Genetic Variation of Growth Hormone Gene and Its Relationship with Milk Production Traits in China Holstein Cows

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ABSTRACT : Associations were analysed between polymorphisms localized in intron 3 of the growth hormone gene (*GH-Msp*I) and milk production traits of 543 China Holstein cows. A PCR-RFLP method was used for identification of genotypes. The following frequencies of genotypes and alleles were found: 0.77, 0.21 and 0.02 for +/+, +/- and -/-, respectively, and 0.87 and 0.13 for GH^+ and GH^-, respectively. Significant differences between herds were observed in the frequencies of both genotypes and alleles. The results of least squares analysis showed that in all three lactation phases the *GH* +/+ cows yielded most milk ($p<0.01$ for lactation I and $p<0.05$ for lactations II and III), whereas +/- cows showed higher milk fat content than +/+ individuals ($p<0.05$ for lactation I and II, and $p<0.01$ for lactation III). The +/+ cows yielded more fat than +/- individuals ($p<0.01$ only in lactation I). The +/+ cows yielded more milk protein than +/- individuals ($p<0.01$ for lactation I, II, and III). The +/+ cows produced milk of higher protein content than that of +/- individuals ($p<0.05$ only in lactation II). Based on these results, we conclude that the +/+ of *GH* locus should be the favored genotype in China Holstein cow breeds for use in marker-assisted selection programmes. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 3 : 315-318)***

Key Words : China Holstein Cows, Growth Hormone Gene, PCR-RFLP, Genetic Polymorphism, Milk Production Traits

INTRODUCTION

Wallis (1973) reported that bovine growth hormone (bGH) is a single peptide of about 22kDa molecular weight. Lingappa et al. (1977) and Wallis et al. (1973) reported, respectively, that it is composed of 190 or 191 amino acids, containing Ala or Phe at the N-terminus, due to alternative processing of bGH precursors. Moreover, Leu or Val amino acid substitutions at residue 127 exist due to allelic polymorphism (Seavey et al., 1971).

Hediger et al. (1990) reported that bovine GH gene *(GH)* is localized in chromosome 19, and Gordon et al. (1983) and Woychick et al. (1982) reported that it consists of five exons separated by introns. Several polymorphisms were identified in the *GH* gene. Cowan et al. (1989) and Hilbert et al. (1989) detected a polymorphic site for *Msp* I restriction endonuclease, the polymorphism being localized in intron 3 of the *GH* gene in position 1,547 (Zhang et al., 1993). Wang et al. (2003) detected a polymorphic site for Apa I restriction endonuclease. Biswas et al. (2003) and Aruna Pal (2004) detected a polymorphic site for Alu I restriction endonuclease.

Studies on the effect of the *GH-Msp* I polymorphism on production traits in cattle are quite advanced, but the results obtained by various authors are not always in agreement. Hojet al. (1993) and Lee et al. (1993) showed that $GH^{(Msp-)}$ allele was more frequent in the lines of cows with high milk fat content. Similar results were reported by Falaki et al. (1996), who demonstrated that $GH^{(Msp-)}$ allele was associated with increased milk fat content in Holstein Friesian cows, the effect, however, being not significant. Lagziel et al. (1996, 1999) reported that heterozygous cows produced milk with higher protein content than +/+ individuals. Different results were published by Yao el al. (1996), who showed that $GH^{(Msp+)}$ allele positively affected milk (+300 kg), fat (+8 kg) and protein (+7 kg) yields.

The aim of this study was to estimate the allelic frequencies at the *GH-Msp* I *loci* and to investigate the relationship between these polymorphisms and milk production traits of China Holstein cows.

MATERIALS AND METHOD

Materials

A total of 543 China Holstein cows were used at five dairy cattle farms (farms 1, 2, 3, 4 and 5) in the cow centre of Beijing, P. R. China. Number of cows per farm was 93, 62, 85, 117 and 186, respectively. Lactations of cow were divided into three phases (I, II and III) in a year, which denoted 20-120 d, 121-210 d and 211-305 d after parturition, respectively. Only cows with a complete lactation were included in the statistical analysis (only 450 cows with lactation I, 300 cows with lactation I and II and 183 cows with lactation I, II and III). Blood samples were collected from all 543 China Holstein cows; to each these was added an anticoagulant (ACD = 0.48 g citric acid, 1.32 g citrate sodium, 1.47 g dextrose, and deionised H_2O to a final volume of 100 ml). Blood samples were stored at -20°C. The EDTA, xylene cyanol FF, bromophenol blue, and

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Table 1. Frequencies of genotypes and alleles of the *GH* gene (*RFLP-Msp* I)

Herd	No.of cows	Genotype			Allele	
		+/+	+/-	-/-	GH^+	GH^-
1	93	0.76^{aB}	0.23^{bA}	0.02	0.87^{aB}	0.13^{aB}
2	62	0.73^{eC}	0.26^{fD}	0.02	0.86^{bc}	0.15^{bc}
3	85	0.86^{aCG}	0.12^{ADG}	0.02	0.92^{abC}	0.08^{abC}
4	117	0.63^{BGH}	0.34^{bGH}	0.03	0.80^{BCD}	0.20^{BCD}
5	186	0.83^{eH}	0.16^{fH}	0.01	0.91^{cD}	0.09^{cD}

$^{A-C, a-c}$ Within columns frequencies bearing the same superscripts differ significantly at: small letter: $p<0.05$, capitals: $p<0.01$.

agarose were from Sino-America Biotechnology Ltd. The acrylamide, TEMED, ammonium persulphate, ethanol, $AgNO_3$ and citrate sodium were from Beijing Chemical Reagent Company. The proteinase K was from Merck Co Ltd of Germany and dNTPs from Gibco BRL Co Ltd. The *Taq* polymerase was from TaKaRa Co Ltd (Dalian P. R. China).

DNA samples

Genomic DNA was extracted from blood samples by the phenol/chloroform method followed by ethanol precipitation (Sambrook, 1989) and dissolved in TE solution at -20°C.

Detection of *GH* gene polymorphisms

The *GH-Msp* I genotypes were analysed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. A 329 bp fragment of intron 3 of *GH* gene was amplified by PCR using forward (5'-CCCACGGGCAAGAATGAGGC-3') and reverse (5'-TGAGGAACTGCAGGGGCCCA-3') primers (Mttra et al., 1995). The following cycles were applied: denaturation-94°C/5 min, followed by 30 cycles: denaturation-94°C for 1min, primer annealing -60°C for 50 s, PCR products synthesis -72°C for 1 min, and final synthesis -72°C/10 min.

Amplified DNA was digested by *Msp* I enzyme at 37°C for 2 h with the following reaction mixture: PCR product 7.5 µl, buffer 1 µl, *Msp*I 0.2 µl (1U), ddH_2O 1.3 µl. The digestion products were separated by horizontal electrophoresis (90 volts, 50 minutes) in 2% agarose gels in 1×TBE and 1.0 µM ethidium bromide.

Statistical analysis

Data for 305 day milk production in lactation I, II and III, including overall yield of milk, milk fat, milk protein, per cent of milk fat and per cent of milk protein, were obtained from the farm record. Statistical calculations were performed using SAS procedures. Frequencies of distribution of alleles within the herds were compared with the *Chi-square* test. The effect of *GH* genotypes on the milk production traits of cows were analysed using the GLM procedure of SAS. The following model was used:

$$Y_{ijklmn} = \mu + G_i + S_j + YS_k + H_l + b_1(x_1 - DD)_m + e_{ijklmn}$$

Where Y_{ijklmn} is trait analysed in lactation I, II and III of cow O; µ is the overall mean of population; G_i is fixed effect of *GH* genotype (i = 1, 2 and 3); S_j is fixed effect of the sire; YS_k is fixed effect of year-season of calving class; H_l is fixed effect of the herd; DD_m is days in milk; b_1: linear regression coefficient of days in milk; x_1 is days in milk of cow m; e_{ijklmn} is the random residual error.

RESULTS

Detection of *GH* gene polymorphsim

The following DNA restriction fragments were obtained for the *GH-Msp* I polymorphism: 224 bp and 105 bp for the +/+ genotype, 329 bp, 224 bp and 105 bp for the +/- and 329 bp (no digestion) for the -/- genotype.

The *GH* +/+ genotype was found most frequent in all the herds studied (0.63-0.86), followed by the +/- (0.12-0.34). The least frequent was the -/- genotype (0.01-0.03). The frequency of the GH^+ allele ranged from 0.80 to 0.92 (Table 1).

The effects of different genotypes on milk production traits

Table 2 shows the effect of the *RFLP-Msp* I polymorphism of the *GH* gene on milk production traits in cows studied.

In all lactations the cows with *GH* +/+ genotype had higher milk yield than +/- individuals ($p<0.01$ for lactation I and $p<0.05$ for lactations II and III).

Differences ($p<0.01$) between cows with different *GH-Msp* I genotype were observed only in lactation I. The +/+ cows yield more fat than +/- individuals.

Differences between cows of different *GH-Msp* I genotypes were found in all lactations ($p<0.05$ for lactation I and II, and $p<0.01$ for lactation III). In all three lactation, the +/- cows had higher milk fat content than those with +/+ genotype.

Differences between cows of different *GH-Msp* I genotypes were found in all lactations ($p<0.01$). The +/+ cows yielded more milk protein than +/- individuals.

Differences between cows of different *GH-Msp* I genotypes were found in lactation II ($p<0.05$). The +/+

Table 2. Means and their standard deviations for milk production traits in cows with different GH-Msp I genotypes

Lactation	Genotype	No. of cows	Least squares means*±standard deviation				
			Milk yield (kg)	Fat		Protein	
				kg	%	kg	%
I	+/+	343	5,310^{A}±1,302	229.1^{A}±63.4	4.17^{a}±0.40	168.7^{A}±47.3	3.15±0.19
	+/-	99	4,957^{A}±1,364	217.2^{A}±59.6	4.25^{a}±0.43	159.5^{A}±44.8	3.16±0.20
	-/-	8	5,291±1,253	223.8±48.9	4.20±0.46	165.1±40.3	3.12±0.21
II	+/+	225	5,726^{a}±1,425	241.2±61.7	4.19^{a}±0.51	187.2^{A}±49.0	3.23^{a}±0.22
	+/-	68	5,509^{a}±1,387	238.0±60.9	4.24^{a}±0.49	175.4^{A}±46.6	3.19^{a}±0.21
	-/-	7	5,660±1,312	230.9±57.1	4.15±0.47	178.7±44.9	3.15±0.20
III	+/+	136	6,030^{a}±1,520	246.6±70.3	4.09^{A}±0.51	196.9^{A}±50.9	3.20±0.19
	+/-	43	5,823^{a}±1,506	247.7±71.6	4.21^{A}±0.53	185.1^{A}±50.4	3.17±0.18
	-/-	4	6,012±1,921	247.2±77.6	4.15±0.50	191.1±64.2	3.19±0.29

$^{A-C, a-c}$ Within columns frequencies bearing the same superscripts differ significantly at: small letter: $p<0.05$, capitals $p<0.01$.

cows produced milk of higher protein content than that of +/- individuals.

DISCUSSION

GH gene frequency

Frequencies of *GH-Msp* I alleles obtained in this study are similar to those reported earlier for the Black-and-White cattle. The highest frequency of the GH^{+} (1.00) in Holsteins was reported by Langziel et al. (2000). GH^{+} frequencies higher (0.90 and 0.91) than obtained in this study were also found, respectively, by Sabour et al. (1997) and Vukasinovic et al. (1999), while Yao et al. (1996) and Falaki et al. (1996) reported somewhat smaller values (0.86 and 0.83, respectively).

Milk production traits

In this study, in all lactations the cows with +/+ genotype had higher milk yield. Similar results were obtained by Yao et al. (1996) who showed that $GH^{(Msp+)}$ allele increased milk yield by 300 kg. In lactation I the cows with +/+ genotype yielded more milk fat. Similar results were reported by Yao el al. (1996) who demonstrated an increased milk fat yield (+8 kg) in +/+ cows. In all lactations the cows with +/- genotype had higher milk fat per cent. Similar results were obtained by Hoj et al. (1993) and Lee et al. (1993), who reported that $GH^{(Msp-)}$ allele was more frequent in the lines of cows with high milk fat per cent. Falaki et al. (1996) reported that GH $GH^{(Msp-)}$ allele increased milk fat content in Holstein-Friesian cows, but the effect was not significant. Chrenek et al. (2003) reported that *GH* L/L genotypes were significantly associated with better milk production traits, mainly the fat content. In all lactations the cows with +/+ genotype yielded more milk protein. Similar results were reported by Lagziel et al. (1999) and Falaki et al. (1996), who showed that +/- cows produced more milk protein than +/+ animals. However, Yao et al. (1996) demonstrated that $GH^{(Msp+)}$ allele increased milk protein yield by 8 kg in cows. In lactation II the cows with +/+ genotype had more milk protein content. Different results were obtained by Lagziel et al. (1996, 1999) who reported that heterozygotes produced milk with higher protein per cent than +/+ cows.

Summarizing, it appears that in the improvement of milk, milk fat and milk protein yield in dairy cattle, the $GH^{(Msp+)}$ allele should be promoted. On the other hand, the improvement of fat content (%) in milk should prefer $GH^{(Msp-)}$ allele rather, which, however, decreased milk yield in the cows examined.

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