# Mapping of the bovine growth hormone secretagogue receptor (GHS-R) and polymorphism study in cattle

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## Introduction

A third control pathway of the Growth Hormone (GH) secretion has come into picture since the development of synthetic compounds known as Growth Hormone Secretagogues (GHSs). The GHS Receptor (GHS-R) and its subtype are abundantly located in the hypothalamus-pituitary unit, but are also distributed in other central areas and peripheral tissues. The GHS-R belongs to the G-protein coupled receptor family with seven transmembrane domain architecture. Ghrelin, a growth hormone(GH)-releasing peptide, was isolated from rat and human stomach as an endogenous ligand for the GHS-R in 1999. Ghrelin has been also identified in bovine oxyntic glands of the abomasum. Rat ghrelin stimulates GH release from bovine pituitary cells *in vitro* and *in vivo*. It circulates at considerable plasma concentrations in cattle. It has been reported that plasma ghrelin levels decrease 1h after feeding and then return to the prefeeding levels in cow. This peptide may function in the regulation of feeding or energy balance in ruminants.

## Aim

Given the influences of ghrelin on the growth hormone axis and the regulation of feeding, this work aims at the study of the bovine GHS-R gene in order to evaluate the potential involvment of GHS-R in genetic variation for growth performances or milk yield.

## Results

### Sequencing and identification of polymorphisms

**Principle**: Total RNA was extracted from bovine abomasum by TriPure Isolation Reagent (Roche Applied Science). Partial GHS-R mRNAs were sequenced by rapid amplification of cDNA ends (BD Smart™ RACE cDNA Amplification Kit, Clontech). Two types of GHS-R cDNA were identified, these 2 types are transcript variants of the same gene. The bovine GHS-R gene contains 2 exons. Primers were designed to sequence these 2 exons. Ten Belgian White Blue bulls, ten Holsteins bulls and ten Limousin bulls were screened for polymorphisms.

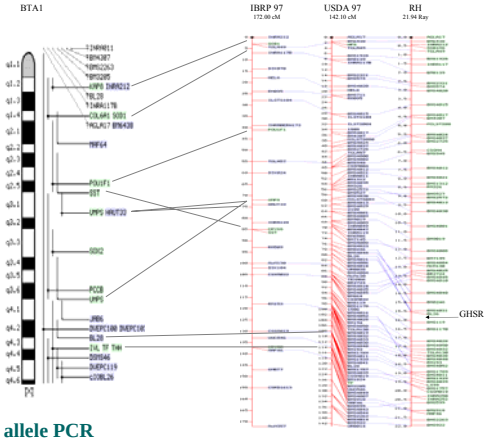
The alignement of the 30 sequences revealed a total of 4 polymorphisms : 3 SNPs (Mut 1, 3 and 5) are on the first exon and 1 SNP (Mut 2) on the second exon.

### Mapping of bovine GHS-R gene

Using a radiation hybrid panel, the gene was mapped to *Bos taurus* autosome 1 (BTA1). This localization on BTA1 agrees totally with comparative data between cattle and human since BTA 1 corresponds to part of human chromosome 3 where human GHS-R is also mapped. By two-point analysis, most significantly linked marker are BL26 and BMS4031 (both LOD score : 5,66) (Figure 1). Some studies detected different QTLs near these markers like for growth rate, carcass yield, milk protein and milk yield.

In order to evaluate the potential involvement of GHS-R in genetic variation for growth rate, carcass yield, milk protein and milk yield, an association study between SNPs on GHS-R gene and these traits could be performed in 2 cattle populations (125 Holstein and 86 Belgian White Blue).

**Figure 1**. Ideogram, 2 comprehensive linkage maps and comprehensive radiation hybrid map of BTA1. Radiation hybrid mapping of GHSR gene to BTA1
(adapted from http://projects.roslin.ac.uk/comrad/maps/bta1map.html)




### Genotyping by single-base extension (SBE) and specific allele PCR

**Principle**: For genotyping of polymorphic sites (Mut 1, 2 and 3, Figure 2), amplifying and extension primers were designed for single-base extension (SBE). Primer extension reactions were performed with the SNaPshot Multiplex Kit (Applied Biosystems). The DNA samples, containing extension products, and Genescan 120 LIZ size standard solution was added to Hi-Di formamide (Applied Biosystems) according to the recommendations of the manufacturer. The mixture was incubated at 95 °C for 5 min, followed by 5 min on ice, the electrophoresis was performed on an ABI Prism 3100 Genetic Analyzer. The results were analyzed using the program of GeneScan Analysis Software v3.7 (Applied Biosystems). For genotyping of Mut 5, allele-specific primers were used for a PCR amplification (Figure 3). Results of the 2 populations genotyping are presented in Table 1.

**Table 1**. Genotype and allele frequencies observed in two different bovine breeds. The Holstein group include 125 commercially available bulls used in Walloon Region and the Belgian White Blue group include 86 bulls from a bull-fattening entreprise.

| | | | Holstein | | Belgian White Blue | |
|---|---|---|---|---|---|---|
| | | | n | frequence | n | frequence |
| Mut 1 | Genotypic classis | CC | 125 | 100,0% | 81 | 98,8% |
| | | CG | 0 | 0,0% | 1 | 1,2% |
| | | GG | 0 | 0,0% | 0 | 0,0% |
| | Allele | C | | 100,0% | | 99,4% |
| | | G | | 0,0% | | 0,6% |
| Mut 2 | Genotypic classis | CC | 125 | 100,0% | 86 | 100,0% |
| | | CT | 0 | 0,0% | 0 | 0,0% |
| | | TT | 0 | 0,0% | 0 | 0,0% |
| | Allele | C | | 100,0% | | 100,0% |
| | | T | | 0,0% | | 0,0% |
| Mut 3 | Genotypic classis | GG | 95 | 76,0% | 56 | 67,5% |
| | | AG | 28 | 22,4% | 25 | 30,1% |
| | | AA | 2 | 1,6% | 2 | 2,4% |
| | Allele | G | | 87,2% | | 82,5% |
| | | A | | 12,8% | | 17,5% |
| Mut 5 | Genotypic classis | AA | 91 | 72,2% | 66 | 76,7% |
| | | AC | 31 | 24,6% | 17 | 19,8% |
| | | CC | 4 | 3,2% | 3 | 3,5% |
| | Allele | A | | 84,5% | | 86,6% |
| | | CC | | 15,5% | | 13,4% |

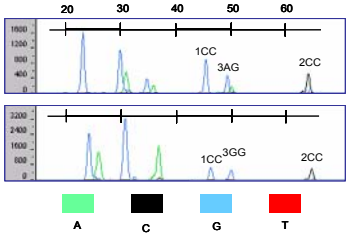



**Figure 2**. Electropherograms of 2 different genotypes. The snapmut1 is antisense, the nucleotide must be changed : G →C. The X-axis indicates the length of the DNA fragment. The Y-axis indicates relative fluorescence units (RFU).

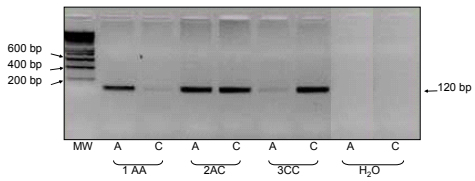



**Figure 3**. Results of Mut 5 allele specific PCR amplification for 3 different genotypes. Lane 1 contains the molecular weight. Lanes 2, 4, 6 and 8 show the 120 bp size PCR product with allele A primer and lanes 3, 5, 7 and 9 show the 120 bp size PCR product with allele C primer.

Genotype and allele frequencies seem to be similar between the two breeds. Neither Mut 1 CG, Mut 1 GG, Mut 2 CT nor Mut 2 TT genotypes were found in the studied Holstein population. The Mut 3 and Mut 5 statistical analysis were performed on the Holstein population because the Belgian White Blue bulls were still in fattenning.

### Statistical analysis

Statistical analysis was performed using the GLM procedure of SAS. The model used was a fixed model :

$$\mathbf{y} = \mathbf{X}\,\mathbf{b} + \mathbf{e}$$

Where $\mathbf{y}$ = vector of estimated breeding value of bulls;

$\mathbf{b}$ = unknown vector of mean effect and regression coefficient;

$\mathbf{X}$ = known design matrix of fixed genotype effect, matrix linking $\mathbf{y}$ and $\mathbf{b}$;

$\mathbf{e}$ = unknown vector of random residual effects.

The regression coefficient represented the gene substitution effect α. This model was solved using the following fixed model equations :

$$\mathbf{X'\,R^{-1}X\hat{b}} = \mathbf{X'\,R^{-1}y} \iff \hat{\mathbf{b}} = \mathbf{(X'\,R^{-1}X)^{-1}X'\,R^{-1}y}$$

where $\mathbf{R^{-1}} = \mathbf{D} / \sigma^2_e$ where $\mathbf{D}$ = a diagonal matrix divided by the estimate of the residual variance $\sigma^2_e$ . The diagonal element of $\mathbf{D}$, representing the weight given to every bull, was computed as weight = reliability. The statistical results are presented in Table 3.

**Table 2**. Regression coefficient on the number of the allele and standard errors observed on 125 Holstein bulls

| Trait | Mutation 3 G/A Allele A | | | Mutation 5 A/C Allele C | |
|---|---|---|---|---|---|
| | α | SE | | α | SE |
| **Production** | | | | | |
| Milk (kg) | 24,6 | 89,3 | | -13,5 | 76,5 |
| Fat (kg) | 0,22 | 3,52 | | 2,27 | 3,01 |
| Protein (kg) | -1,8 | 2,74 | | 1,06 | 2,35 |
| Fat (%) | -0,008 | 0,053 | | 0,035 | 0,045 |
| Protein (%) | -0,035 | 0,023 | | 0,020 | 0,020 |
| **Functional** | | | | | |
| Somatic Cell Score | 0,05 | 0,08 | | -0,05 | 0,07 |
| Herd Life | -0,10 | 0,06 | * | 0,008 | 0,047 |

* $P < 0,10$

Mut 3 shows marginal association with a decrease in herd life so it wouldn't be interesting to use Mut 3 A allele through selection for this factor. Finally, greater numbers of Holstein bulls with missing pattern would be helpful.

## Conclusion

These results suggest that polymorphisms in bovine GHS-R gene are not convenient for milk traits selection. Further genetic study is underway to investigate the GHS-R effet on performances in Belgian White Blue bulls.

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