

Full Length Research Paper

# Association of IGF- 1 gene polymorphism with milk production traits and paternal genetic trends in Iranian Holstein bulls

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The effects of Insulin-Like Growth Factor-1 polymorphism were examined on milk production traits and genetic trends in the Iranian Holstein bulls. A total of 282 bulls were genotyped for *SnaBI* restriction site in the 5' flanking region of IGF-1 by applying PCR-RFLP method. The allele frequencies for C and T alleles were 0.562 and 0.438, respectively. The results indicated that genetic variants at the 5'-noncoding region of the bovine IGF-1 gene had a marked effect on estimated breeding values of milk (EBVM) and fat yields (EBVF). The heterozygous bulls (CT genotype) had higher EBVM and EBVF than homozygous bulls ( $P < 0.1$ ). The average substitution effects of alleles were not significant for none of studied traits ( $P > 0.1$ ). The studied polymorphism of IGF-1 showed no significant association with genetic trends of milk related traits ( $P > 0.1$ ). These results suggest that allelic interaction in IGF-1 polymorphism as over dominance and might be effectiveness in animal improving by crossbreeding.

**Key words:** IGF-1, polymorphism, Holstein, genetic trend.

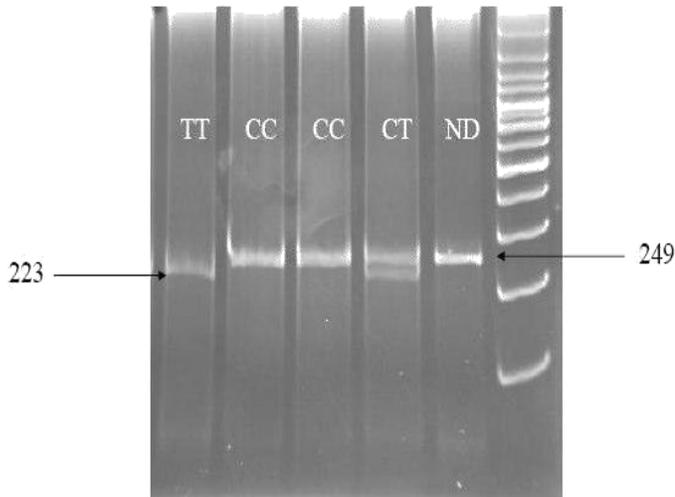
## INTRODUCTION

Holstein is major breed of industrial dairy cattle in Iran, and recording for milk production traits is done for all industrial dairy cows, and breeding values are annually estimated for them. The bulls are also selected based on parent EBVs, then some of them are proofed based on progeny test (at least 50 daughters). The problems associated with phenotypic data recording such as long time and high expense of recording and also low cooperation of some Iranian dairymen in recording process cause less accurate estimation of breeding values and sequently less improvement in the selection programs. Thus, collection of genotypic data by molecular methods in addition to phenotypic data is an essential work to improve the selection procedure.

Insulin-like growth factors 1 and 2 are structurally related proteins playing a key role in cell differentiation,

embryogenesis, growth, and regulation of metabolism. IGF-1 is a polypeptide of the molecular weight 7.5 kDa built of 70 amino acids (Daughaday and Rotwein 1989). The IGF-1 gene contains 6 exons and is about 90 kb-long (Steenbergh et al. 1991). Because of an alternative splicing of exons 1 and 2, two different transcripts are formed. The transcript of exon 1 contain 1155 nucleotides (nt), while the other one, with exon 2, is shorter and contains 750 nt. Production of these transcripts is controlled by two different promoters both containing canonical regulatory sequences - TATA-box and CCAAT-box (Jansen et al. 1991). It was shown that transcripts of both classes are differentially expressed in various tissues, being, however, most abundant in liver (Wang et al. 2003). In cattle, the IGF -1 gene was localized in chro-mosome 5 (Bishop et al. 1991). Nucleotide sequence polymorphisms were identified in the bovine IGF-1 gene. The STR (short tandem repeat) polymorphism in the 5'-flanking region, and the SSCP (single strand conformation polymorphism) in intron 3 of the IGF-1 were

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**Figure 1.** Acryl amid gel (8%) electrophoresis showing RFLP-SnaBI in 5' -noncoding region of the bovine IGF-1 gene. TT = 223 bp, CC = 249 bp, CT = 249 and 223 bp, ND = undigested product and M = 100 bp DNA marker. The 26 bp band was not seen in gel.

reported by Kirkpatrick (1992, 1993). Other polymorphisms namely, RFLP-*SnaBI* (Ge et al., 2001), and the TTTG insertion/deletion (InDel) in intron 4 and the RFLP-*DpnI* in intron 5 (Lien et al., 2000) have been detected.

Paternal genetic trends is defined as change trend of EBVs of traits through years, that is affected by changes of allele frequencies of corresponding genes through years and the rate of their effects on trait. Obviously, genes may have a noticeable effect on genetic trends. It has not been subject of any studies so far.

The objective of this study was to evaluate the relationships between the polymorphism of the IGF-1 gene and milk production traits and its effect on paternal genetic trends of Iranian Holstein bulls.

## MATERIALS AND METHODS

### Samples

Semen samples were collected from all the proven Holstein bulls (282 bulls) born from 1990 - 2006. They were obtained from Animal Breeding Center of Iran. Genomic DNA from semen was extracted as previously described by Zadworny and Kuhlein (1990).

### Genotyping

Detection of IGF-1 polymorphism was carried out according to Ge et al. (2001). Briefly, the 249 bp fragment of the IGF-1 gene (GenBank ID: AF210383) was amplified using following primers: (Forward): 5'-ATTACAAAGCTGCCTGCCCC-3', and (Reverse): 5'-ACCTTACCCGTATGAAAGGAATATACGT-3'. The PCR amplification cycles were: 94°C for 1 min, 64°C for 45 s and 72°C for 1 min (31 cycles). The PCR amplified DNA fragment of the IGF-1 was digested at 37°C for 12 h with 5 units of *SnaBI* restriction endonuclease enzyme. The digestion products were separated on 8% acryl amid gels in 1x TAE buffer and visualized in UVIDOC Imager.

## Statistical analysis

The allelic frequencies were calculated by simple allele counting according to the Hardy-Weinberg equilibrium (Falconer and Mackay, 1996); the possible deviations of genotype frequencies from expectations were tested by chi-square ( $\chi^2$ ) test.

The effect of IGF-1 genotypes on milk production traits, namely, milk yield (kg), fat content (%), fat yield (kg), protein yield (kg) and protein content (%) were analyzed by GLM procedure of SAS (2002). The following statistical model was used:

$$EBV_{ijk} = \text{year}_i + G_j + e_{ijk}$$

Where,  $EBV_{ijk}$  = the estimated breeding values for milk related traits adjusted for number of daughters (10 to 60); = the overall mean;  $\text{year}_i$  = the fixed effect of birth years of bulls (genetic trends) ( $i = 1990, \dots, 2006$ ),  $G_j$  = the fixed effect of IGF-1 genotypes ( $j = 1, 2$  and 3);  $e_{ijk}$  = the residual effect.

Breeding values of the bulls for milk production traits were obtained from the September, 2008 Iranian Animal Breeding Center evaluations, which were based on an animal model. This model included animal effect as random effect, age of calving as covariate factor and fixed effects of herd-year-season. The reliabilities of EBVs for all bulls were high and on average 92%.

Average effect of allele substitution was determined by coding genotypes as 0 (CC), 1 (CT), and 2 (TT) to represent the number of (T) alleles in IGF-1 polymorphism. As described by Falconer and Mackay (1996), the regression coefficient ( $b_2$ ) in below model estimates the average effect of allele substitution, or the average effect of replacing a (T) allele with (C) allele.

$$EBV = b_0 + b_1 (\text{year}) + b_2 (\text{Genotype code}) + e$$

Where,  $EBV$  = the estimated breeding values as dependent variable;  $b_0$ ,  $b_1$  and  $b_2$  representing the intercept, genetic trend and Average effect of allele substitution, respectively;  $\text{year}$  = the effect of birth years of bulls as independent variable (genetic trends)  $\text{Genotypecode}$  = assigned codes for genotypes.

The frequency of CT genotype in each year was calculated (Ratio). Then, the changes of these frequencies during years were estimated by linear regression model:

$$\text{Ratio} = b_0 + b_1 (\text{year}) + e$$

The association of studied polymorphism with genetic trends of traits was investigated by below model

$$mEBV_i = b_0 + b_1 (\text{Ratio}) + e$$

Where,  $\text{Ratio}$  = The frequency of CT genotype in each year ( $i=1990$  to 2006),  $b_0$  and  $b_1$  representing the intercept and association rate between paternal genetic trend and the studied polymorphism and

$mEBV_i$  = mean of EBV for bulls born in  $i^{\text{th}}$  year. In this study, the paternal path genetic trends (change trend of yearly EBV means of bulls during years 1990 - 2006) were estimated based on only proven Iranian Holstein bulls (on average 15 proven bulls per year).

## RESULTS

The C/T transition at position -472 in the 5'-noncoding region of the IGF-1 was first reported in Angus cattle by Ge et al. (1997) as SSCP. This mutation is at position 512 bp upstream from the ATG codon. The C T substitution creates a *SnaBI* restriction site and digestion of the 249 bp PCR product with the restriction *SnaBI* nuclease resulted in two DNA bands (223 and 26 bp) for homozy-

**Table 1.** The observed and expected genotypic and allelic frequencies of IGF-1 gene polymorphism.

Genotypes	TT	CT	CC	Chi-square test
Number of animals	45	157	80	
Observed frequency	0.159	0.557	0.284	$\chi^2 = 4.878$
Expected frequency	0.192	0.492	0.316	Critical Value=3.841
Alleles	T = 0.438		C = 0.562	

**Table 2.** Least square means for milk, fat, and protein yields and fat content in Iranian Holstein bulls with different IGF-1 genotypes.

Traits (EBV)	Genotypes			P-Values
	CC	CT	TT	
Milk Yield (Kg)	9.8355 <sup>b</sup>	118.8269 <sup>a</sup>	-46.819 <sup>b</sup>	0.072
Fat Yield (Kg)	1.5159 <sup>b</sup>	5.4918 <sup>a</sup>	1.1288 <sup>b</sup>	0.092
Protein Yield (Kg)	2.0755	3.5566	1.2167	0.302
Fat Content (%)	0.0059	0.02815	0.02599	0.4779
Protein Content (%)	0.0150	0.0099	0.0181	0.4132

<sup>a</sup> Lsmeans with the different letter within one row differ significantly ( $p < 0.1$ ).

**Table 3.** Average allele substitution effects of IGF-1 polymorphism in EBVs of milk related traits.

Trait (EBV)	b	SE	P-Value
Milk Yield (Kg)	28.88	42.806	0.500
Fat Yield (Kg)	0.09622	1.1636	0.9358
Protein Yield (Kg)	0.46796	0.9269	0.6141
Fat Content (%)	-0.01406	0.01166	0.229
Protein Content (%)	-0.00196	0.00371	0.5984

b: Linear regression coefficient estimating average substitution effects of T allele.

gote (TT) and three bands (249, 223 and 26 bp) for the heterozygote. The DNA amplified from homozygous (CC) animals remained undigested with *SnaBI* restriction endonuclease (Figure 1).

Based on Table 1, the expected genotype frequencies were not similar to observed, suggesting that genotype distribution was not in the Hardy-Weinberg equilibrium ( $p < 0.05$ ).

Based on Table 2. Bulls with CT genotype had higher estimated breeding values of milk and fat yield compared to CC and TT genotypes ( $P < 0.1$ ). The heterozygous bulls had higher protein yield, fat and protein content (%), but the differences between genotypes for these traits were not statistically significant ( $p > 0.1$ ).

The average effect of the T allele substitution was not statistically significant and that was 28.88, 0.0962, -0.468 kg for EBVs of milk, fat and protein yields, and 0.014 and 0.0019 % for fat and protein content, respectively (Table 3).

The regression coefficient of yearly frequencies of heterozygous genotype on birth years of bulls were -0.0048

and this coefficient was not statistically significant ( $p < 0.1$ ). So, the change trend of CT genotype frequencies was not linear. No significant relations were shown between yearly means of estimated breeding values of milk related traits and yearly frequencies of CT genotype that is no significant relation was seen between genetic trends and IGF-1 gene (Table 4).

## DISCUSSION

Similar frequencies of alleles C and T in bovine IGF-1 gene were found by Hines et al. (1998), who reported an estimate of 0.55 and 0.45 for the frequency of C and T alleles in a population of Holstein cattle. Also, Li et al. (2004) reported estimates of 0.56 and 0.44 in two commercial lines of dairy cattle; respectively. However, different estimates of frequencies (0.64 for (C) and 0.36 (T) alleles) were reported by Ge et al. (2001) in Angus cattle.

The association between RFLP-*SnaBI* of the IGF-1

**Table 4.** Rate of genetic trends of milk related traits and effects of IGF-1 gene on genetic trend in Iranian Holstein bulls.

Trait	Genetic trend	IGF-1		P-Value
		b	SE	
Milk yield (kg)	63.761	-0.00007	0.000087	0.4328
Fat yield (kg)	1.507	-0.00331	0.00377	0.3925
Protein yield (kg)	1.418	-0.00285	0.00382	0.4665
Fat percent (%)	-0.011	0.2852	0.0337	0.5366
Protein Percentage (%)	-0.0057	0.7685	0.9638	0.4369

b: the estimated coefficient of regression representing rate effect of IGF-1 on genetic trends.

gene and milk traits was studied by Siadkowska et al. (2006), using 262 Polish Holstein-Friesian cows. They did not find any differences between genotypes in the daily milk yield, but CT cows yielded significantly more daily fat (+20 g) and protein (+14.5 g) than the cows with CC genotype ( $P < 0.05$ ). The CT genotype also appeared favorable for fat and protein content of milk. Hines et al. (1998) reported no association between IGF-1 gene RFLP-SnaBI and dairy production traits in Holstein dairy cattle. No other papers were found in the literature concerning effects of IGF-1 polymorphism on milk production traits. The effect of this SNP has been generally tested in relation to meat production traits in previous studies.

The analysis of this study confirmed that IGF-1 could be a candidate gene for application in marker assisted selection. The results proved a significant effect of IGF-1 polymorphism on EBVs for milk production traits. However, the important role of IGF-1 in the meat production process is well known, thus its polymorphism effects on other traits specially conformation traits of bulls should be the subject of further research. Also, the association of gene polymorphism with genetic trend was studied in first time in this study, and understanding of molecular mechanism of genetic trend needs to additional researches.

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