

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 (Olenski *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'-CCG TGT CTA TGA GAA GC-3') and reverse (5'-GTT CTT GAG CAG CGC GT-3') primers (Lucy *et al.*, 1993). The PCR was carried out in a final volume of 25 μ L reaction mixture containing 60 ng DNA, 2.5 μ L 10 X PCR assay buffer, 200 μ M of each dNTP, 1 unit *Taq* DNA polymerase (MBI Fermentas, Germany), 0.40 μ M 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'-GCT AAC TTC ATC GTG GAC AAC-3') and reverse primers (5'-CTA TGG CAT GAT TTT GTT CAG-3') suggested by Di Stasio *et al.* (2005) were used. PCR was carried out in a final volume of 25 μ L reaction mixture containing 60ng DNA, 2.5 μ L 10 X PCR assay buffer, 200 μ M of each dNTP, 1 unit *Taq* DNA polymerase (MBI Fermentas, Germany), 0.55 μ M 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 μ L 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:

$$y_{ijl} = \mu + hys_i + p_j + g_k + b_1(FCA_{ijl}) + a_1 + e_{ijl}$$

where

y_{ijl} : Dependent variable on l^{th} animal, at j^{th} parity belonging to the i^{th} combination of herd-year-season of calving

μ : Overall mean

hys_i : The fixed effect of the i^{th} combination of herd-year-season of calving

p_j : The fixed effect of the j^{th} parity

g_k : The fixed effect of the k^{th} SNP genotype

b_1 : Regression coefficient of the dependent variable on age at first calving

FCA_{ijl}: The covariate effect of first calving age

a_1 : The random effect of the l^{th} animal

e_{ijl} : Residual of the model with a mean of 0 and a normal distribution

The relationship between the SNPs and categorical performance traits (e.g. birth number, stillbirth, dystocia) was quantified using multivariable logistic regression models through the maximum likelihood method of Proc GENMOD in SAS (1999) using the following statistical model:

$$y_{ijkl} = \mu + h_i + y_j + s_k + p_l + g_m + b_1(FCA_{ijkl}) + s_n + e_{ijkl}$$

where;

$$y_{ijkl}: \text{Dependent categorical variable expressed as } \left(\ln \left(\frac{p}{1-p} \right) \right)$$

when;

p : The probability of twin birth, stillbirth or difficult calving

The dependent variables were birth number (0 for single and 1 for double), stillbirth (0 for livebirth and 1 for stillbirth), and dystocia (0 for easy calving and 1 for difficult calving). Independent variables were herd, calving year, calving season, parity, FCA, and random effect of service sire. To analyze stillbirth and dystocia, the effect of calf sex was included in the model as a fixed effect. Reference categories used to compare odds ratios for each effect were spring, primiparous, male, and genotype GH-LL and GHR-AA, respectively. For each trait, 452 records on 130 cows in two herds were used in the association analysis and p-values less than 0.05 were considered as significant.

Results

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).

Table 1: Least squares means (\pm SE) of calving interval, days open, first postpartum estrus, milk yield, and number of inseminations for *GH/AluI* and *GHR/AluI* genotypes

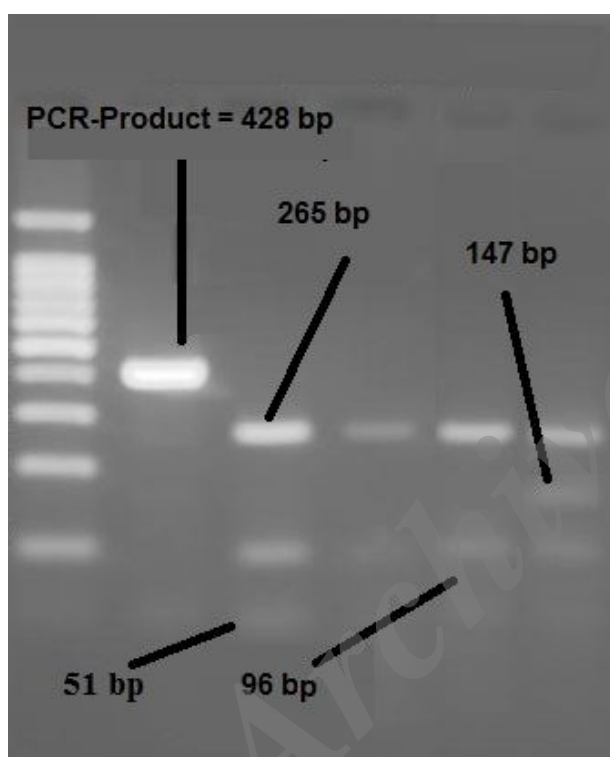
Gene	Genotypes	CI (day)	Days open (day)	The first postpartum estrus (day)	Milk yield (kg)	Number of inseminations
<i>GH/AluI</i>	LL	433 \pm 14 ^a	152 \pm 18 ^a	91 \pm 27 ^a	9317 \pm 542 ^a	3 \pm 0.3 ^a
	LV	427 \pm 14 ^a	136 \pm 17 ^a	94 \pm 27 ^a	8979 \pm 518 ^a	3 \pm 0.3 ^a
<i>GHR/AluI</i>	AA	422 \pm 15 ^a	142 \pm 19 ^a	95 \pm 27 ^a	8932 \pm 555 ^a	2.7 \pm 0.4 ^a
	AG	434 \pm 15 ^a	144 \pm 17 ^a	90 \pm 27 ^a	9142 \pm 519 ^a	2.8 \pm 0.4 ^a

Different letters indicate significant differences ($P < 0.05$) within columns for each gene

Table 2: Relationship between the *GH/AluI* and *GHR/AluI* polymorphism genotypes and categorical traits (Odds ratio \pm (95% confidence interval))

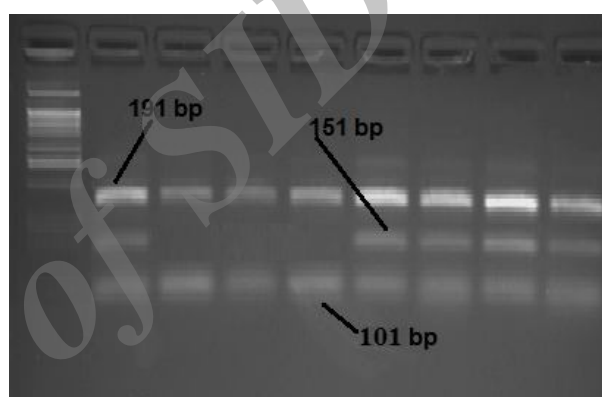
Locus	Dystocia	Stillbirth	Birth-number
<i>GH/AluI</i>	0.44 \pm (0.2-0.98)*	0.46 \pm (0.1-1.99) ^{ns}	0.26 \pm (0.05-1.28) ^{ns}
<i>GHR/AluI</i>	1.67 \pm (0.69-4.06) ^{ns}	1.28 \pm (0.25-6.58) ^{ns}	0.84 \pm (0.19-3.78) ^{ns}

* Significant at $P < 0.05$. ^{ns} Not significant $P \geq 0.05$

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

Discussion

Frequencies of the *GH-LL*, *GH-LV* and *GH-VV* genotypes were 39, 61 and 0%, and the *GH-L* allele had higher frequency (69%) than *GH-V* allele (31%). Lucy *et al.* (1993) demonstrated that dairy cow breeds with the largest mature size (e.g. Holstein) had a higher *GH-L* allele frequency, whereas dairy cattle with a smaller mature size (e.g. Jersey) had a higher *GH-V* allele. Dario *et al.* (2008) reported the frequencies of *GH-LL*, *GH-LV* and *GH-VV* genotypes to be 22, 61 and 17%, respectively. Balogh *et al.* (2009) reported the frequencies of *GH-LL*, *GH-LV* and *GH-VV* genotypes to be 83, 17 and 0%, respectively.

**Fig. 2:** PCR-RFLP patterns of bovine growth hormone receptor exon 10 digested with *AluI* restriction enzyme (PCR product size = 342 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*, *GH-AG* and *GH-GG* genotypes to be 24, 50 and 26%, respectively. Kovacs *et al.* (2006) reported the frequencies of *GHR-AA*, *GH-AG* and *GH-GG* genotypes to be 78, 20 and 2%, respectively. Hradecka *et al.* (2008) reported the frequencies of *GHR-AA*, *GH-AG* and *GH-GG* genotypes to be 91, 7 and 2%, respectively.

The results of the statistical analyses showed no relationship between the examined traits with the *GH/AluI* or *GHR/AluI* genes. Several other researches have also investigated the association between *GH/AluI* and *GHR/AluI* 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; Olenski *et al.*, 2010). Similar to the present work, many studies showed no association between *GH/AluI* 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 *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 *GHR/AluI* polymorphism. Kovacs *et al.* (2006) reported no relationship between *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 *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 *GH/AluI* gene and dystocia, whereas the presence of the *GH-L* allele reduced the incidence of dystocia. According to Fohlenhag *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 *GH-LL* genotype released more GH than those with *GH-LV* or *GH-VV* genotypes. The results of the present study suggest that the *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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مقاله کامل:

همراهی چند ریختی ژن هورمون رشد و گیرنده آن با عملکرد تولیدی و تولید مثلی در گاوهای هلستاین

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هدف این پژوهش بررسی همراهی چند ریختی جایگاه ۵ ژن هورمون رشد و چند ریختی جایگاه ۱۰ ژن گیرنده هورمون رشد، با صفات تولیدی و تولید مثلی در گاوهای هلستاین بود. در این پژوهش از ۱۵۰ گاو هلستاین خونگیری شد و سپس DNA ژنومیک آن‌ها با استفاده از کیت استخراج DNA شرکت ژن فناوران استخراج شد. ژن‌های مورد مطالعه، با استفاده از پرایمرهای اختصاصی و با واکنش‌های زنجیره‌ای پلیمرز تکثیر شدند. برای تعیین ژنوتیپ در دو ژن، آنزیم محدود کننده *AluI* استفاده شد، و سپس نمونه‌ها روی ژل آگارز ۳٪ الکتروفورز شدند. داده‌های صفات پیوسته با رویه MIXED و داده‌های صفات گسسته با رویه GENMOD در نرم افزار SAS واکاوی شدند. نتایج نشان داد که چند ریختی *AluI* در جایگاه ۵ ژن هورمون رشد با سخت‌زایی همراهی معنی‌دار داشت، اما با دیگر صفات همراهی نداشت. چند ریختی *AluI* در جایگاه ۱۰ ژن گیرنده هورمون رشد با صفات تولید شیر و صفات تولید مثلی همراهی نداشت. اگرچه تشریح همراهی سخت‌زایی و ژنوتیپ‌های ژن هورمون رشد به مطالعات بیشتری نیاز دارد، اما می‌توان بیان کرد که ژنوتیپ *GH-LL* احتمالاً با اثر بر آزادسازی هورمون رشد سخت‌زایی را کاهش می‌دهد.

واژه‌های کلیدی: سخت‌زایی، رگرسیون لجستیک، مرده‌زایی