Association of TG-repeats in the 5’-flanking region of bovine growth hormone receptor (GHR) gene with milk production traits and somatic cell count in Holstein cattle

M. Muhaghegh-Dolatabady, J. Habibizad and M. R. Bahreini Behzadi

Department of Animal Science, Faculty of Agriculture, Yasouj University, Yasouj, Iran

* Corresponding author, E-mail address: mmuhaghegh@yu.ac.ir

Abstract  The growth hormone receptor (GHR) is a member of cytokine/hematopoietin family that mediates the biological actions of growth hormone (GH) on target tissues. Therefore, the purpose of this study was to examine the association of TG-repeat polymorphisms in the 5’-flanking region of bovine GHR gene with milk production traits and somatic cell score (SCS) in Holstein cattle of Iran. The part of 5’-flanking region of GHR gene that encompassed TG-repeat was screened by single strand conformation polymorphism (SSCP) method and DNA sequencing. Five hundred eighteen Iranian Holstein cows were genotyped, giving 3 distinct SSCP patterns (A, B, and C). Frequencies of these patterns for the amplified fragment were 0.21, 0.26 and 0.53, respectively. Statistical analysis revealed that TG-repeat had a significant effect on average daily milk production (P < 0.05) and tended to associate with fat and protein percentage. No significant difference was observed between TG-genotypes and SCS. The association identified in the TG-repeat of GHR gene may have potential to serve as candidate genetic marker for marker assisted selection (MAS) in cattle.

Keywords: GHR gene, 5’-flanking region, SSCP, microsatellite, cattle

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Introduction

Bovine growth hormone (GH) plays important roles in lactation and growth (Parmentier et al., 1999). An important factor in relation to GH action is growth hormone receptor (GHR) which facilitates growth and development of mammary glands (Feldman et al., 1993). The GHR acts as a biological mediator of GH on target cells by transducing a signal across the cell membrane and inducing transcription of many genes, including insulin like growth factor1 (IGF-I) (Argentsinger and Carter-Su, 1996). The gene coding for bovine GHR consists of nine exons (numbered 2 to 10) in the translated part, and a long 5’-noncoding region that includes several alternative untranslated exons, but only exons 1A, 1B, and 1C have been studied in detail (Jiang and Lucy, 2001). Distinct promoters regulate transcription from each of the alternative exons. The P1 promoter, which regulates GHR expression in the liver, is associated with exon 1A (Jiang et al., 1999).

DNA polymorphisms, observed in the 5’-flanking region of genes such as promoter, are known to influence transcription rate, thus, expression of protein products. So far, several DNA variants in the 5’-flanking region have been identified in different candidate genes for production traits in cattle, sheep and goat. For example, a TG-repeat occurs in or near the P1 promoter of the GHR gene in mouse (Menon et al., 1995), human (Pekhletsy et al., 1997), sheep (O’Mahoney et al., 1994), goat (Maj et al., 2007), European bison (Flisikowski et al., 2007) and cattle (Heap et al., 1995). In Bos taurus and Bos indicus, the TG-repeat is located 90 bp upstream of the initiation codon and 64 bp upstream of the TATA sequence of the GHR gene (chromosome 20), and is polymorphic (Lucy et al., 1998). Alleles with 16-20 consecutive TGs commonly occur in Bos taurus, whereas the 11-TG-repeat allele is most common in Bos indicus.

Although microsatellite markers are usually considered just as neutral DNA markers, several studies indicated that microsatellites located in the promoter regions may affect gene activity (Sandaltzopoulos et al., 1995; Punt et al., 1990; Chen and Roxby, 1997). In many cases, number of repeats in a microsatellite appears to be a key factor for gene expression and expression level (Liu et al., 2000). These findings suggest that variations of TG-repeats in the promoter region of bovine GHR gene would be of interest because of GHR...
role in mammary development. Based on above consideration, the aims of this study were to estimate the genotype frequencies of TG-repeats in the 5′-flanking region of bovine GHR, and to evaluate their influence on milk production traits and somatic cell count in Holstein cattle of Iran.

Materials and methods

To study the possible association between different genotypes of GHR TG-repeats with milk production traits and somatic cell count (SCC), data recorded on 601 Holstein cows at single farm (Qyam) were used. Genomic DNA was extracted from 200 μl peripheral blood samples, collected in tubes in the presence of EDTA, by AccuPrep® Genomic DNA extraction kit.

For genotyping of the TG-repeat, two new primers (Forward: 5′-gcaatgtgtgtgtgctca-3′ and Reverse: 5′-gtttctcagctttagt-3′) were designed from the GHR sequence (GenBank Accession No. U157312), using Primer3 Software (http://frodo.wi.mit.edu/primer3/).

The 324 bp of P1 promoter region (-159 to +165) of the GHR gene was amplified using the following polymerase chain reaction (PCR) conditions: one 5 min denaturing cycle at 95 °C, 35 cycles of 95 °C for 45 s, 63 °C for 30 s and 72 °C for 30 s, and one elongation cycle at 72 °C for 7 min. Twenty five ml of PCR mixture was carried out 0.2 ml PCR tubes, using a PCR kit with the lyophilized components. Each tube contained 1.5 units of Taq DNA polymerase, 10 mM Tris-HCl (pH 9), 50 mM KCl, 1.5 mM MgCl2, 200 mM of each dNTP, 20 pmol of each primer and 50 ng genomic DNA.

The SSCP analysis parameters, including the amount of PCR product, acrylamide concentration (8-10%) and the presence or absence of glycerol were optimized for this fragment. For SSCP analysis, 4 μl of each amplification product was added to 8 μl of stop solution (95% formamide, 10mM NaOH, 0.05% xylene cyanol and 0.05% bromophenol blue). The samples were heat-denatured at 95 °C for 10 min, chilled on ice for 10 min, and the total volume was loaded onto 8% polyacrylamide gel (38:1 acrylamide/bisacrylamide).

Electrophoresis was carried out at room temperature in 0.5X TBE buffer for 20 h. The gels were subsequently fixed in 10% acetic acid, stained with 0.15% AgNO3 and revealed with 1.5% Na2CO3.

Statistical analysis

Frequencies of genotypes, alleles and Hardy-Weinberg test were computed using TPFPA version 1.3. The data on milk production traits and somatic cell count were analyzed using the PROC MIXED (SAS Institute Inc. 2001). To test the association between the TG-repeats and the traits, the following model was fitted to the phenotypic data:

\[
Y_{ijklm} = \mu + G_i + S_j + L_k + D_{im} + e_{ijklm}
\]

where: \(Y_{ijklm}\): Phenotypic value of the milk production traits and somatic cell count of the animal \(k\) with a genotype \(I\), \(\mu\): overall mean, \(G_i\): fixed effect of the TG genotype, \(S_j\): fixed effect of season, \(L_k\): fixed effect of lactation, \(D_{im}\): random effect of sire, \(D_{im}\): random effect of dam and \(e_{ijklm}\): random error.

Traits analyzed with this model were average of daily milk yield during lactation, fat percentage, protein percentage and SCC. The distribution frequency of SCC values was highly skewed; therefore, the SCC values (expressed in thousands) were transformed to the natural logarithm scale (Shooke, 1993), and converted to somatic cell score (SCS).

Results

Three different SSCP patterns, A, B and C (Figure 1), were observed for the amplified fragment of 5′-flanking region of bovine GHR gene in 518 animals. The samples revealing other SSCP patterns were excluded from further analysis. Sequence analysis of different SSCP patterns revealed that the TG-repeats contained only two alleles differing by 4 repeat units (Figure 2). According to sequence analysis, the SSCP patterns of A, B and C equaled to homozygous for 20-TG-repeats (TG\textsubscript{20/20}), homozygous for 17-TG-repeats (TG\textsubscript{17/17}) and heterozygous (TG\textsubscript{17/20}), respectively.

**Figure 1.** Different SSCP patterns of amplified fragment at the 5′-flanking region of GHR gene (A = TG\textsubscript{20/20}, B = TG\textsubscript{17/17}, C = TG\textsubscript{17/20}).

Genotypes of TG-repeats in the 5′-flanking region of GHR had a significant effect on the average daily milk yield \((P < 0.05)\) and tended to associate with fat \((P = 0.058)\) and protein percentage \((P = 0.089)\). No significant association was found between these geno-
5'-flanking region of bovine growth hormone gene

![Figure 2. Sequence chromatographs of TG-repeats (CA-repeat) in the 5'-flanking region of GHR gene obtained by reverse primer. a) Short allele with 17-TG-repeats, b) Long allele with 20-TG-repeats.](image)

types and the SCC ($P = 0.245$). The TG_{17/17} genotype had significantly lower average daily milk yield compared to TG_{17/20} and TG_{20/20} genotypes. The short allele (TG_{17}) had negative effect on milk yield and positive effect on milk fat and protein percentage (Table 1). The expected genotypic frequencies were similar to the observed ones, suggesting that genotypic distributions were at Hardy-Weinberg equilibrium (Table 2).

**Discussion**

Many studies have already reported on the association between length variation in TG-microsatellite of GHR gene and performance traits in livestock. For example, the short allele (TG_{11}), which was identified in Bos taurus and Bos indicus cattle, was associated with growth traits in Angus steers (Hale et al., 2000). Angus steers having the short length of TG-repeats had lower weaning weight, carcass weight, and marbling scores in comparison with steers with the longer allele. Similar results were observed in Bos indicus-influenced composite cattle (Curi et al., 2005) and in Japanese Black cattle (Ohkubo et al., 2006). In addition, the TG-repeat polymorphism in the 5'-noncoding region of the bovine gene GHR was significantly associated with mean body live weight, daily live weight gain, cold carcass weight and weight of lean and fat in valuable cuts in Polish Black and White cattle. The longest allele with 21 TG repeats was superior for most carcass traits, but when growth rate was considered, the allele homozygote for 21-TG-repeats was inferior to other genotypes of TG-repeat (Maj et al., 2004). On the other hand, no associations were found between TG-repeat variants and milk production traits in Polish goat breeds (Maj et al., 2007). However the results of our study revealed that the TG-repeat polymorphisms were significantly associated with average daily milk yield and tended to associate with fat and protein percentage.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Genotype frequency (%)</th>
<th>Number of Genotypes</th>
<th>Allele frequency (%)</th>
<th>P value of HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG_{20/20}</td>
<td>0.21</td>
<td>126</td>
<td>0.475</td>
<td>0.61</td>
</tr>
<tr>
<td>TG_{17/20}</td>
<td>0.53</td>
<td>319</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TG_{17/17}</td>
<td>0.26</td>
<td>156</td>
<td>0.525</td>
<td></td>
</tr>
</tbody>
</table>

Such associations would suggest the existence of a direct effect of the TG- microsatellite on traits by changing the expression of GHR gene. In general, microsatellites located in intragenic regions (promoters, 5'- and 3'-untranslated regions, and introns) can be important regulators of gene expression by influencing the transcription rate, RNA stability, splicing efficiency, and RNA-protein interactions. Several lines of evidence indicated that microsatellite length polymorphisms were involved in transcriptional regulation and, therefore, they are an important source of variation in quantitative traits (Kashi et al., 1997; King et al., 1997; Li et al., 2002). For example, variation in the number of CA-repeats causes differing levels of epidermal growth factor receptor gene expression and concomitant protein production (shorter alleles produced greater protein), which may have potential as a predictor of clinical outcome in human breast cancer (Brandt et al., 2006). In addition, variable expression of NadA, an outer membrane protein and adhesion of the pathogen Neisseria meningitidis, is mediated by changes in the number of TAAA repeats located upstream of the core promoter of nadA gene (Martin et al., 2005). Furthermore, GT- repeat polymorphisms in the promoter region of Tilapia prolactin 1 (prl 1) are associated with
differences in *prl* 1 gene expression and the growth response of salt-challenged fishes (Streelman and Kocher, 2002). Also, transcription rates were incrementally decreased by stepwise increases in the repeat number from 16 to 20 for a CA-microsatellite located near an enhancer element in intron 1 at the human epidermal growth factor receptor gene (Gebhardt et al., 1999). It has been reported that stepwise increases in the repeat number from 0 to 21 for a CA-microsatellite, located in the promoter region of the human matrix metalloproteinase-9 gene, produced incremental increases in transcription rates (Shimajiri et al., 1999).

The effect of variable number of TG-microsatellite in the 5'-flanking region of bovine *GHR* gene was studied using reporter gene *luc* constructs. No effect of TG-variants was shown, various numbers of TG-repeats had similar transcriptional activity (Zhou and Jiang, 2005; Muhaghegh-Dolatabady et al., 2006). Maj and Zwierzchowski (2008), the present study provides evidence that in Holstein cattle QTL(s) segregate on the chromosomal region including *GHR* affect milk production traits on chromosome 20.

### Conclusions

The current results showed that the GHR TG-variants can be selected for genetic improvement of milk production traits in Holstein cattle. Although, variations of GHR TG-repeats are indicated to have similar transcriptional activity (Zhou and Jiang, 2005; Maj and Zwierzchowski, 2008), the present study provides evidence that in Holstein cattle QTL(s) segregate on the chromosomal region including GHR affect milk production traits on chromosome 20.

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