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# Molecular characterization and chromosomal assignment of the bovine glycinamide ribonucleotide formyltransferase (*GART*) gene on cattle chromosome 1q12.1–q12.2

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**Abstract**

The mammalian glycinamide ribonucleotide formyltransferase (*GART*) genes encode a trifunctional polypeptide involved in the de novo purine biosynthesis. We isolated a bacterial artificial chromosome (BAC) clone containing the bovine *GART* gene and determined the complete DNA sequence of the BAC clone. Cloning and characterization of the bovine *GART* gene revealed that the bovine gene consists of 23 exons spanning approximately 27 kb. RT-PCR amplification of bovine *GART* in different organs showed the expression of two *GART* transcripts in cattle similar to human and mouse. The *GART* transcripts encode two proteins of 1010 and 433 amino acids, respectively. Eleven single nucleotide polymorphisms (SNPs) were detected in a mutation scan of 24 unrelated animals of three different cattle breeds, including one SNP that affects the amino acid sequence of *GART*. The chromosomal localization of the gene was determined by fluorescence in situ hybridization. Comparative genome analysis between cattle, human and mouse indicates that the chromosomal location of the bovine *GART* gene is in agreement with a previously published mapping report.


*Keywords:* GART; Cattle; Gene structure; BAC clone; FISH; SNP

## 1. Introduction

The glycinamide ribonucleotide formyltransferase (*GART*) gene is part of a gene family that encodes multifunctional enzymes (Aimi et al., 1990). These enzymes have been found in several metabolic pathways in eukaryotes. In mammals and birds, the *GART* gene encodes enzymes of purine synthesis, which catalyze three steps of this pathway (glycinamide ribonucleotide synthetase (GARS), glycinamide ribonucleotide formyltransferase (GART) and aminoimidazole ribonucleotide synthetase (AIRS)) (Kan et al., 1993). The enzymes GARS, GART and AIRS catalyze the second, third and fifth step of the de novo purine biosynthetic pathway, respectively (Daubner et al., 1985). The human gene encodes not only the trifunctional protein of 110 kDa, but also a monofunctional GARS protein of 50 kDa (Brodsky et al., 1997). Previous studies on the human and mouse *GART* locus demonstrated that the mRNA for monofunctional GARS is produced by the use of polyadenylation signals present in intron 11 (Kan and Moran, 1995, 1997). The expression of the monofunctional and trifunctional proteins is regulated during development of the human cerebellum (Brodsky et al., 1997). All three proteins are expressed at a high level in the cerebellum during normal prenatal development and become undetect-

*Abbreviations:* BAC, bacterial artificial chromosome; bp, base pair; BLASTN, basic local alignment search tool nucleotide; BTA, bovine chromosome; E2F, E2 transcription factor; EST, expressed sequence tag; FISH, fluorescence in situ hybridization; GART, glycinamide ribonucleotide formyltransferase; HSA, human chromosome; kb, kilobase; kDa, kilodalton; MMU, mouse chromosome; nt, nucleotide; Rb, retinoblastoma; RH, radiation hybrid; RT-PCR, reverse transcriptase polymerase chain reaction; SNP, single nucleotide polymorphism; Sp1, stimulating protein 1.

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able in the cerebellum shortly after birth. In individuals with Down syndrome the expression of these proteins continues in the postnatal development of the cerebellum (Brodsky et al., 1997). The human *GART* locus maps to human chromosome (HSA) 21q22.1 (Chadefaux et al., 1984). The human *GART* gene consists of 22 exons and spans 38.1 kb (NCBI map viewer, human genome build 34.3). The murine *Gart* gene also consists of 22 exons spanning about 25.5 kb on chromosome MMU 16C3-C4 (NCBI map viewer, mouse genome build 32.1). Because the bovine genome represents an evolutionary clade distinct from primate or rodent genomes we selected the bovine GART gene for sequencing. Until now no sequence information of the orthologous bovine gene has been reported.

In this report, we provide the cloning, chromosomal assignment, genomic organization and the complete sequence of the bovine *GART* gene, respectively. Additionally, we present data on new single nucleotide polymorphisms (SNPs) in this gene.

## 2. Materials and methods

### 2.1. Cloning and sequencing of the bovine GART gene

For the isolation of a bovine BAC clone with the *GART* gene the bovine BAC library RPCI-42 (Warren et al., 2000) was initially screened with a $^{32}$P-labeled insert of a human IMAGE cDNA clone (IMAGp998J037162Q2) provided by the German Human Genome Resource Center/Primary Database (http://www.rzpd.de/) from the orthologous human gene. DNA from the clone RP42-564N14 was isolated using the Qiagen Large Construct kit (Qiagen, Hilden, Germany). BAC DNA was mechanically sheared to obtain fragments of approximately 2 kb. Sheared BAC DNA was used to construct a shotgun plasmid library. Plasmid subclones were sequenced with the ThermoSequenase kit (Amersham Biosciences, Freiburg, Germany) and a LICOR 4200 automated sequencer (MWG Biotech, Ebersberg, Germany).

Sequence data were analyzed with Sequencher 4.1.4 (GeneCodes, Ann Arbor, MI). Remaining gaps were closed by a primer walking strategy until both strands were completely sequenced. Repetitive elements were detected with Repeatmasker 2 (http://www.repeatmasker.genome.washington.edu/). The genomic structure of the bovine *GART* gene was determined by using the genomic DNA sequence as query in BLASTN (basic local alignment search tool nucleotide) analyses of the bovine expressed sequence tag (EST) databases (http://www.ncbi.nlm.nih.gov/BLAST/). The ESTs which were used to determine the exon organization of the bovine *GART* gene are given in Table 1. Only when no corresponding bovine EST could be detected the human *GART* mRNA (Table 1) was used to annotate the *GART* exons on the genomic sequence. For the exact localization of the exon/intron boundaries the mRNA-to-genomic alignment program Spidey (http://www.ncbi.nlm.nih.gov/IEB/Research/Ostell/Spidey/index.html) was used. The putative promoter sequence was analyzed in silico with the program MatInspector from Genomatix (http://www.genomatix.de/cgi-bin/matinspector_prof/). GC content was calculated with the EBI toolbox CpG Plot/CpGreport (http://www.ebi.ac.uk/Tools/sequence.html).

Table 1
Sequences for annotation of bovine *GART* exons

| Exon no. | EST accession nos. | Experimental bovine cDNAs | Human mRNA isoform 1 |
|---|---|---|---|
| 1 | BE487216 | | |
| 2–4 | BE487216 | | NM_000819 |
| 5 | BI681173 | | NM_000819 |
| 6 | | | NM_000819 |
| 7 | CN789820 | | NM_000819 |
| 8–11 | CN789820 | AJ783707 | NM_000819 |
| 12–14 | | AJ783707 | NM_000819 |
| 15–17 | BM967887 | | NM_000819 |
| 18–20 | CK837096 | | NM_000819 |
| 21 | CB457481 | | NM_000819 |
| 22 | CB457481 | AJ783709 | NM_000819 |
| 23 | | AJ783709 | |

### 2.2. cDNA synthesis, RT-PCR

RNeasy™ 96 Universal Tissue kit (Qiagen) was used to extract total RNA from bovine liver, heart muscle, spleen, kidney, brain, small intestine, pituitary, lung and skin, respectively, from a normal German Holstein calf according to the manufacturer's protocol. Aliquots of 1 μg total RNA were reverse transcribed into cDNA using 20 pmol $(T)_{24}V$ primer and Omniscript™ Reverse Transcriptase (Qiagen) in 20 μl reactions. One microliter of the cDNA was used as template in a reverse transcriptase polymerase chain reaction (RT-PCR) reaction. The reaction was performed in a total of 25 μl containing 100 μM dNTPs, 25 pmol of each primer, the reaction buffer supplied by the manufacturer (Qiagen) and 1 U *Taq* polymerase. After a 5 min initial denaturation at 94 °C, 35 cycles of 30 sec at 94 °C, 1 min at 58 °C and 45 sec at 72 °C were performed in a MJ Research thermocycler (Biozym, Hess. Oldendorf, Germany). To detect the two possible splice variants, we created a single forward primer overlapping the exon 10/11 junction (Ex10-11_F 5′-AGA TAA CAG GGT TTC CTG AG-3′). The *GART* isoform 1 was detected by a combination of this forward primer with a reverse primer situated at the junction of exon 13/14 (Ex13-14_R 5′-ACT GCT GGG CAA TCT TCA GT-3′) and the *GART* isoform 2 by a combination with a reverse primer located in intron 11 (GEx11_R 5′-CCT TTG TTC AGG TTC AGT GG-3′). For detection of the 3′ end of *GART* isoform 1, we used a forward primer situated at the junction of exon 21/22 (Ex21-22_F ACT TTG TAG CTG AAG ATG TAG ATG C) and a reverse primer (poly A_R ACC ATC TGT GTG GGT TTT CC) near the predicted polyadenylation signal.