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Analysis of structure and gene expression of bovine CCDC3 gene indicates a function in fat metabolism

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ABSTRACT

Our study reports the molecular analysis of the bovine gene encoding the coiled-coil domain-containing protein 3 (*CCDC3*). Based on comparative sequence analysis and *in silico* sequence merging of predicted gene models, a new full-length gene model for the bovine *CCDC3* gene was predicted and confirmed experimentally. The *CCDC3* gene was assigned to bovine chromosome 13. It consists of three exons comprising 2599 bp encoding for a respective protein of 274 amino acids. The strong CCDC3 sequence homology on amino acid level between species suggests a conserved universal function of this protein. In mice, the *CCDC3* gene had been found to be highly expressed in adipocytes and regulated by hormonal-nutritional alternations and in obesity. The tissue expression pattern of bovine *CCDC3* mRNA indicates a ubiquitous physiological function of the gene. Significant differences in *CCDC3* mRNA expression in skeletal muscle between individuals characterized by divergent intramuscular fat deposition support the potential function of the gene in fat or energy metabolism, which possibly could also be inferred for other mammalian species. This first report of structural analysis and molecular characterization of the *CCDC3* gene in cattle will contribute to a better understanding of the yet unknown physiological role of the respective protein in mammals.

1. Introduction

Comparative genomics provides a powerful tool to deliver insights regarding genome function and evolution, which could serve as a starting point to understand how the genomic variation contributes to diverse phenotypes and diseases (Womack, 2005). Comparing the finished reference sequence of the human genome with insufficiently annotated genome sequences of other organisms can allow the identification of regions of structural similarity and help to improve the genome assemblies of these species (Dalrymple et al., 2007; Derrien et al., 2009). Vice versa, for human genes, cross-species comparative sequence and expression analyses are valuable tools to identify potential regulatory elements and affected metabolic pathways not yet detected. Thus, the information retrieved from other species can also assist to provide better understanding of the structure and function of human genes, and thereby develop new strategies to analyze the molecular background of human diseases and disorders (Switonski et al., 2004).

In cattle, more than 4000 genes out of the approximately 22,000 protein coding genes have hitherto been annotated manually (Elsik et al., 2009). Accordingly, by far not all bovine transcripts are sufficiently annotated, and there are still gaps in the current bovine genome sequence assembly. However, for a subsequent investigation of the functional relevance of genes and transcripts, a correct and conclusive structural gene annotation is a prerequisite.

In this study we validated the computational gene prediction model of bovine locus *LOC509875* that previously had shown indication on divergent expression in skeletal muscle of bovine individuals kept under carefully monitored identical environmental conditions, but exhibiting extreme differences in intramuscular fat content (Kalbe et al., unpublished results). This bovine transcript is a member of the UniGene cluster Bt.3946, for which similarity to the human coiled-coil domain-containing protein 3 (*CCDC3*) was predicted. Qualitative and quantitative gene expression studies in tissues relevant in energy and fat metabolism were performed to elucidate the potential physiological function of the bovine *CCDC3* gene.

2. Materials and methods

2.1. In silico sequence analyses

To identify sequences highly similar to the target transcript representing the probe sets Bt.3946.1.S1_at and Bt.3946.2.S1_at located

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Table 1
Summary of PCR primers.

Primer	Sequence	Location	Amplicon (bp)	Application
CCDC3_F1	GGGCTACTTCTCCTGTCACTC	exon 1		Gene structure, expression
CCDC3_R1	TGCTTGTTCCGCTTCTCC	exon 3	336[a]	Gene structure, expression
CCDC3_R2	AGAAGCGTGGCAGTGAAG	exon 3	1858[a]	Gene structure
CCDC3_R3	GACCATACTGCCTTTCCTTG	exon 3	–	Reverse transcription
CCDC3_HF1	GTC TCC TGT CTC CTT GGC TG	exon 3	181	Physical mapping
CCDC3_HR1	CTGAGACCATACTGCCTTTCC			
CCDC3_HF3	CCCGCCCATACCATCCTTTCC	intron1	171	Physical mapping
CCDC3_HR3	CTCCCAAGATCTCTGTAGACAC			

[a] Amplification with primer CCDC3_F1. Primers refer to the revised gene model of the bovine *CCDC3* gene (GenBank accession number GU140072).

on the 24 K GeneChip® Bovine Genome Array (Affymetrix), *in silico* similarity searches with the corresponding representative transcript sequences of bovine locus *LOC509875* (GenBank accession no. XM_586935.4) and the orthologous human *CCDC3* mRNA (NM_031455.3) were performed against available versions of bovine genome sequence assemblies (Btau4.0, Btau3.1 http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid=9913), and UMD 3.0 (ftp://ftp.cbcb.umd.edu/pub/data/assembly/Bos_taurus/Bos_taurus_UMD_3.0/) as well as the nucleotide and protein databases at the National Center for Biotechnical Information (NCBI) and Ensembl database using BLAST tools (Altschul et al., 1997; Zhang et al., 2000). For structural and functional sequence analysis, further bioinformatics analysis tools were used including ORF (open reading frame) finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html), CDD (conserved domain database) search (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml), the Simple Modular Architecture Research Tool SMART (http://smart.embl-heidelberg.de/), and the database of protein domains, families and functional sites (PROSITE) available at the DKFZ, Heidelberg (http://genius.embnet.dkfz-heidelberg.de).

2.2. Structural analysis of the bovine CCDC3 gene

The *in silico* assembly of the bovine locus similar to the human *CCDC3* gene was constructed by merging information on the bovine prediction models *LOC509875* and *LOC509985* detected in the current bovine genome assembly of the NCBI database (Btau4.0) and the respective predicted bovine mRNA sequences (GenBank accession no. XM_586935.4 and XM_587062.4) as well as the orthologous human mRNA sequence (NM_031455.3). To provide evidence for our revised bovine *CCDC3* gene model and to confirm that the target sequence Bt.3946.1.S1_at on the Affymetrix expression microarray indeed represents a *CCDC3* transcript, reverse transcription (RT)-PCR was performed on cDNA from bovine liver and skeletal muscle with primer combinations CCDC3_F1 and CCDC3_R1 as well as CCDC3_F1 and CCDC3_R2, respectively (Table 1), which were derived from the two different gene models, *LOC509875* and *LOC509985* (Fig. 1). The cDNA was prepared as described in section 2.4 Sequencing of PCR products was performed with BigDye© sequencing chemistry on a capillary sequencer (ABI 3130, Applied Biosystems) with primers used for PCR.

2.3. Physical mapping of the bovine CCDC3 gene

Chromosomal assignment of *CCDC3* was performed by synteny mapping using the rodent/bovine somatic cell hybrid panel SHC (Womack and Moll, 1986). PCR has been carried out as described (Goldammer et al., 2002) using two bovine gene-specific primer sets (Table 1) that were derived from intron 1 and exon 3 of the *CCDC3*

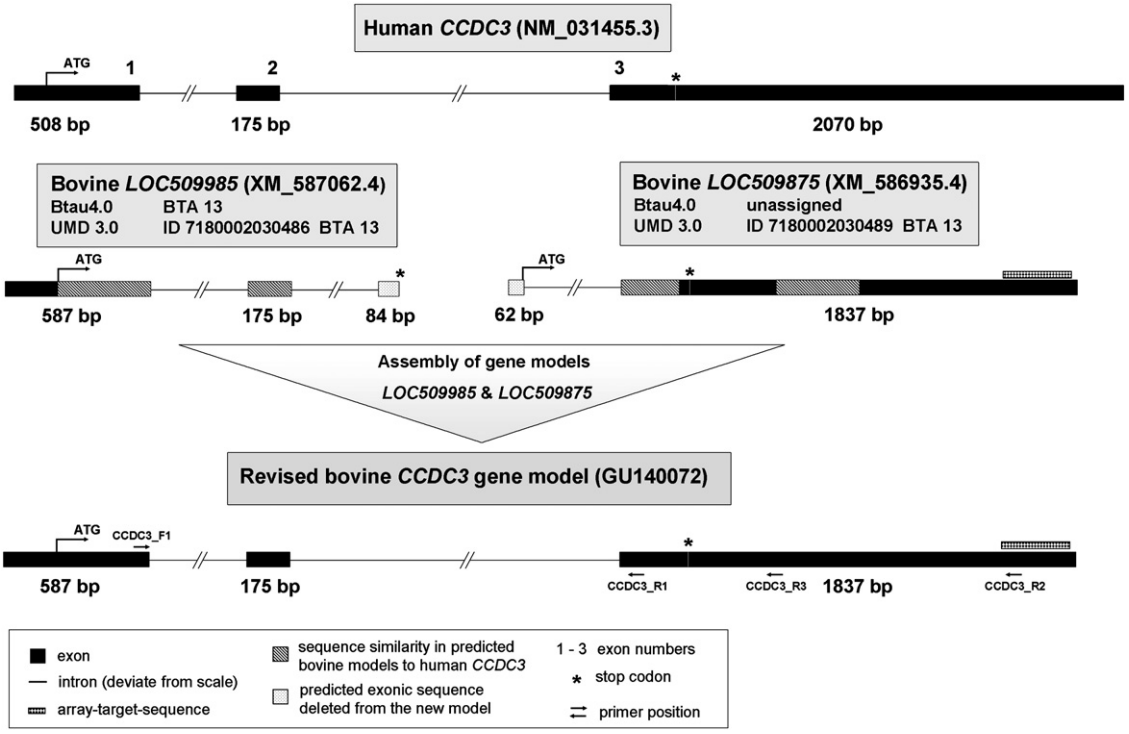

Fig. 1. Construction of the gene model for bovine *CCDC3* based on two predicted bovine loci and comparative sequence alignment to the orthologous human gene. *LOC509875* and *LOC509985*: predicted bovine loci similar to human coiled-coil domain-containing protein 3 (*CCDC3*) gene, XM_587062.4 and XM_586935.4: GenBank accession numbers for predicted bovine mRNAs models similar to human *CCDC3*, NM_031455.3: GenBank accession number for human *CCDC3*, GU140072: GenBank accession number for the revised gene model of bovine *CCDC3*.