA extraction was carried out by Diatom DNA Prep Kit (Cinagen, Iran) as follows: briefly, to an aliquot of 100 µL blood (after thawing), 400

µL of lysis buffer (Guanidin Thiocyanate, 20 m*M*; EDTA, 20 m*M*; Tris-HCl, 10 m*M*; Triton X100, 40 g/L; DTT, 10 g/L) 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 12000 × g. The pellet was re-suspended 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 m*M*; KCl, 1M and EDTA, 20 m*M*), the mixture was vortexed and then spun for 10 sec at 5000 × 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 gel electrophoresis on 1% agarose gel.

The sequences of the forward and reverse primers for the amplification of the PIT1 gene were: 5' –AAA CCA TCA TCT CCC TTC TT-3' and 5'-AAT GTA CAA TGT GCC TTC TGA G-3'.

The polymerase chain reaction for the PIT1 gene was performed in a 25 µL reaction mixture, containing 1.5 m*M* $MgCL_2$, 200 µ*M* of each dNTPs, 0.3 µ*M* of each primers, 1X PCR buffer, 1U *Taq* polymerase (Cinagen, Iran) 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 95 °C for 4 min followed by 35 cycles of 94 °C for 45 sec, 59.7 °C for 45 sec, 72 °C for 1 min and a final extension at 72 °C for 10 min. A 20 µL aliquot of the PCR products was digested with 7 U of *HinfI* restriction enzyme at 37 °C overnight in incubator for 16 h. Restriction fragments from the above PCR reactions were electrophoresed on 2% agarose gels and stained with ethidium bromide. The allele and genotype frequencies were estimated by direct counting. The heterozygosities (as gene variation indicates) were calculated using the PopGene software version 1.31 (Yeh *et al.* 1999), according to Nei procedure (1978). The Chi-square test whether the distribution of the genotype frequencies was in the Hardy-Weinberg equilibrium was carried out by PopGene Version 1.31 (Yeh *et al.* 1999) and also heterozigosity value was determined (Nei, 1973).

The next stage involved an analysis of associations between the PIT1 genotypes and milk fat percentage. The following linear model was used in this study:

$$Yij= \mu + Gi + Sj + \varepsilon ij$$

Where:
Yij: observed milk fat percentage in ij^{th} animal.
µ: mean of milk fat percentage for population.
Gi: fixed effects of genotype PIT1 (AA, AB, BB).
Sj: fixed effects of sires (fathers).
εij: random error.

PROC GLM (General Linear Model) in the computer program SAS (SAS, 2002) was used to determine the associations between PIT1 genotypes and milk fat percentage. After statistically analyses, the differences between any two least squares means of the genotypes were compared with Least Significant Difference (LSD) for milk fat percentage by using adjusted Tukey's procedure.

RESULTS AND DISCUSSION

Extracted DNA had good quality and had not any protein and phenol contamination (Figure 1). All tested DNA from Holstein cattle in Khorasan Razavi province of Iran used in the present study were amplified using these primers and gave PCR products at the expected size, 451-bp (Figure 2). The amplified DNA fragments (451 bp) were digested with *HinfI* enzyme and separated electrophoretically to detect the genetic polymorphisms of PIT1 gene. The point mutation (A→G) in exon VI, affecting a *HinfI* restriction site, was used to differentiate between two alleles, A and B. The restriction fragments obtained for the PIT1 gene polymorphism were: 244 and 207 bp for BB genotype; 451, 244 and 207 bp for AB genotype and 451 bp (undigested fragment) for AA genotype (Figure 3). The genotype and allele frequencies of PIT1 polymorphisms (*HinfI*, 451 bp) in Holstein cattle in Khorasan Razavi province of Iran has shown in Table 1.

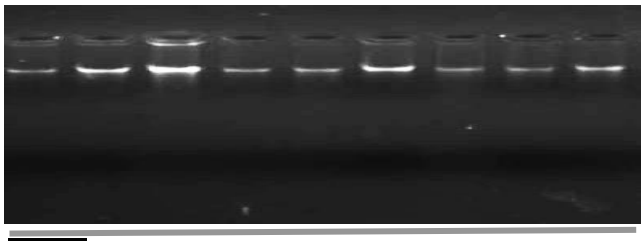

Figure 1 Some samples of extracted DNA from studied animals on 1% agarose gel

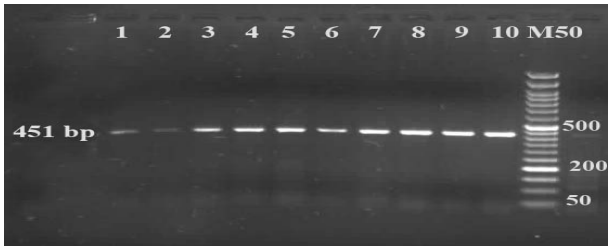

Figure 2 Ethidium bromide-stained agarose gel of amplified PCR products representing amplification of PIT1 gene in Holstein cattle in Khorasan Razavi province of Iran. Lane M50: 50 bp ladder marker. Lanes 1-10: 451 bp PCR products amplified from DNA of studied cattle

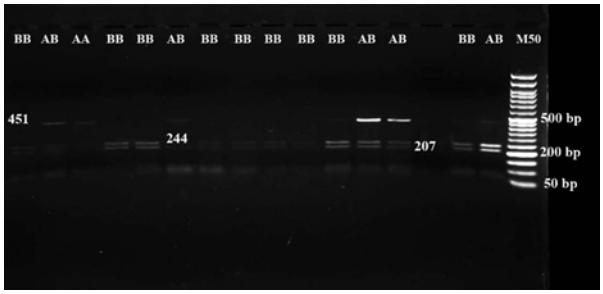

Figure 3 PCR products digested with *HinfI* on 2% agarose gel electrophoresis stained with ethidium bromide. AA: undigested PCR product, AB: digested PCR product (451, 244 and 207 bp) and BB: digested PCR product (244 and 207 bp).

Table 1 The genotype and allele frequencies of PIT1 *HinfI* polymorphism in Holstein cattle in Khorasan Razavi province of Iran

Genotype	Number of genotypes	Genotype frequencies	Allele	Allele frequencies
AA	6	0.06	A	0.26
AB	40	0.40		
BB	54	0.54	B	0.74
Total	100	1		

As shown in 40 and 54 heads of 100 Holstein cattle in Khorasan Razavi province of Iran were determined as AA, AB and BB genotypes, respectively. Allele A and B frequencies in studied cattle were found as 0.26 and 0.74, respectively. The population was found in the Hardy-Weingberg equilibrium with respect to *HinfI* polymorphism in the present study (P>0.05).

Number of observed alleles, number of effective alleles, expected heterozygosity, observed heterozygosity, mean of heterozygosity, expected hemozygosity, observed hemozygosity, Nei's index and Shanon's index were 2, 1.6, 0.37, 0.40, 0.37, 0.62, 0.59, 0.37 and 0.56 respectively. Results of different studies on genotype and allele frequencies of PIT1 (*Hinf I*) polymorphism are shown in Table 2.

As shown in Table 2, in the most breeds in terms of PIT1 polymorphisms (*HinfI*_451 bp), A allele seems to have a lower frequency values than B allele. This situation is similar to our study. In the present study, the frequency of AA genotypes was lower than frequency of BB genotypes that it was confirmed by the results of other researchers (Table 2).

The value of expected heterozygosity was calculated to be 0.37 in Holstein cattle in Khorasan Razavi province of Iran. When compared with literature, this value was lower than findings to be as, 0.50 in Nanyang cattle (Xue *et al.* 2006), 0.43 in East Anatolian Red breed cattle (Özdemir 2012), 0.47 in Brown Swiss cattle (Aytekin and Boztepe, 2013), 0.48 in East Anatolian Red cattle (Özdemir, 2012), 0.38 in Holstein cows (Edriss *et al.* 2008), 0.44 in Angus × Qinchuan cattle (Zhang *et al.* 2009), 0.39 in Charolais × Nelore cattle (Carrijo *et al.* 2008), 0.47 in Manzadrani cattle (Zakizadeh *et al.* 2007), 0.40 in Sarabi cattle (Zakizadeh *et al.* 2007), 0.45 in Golpayegani cattle (Zakizadeh *et al.* 2007), 0.47 in Sarabi cattle (Javanmard *et al.* 2005), 0.38 in Golpayegani cattle (Javanmard *et al.* 2005), 0.47 in Dashtiyari cattle (Javanmard *et al.* 2005), 0.47 in Golpayegani × Brown Swiss F1 cattle (Javanmard *et al.* 2005), 041 in Holstein-Friesian cattle (Vargas *et al.* 2004), 0.44 in Angus beef cattle (Zhao *et al.* 2004), 0.48 in Belgian Blue cattle (Renaville *et al.* 1997b) and 0.43 in 1/2 Angus cattle (Curi *et al.* 2006).

This value was higher than findings to be as, 0.32 in Holstein cattle (Özdemir, 2012), 0.30 in Najdi cattle (Beigi Nassiri *et al.* 2010), 0.16 in Jordan native cattle (Jawasreh *et al.* 2009), 0.29 in Holstein-Friesian cattle (Jawasreh *et al.* 2009), 0.36 in Qinchuan cattle (Zhang *et al.* 2009), 0.30 in Limousin×Qinchuan (Zhang *et al.* 2009), 0.29 in Germany Yellow×Qinchuan cattle (Zhang *et al.* 2009), 0.12 in distinct Indian native cattle (*Bos indicus*) (Mukesh *et al.* 2008), 0.23 in Charolais × Zebu cattle (Carrijo *et al.* 2008), 0.34 in Simmental cattle (Viorica *et al.* 2007), 0.33 in Holstein (Zakizadeh *et al.* 2007), 0.19 in Nellore cattle (Curi *et al.* 2006), 0.21 in Canchi cattle (Curi *et al.* 2006), 0.23 in 1/2 Simmental cattle (Curi *et al.* 2006), 0.36 in Qinchuan cattle, 0.23 in China Holstein-Friesian cattle (Yan *et al.* 2006), 0.14 in Sistani cattle (Javanmard *et al.* 2005), 0.35 in Taleshi cattle (Javanmard *et al.* 2005), 0.29 in Manzadrani cattle (Javanmard *et al.* 2005), 0.10 in Gry bulls (De Mattos *et al.* 2004), 0.26 in Holstein cattle (Hori-Oshima and Barreras-Serrano, 2003), 0.31 in Italian Holstein-Friesian bulls (Renaville *et al.* 1997a).

But, similar results such as 0.37 in black-and-white bulls (Oprzadek *et al.* 2003), 0.37 in Holstein-Friesian cattle (Misrianti *et al.* 2010), 0.37 in Poland black-and-white cows (Dybus *et al.* 2004).

High heterozygosity value in a population is based on parental choice of increasing the frequency of heterozygotes in terms of the relevant gene. Especially, the bulls used in artificial insemination according to genes in relation with the economic traits such as milk yield and components are not pre-tested yet. This situation may alter the genetic makeup of the population by chance and also can lead to deflection of the balance. Arora and Bhatia (2004) denoted that the high mean heterozygosity values could be attributed to low level of inbreeding, low selection pressure and large number of alleles present in a population.

The number of alleles and their frequencies determine the value of heterozygosity in a population. Arora and Bhatia (2004) stated that the level of variation depicted by number of alleles at each locus serves as a measure of genetic variability having direct impact on differentiation of breeds within a species. Also, it may cause some variations in phenotypes. High heterozygosity value in a population is based

Table 2 Results of different studies on genotype and allele frequencies of Pit-1 (*HinfI*) polymorphism

Breed	Genotype frequency			Allele frequency		Expected heterozygosity	Length of PCR fragment	Reference
	AA	AB	BB	A	B			
Brown Swiss cattle	0.12	0.51	0.37	0.37	0.63	0.47	451 bp	Aytekin and Boztepe, 2013
East Anatolian Red	0.14	0.54	0.32	0.41	0.59	0.48	260 bp	Özdemir, 2012
Holstein	0.04	0.31	0.65	0.20	0.80	0.32		
Najdi	0.36	0.30	0.67	0.18	0.82	0.30	451 bp	Beigi Nassiri *et al*. 2010
Holstein-Friesian	0.02	0.45	0.53	0.24	0.76	0.37	611 bp	Misrianti *et al*. 2010
Jordan native cattle	0	0.18	0.82	0.09	0.91	0.16	422 bp	Jawasreh *et al*. 2009
Holstein-Friesian	0.05	0.25	0.70	0.17	0.83	0.29		
Qinchuan	0.03	0.40	0.57	0.23	0.77	0.36	451 bp	Zhang *et al*. 2009
Limousin × Qinchuan	0.04	0.28	0.68	0.18	0.82	0.30		
Angus × Qinchuan	0.11	0.44	0.45	0.33	0.67	0.44		
Germany Yellow × Qinchuan	0.07	0.21	0.72	0.18	0.82	0.29		
Holstein cows (4 herds)	0.03	0.45	0.52	0.26	0.74	0.38	451 bp	Edriss *et al*. 2008
16 distinct Indian native cattle (*Bos indicus*)	0	0.12	0.88	0.06	0.94	0.12	1350 bp	Mukesh *et al*. 2008
5/8 Charolais ve 3/8 of Zebu	-	-	-	0.13	0.87	0.23	1301 bp	Carrijo *et al*. 2008
21/32 Charolais ve 11/32 Nelore	-	-	-	0.27	0.73	0.39		
Simmental	0.12	0.20	0.68	0.22	0.78	0.34	1350 bp	Viorica *et al*. 2007
Manzadrani	0.17	0.41	0.42	0.37	0.63	0.47	451 bp	Zakizadeh *et al*. 2007
Sarabi	0.08	0.38	0.54	0.27	0.73	0.40		
Golpayegani	0.11	0.45	0.44	0.34	0.66	0.45		
Holstein	0.06	0.30	0.64	0.21	0.79	0.33		
Nellore	0.79	0.21	0	0.90	0.10	0.19	1301 bp	Curi *et al*. 2006
Canchim	0.80	0.17	0.03	0.88	0.12	0.21		
1/2 simmental	0.73	0.27	0	0.87	0.13	0.23		
1/2 angus	0.30	0.69	0.01	0.64	0.36	0.43		
Qinchuan	-	-	-	0.23	0.77	0.36	451 bp	Yan *et al*. 2006
China Holstein-Friesian	-	-	-	0.13	0.87	0.23		
Nanyang	0.21	0.51	0.28	0.47	0.53	0.50	451 bp	Xue *et al*. 2006
Sarabi	0.45	0.34	0.21	0.62	0.38	0.47	600 bp	Javanmard *et al*. 2005
Golpayegani	0.61	0.26	0.13	0.74	0.26	0.38		
Sistani	0.84	0.16	0	0.92	0.08	0.14		
Taleshi	0.61	0.32	0.07	0.77	0.23	0.35		
Manzadrani	0.69	0.27	0.04	0.83	0.17	0.29		
Dashtiyari	0.62	0	0.38	0.62	0.38	0.47		
Golpayegani × Brown Swiss F1	0	0.77	0.23	0.39	0.61	0.47		
Holstein-Friesian	0.10	0.35	0.55	0.28	0.72	041	451 bp	Vargas *et al*. 2004
Poland Black-and-White cows	0.05	0.38	0.57	0.24	0.76	0.37	451 bp	Dybus *et al*. 2004
Gry bulls	0.90	0.10	0	0.95	0.05	0.10	~1.355 bp	De Mattos *et al*. 2004
Angus beef cattle	0.11	0.44	0.45	0.33	0.67	0.44	451 bp	Zhao *et al*. 2004
Black and White bulls	0.06	0.37	0.57	0.25	0.75	0.37	451 bp	Oprzadek *et al*. 2003
Holstein	0.03	0.26	0.71	0.16	0.84	0.26	451 bp	Hori-Oshima and Barreras-Serrano, 2003
Belgian Blue	0.20	0.44	0.36	0.42	0.58	0.48	451 bp	Renaville *et al*. 1997a
Italian Holstein-Friesian bulls	0.02	0.32	0.66	0.18	0.82	0.31		

on parental choice of increasing the frequency of heterozygotes in terms of the relevant gene. Especially, the bulls used in artificial insemination according to genes in relation with the economic traits such as milk yield and components are not pre-tested yet.

This situation may alter the genetic makeup of the population by chance, and also can lead to deflection of the balance. Arora and Bhatia (2004) denoted that the high mean heterozygosity values could be attributed to low level of inbreeding, low selection pressure and large number of alle-

les present in a population. The association of PIT1 gene polymorphism with milk fat percentage in the studied population was analyzed (Table 3). As seen in Table 2, F statistic was significant (α=0.01) thus there is association between genotype and milk fat percentage.

Table 3 The association of Pit-1 gene polymorphism with milk fat percentage in Holstein cattle in Khorasan Razavi province of Iran

S.O.V.	df	Sum of square	Mean of square	F statistic	Significant level
Model	4	3.62	0.90	3.25	** (α=0.01)
Error	93	25.93	0.27		

SOV: source of variation.

Table 4 Least square means and standard error of the means of 3 genotypes (AA, AB and BB) on milk fat percentage in Holstein cattle in Khorasan Razavi province of Iran

Genotype	Least square mean	Standard error	Significant level
AA	3.22	0.21	**(α=0.01)
AB	3.62	0.08	**(α=0.01)
BB	3.25	0.07	**(α=0.01)

Comparison between 3 genotypes (AA, AB and BB) with milk fat percentage, by using adjusted Tukey's procedure showed that association between genotypes (AA, AB and BB) and milk fat percentage is significant (Table 4). AB genotype was more significant than AA and BB genotypes, thus is more effective on milk fat percentage and can conclude that cattle with AB genotype have milk with more fat percentage in comparison with AA and BB genotypes. BB genotype is more effective than AA genotype on milk fat percentage.

Our results confirmed results of other researchers. Renaville *et al*. (1997a) stated that the A allele showed significant superiority over the B allele for milk yield (P<0.10), protein yield (P<0.05), some conformation traits such as body depth (P<0.10), angularity (P<0.10), rear leg set (P<0.10) and also less fat percentage (P<0.10) in Italian Holstein-Friesian bulls. De Mattos *et al.* (2004) reported *HinfI* variants is associated with only the fat yield of milk yield traits and bulls carrying AB genotype (16.6 kg) were superior than AA genotype (6.5 kg) for fat yield (P<0.05). They stated that this superiority resulted from the influence of allele B on these genotypes. Although this superiority could result from the non additive heterosis effect and could affect the milk fat percentage.

Parmentier *et al.* (1999) demonstrated significant superiority of the HinfI B allele for milk (+222.4) and protein (+9.17) yields, but an inferiority for fat yield (-2.29%). The results can be interpreted as a single positive action of the A allele on protein yield and, to a lesser extent, on milk yield and fat content. This interpretation declared the milk production performance for PIT1 which is characterized by higher fat content other than milk yield or milk protein content.

Based on our results and literature investigating the relationship between PIT1-*HinfI* polymorphism and milk production traits, it is mentionable that A allele and AA genotype should be exploited for selection of dairy traits except for Gry bulls (*Bos indicus*) due to different genomic background (De Mattos *et al.* 2004). Distribution differences of allele frequencies between different populations may indicate genetic differences in the base populations (Carrijo *et al.* 2008).

In contrast to this, association analysis to be made with phenotypic values in different populations with the same distribution of allele frequencies may be varied.

Increasing the number of animal and data, especially taking into consideration the genotype × environment interactions and other genes affecting milk yield and components should be made more comprehensive studies. Besides, it would be more informative that different alleles in other genes connection with the PIT1 gene could be evaluation.
A heterozygosity of less than 0.5 indicated low variation for these genes in studied population.

CONCLUSION

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.

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