



Effect of growth hormone receptor gene polymorphism on breeding values of milk production traits and paternal genetic trends in Iranian holstein bulls

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ABSTRACT

The effects of *growth hormone receptor (GHR)* gene polymorphism were examined on milk production traits in Iranian Holstein bulls. Bulls (282) born from 1989 to 2006 were genotyped for *AluI* restriction site in the 5' flanking region of *GHR* by applying PCR-RFLP method. The genotypic frequencies for *AluI* (-/-), *AluI* (+/-) and *AluI* (+/+) were 0.237, 0.465 and 0.298, respectively. The results indicated that genetic variants at the 5'-noncoding region of the bovine *GHR* gene had a marked effect on estimated breeding values of fat per cent (EBVFP). The *AluI* (+/+) bulls had lower than *AluI* (-/-) bulls and average effect of allele *AluI* (+) to *AluI* (-) substitution was -0.0548 for EBVFP. Study of yearly trend in ratio of allele *AluI* (+) to *AluI* (-) frequencies based on birth years of bulls showed that the ratio had a linear and additive trend as 0.01424/year. This trend showed that the studied SNP on *GHR* may be linked with paternal genetic trends. These results suggested that *GHR* polymorphism may be useful for selection at the DNA level for milk production traits.

Key words: Bovine, GHR, Genetic trend, Milk, Polymorphism

In dairy cattle, promising candidate genes for many traits are in the growth hormone axis (Aggrey *et al.* 1999). The growth hormone plays a critical endocrine role in controlling nutrient metabolism in dairy cattle. Growth hormone actions on target cells depend on the growth hormone receptor (GHR) (Burton *et al.* 1994). In liver *GHR* is dynamically regulated by lactation and energy balance (Rhoads *et al.* 2008).

The *GHR* contains a single transmembrane domain comprising 24-amino acids, extracellular hormone binding domain, and a long cytoplasmic domain (Kopchick *et al.* 2000). In cattle, the *GHR* gene is encoded by a single gene located on chromosome 20 (Moody *et al.* 1995). The gene coding for bovine *GHR* consists of 9 exons (from 2 to 10) in

the translated part and of a long 5'-noncoding region that includes 9 untranslated exons (Jiang *et al.* 2001). Exons from the untranslated region are spliced alternatively and each of them has its own transcription start site. A LINE-1 element from the family of retrotransposons, about 1.2 Kbp-long, was found upstream from exon 1A (Lucy *et al.* 1998). Several polymorphic sequences were identified in the bovine *GHR* gene, RFLP-*TaqI* (Falaki *et al.* 1996), RFLP-*AluI*, RFLP-*AccI*, and RFLP-*StuI* (Aggrey *et al.* 1999), TG-repeat microsatellite (Hale *et al.* 2000), RFLP-*NsiI* (Ge *et al.* 2003) and RFLP-*Fnu4HI/TseI* (Maj *et al.* 2004).

The objective of this study was to evaluate the relationships between the polymorphism of the *AluI*-RFLP on *GHR* gene and milk production traits and its association with paternal genetic trends of Iranian Holstein bulls.

MATERIAL AND METHODS

Samples: Semen samples were collected from 282 proven Holstein bulls that were born between 1989 and 2006. Genomic DNA from semen was extracted as per Zadworny and Kuhenlein (1990).

Genotyping: A 836 bp fragment of 5'-flanking region of *GHR* (position -1866 to -1031) was amplified according to Aggrey *et al.* (1999). Briefly, the sequences of the forward and reverse primers were 5' TGC GTGCACAGCAGCTCAACC-3' and

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5'-AGCAACCCCACTGCTGGGCAT-3', respectively. The PCR amplification was carried out in 37 cycles at 95°C for 35 s, 66°C for 45 s, and 72°C for 60 s. The amplified DNA was digested for 12 h at 37°C with 5 units of *AluI* restriction endonuclease. The digested DNA fragments were separated by electrophoresis in 8% acrylamid in 1X TAE buffer and visualized under UV light.

Statistical analysis: The allele 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 (χ^2).

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

$$EBV_{ijk} = \mu + year_i + G_j + e_{ijk}$$

where, EBV_{ijk} , the estimated breeding value for milk related traits adjusted for number of daughters; μ , the overall mean; $year_i$, the fixed effect of birth year of bulls (for genetic trends), G_j , the fixed effect of *GHR* genotypes; 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. The 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 the bulls were high and on average 92%.

Average effect of allele substitution was determined by coding genotypes as 0 *AluI* (-/-), 1 *AluI* (+/-), and 2 *AluI* (+/+) to represent the number of *AluI* (+) alleles in *GHR* polymorphism. As described by Falconer and Mackay (1996), the regression coefficient estimates the average effect of allele substitution, or the average effect of replacing a *AluI* (+) allele with *AluI* (-) allele:

$$EBV = b_0 + b_1 (year) + b_2 (Genotypecode) + e$$

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; year, the effect of birth years of bulls as independent variable (genetic trends), genotypecode, assigned codes for genotypes.

For study of change trend of allelic frequencies in 18 years, the ratio of frequency of allele (+) to allele (-) for *GHR* gene in each year was estimated. Then, the regression coefficient (b_1) of these yearly ratios on birth years of bulls was estimated:

$$Ratio = b_0 + b_1 (year) + e$$

RESULTS AND DISCUSSION

There were 3 *AluI* sites in the 836 bp fragment of 5' flanking region of the bovine *GHR* gene. The digested *AluI* (-/-) PCR product exhibited 3 fragments of 747 bp, 75 bp,

Table 1. Genotype and allele frequencies of *GHR* gene

genotypes	<i>AluI</i> (+/+)	<i>AluI</i> (+/-)	<i>AluI</i> (-/-)
Number of animals	84	131	67
Observed frequency	0.298	0.465	0.237
Expected frequency	0.282	0.498	0.220
Allele frequencies	<i>AluI</i> (+)=0.531		<i>AluI</i> (-)=0.469
Calculated $\chi^2=1.282$	Critical value of $\chi^2=3.841$		

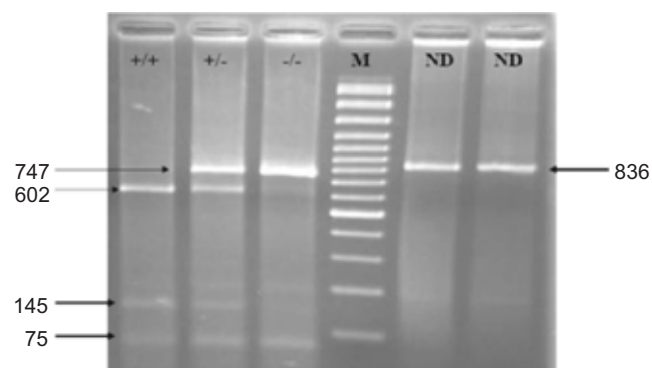


Fig 1. Digestion products of the 836 bp fragment in the 5' UTR region of growth hormone receptor gene with enzyme *AluI*, loaded on 8% acrilamid gel. The genotypes of *AluI* (-/-), *AluI* (+/+) and *AluI* (+/-) were shown in left side of gel, respectively. M: Marker (100bp), ND: undigested PCR product.

and 14 bp (not detected on the gel). For the *AluI* (+/+) PCR product, the 747 bp fragment was cleaved into 2 fragments of 602 and 145 bp (Fig. 1). The polymorphic *AluI* site revealed a mutation at position -1182 (A-to-T transversion).

The genotype and allele frequencies at this SNP are shown in Table 1. The calculated χ^2 was 1.282 and it was lower than critical value of χ^2 table ($\chi^2_{\alpha=0.05, df=1}=3.841$), then the null hypothesis did not rejected, suggesting that genotypes distributions were in the Hardy-Weinberg equilibrium.

Least square means of genotypes are presented in Table 2. Bulls with *AluI* (+/+) genotype had best EBV for milk yield, fat yield and protein yield, but the differences between genotypes were not statistically significant ($P>0.05$). On the

Table 2. Least square means and p-value for estimated breeding values of milk related traits in Iranian Holstein bulls based on different *GHR* genotypes

Traits	Genotypes			P-value
	<i>AluI</i> (-/-)	<i>AluI</i> (+/-)	<i>AluI</i> (+/+)	
Milk yield	-251.85	-210.34	-85.93	0.2828
Fat yield	-1.89	-2.76	-0.545	0.7411
Protein yield	-8.87	-1.73	-1.22	0.3869
Fat %	0.113 ^a	0.057 ^{ab}	0.003 ^b	0.0155
protein %	0.0488	-0.037	-0.0253	0.283

^{a,ab,b}: Ls means signed with the different letter within any row, differ significantly ($P<0.05$).

Table 3. Average allele substitution effects of GHR polymorphism on EBVs of milk related traits

Traits	α	SE	P-value
Milk yield (kg)	80.62	53.204	0.1336
Fat yield (kg)	0.588	1.573	0.7096
Protein yield (kg)	2.022	2.870	0.4829
Fat (%)	-0.0548*	0.01838	0.0038
Protein (%)	-0.007566	0.00499	0.1338

α : Average allele substitution effects of allele *AluI* (+) instead of *AluI* (-).

contrary, the highest fat % and protein % EBVs were observed in bulls with *AluI* (-/-) genotypes that its difference in compared to other genotypes was significant only for fat percent ($P < 0.05$).

The average allele substitution effect *AluI* (+) instead of allele *AluI* (-), estimated by Falconer and McKay method (1996) was 80.62, 0.588, 2.022, -0.0548 and -0.007566 for EBVs of milk yield, fat yield, protein yield, fat percent and protein percent, respectively (Table 3). *AluI* (-) allele have increment effect on fat and protein percent that only the average effect of the *AluI* (-) allele on fat % was statistically significant ($P < 0.05$).

Change in ratio of *AluI* (+) to *AluI* (-) frequencies based on birth years of bulls (18 years) was studied by fitting of a linear regression. The estimated regression coefficient and its p-value were 0.01424 and 0.017, respectively. Therefore it can be said that frequency of allele *AluI* (+) increased averagely as 1.424% per year. Based on obtained p-value (0.017) and lack of fit test, the linear relationship between allele frequency and birth years of bulls was confirmed in the Iranian Holstein bulls.

The *AluI* SNP was located within the 1.2-kb LINE-1 element, a retrotransposon of viral origin, inserted in the bovine GHR gene 5' region. The frequency of *AluI* (-) allele in present study was 0.469, that has been reported 0.473 in Canadian Holstein cattle (Aggrey *et al.* 1999); 0.45 and 0.64 in Lithuanian Black and White and Lithuanian Red cattle, respectively. Heterozygosity rate (frequency of heterozygote genotype) in Iranian Holstein bulls was 0.465 and these rates were 0.45, 0.59 and 0.43 for Canadian Holstein bulls, Lithuanian Black and White and Lithuanian Red cattle, respectively. Comparison of allele frequencies and heterozygosity rate in Canadian and Iranian Holstein bulls showed these populations are similar that may be caused by similarity in selection programs of 2 populations. Observed differences between Iranian and Lithuanian cattle population may be due to the studied cows in the Iranian population included bulls that were used in the selection program, while the Lithuanian cows had been selected from commercial herds.

As reported by Aggrey *et al.* (1999), the *AluI* (+/+) bulls had higher fat EBV than the *AluI* (-/-) bulls. No significant

differences were observed between GHR genotypes for fat EBV, but the *AluI* (-) allele is a favorable allele for fat % EBV. However, it seems that this polymorphism have marked effect on milk fat (fat % and/or fat production), and observed differences between 2 results may be due to fat recording accuracy in Canada and Iran. The 5' region of the GHR gene contains regulatory sequences which control the expression of GHR and interact with a large number of cis-acting and trans-acting factors (Heap *et al.* 1995). Modulation of the affinity of binding of any of these factors may affect GHR transcription and consequently its binding ability with GH.

The significant relation between birth years and yearly ratio of allele frequencies may be resulted of association between *AluI* (+) allele and paternal genetic trends of economic important traits in Iranian Holstein selection program. Although, the significant correlation of allele *AluI* (+) with milk traits was not confirmed in this study, but its positive effect was observed, and also its likely associations with other important traits in dairy cattle breeding, such as body conformation traits is not impossible (under studing) that these reasons may be justifying of the allele frequency change during Holstein breeding program in Iran. The decreasing trend of allele frequency *AluI* (-) in selected years is probably due to fat percent trait is not included in the selection program of dairy cows in Iran.

One of the advantages of breeding values as the dependent variable in the association studies of polymorphism with traits is the possibility of investigation of major gene effect on genetic trends. It is recommended that the various statistical models and further research are required for better understanding of molecular mechanisms of genetic trend.

The analysis of this study confirmed that GHR could be a strong candidate for application in marker-assisted selection. Thus its polymorphism effects on other traits especially conformation traits of bulls should be the subject of further research.

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