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Full Length Research Paper

Identification of polymorphism in promoter region of growth hormone receptor (GHR) gene and its association with milk related traits in Holstein cows

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It is widely accepted that hormones, growth factors and other agents exert their biological effects on target tissues by binding to specific receptors on the plasma membrane. The variability in constituent sequences of growth hormone receptor (GHR) gene is very important because of its major role in mammary gland development. The aim of the present study is to detect polymorphism in promoter region of GHR gene and its association with milk related traits in Holstein cows. Blood samples were randomly collected from 93 Holstein cows, transported to the laboratory and stored at -20°C for further analysis. DNA was extracted using modified salting-out method and a fragment of 836 base pair from promoter region of GHR gene was amplified by a specific primer pairs using polymerase chain reaction (PCR). The PCR products were digested by *AluI* restriction enzyme and electrophoresed on 2% agarose gel. Result of enzyme digestion for GHR gene, showed allele *AluI*(-) with the fragment sizes of 14, 75 and 747, and allele *AluI*(+) with the fragment sizes of 14, 75, 145 and 602 base pairs. Frequency estimation of *AluI*(-) and *AluI*(+) alleles were 56 and 44%, respectively, and mean of heterozygosity was 0.49. Statistical analysis showed that cows with *AluI*(+) allele had significantly higher milk protein and fat percentage at first lactation compared with cows with *AluI*(-/-), while there was no significant relation between different genotypes and other traits.

Key words: Growth hormone receptor, polymorphism, milk, Holstein.

INTRODUCTION

Nowadays, new molecular techniques such as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) are used in animal breeding domain as new and powerful tools with the aim of providing breeders with the opportunity to carefully identify better animals immediately. Growth hormone contains many metabolic and physiological actions (Chagas et al., 2007). Growth hormone is a well-known somatotropin, with a main motive of growth traits and milk production (Etherton and Bauman, 1998). In several studies, it has been indicated that there are association between variants of growth hormone gene and milk production (Hoj et al., 1993; Lagziel et al., 1996; Yao et al., 1996). One of the important factors in relation to growth hormone action is growth hormone receptor which facilitates growth and development of udder glands (Feldman et al., 1993).

The connection of growth hormone to receptor is caused as a result of change in the structure of the cell outside the region of the receptor as it acts on janus kinase2 (Jak2), which is one of the cytoplasm kinase tyrosine. Mutation in growth hormone receptor prevents Jak2 from

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Abbreviations: GHR, Growth hormone receptor; **PCR-RFLP,** polymerase chain reaction-restriction fragment length polymorphism; **Jak2,** janus kinase2; **STAT5,** signal transducer and activator of transcription 5; **T,** thymine; **A,** adenine; **G,** guanine; **SNP,** single nucleotide polymorphism; **EDTA,** ethylenediaminetetraacetic acid; **IGF-I,** insulin-like growth factor I; **SCC,** somatic cell count; **BW,** body weight; **DW,** dressed weight; **SFT,** subcutaneous fat thickness; **HW,** hatch weight.

acting and this affects the cascade transportation of growth hormone message (Argetinger and Carter-Su, 1996). Jak2 and signal transducer and activator of transcription 5 (STAT5) proteins phosphorylation is caused to act once and so phosphorylated STAT5 is transported from the cytoplasm to the nucleus. The proteins are banded to special region of DNA in the nucleus and these, therefore, cause the increment of gene copy number (Herrington and Carter-Su, 2001). Growth hormone receptor gene, which may be accounted as quantitative trait loci (QTL) for milk production trait and its composition, is found in chromosome 20 in cows (Arranz et al., 1998). Falaki et al. (1996) reported the correlation between TaqI polymorphism in growth hormone receptor gene and protein percentage in Italian Holstein cattle.

Aggry et al. (1999) introduced *Alu*I(+) allele as a marker for milk fat percentage in Holstein breed. Lucy et al. (1998) found micro satellite polymorphism in growth hormone receptor gene promoter and indicated $(TG)_{16-20}$ as a general allele in European cows. Viitala et al. (2006) studied growth hormone receptor gene role in milk, milk fat and milk protein production of Arshire breed. They showed that the polymorphism of gene had more effect on fat and protein percentage. Zhou and Jiang, (2006) showed that there was a nucleotide replacement of thymine (T) to adenine (A) in exon 8 of the growth hormone receptor gene and it had more effect on milk production of cow. Sherman et al. (2008) identified the polymorphisms in the growth hormone receptor gene and studied their asso-ciation with growth size, function, food efficiency and carcass quality of beef cattle. They indicated that single nucleotide polymorphism (SNP) (A to G) is placed either in exon 4 or promoter gene and both of them had a signi-ficant association with body weight and carcass quality. Garret et al. (2008) studied association between SNPs in growth hormone receptor promoter gene and body fat amount in Brangus beef cattle. They found that there are significant association between genotypes and rib fat amount. The aim of this study is to identify polymorphism in the promoter region of growth hormone receptor gene and its association with milk related traits in Holstein cows.

MATERIALS AND METHODS

Sampling and extraction of DNA

Blood samples were randomly collected from 93 Holstein cows from Astan–e–Ghods Razavi's farm. The collection of 5 ml blood was done from the sub tail vessel and through the venoject container holding the opposed material (ethylenediaminetetraacetic acid (EDTA) 0.5 M, pH = 8). Blood samples were placed on ice plate and transported to the laboratory. They were stored at -20°C for further analyses. DNA extracted using modified salting-out method was stated by Miller et al. (1988).

Primers designing and PCR

The promoter region of the cattle GHR gene containing one fragment of 836bp in length (position - 1871 to -1036) was amplified (Aggrey et al., 1999). PCR was performed in a reaction volume of 25 µl using 200 ng of DNA, 10 µM of each primer, 1 x PCR buffer (50 mM KCL, 2.5 mM $MgCl_2$, and 10 mM Tris - Hcl PH = 9.0), 200 µM dNTP, and 1 units of Taq DNA polymerase. Amplification was carried out for 35 cycles at 92°C for 60 s, 66°C for 80 s and 72°C for 120 s after the preheating at 94°C for 10 min using a thermal cycle.

Digestion and genotype detection

For RFLP analysis, 7 µl of the PCR products were digested with 4 units of AluI restriction enzyme and incubated at 37°C for 3 h. Digested DNA fragments were separated using electrophoresis on 2% agarose gel in 1x TPE (90 mM Tris phosphate, 2 mM EDTA). The gel was stained with ethedium bromide and visualized under UV light.

Statistical analysis

Data for 305 days milk production of lactation I, II, III and IV, including overall yield of milk, milk fat and protein, percentage of milk fat and protein, were obtained from the Astan–e–Ghods Razavi's farm. The records of the fat and protein percentage were divided into three periods of 100 milk days: 0 - 100 milk days for first period; 101 - 200 milk days for second period; and 201-300 milk days for third period. Statistical analysis was performed using statistical analysis system (SAS) procedures (SAS, 2003). The effect of GHR genotypes on the milk production traits were analyzed using general linear model (GLM) procedure of SAS software. The following model was used:

$$Y_{ijklm} = \mu + a_i + b_j + c_k + d_l + e_{ijklm}$$

Where, Y_{ijklm}: analyzed trait in lactation I, II, III and IV cow; µ: the overall mean of population; a_i: fixed effect of GHR genotype (I = 1, 2 and 3); b_j: fixed effect of the lactation; c_k: fixed effect of milk month; d_l: fixed effect of year-season of calving class; e_{ijklm}: the random residual error.

Average effect of allele substitution was determined by coding genotypes as 0 *Alu*I(-/-), 1 *Alu*I(+/-) and 2 *Alu*I(+/+) to represent the number of B alleles present for the GHR polymorphism. As described by Falconer and Mackay (1996), the regression coefficient (α) estimates the average effect of allele substitution, or the average effect of replacing a *Alu*I(-) allele with a *Alu*I(+) allele. The y-intercept (a) estimates the average value of the *Alu*I(-/-) genotype and dominance deviations (b) estimates the difference between observed and predicted values at the *Alu*I(+/-) genotype.

RESULTS

The digested *Alu*I(-/-) PCR product exhibited three fragments of 747, 75 and 14 bp (was not detected on the gel). For the *Alu*I(+/+) PCR product, the 747 bp fragment was cleaved into two fragments of 602 and 145 bp. Figure 1 shows the restriction patterns of the three genotypes- *Alu*I(+/+), *Alu*I(+/-) and *Alu*I(-/-) upon digestion of the PCR products. The frequency of *Alu*I(-) and *Alu*I(+) alleles were 0.56 and 0.49, respectively. Also, genotype frequencies of *Alu*I(-/-), *Alu*I(+/-) and *Alu*I(+/+) were 29.03, 53.76 and 17.2, respectively.

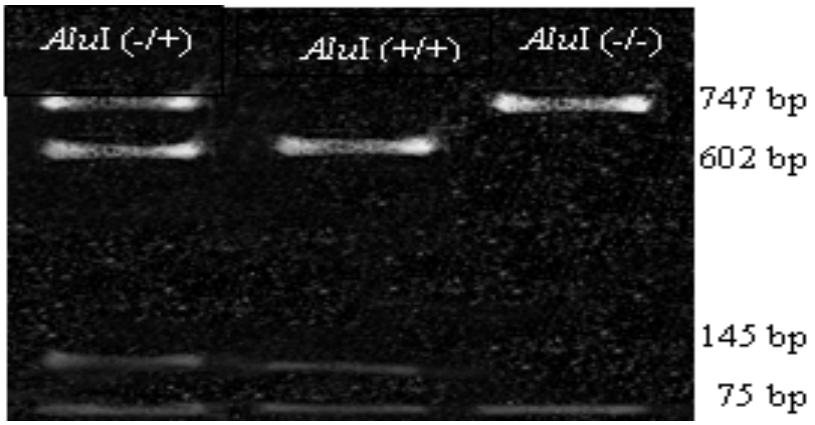

Figure 1. Restriction fragment length polymorphisms within the promoter region of the cattle growth hormone receptor PCR product separated on a 2% agarose gel.

Table 1. Means and standard deviations of milk production traits in cattle with different GHR-*Alu*I genotypes.

Genotype	LSR ± SE					
	Fat (%)			Protein (%)		
	First period	Second period	Third period	First period	Second period	Third period
*Alu*I (-/-)	2.55±0.092[b]	2.72±0.091	2.86±0.067	2.75±0.052[b]	2.8±0.048	2.99±0.036
*Alu*I (-/+)	3.02±0.088[a]	2.91±0.059	2.96±0.069	3.16±0.032[a]	3.08±0.034	3.13±0.032
*Alu*I (+/+)	2.9±0.11[a]	2.91±0.11	3.03±0.079	3.24±0.052[a]	3.25±0.058	3.13±0.051

Table 2. Means and their standard deviations for milk production traits in cattle.

Genotype	LSR ± SE			
	Total F (%)	Total P (%)	305 yield	SCS[1]
*Alu*I (-/-)	3.02±0.088	3.02±0.048	7064±356.9	21.34±0.17
*Alu*I (-/+)	3.13±0.036	2.99±0.032	7120±279.4	21.24±0.15
*Alu*I (+/+)	3.23±0.036	2.9±0.048	7038±339.3	20.81±0.19

[1] SCS = Somatic cell score; SCS = $3+\text{Log}_2$ SCC; SCC = somatic cell count.

The effects of different genotypes of GHR on milk production traits

Results are shown in Tables 1 and 2 of the RFLP-*Alu*I polymorphism of the GHR gene on milk production traits. Statistical analysis showed that cows with *Alu*I(+) allele had higher milk protein and fat percentage at first lactation (P<0.05) compared with cows with *Alu*I(-/-) (Table1).

There was no significant relation between different genotypes and other traits. The average effects of replacing a *Alu*I(-) with a *Alu*I(+) are given in Table 3. The average effects of the gene substitution for GHR amounted to -118.19 kg for 305 milk yield and -0.018 and -0.008 for total fat and protein percentage, respectively.

DISCUSSION

In this study, part of the GHR promoter region including a fragment of 836 bp length was amplified by special primers. Three genotypes, *Alu*I(-/-), *Alu*I(-/+) and *Alu*I(+/+) were detected and their frequency were 29.03, 53.76 and 17.2, respectively, while the frequency of the *Alu*I(-) and *Alu*I(+) alleles were 0.56 and 0.44, respectively. Aggrey et al. (1999) developed a PCR-based method for detecting *Alu*I, *Acc*I and *Stu*I RFLPs in the 5´ franking region of the bovine growth hormone receptor gene and it has been tested for association with milk-related traits of Holstein bulls. They reported that allele frequency of the *Alu*I(-) allele was 0.63 and 0.42 in the bulls from 1950 to

Table 3. Estimates of the allele substitution effect of GHR.

Trait	[a]α	a[b]	d[c]
Total fat (%)	-0.018±0.004	3.02±0.0044	-0.52±0.048
First period fat (%)	-0.122±0.009	3.07±0.01	-0.48±0.063
Second period fat (%)	-0.103±0.07	3.09±0.076	0.56±0.012
Third period fat (%)	-0.01±0.005	3.01±0.005	-0.11±0.01
Total protein (%)	-0.008±0.002	3.18±0.027	-0.86±0.079
First period protein (%)	0.026±0.0036	3.18±0.0039	-0.23±0.051
Second period protein (%)	0.011±0.036	3.14±0.0039	-0.99±0.051
Third period protein (%)	-0.021±0.003	3.15±0.0028	-0.4±0.054
305 milk yield	-118.193±21.36	7131.95±149.92	57.67±24.66

[a]Average effect of allele substitution; [b]average breeding value of the *Alu*I(+/+) genotype; [c]dominance deviation.

1970 and in the 1980s, respectively. Also, they showed that bulls with *Alu*I(+/+) genotype had higher fat content (P ≤ 0.016) compared with *Alu*I(-/-) and *Alu*I(+/-) bulls. However, in this study, the frequency of the *Alu*I(-) allele was 0.56. Also, statistical analysis has shown that cows with *Alu*I(+) allele had significantly higher milk protein percentage and milk fat percentage at first lactation period compared with *Alu*I(-/-) cows. Ge et al. (2000) detected SNP in exon10 of the bovine growth hormone receptor gene. They amplified a 528 bp fragment from genomic DNA by PCR. DNA fragments amplified from individuals of the two homozygous genotypes were sequenced and four single nucleotide polymorphisms were identified by aligning the sequences. Four SNPs were at positions of 76 (T→C), 200 (G→A), 229 (T→C), and 257 (A→G) bp from the 5´ end of the fragment. They reported that the SNP at 200 and 257bp from the 5´ end changed amino acid encoding from alanine (Ala) to threonine (Thr) and from serine (Ser) to glycine (Gly), respectively. Hale et al. (2000) compared growth and carcass traits between Angus steers that had two of the longer growth hormone receptor alleles, with their half-siblings that had one short allele and one of the longer alleles. They found that contrasts for long/long homozygotes (the longer 16 to 20-TG-repeat alleles) vs the short/long heterozygotes (a short 11-TG allele) were significant for weaning weight (17±4 kg; P<0.001) and carcass weight (14 ± 5 kg; P < 0.01). Ge et al. (2003) studied polymorphisms in the promoter and coding regions of growth hormone receptor gene and its association with growth traits and serum insulin-like growth factor I (IGF-I) concentration in Angus cattle. They reported that there were a significant relation between SNP (A to G) in promoter region of growth hormone receptor gene and serum IGF-I concentration. Maj et al (2006) detected association of the polymorphism in the 5′-non-coding region of the bovine growth hormone receptor gene with meat production traits in polish black-and-white cattle. They found that the (-/-) genotype at RFLP-*Alu*I appeared favorable for weight of carcass, carcass dressing percentage, and weight of lean invaluable cuts. Curi et al. (2006) searched for the effects of GHR gene polymorphisms on growth and carcass traits in zebu and crossbred beef cattle. Significant associations (P<0.05) were observed between the *Alu*I(N/N) genotype of the GHR/*Alu*I polymorphism and lower weight gain and body weight during slaughter. Fontanesi et al. (2007) researched on milk production QTL and candidate gene analysis in the Italian Holstein-Friesian breed. The effect of the known GHR F279Y and PRLR S18N mutations were for the most part confirmed. Marchitelli et al. (2007) reported the characterization of single-nucleotide polymorphisms in 20 genes affecting milk quality in cattle sheep, goat and buffalo. They indicated that major allele frequencies ranged from 0.509 (GHR) to 0.0996 (PRLR). Also preliminary statistical analysis suggests association of some polymorphisms and milk quality. Maj et al. (2007) studied a TG-repeat polymorphism in the 5′-noncoding region of the goat growth hormone receptor gene and search for its association with milk production traits. No associations were found with dairy traits-milk yield and content of the major milk components (fat, protein, and lactose). Also, no effect was shown of the GHR genotype on the somatic cell count (SCC). Garrett et al. (2008) studied the association between SNP discovery in promoter region of the bovine growth hormone receptor gene and performance in Brangus bulls. Two A and G alleles were identified in the promoter region of the gene. They found that bulls of the GG genotype had rib fat of 6.1% more than bulls of the AA and AG genotypes, respectively. Ouyang et al. (2008) studied SNP at the GHR gene and its associations with chicken growth and fat deposition traits. They found that the SNP, G6631778A, was associated with body weight at 63 d (BW63), dressed weight (DW) and subcutaneous fat thickness (SFT), BW35 and BW49 (P<0.01) as well as hatch weight (HW) and BW42 in the male population. Signorelli et al. (2009) studied the exploration of polymorphisms and its effects on milk traits of the DGAT1, SCD1 and GHR genes in four cattle breeds. They confirmed the positive effect of GHR F279Y variant on milk fat and protein content.

Conclusion

In summary, it appears that there is improvement of fat and protein content (%) of milk at first lactation period. Therefore the GHR *Alu*I(+) allele should be promoted in Holstein cows.

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