The relationship between growth hormone polymorphism and growth hormone receptor genes with milk yield and reproductive performance in Holstein dairy cows

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Summary

The aim of this study was to investigate the potential association between growth hormone GH/AluI and growth hormone receptor GHR/AluI polymorphisms with milk yield and reproductive performances in Holstein dairy cows in Iran. Blood samples of 150 Holstein cows were collected and their genomic DNA was extracted using Gene Fanavaran DNA extracting kit. Fragments of the 428 bp of exon 5 growth hormone (GH) gene and the 342 bp of exon 10 growth hormone receptor (GHR) gene were amplified using the polymerase chain reaction (PCR) method. PCR products were digested by the AluI restriction enzyme and electrophoresed on 3% agarose gel. Continuous and categorical data were analyzed using linear mixed models through Proc MIXED and logistic regression models through Proc GENMOD of SAS software, respectively. The results showed no relationship between the examined traits and GH/AluI or GHR/AluI genes. A significant relationship was found between GH/AluI polymorphism and dystocia, but the presence of the GH-L allele reduced the incidence of dystocia. The results suggest that the GH-LL genotype reduces dystocia probably by affecting the release of growth hormone; nevertheless, further studies will be needed to examine the relationship between dystocia and GH genotypes.

Key words: Dystocia, Logistic regression, Stillbirth

Introduction

Growth hormone (GH), also known as somatotropin, is a peptide hormone of about 190 amino acids that is synthesized and secreted by cells known as somatotrophs in the anterior pituitary (Hediger et al., 1990). This hormone is a major regulator of postnatal growth and metabolism in mammals and plays critical roles in the control of lactation, mammary gland development, growth processes, and fertility in cows (Renaville et al., 2002; Lucy, 2008). Previous research shows a significant relationship between polymorphisms in the bovine growth hormone (bGH) gene and lactation performance (Ge et al., 2003; Zhou et al., 2005; Mullen et al., 2010). Bovine GH is a single-chain polypeptide of approximately 22 kDa, composed of 190 or 191 amino acids, located on chromosome 19 (Hediger et al., 1990). It consists of five exons separated by four introns (Gordon et al., 1983).

Several authors have identified polymorphisms in the promoter, third and fourth introns, and the fifth exon of bovine GH (Lucy et al., 1993; Yao et al., 1996; Ge et al., 2003). Lucy et al. (1993) reported that a substitution of cytosine (C) to guanine (G) at position 2141 caused an amino acid change from leucine to valine at residue 127 of the GH polypeptide. The GH exerts its effects on growth and metabolism by interacting with a specific receptor (GHR) on the surface of the target cells. Consequently, changes in the functional regions of the GHR affect its binding capacity and signaling pathway, and result in the alteration of GH activity in the target tissues (Olenksi et al., 2010). One polymorphism has been described in the bovine GHR promoter region, and at least four single nucleotide polymorphisms (SNP) have been found in its exon 10, coding for the cytoplasmic domain of GHR. The SNPs are located at positions 76 (T/C), 200 (G/A), 229 (T/C) and 257 (A/G) (Ge et al., 1999; Ge et al., 2000). The SNPs at positions 200 and 257 induce amino acid substitutions, alanine to threonine and serine to glycine, respectively, whereas the other two are synonymous mutations.

The aim of this study was to investigate the potential relationship between GH/AluI and GHR/AluI polymorphisms and milk yield and reproductive performances in Holstein dairy cows in Iran.

Materials and Methods

The study was carried out on 150 Holstein dairy cows reared in two herds located in Fars province, Iran. The herds were purebred Holsteins, medium in size, each consisting of about 450 male and female calves, heifers and cows. The herds were managed under conditions similar to those of most developed countries, and were
under official performance and pedigree recording. The diet was fed as a TMR and consisted of corn silage, alfalfa hay, barley grain, fat powder, beet pulp, soybean meal, and feed additives. Monthly milk recording was performed by trained technicians of the Iranian Animal Breeding Center, according to the guidelines of the International Committee for Animal Recording (ICAR, 2011).

Genomic DNA was extracted from fresh blood samples using commercially available DNA extraction kits (Gene-Fanavar Co., Tehran, Iran), whose quality was assessed using a spectrophotometer. Samples with an optical density (OD) ratio (260 nm/280 nm) between 1.7 and 1.9 were used for the PCR process (n = 130).

PCR amplification was performed to obtain the 428-bp fragment of the GH gene with forward (5’-CGCTTAGCAAACGC-3’) and reverse (5’-GTTCTTGGACCCGGCT-3’) primers (Lucy et al., 1993). The PCR was carried out in a final volume of 25 µL reaction mixture containing 60 ng DNA, 2.5 µL 10 X PCR assay buffer, 200 µM of each dNTP, 1 unit Taq DNA polymerase (MBI Fermentas, Germany), 0.40 µM of each primer, and 1.2 mM MgCl₂. Thermal cycling conditions included an initial denaturation step at 94°C for 10 min followed by 30 cycles of 94°C for 30 s, 56°C for 60 s, 72°C for 35 s and a final extension at 72°C for 10 min.

In order to obtain a 342-bp fragment of the GHR gene, forward (5’-GCTAACATCTGCTGACACAC-3’) and reverse primers (5’-CTAGTTGGCATGTTTGGACGACCCGGCTA-3’) suggested by Di Stasio et al. (2005) were used. PCR was carried out in a final volume of 25 µL reaction mixture containing 60ng DNA, 2.5 µL 10 X PCR assay buffer, 200 µM of each dNTP, 1 unit Taq DNA polymerase (MBI Fermentas, Germany), 0.55 µM of each primer and 2 mM MgCl₂. Thermal cycling conditions included an initial denaturation step at 94°C for 5 min followed by 35 cycles of 94°C for 45 s, 50°C for 30 s, 72°C for 50 s and a final extension at 72°C for 10 min.

PCR tubes were kept in a pre-programmed thermocycler (Eppendorf AG Master cycler Gradient, USA) for amplification. For both genes, a volume of 20 µL of PCR product was digested with 5 units of the AluI restriction enzyme (Metabion, Germany) and 10 X assay buffer at 37°C overnight. The digested product was separated on a 3% agarose gel at 70 V for 1.5 h, stained with ethidium bromide and visualized under UV light.

The relationship between the SNPs and continuous performance traits (e.g. milk yield, calving interval, days open, number of inseminations per conception, and days to first service) was quantified using multiple regression mixed models through Proc MIXED in SAS (1999) using the following statistical model:

\[ y_{ijkl} = \mu + h_1y + g_k + p_j + a_l + r_{ijkl} \]

where

- \( h_1y \): Dependent variable on l\textsuperscript{th} animal, at j\textsuperscript{th} parity belonging to the k\textsuperscript{th} combination of herd-year-season of calving
- \( \mu \): Overall mean
- \( r_{ijkl} \): Residual of the model with mean of 0 and a normal distribution

The following DNA restriction fragments were obtained for the GH/AluI polymorphism (Fig. 1): 265, 147 and 16 bp for the GH-VV genotype, 265, 96, 51, and 16 bp for the GH-LL genotype, 265, 147, 96, 51 and 16 bp for the GH-LV genotype (16 bp was not found in the gel). Frequencies of the GH-LL, GH-LV and GH-VV genotypes were 39, 61 and 0%, and the GH-L allele had a higher frequency (69%) than the GH-V allele (31%). The following DNA restriction fragments were obtained for the GHR/AluI polymorphism (Fig. 2): 191 and 151 bp for the GHR-GG genotype, 191, 151, 101, and 50 for the GHR-AG genotype, 191, 101, and 50 bp for the GHR-AA genotype. Frequencies of the GHR-AA, GHR-AG and GHR-GG genotypes were 30, 70 and 0%, and the GHR-A had higher frequency (64%) than GHR-G allele (36%).

The results of the statistical analyses showed no relationship between the examined traits and GH/AluI or GHR/AluI genes (Tables 1 and 2). A significant relationship was found between the GH/AluI gene and dystocia, whereas the presence of GH-L allele reduced the incidence of dystocia (Table 2).
Statistical analyses showed no association between the GHR/Alu and GHR/Alu polymorphism genotypes and categorical traits (Odds ratio ± (95% confidence interval)).

**Table 2:** Relationship between the GHR/Alu and GHR/Alu polymorphism genotypes and categorical traits (Odds ratio ± (95% confidence interval))

<table>
<thead>
<tr>
<th>Locus</th>
<th>Dystocia</th>
<th>Stillbirth</th>
<th>Birth-number</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHR/Alu</td>
<td>0.44 ± (0.2-0.98)</td>
<td>0.46 ± (0.1-1.99)*</td>
<td>0.26 ± (0.05-1.28)*</td>
</tr>
<tr>
<td>GHR/Alu</td>
<td>1.67 ± (0.69-4.06)*</td>
<td>1.28 ± (0.25-6.58)*</td>
<td>0.84 ± (0.19-3.78)*</td>
</tr>
</tbody>
</table>

Significant at P<0.05. *Not significant P≥0.05

Fig. 1: PCR-RFLP patterns of the bovine growth hormone receptor exon 5 digested with AluI restriction enzyme (PCR product size = 428 bp)

Fig. 2: PCR-RFLP patterns of bovine growth hormone receptor exon 10 digested with AluI restriction enzyme (PCR product size = 428 bp)

Frequencies of the GHR-AA, GHR-AG and GHR-GG genotypes were 30, 70 and 0%, and the GHR-A had higher frequency (64%) than GHR-G allele (36%). Di Stasio et al. (2005) reported the frequencies of GHR-AA, GHR-AG and GHR-GG genotypes to be 24, 50 and 26%, respectively. Kovacs et al. (2006) reported the frequencies of GHR-AA, GHR-AG and GHR-GG genotypes to be 78, 20 and 2%, respectively. Hradecka et al. (2008) reported the frequencies of GHR-AA, GHR-AG and GHR-GG genotypes to be 91, 7 and 2%, respectively.

The results of the statistical analyses showed no relationship between the examined traits with the GHR/Alu or GHR/Alu genes. Several other researches have also investigated the association between GH/Alu and GHR/Alu with lactation and reproductive performance in Holstein dairy cows and come up with very inconsistent results (Lucy et al., 1993; Lee et al., 1996; Shariflou et al., 2000; Dybus, 2002; Sørensen et al., 2002; Kovacs et al., 2006; Balogh et al., 2009; Oleniski et al., 2010). Similar to the present work, many studies showed no association between GH/Alu polymorphism and estimated breeding value for milk (EBV-Milk), releasing of GH and days to first service (Lee et al., 1996; Sørensen et al., 2002; Balogh et al., 2009). In contrast to the present study, other researchers reported relationships between GH/AluI and milk yield,
and the \textit{GH-L} allele and higher milk yield in Holstein dairy cows (Lucy et al., 1993; Shariflou et al., 2000; Dybus, 2002). Varvio et al. (2008) reported no significant relationship between milk yield and its composition and \textit{GHR/AluI} polymorphism. Kovacs et al. (2006) reported no relationship between \textit{GHR-AluI} polymorphism and reproductive measures such as first calving age or calving interval. On the other hand, several studies including Di Stasio et al. (2005) and Olenski et al. (2010) have reported relationships between \textit{GHR-AluI} polymorphism and meat characteristics and lactation performance. In order to explain such inconsistencies, multiple genetic and environmental factors influencing most quantitative traits in dairy cows, including milk yield and reproductive performance must be taken into account. As such, alleles associated with these traits seem to be less deterministic and more probabilistic. In addition, it is important to note that genetic associations are not consistently reproducible due to the possibility of gene-gene interactions, gene-environment interactions, weak genetic effect, linkage disequilibrium, genetic structures of the examined population and sample size.

The results of the present study indicate a significant relationship between the \textit{GH/AluI} gene and dystocia, whereas the presence of the \textit{GH-L} allele reduced the incidence of dystocia. According to Fholenhag et al. (1994), growth hormone does not cross the placenta; nevertheless, elevated maternal GH concentrations may improve nutrient supplies to the gravid uterus by inhibiting the lipogenic actions of insulin and increasing glucose concentrations in maternal and fetal circulations, causing the fetus to become larger in size. Wallace et al. (2006) reported fetal weight to elevate in ewes treated by bGH at day 130 of gestation. Schlee et al. (1994) showed that Holstein cows with the \textit{GH-LL} genotype released more GH than those with \textit{GH-LV} or \textit{GH-VV} genotypes. The results of the present study suggest that the \textit{GH-LL} genotype can reduce the incidence of dystocia by affecting the release of growth hormone. Nevertheless, further research is required to explain the relationship between dystocia and GH genotypes.

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