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# GENOMIC LOCATION OF THE BOVINE GROWTH HORMONE SECRETAGOGUE RECEPTOR (GHSR) GENE AND INVESTIGATION OF GENETIC POLYMORPHISM

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***The growth hormone secretagogue receptor (GHSR) is involved in the regulation of energetic homeostasis and GH secretion. In this study, the bovine GHSR gene was mapped to BTA1 between BL26 and BMS4004. Two different bovine GHSR CDS (GHSR1a and GHSR1b) were sequenced. Six polymorphisms (five SNPs and one 3-bp indel) were also identified, three of them leading to amino acid variations L24V, D194N, and Del R242. These variations are located in the extracellular N-terminal end, the exoloop 2, and the cytoloop 3 of the receptor, respectively.***

***Keywords***: Cattle; GHSR; Polymorphism

The GHSR is activated by small, synthetic peptides and nonpeptidyl molecules and stimulates the secretion of GH (1, 2). Ghrelin is the endogenous ligand for GHSR (3)

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and is involved in energetic homeostasis (4). It was suggested that the *GHSR* gene expression is regulated by GH and other hormones (5). Moreover, ghrelin downregulates its own receptor (6).

Human, rat, and swine *GHSR* cDNAs were previously sequenced (7, 8) and two types were identified: GHSR1a, encoding a functional receptor, and GHSR1b, encoding a receptor without measurable functional activity (8). Interestingly, GHSR1a exhibits a high constitutive activity signal (50% activity in the absence of ligand; 9).

The human *GHSR* gene is located on HSA 3q26.2 (8) and some QTLs (for obesity and metabolic syndrome) were also detected on HSA 3q26–29 (10). Moreover, several polymorphisms were detected in the human *GHSR* gene. Two of them lead to substitutions A204E and F279L and were associated with obesity and/or short stature (11, 12).

Given the importance of these polymorphisms found in human and the involvement of ghrelin in the regulation of food intake and energetic homeostasis, the present study aims at characterizing the bovine *GHSR* gene.

## MATERIALS AND METHODS

Rapid amplification of cDNA ends (RACE) were carried out on cDNA sample from bovine abomasum with BD Smart RACE cDNA Amplification Kit (Clontech, Palo Alto, USA). Primers P4R, nested P4R, P5F, and nested P5F used in RACE-PCR are presented in Table 1. The amplification products were directly sequenced

**Table 1** List of the used Primers

| Primer name | Primer sequence | Binding region | Ta (°C) | Use |
|---|---|---|---|---|
| P4R | CTC TCG CTG ACA AAC TGG | Exon 1 | | 5′-RACE |
| nested P4R | GGA AGA AGA AGA CGC TGG | Exon 1 | | 5′-RACE |
| P5F | GCT CAG AGA CCA GAA CCA CAA ACA GAC | Exon 1 | | 3′-RACE |
| nested P5F | ATC AGC CAA TAC TGC AAC C | Exon 2 | | 3′-RACE |
| P6F | TAG GAA TGG GGA AGA GC | Intron 1 | 52 | Screening of BAC Library |
| P6R | CGA AAG AGA CGA GGT TG | Exon 2 | | RH mapping |
| P7F | CTC CTC CCT CGC ACT CT | 5′UTR | 56 | Exon 1 sequencing |
| P7R | CTC TCG CTG ACA AAC TGG | Exon 1 | | |
| P8F | GGA ACT TGG GCG ACC TG | Exon 1 | 56 | Exon 1 sequencing |
| P8R | GGA AGA AGA AGA CGC TGG | Exon 1 | | |
| P9F | CTG GTC GGA GTG GAG CA | Exon 1 | 56 | Exon 1 sequencing |
| P9R | GGA GAG AAT AAT TGA GAC A | Intron 1 | | |
| P10F | AGA GAT GAT GGT TTG CTA TG | Intron 1 | 54 | Exon 2 sequencing |
| P10R | CTT CCT CCC AAG TTC CG | 3′UTR | | |
| P11F | GTG CTC TAC AGC CTC ATC G | Exon 1 | 60 | FAFLP (g.1063–1065) |
| P11R | (6-FAM) CGC CCA CCG CCG CCT C | Exon 1 | | |
| P12R A | CAT TCC ACA TGC TGC C**T** | Exon 1 | 56 | AS-PCR with P7F (g.333A) |
| P12R C | CAT TCC ACA TGC TGC C**G** | Exon 1 | | AS-PCR with P7F (g.333C) |
| P13R G | CGG CTC TCG TTG GTG T**C** | Exon 1 | 56 | AS-PCR with P7F (g.919G) |
| P13R A | CGG CTC TCG TTG GTG T**T** | Exon 1 | | AS-PCR with P7F (g.919A) |
| P14R | $(GATC)_6$ GAG TCA TTG TCG GGG A | Exon 1 | | SBE (g.409) |
| P15F | $(GATC)_8$ CCG CTG CGG GCC AA | Exon 1 | | SBE (g.795) |
| P16F | $(GATC)_{11}$ CTC TTC TAC CTC AGT GC | Exon 2 | | SBE (g.3429) |

using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Sequences were compared to human mRNA of GHSR1a (NM_198407) and GHSR1b (NM_004122) by alignment with ClustalW (13).

Thirty unrelated bulls (10 Limousin, 10 Holstein, and 10 double-muscled Belgian Blue) were used in the screening for polymorphisms within the *GHSR* gene. Four sets of primers (P7F to P10R) were designed to amplify genomic DNA sequence of the two exons, and PCR products were directly sequenced.

Another group of 124 Holstein bulls was genotyped. Polymorphisms at g.409, g.795 and g.3429 were determined by Single Base Extension (SBE) with the SNaP-shot Multiplex Kit (Applied Biosystems, USA) using primers P14R, P15F and P16F, respectively (Table 1). Due to hairpin structure interfering with SBE reaction, two differents methods were then designed to genotype the other polymorphisms. The indel at g.1063–1065 was determined by Fluorescent Amplified-Fragment Length Polymorphism (FAFLP) analysis with primers P11F-P11R. Genotypes at g.333 and g.919 were both determined by allele-specific PCR (AS-PCR) with P7F as forward primer and one of the following reverse primers (P12RA, P12RC, P13RG, and P13RA). Using an unitrait animal mixed model, a first association study was then performed on deregressed breeding values of the 124 Holstein bulls.

The *GHSR* gene was also mapped using the 3000 Rad bovine panel (14) and primers P6F-P6R. Then, the Carthagen software (15) was used to perform two-point and multipoint analysis of the radiation hybrid data. The same primers were also used for screening a bovine BAC library (16).

## RESULTS AND DISCUSSION

Two CDS were detected for the bovine GHSR and called GHSR1a and 1b by analogy to human GHSR. The GHSR1b is 879 bp long and encodes a protein of 292 residues. The GHSR1a consists of 1,101 bp encoding a 366 amino acid protein. This protein shares 95% and 94% identity with ovine (NM_001009760) and human (Q92847) GHSR, respectively. The GHSR1a has a seven transmembrane domain (TM) architecture, typical of members of G protein-coupled receptor family.

These two CDS originate from alternative mRNA processing of a single gene (Fig. 1). Our analysis of genomic structure revealed that the *bGHSR* gene encompasses two exons and one intron. The intron-exon boundary is located in TM6. The *bGHSR* gene sequence was submitted to The European Molecular Biology Laboratory (accession number AM931584).

In this study, we detected five SNPs and one trinucleotide indel (positions and alleles are listed in Table 2). The g.3429T variant was found in only one heterozygous Limousin bull. Surprisingly, separetely examined genotype at g.795 and g.1063–1065 for each bull showed that the genetic content at both sites were identical; however, SNPs at g.333 and g.795 were previously detected in cattle breeds in China (17) whereas the deletion was not detected.

The first association study on deregressed breeding values of the 124 Holstein bulls was performed for polymorphisms at g.333 and haplotype formed of g.795 and g.1063–1065; however, no effect on milk production traits were found (data not shown).

Polymophisms at g.409, g.919, and g.1063–1065 lead to amino acid substitutions L24V, D194N, and deletion of R242, respectively. These three variations occur

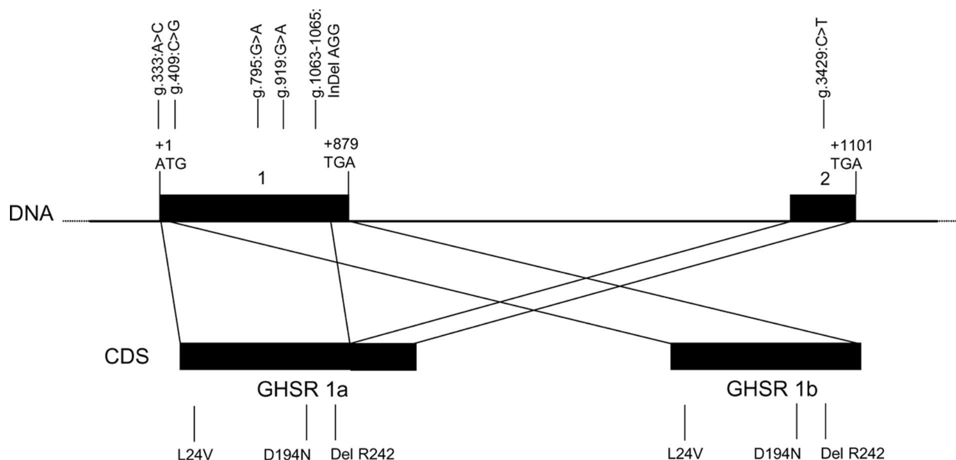


**Figure 1** Genomic structure of the bovine *GHSR* gene. Translated exons are shown as two solid boxes. The position of the six described polymorphisms are located at the top. In the lower part, the position of the amino acid variations are indicated on both transcripts, GHSR1a and GHSR1b.

**Table 2** Polymorphisms within the bovine *GHSR* gene and genotypes of all the studied bulls

| Polymorphism position[1] | Breed | Genotypes | | | Minor allele frequency (%) |
|---|---|---|---|---|---|
| g.333 | | A/A | A/C | C/C | C |
| | Limousin[2] | 8 | 2 | 0 | 10 |
| | Belgian Blue[2] | 8 | 1 | 1 | 15 |
| | Holstein[3] | 97 | 33 | 4 | 15 |
| g.409 | | C/C | C/G | G/G | G |
| | Limousin[2] | 8 | 2 | 0 | 10 |
| | Belgian Blue[2] | 9 | 1 | 0 | 5 |
| | Holstein[3] | 134 | 0 | 0 | 0 |
| g.795 | | G/G | A/G | A/A | A |
| | Limousin[2] | 8 | 2 | 0 | 10 |
| | Belgian Blue[2] | 7 | 1 | 2 | 25 |
| | Holstein[3] | 103 | 28 | 3 | 13 |
| g.919 | | G/G | A/G | A/A | A |
| | Limousin[2] | 9 | 1 | 0 | 5 |
| | Belgian Blue[2] | 10 | 0 | 0 | 0 |
| | Holstein[3] | 134 | 0 | 0 | 0 |
| g.1063–1065 | | AGG/AGG | AGG/— | —/— | — |
| | Limousin[2] | 8 | 2 | 0 | 10 |
| | Belgian Blue[2] | 7 | 1 | 2 | 25 |
| | Holstein[3] | 103 | 28 | 3 | 13 |
| g.3429 | | C/C | C/T | T/T | T |
| | Limousin[2] | 9 | 1 | 0 | 5 |
| | Belgian Blue[2] | 10 | 0 | 0 | 0 |
| | Holstein[3] | 134 | 0 | 0 | 0 |

[1]Positions refer to the genomic sequence (EMBL: AM931584).
[2]Genotypes determined by direct sequencing (n = 10).
[3]Genotypes determined by direct sequencing (n = 10) or by SBE, FAFLP and AS-PCR (n = 124).

respectively in the extracellular N-terminal end, exoloop 2 (in the turn of a β-sheet hairpin structure), and cytoloop 3 of the receptor.

The *bGHSR* gene is located on a BAC contig which corresponds to part of HSA3. Furthermore, this gene is also located at BTA1 between markers BL26 and BMS4004 with significant LOD scores (5.7 and 3.1, respectively). These results agree with comparative mapping data between cattle and human because BTA1 corresponds to a part of HSA3 (18). In addition, an alignment on the Btau_4.0 assembly by BLAT on UCSC genome browser website showed that the *bGHSR* gene and the two markers are located on BTA1 in the same order as expected by RH mapping.

In conclusion, the bovine GHSR gene and protein sequence were characterized and six polymorphisms were detected. This characterization and the mapping on BTA1 are useful for ongoing annotation of the bovine genome.

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