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Molecular Cloning of Bovine *FABGL* Gene and Its Effects on Bovine Bioeconomic Traits

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Abstract: The complete CDS sequence of the bovine *FABGL* gene was determined by homology cloning approach combined with RT-PCR and 3′- and 5′-RACE. The results of sequence analysis and bioinformatics study showed that this cDNA contained 994 nucleotides, with a 780 bp open reading frame (ORF) flanked by a 16 bp 5′-UTR (incompletely) and a 198 bp 3′-UTR. The deduced amino acid sequence (260 AA) shows 88% identity with the corresponding sequence in humans. Two single nucleotide substitutions, one located in intron 5 (I5) at position 1 065 bp (Y = C/T) (GenBank: DQ409814) and the other in intron 8 (I8) at position 1 792 bp (R = A/G), were detected using the PCR-SSCP method. Analysis of the allele frequencies of the two polymorphic sites in three different cattle breeds (Angus, Hereford, and Simmental) with different genotypes showed large differences: in locus I8, cattle with the *GG* genotype showed higher beef performance index (BPI) (4.283 ± 0.475 kg/cm) in comparison with cattle with the *AA* genotype (4.008 ± 0.465 kg/cm) ($P \leqslant 0.01$). Regarding the ribeye area, cattle with the *GG* genotype showed significantly higher ribeye area (73.380 ± 13.005 cm^2) compared with cattle with the *AA* genotype (67.744 ± 12.777 cm^2) ($P \leqslant 0.05$). In locus I5, some associations for the average daily gain (ADG) were found at the significance level of 0.01 between three different genotypes (*CC*, *CT*, *TT*): cattle with the *TT* genotype showed the highest ADG (0.652 ± 0.330 kg/d), whereas cattle with the *CC* genotype showed the lowest ADG value (0.421 ± 0.178 kg/d).

Key words: cattle; SNPs; *FABGL* gene; SSCP; relationship; BPI; ribeye area; ADG

The *FabG*-like (*FABGL*) gene, also known as 17-beta-*HSD*8, estrogen 17-oxidoreductase; 17-beta-hydroxysteroid dehydrogenase 8, H2-Ke6 or RING 2; encodes a nicotinamide adenine dinucleotide (NAD)-dependent 17β-hydroxysteroid dehydrogenase (*17β-HSD*)[1]. The protein encoded by this gene is a member of the short-chain dehydrogenase superfamily. This protein efficiently catalyses the oxidation of oestradiol, testosterone, and dihydrotestosterone as well as the reduction of oestrone to biological active oestradiol. In gonadal tissue, it functions as a regulator of sex steroids [2]. Female sex steroids are known to regulate adipose tissue metabolism[3], whereas testosterone is known to inhibit lipoprotein lipase (*LPL*) thereby markedly stimulating lipolysis [4].

Several researchers have isolated the cDNA and genomic clones of the *FABGL* gene in humans, rat and swine; it is especially noteworthy that the porcine *FABGL* gene, coding for 17β-hydroxysteroid dehydrogenase (*17β-HSD*), is a candidate gene for reproduction, fatness, growth, meat quality traits[5], and immunological traits[6,7], but the whole sequence of bovine *FABGL* has not been described, much less its functions.

In this article, we report – the isolation and sequencing of the ORF of bovine *FABGL* obtained by

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using the information available in the EST database, reverse transcription-polymerase chain reaction (RT-PCR), and rapid amplification of cDNA ends (RACE) approach – polymorphisms and allele distributions in three cattle breeds. This study is the first step to understand the physiological role played by the bovine *FABGL* protein and to investigate whether bovine *FABGL* alleles can serve as genetic markers for both the growth related, meat and the reproduction traits in cattle.

1 Materials and Methods

1.1 Isolation of complete CDS for bovine *FABGL* gene

To obtain the entire cDNA of the bovine *FABGL* gene, we searched the GenBank "EST-others" database with the coding sequences (*CDS*) of the human *FABGL* gene (GenBank accession number: NM 014234) for homology comparison by BLAST (http://www.ncbi.nlm.nih.gov/blast/). ESTs sharing at least 90% identity with the human cDNA were considered meaningful and assembled to form a bovine EST-contig (Fig. 1). Four primers (Table 1) were generated to obtain the entire ORF and UTR of the *FABGL* gene using the RT-PCR and RACE technique. Among these four primers, primers A and B were designed from the bovine contig sequence, whereas primers C and D were designed from the sequence through RT-PCR. Total RNA was isolated from the liver tissue of a mature Angus using the TRIzol reagent kit (GIBCO/BRL, Bethesda, MA, USA). The first-strand cDNA used for RT-PCR was synthesized using 1 μg of Dnase I-treated total RNA, 0.5 μg of oligo dT11, 500 μmol/L dNTPs, 10 U RNAsin (Promega, Madison, WI, USA), 1× murine leukemia virus (MLV) RT buffer, and 300 U M-MLV reverse transcriptase (Promega) in a volume of 50 μL at 37℃ for 1 h. The enzyme was then inactivated at 95℃ for 5 min. The PCR reactions were carried out using 2 μL first-strand product, 1 μmol/L primer, 2.5 mmol/L $MgCl_2$, 150 μmol/L dNTPs, and 2 U *Taq* DNA polymerase. The temperature profile of the PCR was 94℃ for 4 min, followed by 35 cycles at 94℃ for 45 s, annealing for 45 s (temperatures as shown in Table 1), 72℃ for 1 min, and a final extension at 72℃ for 5 min. The synthesis of the first-strand cDNA used for RACE and continued RACE experiments was carried out using BD SMART™ RACE cDNA Amplification Kit, as per manufacturer's instructions.. The touch-down PCR program was applied in RACE trials. The temperature profile of the touch-down PCR was as follows: first, 95℃ for 2 min, followed by four cycles at 94℃ for 30 s, 72℃ for 3 min, then followed by four cycles at 94℃ for 30 s, annealing at 70℃ for 30 s, extension at 72℃ for 3 min; second, 30 cycles at 94℃ for 30 s, annealing at 68℃ for 30 s, 72℃

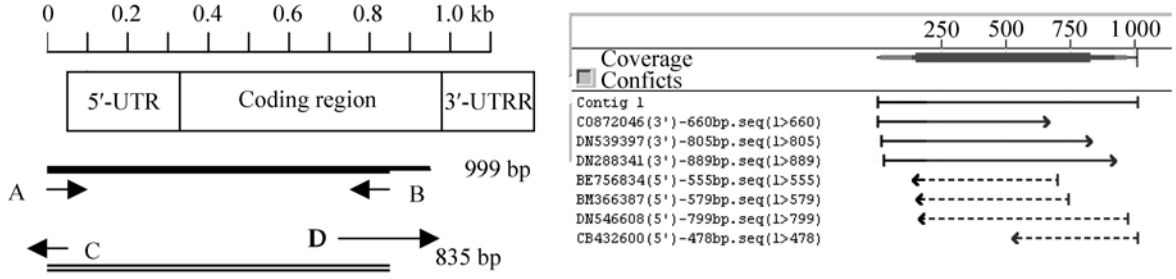

Fig. 1 Schematic representation of the bovine EST-contig assembled by the selected ESTs encoding bovine *FABGL* gene

The left upper line shows the scale in kb. The 999 bp long bovine EST-contig is indicated as a thick line; the region corresponding to the coding sequence is shown as middle box. The arrows indicate the position of the five primers used to obtain ORF. Primer A and B were derived from the bovine EST-contig sequence, whereas the other two specific primers, named C and D, respectively, used for 5′-RACE and 3′-RACE were designed based on the result of sequencing for RT-PCR. Seven overlapping ESTs (lines) used to generate the bovine EST-contig are shown at the right-hand side, and each EST is identified by its accession number.