J. Anim. Breed. Genet. ISSN 0931-2668

ORIGINAL ARTICLE

Relationship of the Bovine Growth Hormone Gene to Carcass Traits in Japanese Black Cattle

K. Tatsuda[1], A. Oka[1], E. Iwamoto[1], Y. Kuroda[1], H. Takeshita[2], H. Kataoka[3] & S. Kouno[4]

1 Hyogo Prefectural Institute of Agriculture, Forestry and Fisheries, Kasai, Japan
2 Shimane Prefectural Livestock Technology Center, Izumo, Japan
3 Okayama Prefectural Center For Animal Husbandry & Research, Kume, Japan
4 Hiroshima Prefectural Technology Research Institute, Shobara, Japan

Keywords
Bovine growth hormone gene; carcass traits; Japanese black cattle; polymorphism.

Correspondence
Ken Tatsuda, 1533 Ko Minaminooka Befucyo Kasai-City Hyogo 679-0198, Japan.
Tel: +81 790 47 2430; Fax: +81 790 47 0549;
E-mail: ken_tatsuda@pref.hyogo.jp

Received: 17 June 2007;
accepted: 12 September 2007

Summary

The bovine growth hormone gene (bGH) possesses three haplotypes, A, B and C, that differ by amino acid mutations at positions 127 and 172 in the fifth exon: (leucine$_{127}$, threonine$_{172}$), (valine$_{127}$, threonine$_{172}$) and (valine$_{127}$, methionine$_{172}$) respectively. The correlation between meat quality or carcass weight and these haplotypes was investigated in Japanese black cattle. Altogether, 940 bGH haplotypes were compared with respect to six carcass traits: carcass weight, longissimus muscle area, rib thickness, subcutaneous fat thickness, beef marbling score and beef colour. The frequency of the B haplotype was higher (0.421) than that of A (0.269) and C (0.311). High carcass weight and low beef marbling were associated with haplotype A ($p < 0.05$ and $p < 0.01$ respectively), whereas beef marbling was increased by haplotype C ($p < 0.05$). Estimated regression coefficient of the A haplotype substitution effect for carcass weight and beef marbling score were 5.55 (13.1% of the phenotypic SD) and −0.31 (17.0%) respectively. That of the C haplotype for beef marbling score was 0.20 (11.0%). The other traits showed no relationship to the haplotypes examined. The results of this investigation suggest that information pertaining to bGH polymorphisms in Japanese black cattle could be used to improve the selection of meat traits.

Introduction

Given that the bovine growth hormone gene (bGH) can play a significant role in growth stimulation and milk production, investigations concerning this gene are critical when considering improvements in cattle production. The gene has been mapped on bovine chromosome 19 (Hediger *et al.* 1990) and the complete DNA sequence has been determined (Gordon *et al.* 1983). The bGH is present as a single copy gene, 1.8 kb in size, and consists of five exons and four introns (Woychik *et al.* 1982; Vukasinovic *et al.* 1999). The protein product consists of 191 amino acid residues. Within the exons, a polymorphism is found only in the fifth exon of the gene, where there is a leucine (Leu)/valine (Val) single-base polymorphism at amino acid residue 127 (Lucy *et al.* 1993). Chikuni *et al.* (1994) reported a new threonine (Thr)/methionine (Met) polymorphism in Japanese black cattle. This is also a single-base polymorphism: it produces two variants of bGH at amino acid residue 172. In Japanese black cattle, the bGH gene has haplotype A (Leu$_{127}$/Thr$_{172}$), haplotype B (Val$_{127}$/Thr$_{172}$) and haplotype C (Val$_{127}$/Met$_{172}$) (Chikuni *et al.* 1994). Haplotype C has not been observed in other breeds.

Investigations attempting to correlate bGH genotype with quantitative traits have mainly addressed milk production. Although previous reports have dealt with the restriction fragment length polymorphism

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(RFLP) polymorphism or the single-strand conformation polymorphism (SSCP) (Lucy *et al.* 1993; Yao *et al.* 1996; Falaki *et al.* 1997; Grochowska *et al.* 2001; Ge *et al.* 2003), the amino acid changes that result in the various observed traits remain unclear. Reports dealing with carcass traits are far fewer in number. Schlee *et al.* (1994) reported that Simmental bulls with Leu/Leu and Leu/Val genotypes had significantly higher breeding values for carcass gain, and that those with the Val/Val genotype for the meat classification score. Beauchemin *et al.* (2006) reported that associations of DNA polymorphisms in bGH (including the Leu/Val polymorphism) relative to growth and carcass characteristics in growing Brahman steers (n = 324 from 68 sires) were not clear. Thomas *et al.* (2007) reported that it was difficult to select advantageous genotype(s) for each trait in similar study using Brangus bulls (n = 434 from 48 sire). Only a single report has been published that addresses the relationship between bGH genotypes and quantitative traits in Japanese black cattle (Chikuni *et al.* 1994). That study used a small sample (n = 59) of meat-producing cattle. No clear relationship between bGH genotype and carcass traits or effect of Thr/Met mutation on carcass traits was found.

We investigated polymorphism of bGH in Japanese black cattle in a much larger data set. Our results could possibly be used to improve carcass traits.

Materials and methods

Sample

In total, 940 Japanese black cattle (718 steers and 222 heifers) originating from 86 sires (more than two offspring per sire) fattened in fourteen Japanese prefectures (Shizuoka, Ishikawa, Toyama, Fukui, Mie, Shiga, Wakayama, Kyoto, Hyogo, Shimane, Okayama, Hiroshima, Kagawa and Nagasaki) were used in this study. All animals had *ad libitum* access to concentrates and roughage in feedlots and were slaughtered at an average age of 29.5 months. The carcasses were dissected and evaluated at the sixth and seventh rib section according to the Japanese meat grading system by a certified grader of the Japan Meat Grading Association (JMGA 1988). The traits determined included carcass weight, longissimus muscle area, rib thickness, subcutaneous fat thickness, beef marbling score and beef colour. Marbling was scored from 1 (poor) to 12 (very abundant) according to the beef marbling standard. Beef colour was scored from 1 (light) to 7 (dark) according to the beef colour standard. Perinephric adipose tissue was collected at carcass competitions. Genomic DNA was extracted from perinephric adipose tissue using a DNeasy™ Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol.

Analysis of bGH polymorphisms

The bGH haplotype was determined by direct DNA sequence analysis using the genotyping method of Chikuni *et al.* (1997). Two single nucleotide polymorphisms (SNP) of bGH are in complete linkage disequilibrium (Leu_{127}-Thr_{172}, Val_{127}-Thr_{172} and Val_{127}-Met_{172}), and they show three haplotypes, A, B and C. A primer pair for PCR (GH6F; 5′-TAG-GGGAGGGTGGAAAATGGA-3′, GH6R; 5′-G ACA-CCTACTCAGACAAT GCG-3′, Yao *et al.* 1996) was designed, based on the bGH sequence information (Gordon *et al.* 1983), in such a way that amplification of the entire fifth exon was ensured. PCR was carried out in 50 μl reaction mixtures containing 100 ng DNA, 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 μM each of four dNTPs, 1.25 units AmpliTaq Gold DNA polymerase (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) and 5 pmol of each primer pairs. PCR consisted of a predenaturation step at 95°C for 9 min, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 65°C for 45 s, extension at 72°C for 45 s and a final extension at 72°C for 10 min. The length of the fragment produced was 404 bp. Sequence reactions were performed using a BigDye Terminator v3.1 Cycle Sequencing Kit (Perkin-Elmer Applied Biosystems). The reaction was carried out in 10 μl reaction mixtures containing 1 μl PCR product, 4 μl Terminator Ready Reaction Mix and 5 pmol of the forward primer (GH6F). Reaction mixtures were subjected to 94°C for 2 min, followed by 35 cycles consisting of 95°C for 30 s, 50°C for 10 s, 60°C for 4 min and finally at 72°C for 10 min. Purifying was carried out using a Gel Filtration Cartridge or a V3 96-well Short Plate (Edge BioSystems, Gaithersburg, MD, USA). Samples were subjected to acrylamide gel (36 cm) electrophoresis on a sequencer (377XL; Perkin-Elmer Applied Biosystems) for 4 h. The data were analysed using Sequencing Analysis software (Perkin-Elmer Applied Biosystems).

Statistical analysis

Data were analysed using the general linear model (GLM) procedure of SAS (SAS Institute 1993). Models of the general least-squares analysis of variance included sex and prefecture as fixed effects, sire as

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random effect, and slaughter age and haplotype substitution as regression variables. Three separate analysis were run, one for each haplotype. The model was

$$y_{ijkl} = \mu + \mathrm{se}_i + \mathrm{pr}_j + \mathrm{si}_k + b(x_{ijkl} - \bar{x}) + \beta z_l + e_{ijkl},$$

where y_{ijkl} = observation for the carcass trait, μ = overall mean, se_i = fixed effect of sex ($i = 1,2$), pr_j = fixed effect of prefecture ($j = 1\ldots14$), si_k = random effect of sire ($k = 1\ldots86$), b = regression coefficient for slaughter age, x_{ijkl} = slaughter age in months, $\bar{x}$ = average of slaughter age, β = regression coefficient of the haplotype substitution effect z_l = number of A, B or C haplotype ($\mathrm{l} = 0,1,2$) e_{ijkl} = random environment effect.

Hardy–Weinberg equilibrium test was performed using ARLEQUIN 3.1.1 (http://anthro.unige.ch/software/arlequin/). The relationships for the random effects of sires were not included.

Results

Distribution of bGH diplotypes

The distribution of bGH diplotypes is shown in Table 1. Frequencies of the A, B and C haplotypes were 0.269, 0.421 and 0.311 respectively. These frequencies well corresponded to values estimated using expectation maximization algorithm (Dempster *et al.* 1977) assuming Hardy–Weinberg equilibrium ($\mathrm{p} = 0.49386$).

Associations of bGH haplotype with production traits

The mean value and SD for six carcass traits are shown in Table 2. Table 3 shows the estimated regression coefficient of the haplotype substitution effect for six carcass traits. The A haplotype was associated with a higher carcass weight ($\mathrm{p} < 0.05$) and a lower beef marbling score ($\mathrm{p} < 0.01$). The C haplotype was associated with a higher beef marbling score ($\mathrm{p} < 0.05$). Estimated regression coefficient of the A haplotype substitution effect for carcass weight and beef marbling score were 5.55 (13.1% of the phenotypic SD) and −0.31 (17.0%) respectively. That of the C haplotype for beef marbling score was 0.20 (11.0%). Although not statistically significant, haplotype B and C showed negative effects on carcass weight. Haplotype A showed a positive effect on subcutaneous fat thickness, whereas haplotype C showed a negative effect.

Table 1 Distribution of bovine growth hormone gene (bGH) diplotypes

Diplotype	AA	AB	AC	BB	BC	CC
Number of animals	72	208	153	175	233	99
Frequency (%)	7.7	22.1	16.3	18.6	24.8	10.5

A, $\mathrm{Leu}_{127}/\mathrm{Thr}_{172}$; B, $\mathrm{Val}_{127}/\mathrm{Thr}_{172}$; C, $\mathrm{Val}_{127}/\mathrm{Met}_{172}$.
Frequencies of the A, B and C haplotype were 0.269, 0.421 and 0.311 respectively.

Table 2 Means and SD of six carcass traits in experimental animals

Trait	Mean	SD
Carcass weight (kg)	436.12	42.45
Longissimus muscle area (cm^2)	55.16	6.91
Rib thickness (cm)	7.37	0.77
Subcutaneous fat thickness (cm)	2.48	0.69
Beef marbling score[a] (BMS No.)	5.54	1.82
Beef colour[b] (BCS No.)	3.91	0.54

[a]Beef marbling score: scored from 1 (poor) to 12 (very abundant).
[b]Beef colour: scored from 1 (light) to 7 (dark).

Discussion

Our investigation showed that the frequency of the B haplotype was high whereas that of A was low. Chikuni *et al.* (1994) investigated the haplotype of 59 Japanese black cattle steers. They found that the frequencies of haplotypes A, B and C were 50.0, 14.4 and 35.6%, respectively. This difference could have resulted from the sampling method used by Chikuni *et al.* (1994). Japanese black cattle possess a particular breeding history because of closed herd breeding at various places in Japan and the haplotype frequency may therefore differ according to the region or strain investigated.

Carcass weight showed a higher value when haplotype A was present. Schlee *et al.* (1994) reported that the carcass weight breeding value of Simmental bulls with Leu/Leu and Leu/Val genotypes at amino acid residue 127 was significantly higher than those with the Val/Val genotype ($\mathrm{p} < 0.01$), which showed a very low carcass weight breeding value. In this study, the estimated regression coefficient of the A haplotype (Leu_{127}) substitution effect for carcass weight was significantly higher than that of the B and C haplotype (Val_{127}). This is consistent with the results of Schlee *et al.* (1994), and this indicates that the effect of haplotype A on growth is greater than that of haplotypes B or C. It is possible that the Leu/Val and Thr/Met mutations could affect the molecular function of the growth hormone gene product and in turn affect muscle formation.

The beef marbling score of animals with the Met (C) haplotype at amino acid residue 172 showed a

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Table 3 Estimated regression coefficients of the haplotype substitution effect and the haplotype effects as a proportion of the phenotypic standard deviation for six carcass traits

	A			B			C		
Trait	Regression coefficient (SE)	p	Proportion[a]	Regression coefficient (SE)	p	Proportion	Regression coefficient (SE)	p	Proportion
Carcass weight	5.55 (2.74)	0.04	0.13	−0.18 (2.28)	0.94	0.00	−3.88 (2.35)	0.10	−0.09
Longissimus muscle area	−0.11 (0.45)	0.81	−0.02	−0.29 (0.37)	0.43	−0.04	0.39 (0.38)	0.31	0.06
Rib thickness	−0.04 (0.06)	0.52	−0.05	0.03 (0.05)	0.48	0.04	−0.01 (0.05)	0.86	−0.01
Subcutaneous fat thickness	−0.02 (0.05)	0.74	−0.03	−0.01 (0.04)	0.90	−0.01	0.02 (0.05)	0.67	0.03
Beef marbling score	−0.31 (0.12)	0.01	−0.17	0.03 (0.10)	0.79	0.02	0.20 (0.10)	0.05	0.11
Beef colour	0.02 (0.05)	0.67	0.04	−0.03 (0.04)	0.47	−0.06	0.02 (0.05)	0.69	0.04

[a]Haplotype effects as a proportion of the phenotypic standard deviation.

higher value than animals with the other haplotypes. Chikuni *et al.* (1994) suggested the high probability of B to C mutations. Furthermore, gene C was only found in Japanese black cattle and Japanese brown cattle, and not found in Holstein, Hereford or Aberdeen Angus. Consequently, they suggested that gene C originated from an ancestor of Japanese native cattle, and that gene C was linked with a high beef marbling score, which is a characteristic of Japanese native cattle. The estimated regression coefficient of the C haplotype (Met_{172}) substitution effect for beef marbling score was significantly higher than that of the A and B haplotype (Thr_{172}) in this study, and supports their suggestion.

Although the difference was not statistically significant, the subcutaneous fat thickness showed a higher value when haplotype A was present and showed a lower value when haplotype C was present. A reverse tendency to the beef marbling score was therefore observed. This indicates that different genotypes of bGH affect muscular tissue lipogenesis and subcutaneous fat deposition. It is possible that the Leu/Val and Thr/Met mutation could affect the molecular function of the growth hormone gene product, which would then in turn affect fat deposition. In particular, when Leu or Met is homozygous, the effect should be stronger.

In conclusion, given the differences in carcass weight and beef marbling score of fattening cattle relative to the bGH polymorphisms, it is likely that bGH mutations influence growth and fat deposition. This gene may therefore be an indicator that could be used for the genetic improvement of bovine quantitative traits. Combining bGH polymorphism data with breeding value information could conceivably improve selection in Japanese black cattle. Marbling could be increased by mating animals possessing haplotype C, and body weight gain could be increased by mating animals possessing haplotype A. By determining the haplotype of feeder cattle from the genotype of sires, efficient fattening could be performed based on the selection of feeder cattle with the same type.

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