Brief communication

Association of genetic polymorphism in *GH* gene with milk production traits in Beijing Holstein cows

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Associations were analysed between polymorphisms of the growth hormone gene (*GH-Msp*I) (localized in intron 3) and milk production traits of Beijing Holstein cows (a total of 543 cows). Polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) method was used for identification of various genotypes. Frequencies of genotypes were 0·77, 0·21 and 0·02 for A/A, A/B and B/B, respectively. The frequency of the GH^A allele is 0·875.

The results of the least squares means show that in all three lactations, the *GH* A/A cows yielded more milk ($P < 0·01$ for lactation I and $P < 0·05$ for lactations II and III), whereas A/B cows showed higher milk fat content than A/A individuals ($P < 0·05$ for lactations I and II, and $P < 0·01$ for lactation III). The A/A cows yielded more fat than A/B individuals ($P < 0·01$ only in lactation I). The A/A cows yielded more milk protein than A/B individuals ($P < 0·01$ for lactations I, II, and III). The A/A cows produced milk of higher protein content than of A/B individuals ($P < 0·05$ only in lactation II).

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1. Introduction

Wallis (1973) reported that bovine growth hormone (bGH) is a single peptide of about 22 kDa molecular weight. Lingappa *et al* (1977) and Wallis *et al* (1973) reported respectively that bGH 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 the allelic polymorphism (Seavey *et al* 1971).

Hediger *et al* (1990) reported that bGH gene (*GH*) is localized in chromosome 19, and Gordon *et al* (1983) and Woychick *et al* (1982) reported respectively that it comprised 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 the intron 3 of the *GH* gene in the position 1547 (Zhang *et al* 1993).

The studies on the effect of the *GH-Msp*I polymorphism on production trait in cattle are quite advanced, but the results obtained by various authors are not always corresponding. However, the general consensus appears to be that the GH^A allele increases milk, protein and fat yield, but decreases fat and protein percent (table 1).

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 Beijing Holstein cows.

Keywords. Beijing Holstein cows; growth hormone gene; genetic polymorphism; milk production traits

Abbreviations used: bGH, Bovine growth hormone; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism.

2. Materials and methods

2.1 *Materials*

A total of 543 China Holstein cows were used at five dairy cattle farms (1, 2, 3, 4 and 5 farms) in cow centre of Beijing, PR 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 denote 20–120 days, 121–210 days and 211–305 days after childbearing, respectively. Only cows with a complete lactation were included in the statistical analysis i.e. 450 cows with lactation I; 300 cows with lactations I and II; and 183 cows with lactations I, II and III. Time frame of all data collection is from 1994 to 2002.

2.2 *Genotyping*

Blood samples were collected from the cows. To the blood samples was added an anticoagulant (ACD = 0·48 g citric acid, 1·32 g citrate sodium, 1·47 g dextrose, H_2O deion was added to a final volume of 100 ml). Blood samples were stored at – 20°C. The EDTA, xylene cyanol FF, bromophenol blue, and agarose were from Sino-America Biotechnology Ltd. (Luoyang, China). The acrylamide, TEMED, ammonium persulphate, ethanol, AgNO3 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. (Grand Island, NY, USA). The Taq polymerase were from TaKaRa Co. Ltd. (Dalian PR China).

Beijing Holstein cows genomic DNA was extracted from blood samples by the phenol/chloroform method followed by ethanol precipitation (Sambrook *et al* 1989) and dissolved in TE solution at – 20°C.

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 (Mitra *et al* 1995). The following cycles were applied: denaturation – 94°C/5 min, followed by 30 cycles: denaturation – 94°C for 1 min, 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 (1 U), and ddH_2O 1·3 µl. The digestion products were separated by horizontal electrophoresis (90 volts, 50 min) in 2% agarose gels in 1 × TBE and 1·0 µM ethidium bromide.

2.3 *Statistical analysis*

Data for 305-day milk production in lactations I, II and III, including overall yield of milk, milk fat, milk protein, percent of milk fat and percent of milk protein, were obtained from the farm record. Statistical calculations were performed using SAS procedures. The effect of *GH* genotypes on the milk production traits of cows were analysed using GLM procedure of SAS. The following model was used:

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

where Y_{ijklmn} is trait analysed in lactations I, II and III of cow m; m is the overall mean of population; G_i is fixed

Table 1. Summary of results from the literature and the present study on frequency of the GH-Msp^A alleles, and on effects of the GH-Msp^A allele on milk production traits.

			Yield		Percent	
Author	Frequency	Milk	Protein	Fat	Protein	Fat
Lee *et al* 1993						D
Hoj *et al* 1993						D
Yao *et al* 1996	0·86	I	I	I		
Falaki *et al* 1996	0·83				D	D
Lagziel *et al* 1996					D	
Sabour *et al* 1997	0·90	I				
Lagziel *et al* 1999					D	
Lagziel *et al* 2000	1·00					
Vukasinovic *et al* 1999	0·91					
Present study	0·875	I	I	I	I	D

I, increasing effect; D, decreasing effect.

Table 2. Least squares means and standard errors (bracketed) for milk production traits in cows according to *GH-Msp*I genotypes.

Lactation	Genotype	No. of cows	Milk yield (kg)	Fat		Protein	
				kg	Percent	kg	Percent
I	A/A	343	5310** (1302)	229·1** (63·4)	4·17* (0·40)	168·7** (47·3)	3·15 (0·19)
	A/B	99	4957 (1364)	217·2 (59·6)	4·25 (0·43)	159·5 (44·8)	3·16 (0·20)
	B/B	8	5291 (1253)	223·8 (48·9)	4·20 (0·46)	165·1 (40·3)	3·12 (0·21)
II	A/A	225	5726* (1425)	241·2 (61·7)	4·19* (0·51)	187·2** (49·0)	3·23* (0·22)
	A/B	68	5509 (1387)	238·0 (60·9)	4·24 (0·49)	175·4 (46·6)	3·19 (0·21)
	B/B	7	5660 (1312)	230·9 (57·1)	4·15 (0·47)	178·7 (44·9)	3·15 (0·20)
III	A/A	136	6030* (1520)	246·6 (70·3)	4·09** (0·51)	196·9** (50·9)	3·20 (0·19)
	A/B	43	5823 (1506)	247·7 (71·6)	4·21 (0·53)	185·1 (50·4)	3·17 (0·18)
	B/B	4	6012 (1921)	247·2 (77·6)	4·15 (0·50)	191·1 (64·2)	3·19 (0·29)

*$P < 0{\cdot}05$, **$P < 0{\cdot}01$; all comparisons between AA and AB genotypes only, as there were too few BB genotypes for statistical analysis.

effect of *GH* genotype (i = 1, 2 and 3); S_j is fixed effect of the sire (j = 1, 2, 3…7); YS_k is fixed effect of year-season of calving class (k = 1, 2, 3 and 4); H_l is fixed effect of the herd; *DD* is average days in milk of the entire population; b_1: linear regression coefficient of trait value on days in milk; x_m is days in milk of cow m; e_{ijklmn} is the random residual error.

3. Results and discussion

The following DNA restriction fragments were obtained for the *GH-Msp*I polymorphism: 224 bp and 105 bp for the A/A genotype, 329 bp, 224 bp and 105 bp for the A/B and 329 bp (no digestion) for the B/B genotype. The *GH* A/A genotype had the highest frequency in all the herds studied (0·63–0·86), followed by the A/B genotype (0·12–0·34). The least frequent genotype was B/B (0·01–0·03). The frequency of the GH^A allele by herds, ranged from 0·80 to 0·92, with average 0·875 (table 1). Frequencies of *GH-Msp*I alleles obtained in this study are in the mid-range to those reported earlier for the black-and-white cattle (table 1).

Table 2 shows the effect of the *RFLP-Msp*I polymorphism of the *GH* gene on milk production traits. In all lactations the cows with *GH* A/A genotype had significantly higher milk and protein yield and lower fat percent than A/B individuals. In addition, protein percent for GH A/a genotypes was significantly greater in second lactation, and fat yield was significantly greater in first lactation. The results for milk, fat and protein yield agree with those obtained by Yao *et al* (1996) and Sabour *et al* (1997). The results for fat percent agree with those obtained by Lee *et al* (1993), Hoj *et al* (1993) and Falaki *et al* (1996), but results for protein percent do not agreee with those obtained by Lagziel *et al* (1999).

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

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