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Nucleotide polymorphisms of the bovine growth hormone secretagogue receptor 1a (*GHSR1a*) gene and their association with growth and carcass traits in Japanese Black cattle

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Introduction

Ghrelin growth hormone secretagogue receptor 1a (GHSR1a) is involved in many important functions, including growth hormone secretion and food intake (Howard et al. 1996). To the best of our knowledge, there have been no reports to date on nucleotide polymorphisms from the 5'-flanking region to the 3'-UTR nor on the transcriptional analysis of the 5'-UTR of the *GHSR1a* gene in cattle. In our previous study (Malau-Aduli *et al*. (2005)), the *GHSR1a* gene was reported as a potential candidate gene when we detected growth trait QTLs in Japanese Black cattle using microsatellite DNA markers and half-sib regression analysis. Here we describe (1) all possible nucleotide polymorphisms from the 5'-flanking region to the 3'-UTR of the *GHSR1a* gene, (2) the transcript sequences of the 5'-UTR of the gene, which allows one to determine whether the microsatellite locus has been transcribed, and (3) an association between nucleotide polymorphisms of the gene and growth and carcass traits in Japanese Black cattle (Komatsu *et al*. (2010)). Finally, we propose a hypothesis that the association is due to differences in RNA secondary structure of the *GHSR 1b* mRNA.

Materials and methods

Animals. (1) We used genomic DNA of a total of 356 individuals of 11 breeds that included 3 Wagyu breeds for fragment analysis of the 5' UTR microsatellite. For the nucleotide polymorphism analysis of the *GHSR1a* gene, we sequenced the genomic DNA from the 5'-flanking region to the 3'-UTR (~6 kb) using 26 individuals belonging to 10 breeds. (2) We used a population of 1,285 steers for progeny testing of 117 sires among the

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Japanese Black cattle used in the projects of the Livestock Improvement Association of Japan (LIAJ).

Sequencing, fragment analyses, 5'-rapid amplification of cDNA ends (RACE) and RNA secondary structure analysis. A *GHSR1a* gene fragment of ~6 kb (from the 5'-flanking region to the 3'-UTR) was divided into 17 blocks, amplified by polymerase chain reaction (PCR) and sequenced. Sequences were analyzed using the Sequencher software and PolyPhred software and verified to identify SNPs and indels. Genomic DNA was extracted from ear tissue and semen using a standard protocol. The 2 microsatellites (5'-UTR Intron1) and DelR242 fragment analyses were carried out as described previously (Komatsu *et al.* (2010)). Brain tissue (arcuate nucleus included) was collected from a 2.5-month-old Holstein-Friesian bull calf at slaughter. Total RNA (1 µg) extracted was reverse-transcribed to single-stranded cDNA (ss-cDNA) by reverse transcriptase (RT) and the 5'-RACE CDS primer according to the manufacturer's instructions. The Vienna RNA secondary structure server (Hofacker (2003)) was used to predict the optimal secondary structure for *GHSR1b* and *GHSR1a* mRNAs.

Statistical analyses. Genetic parameters for a univariate model were estimated using a derivative-free restricted maximum likelihood algorithm as applied in the MTDFREML (Boldman *et al.* (1993)). The following linear mixed animal model was used: $\mathbf{y} = X1\mathbf{b}+X2\mathbf{g}+Z\mathbf{u}+\mathbf{e}$, where **y** is a vector of phenotypic observations; **b** is a vector of fixed effects and includes the year and month of birth, the place of birth, the place of test stations and the linear covariate of age at the beginning of the progeny tests; **g** is a vector of the fixed additive effect of the GHSR1a microsatellite or SNP alleles or haplotypes of the microsatellite and SNP; **u** is a vector of random additive genetic effect of the polygene; **e** is a vector of random residual effects; and X1, X2 and Z are the corresponding incidence matrices.

Results and discussion

The nucleotide sequencing of this gene revealed 47 single nucleotide polymorphisms (SNPs), 4 indels and 2 microsatellites ($(TG)_n$, 5'-UTR and $(GTTT)_n$, Intron 1) (Table 1). The 19 haplotypes were constructed from all nucleotide viability patterns and were divided into 3 major groups. Breed differences in allele frequencies of the 2 microsatellites, nt-7(C>A), L24V and DelR242 loci were found ($P < 0.005$). A DelR242 was found in the Japanese Shorthorn (frequency: ~ 0.44) and Japanese Brown, but none was detected in the

Japanese Black cattle. The 5'-RACE and RT-PCR analyses revealed two kinds of transcripts: spliced, without a microsatellite within 5'-UTR (*GHSR1a*), and non-spliced, with the microsatellite (*GHSR1b*). We carried out a statistical analysis of 5 nucleotide polymorphisms (5'-UTR microsatellite ($(TG)_n$), nt-7(C>A), L24V, DelR242 and Intron 1-microsatellite ($(GTTT)_n$) of the *GHSR1a* gene and growth and carcass traits in Japanese Black cattle, and our analysis revealed that the 5'UTR microsatellite had a significant additive effect on carcass weight (CW) ($P<0.001$) and average daily gain (ADG) ($P<0.005$) but not on the beef marbling score. The 19-TG allele, one of the four major microsatellite alleles (the allele frequency: 0.145), had a desirable effect on these traits ($P<0.0007$) (Table 2). The GHSR1b (the truncated receptor polypeptide) acts as a dominant-negative mutant of the GHSR1a (functional Ghrelin receptor) due to the formation of a GHSR1a/GHSR1b heterodimer (Leung *et al*. (2007)). To test a "translational hypothesis" to explain the association, we predicted the optimal RNA secondary structure and a number of Kozak sequence bases bound in the secondary structure (Kozak (2005)) using the Vienna RNA secondary structure server (Hofacker (2003)). We hypothesize that the association is due to differences in RNA secondary structure of the *GHSR 1b* mRNA among the haplotypes.

Table 1: Summary of nucleotide polymorphisms of the bovine *GHSR1a* gene

Items	Classification	No. of polymorphism			
		Subtotal	*B. taurus* only	*B. taurus* and *B. indicus*	*B. indicus* only
Region					
5'-flanking + 5'-UTR	SNP	16	5	2	9
	1-bp deletion†	1	1	0	0
Exon 1	SNP‡	4	4	0	0
	3-bp deletion ¶	1	1	0	0
Intron 1	SNP	23	6	7	10
	1-bp deletion§	1	0	1	0
	3-bp deletion¦	1	0	0	1
Exon 2	SNP	1	0	0	1
3'-UTR	SNP	3	0	1	2
	Total	51	17	11	23
5'-UTR	Microsatellite $(TG)_n$	(17)¥ (10-33)£	(3) (27-33)	(8) (19-26)	(5) (10-18)
Intron 1	Microsatellite $(GTTT)_n$	(4)¥ (4,5,6,8)¢	(1) (8)	(2) (5, 6)	(1) (4)

† nt-1117(A>－). ‡ L24V(nt70(C>G), nt456(G>A), D191N(nt580(G>A), nt667(C>T).
¶ DelR242 (nt724-726(AGG>－)). § nt2323(T>－). ¦nt1449-1451(TTT>－).
¥Number of alleles. £Number of (TG) repeats. ¢Number of (GTTT) repeats.

Table 2: Mean values of the four traits of each *GHSR1a* 5'UTR microsatellite genotype and allele additive substitution effect (α) of 19-TG for non-19-TG

5'UTR microsatellite ($(TG)_n$) genotype[†]	No. of animals	WS (kg)[‡]	CW (kg)[‡]	ADG (kg)[‡]
19-TG / 19-TG	24	614[d]	367[d]	0.96[b]
19-TG / non −19-TG	323	605[a]	361[a]	0.94[a]
non −19-TG / non −19-TG	935	589[c]	349[c]	0.90[c]
Total mean (SD)	1,282	593 (57)	352 (37)	0.91 (0.11)
	α	11	6.5	0.022
	SE	3.2	2	0.006
	P	<0.0004	<0.0007	<0.0002

[†] 19-TG; non-TG-19: 15-TG, 21-TG, 22-TG, 23-TG, 24-TG, 26-TG, 29-TG, 33-TG [‡] WS, weight at slaughtering time; CW, cold carcass weight; ADG, average daily gain. [§] a,c: P<0.0001; b,c: P<0.05; c,d: P<0.10.

Conclusion

The GHSR1a 5'UTR microsatellite, the 19-TG allele, may be an economically useful nucleotide marker for growth and carcass traits in Japanese Black cattle.

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