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Importance of polymorphism in the promoter region of GH gene for beef production and quality

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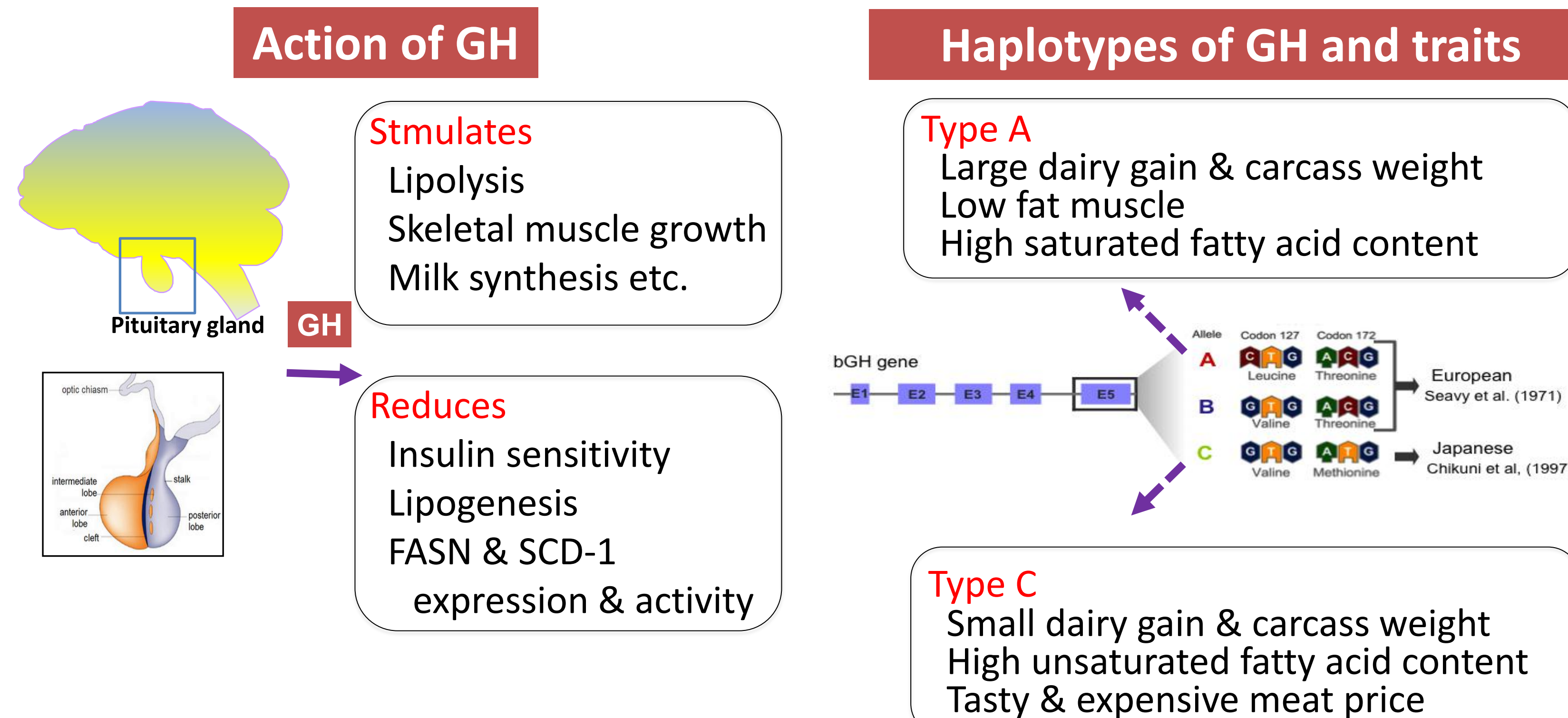
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Introduction

Single nucleotide polymorphism (SNP) in the genes for growth hormone (GH), fatty acid synthase (FASN) and stearoyl-coA desaturase (SCD) are massively involved in performance of meet quality of Japanese Black cattle. For GH SNP, cattle with the allele C showed lower carcass weight (CW) but richer intramuscular oleic acid % (C18:1) than those with other alleles (A and B). The SNPs preferable for high-fat meet production have also been reported for FASN and SCD genes.

Action of GH

Pituitary gland — GH

Stmulates
Lipolysis
Skeletal muscle growth
Milk synthesis etc.

Reduces
Insulin sensitivity
Lipogenesis
FASN & SCD-1
 expression & activity

Haplotypes of GH and traits

Type A
Large dairy gain & carcass weight
Low fat muscle
High saturated fatty acid content

bGH gene — Allele A / B / C (Codon 127 Leucine / Valine / Valine, Codon 172 Threonine / Threonine / Methionine) → European Seavy et al. (1971), Japanese Chikuni et al. (1997)

Type C
Small dairy gain & carcass weight
High unsaturated fatty acid content
Tasty & expensive meat price

Objective

The aims of the present study were
1) to identify SNPs at the promoter region of GH gene, and
2) to analyze the interaction between GH promoter SNPs and meet production and quality, in Japanese Back cattle.

Materials and Methods

1) Animals and Sample collection

Japanese Black cattle (n = 1189) were used for genotyping of the selected SNPs. The cattle used were 798 steers and 391 heifers raised in Miyagi prefecture. Animals were harvested at a slaughterhouse in Miyagi prefecture from year 2009 to 2011 at an average age of 30.5 ± 2.0 months.

A portion of the intramuscular adipose tissue was collected from the cross-sectional location at the 6th-7th thoracic vertebrae of longissimus thoracis muscle, and a portion of skeletal muscle tissue was sampled from carcasses

2) DNA extraction and the analysis of fatty acid composition

The adipose and muscle tissues were stored at -20°C until analyses of fatty acid composition and genomic DNA extraction, respectively.

3) Analysis of SNPs in the genes of GH, GH promoter, FASN and SCD-1

GH and GH promoter : Direct sequencing
FASN and SCD-1: PCR-RFLP

4) Statistical analysis

The statistical analysis was performed by GLM procedure (SAS). The statistical model included fixed effects (sex, sampling year, sire and genetic information of FASN, SCD and GH promoter), interaction effect between GH promoter genotype and sex, and random effect of slaughter age. The statistical model of the genotype of g.253 or g.303 of GH promoter was as follow:

$y_{ijkl} = \mu + sex_i + year_j + sire_k + bx_{ijkl} + FASN + SCD + GH253 + (GH253*sex_i) + e_{ijkl}$ or
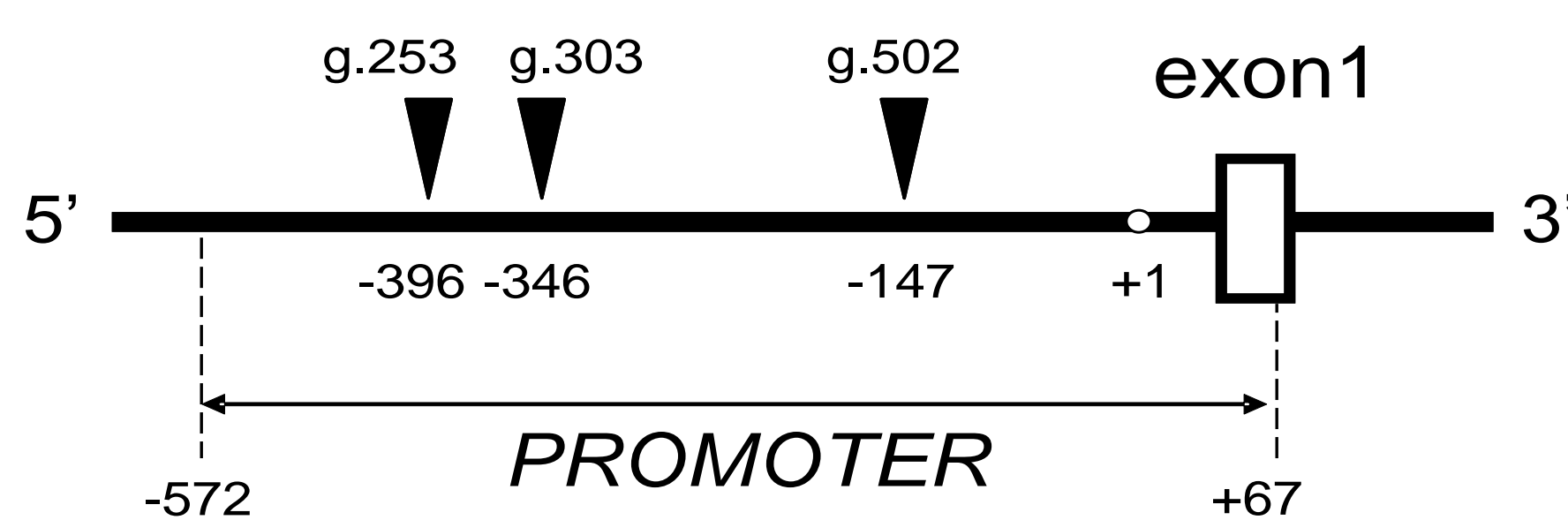
$y_{ijkl} = \mu + sex_i + year_j + sire_k + bx_{ijkl} + FASN + SCD + GH303 + (GH303*sex_i) + e_{ijkl}$

The statistical model of the diplotypes of GH promoter was as follow:

$y_{ijkl} = \mu + sex_j + year_k + sire'_l + bx_{jklm} + FASN + SCD + GHdip + (GHdip*sex_i) + e_{ijklm}$

Results

1. Structure of the promoter region of bGH gene and location of polymorphisms

g.253 g.303   g.502   exon1
-396 -346   -147   +1
5'   PROMOTER   3'
-572   +67

There are three C>T transitions at genomic position 253, 303 and 502 according to Gordon (1983), or -396, -346 and -147 relative to the transcription initiation site in bGH promoter.

2. Haplotype frequency of bGH gene promoter region and exon 5

GH promoter haplotype	PROMOTER			GH promoter frequency	GH haplotype (Chikuni et al., 1994)
	g.253 (-396)	g.303 (-346)	g.502 (-147)		
P	T	C	C	0.14	A
Q	C	C	C	0.13	A
R	C	C	T	0.05	A
S	T	T	C	0.68	B, C

3. Genotypes of bGH promoter region and distribution of allele frequencies

Genomic position	genotype (n)			Allele frequency	
	CC	CT	TT	C	T
g.253	23	232	508	0.18	0.82
g.303	91	309	363	0.32	0.68
g.502	692	70	1	0.95	0.05

4. Diplotype distribution of bGH gene promoter polymorphisms

Diplotype	Genomic position			n	Diplotype frequency
	g.253	g.303	g.502		
PP	TT	CC	CC	21	0.028
PQ	CT	CC	CC	36	0.047
PR	CT	CC	CT	11	0.014
PS	TT	CT	CC	124	0.163
QQ	CC	CC	CC	10	0.013
QR	CC	CC	CT	12	0.016
QS	CT	CT	CC	138	0.181
RR	CC	CC	TT	1	0.001
RS	CT	CT	CT	47	0.062
SS	TT	TT	CC	363	0.476

5. Effects of GH promoter genotypes on carcass traits and fatty acid compositions

Trait	Genotype at g.253			Genotype at g.303		
	CC (n = 23)	CT (n = 232)	TT (n = 508)	CC (n = 91)	CT (n = 309)	TT (n = 363)
Price (yen/kg)	1577.4	1671.0	1673.8	1704.0	1629.4	1588.8
CW (kg)	423.2 [B]	423.2 [b]	438.4 [a]	438.4 [A]	423.2 [A]	399.5 [B]
REA (cm2)	54.9 [b]	59.2 [ab]	61.1 [a]	59.6	58.8	56.9
RT (cm)	7.4 [B]	7.7 [B]	8.0 [A]	8.0 [A]	7.7 [A]	7.4 [B]
SFT (cm)	2.4	2.5	2.5	2.6	2.5	2.4
YE (%)	73.2	73.7	73.9	73.5	73.7	73.6
BMG	3.8	4.1	4.1	4.0	4.0	4.0
BCG	3.6	3.9	3.9	3.9	3.8	3.7
BTG	3.5	3.8	3.8	3.8	3.7	3.7
C14:0	2.2 [C]	2.6 [B]	2.8 [A]	2.9 [A]	2.5 [B]	2.2 [C]
C16:0	24.9 [B]	26.3 [A]	26.0 [A]	26.5 [A]	25.6 [B]	25.1 [B]
C16:1	3.8 [B]	4.1 [B]	4.6 [A]	4.6 [A]	4.3 [B]	3.7 [C]
C18:0	12.4 [A]	12.0 [A]	11.0 [B]	11.3 [B]	11.6 [B]	12.6 [A]
C18:1	54.3 [A]	52.7 [B]	53.1 [AB]	52.5 [B]	53.6 [A]	54.1 [A]
C18:2	2.4	2.3	2.4	2.3 [b]	2.5 [a]	2.4 [ab]
SFA	39.5 [AB]	40.9 [A]	39.8 [B]	40.6 [a]	39.7 [b]	39.9 [ab]
MUFA	58.1 [AB]	56.8 [B]	57.7 [A]	57.1	57.9	57.7

Price = price per weight; CW = carcass weight; REA = rib eye area; RT = rib thickness; SFT = subcutaneous fat thickness; YE = yield estimate; BMG = beef marbling grade; BCG = beef color grade; BTG = beef texture grade. Values without common lettres within one row of the same genomic position highly differ (^{A,B}P<0.01) or differ (^{a,b}P<0.05).

6. Effects of diplotypes of bGH gene promoter region on carcass traits and fatty acid compositions (n=394).

Traits	PP (n = 21)	QQ (n = 10)	SS (n = 363)
Price (yen/kg)	1760.5	1523.4	1649.3
CW (kg)	450.9 [a]	367.4 [b]	423.7 [ab]
REA (cm2)	61.8	53.9	60.4
RT (cm)	8.3	7.6	7.8
SFT (cm)	3.0	2.8	2.5
YE (%)	73.5	73.1	73.9
BMG	4.4	3.7	4.1
BCG	4.3	3.5	3.9
BTG	4.2	3.4	3.8
C14:0	2.8	2.3	2.4
C16:0	26.4 [a]	23.8 [b]	25.2 [ab]
C16:1	4.9 [a]	4.3 [b]	4.2 [b]
C18:0	10.2 [y]	11.8 [x]	11.8 [x]
C18:1	53.6	55.6	54.0
C18:2	2.2	2.4	2.4
SFA	39.4	37.8	39.4
MUFA	58.5	59.9	58.2

Price = price per weight; CW = carcass weight; REA = rib eye area; RT = rib thickness; SFT = subcutaneous fat thickness; YE = yield estimate; BMG = beef marbling grade; BCG = beef color grade; BTG = beef texture grade. Values without common lettres differ (^{a,b}P<0.01) or with tendency (^{x,y}P<0.05).

Summary

1) Single nucleotide polymorphisms in the promoter region of the GH gene locating at g.253, g.303, and g.502 were detected and classified into 4 haplotypes, i.e. haplotype P: T-C-C, haplotype Q: C-C-C, haplotype R: C-C-T, and haplotype S: T-T-C (Results 1 &2).

2) Promotor haplotypes P, Q and R are included in Exon-5 haplotype A, while S is included B and C, respectively (Results 2).

3) At g.253, cattle with genotypes CC showed the highest C18:0 and C18:1 percentage, while those with genotype TT showed the highest growth traits and C14:0, C16:0, C16:1 percentages (Results 5).

4) At g.303, cattle with genotypes CC showed the highest C14:0, C16:0 and C16:1 percentages, while those with genotype TT showed the highest growth traits and C18:0 and C18:1 percentages (Results 5).

Conclusion

Genotypes of g.253 and g.303 and diplotype in the GH promoter region affected carcass traits and fatty acid compositions in Japanese Black cattle, being useful as genetic markers for future improvement of carcass traits and intramuscular fatty acid compositions.