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Characterization and Mapping of the Bovine FBP1 Gene

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ABSTRACT : Fructose-1,6-bisphosphatase (FBP1) is a key regulatory enzyme of gluconeogenesis that catalyzes the hydrolysis of fructose-1,6-bisphosphate to generate fructose-6-phosphate and inorganic phosphate. Deficiency of fructose-1, 6-bisphosphatase is associated with fasting hypoglycemia and metabolic acidosis. The enzyme has been shown to occur in bacteria, fungi, plants and animals. The bovine *FBP1* gene was cloned and characterized in this study. The full length (1,241 bp) *FBP1* mRNA contained an open reading frame (ORF) encoding a protein of 338 amino acids, a 63 bp 5' untranslated region (UTR) and a 131 bp 3' UTR. The bovine *FBP1* gene was 89%, 85%, 82%, 82% and 74% identical to the orthologs of pig, human, mouse, rat and zebra fish at mRNA level, and 97%, 96%, 94%, 93% and 91% identical at the protein level, respectively. This gene was broadly expressed in cattle with the highest level in testis, and the lowest level in heart. An intronic single nucleotide polymorphism (SNP) (A/G) was identified in the 5[th] intron of the bovine *FBP1* gene. Genotyping of 133 animals from four beef breeds revealed that the average frequency for allele A (A-base) was 0.7897 (0.7069-0.9107), while 0.2103 (0.0893-0.2931) for allele B (G-base). Our preliminary association study indicated that this SNP is significantly associated with traits of Average Daily Feed Intake (ADFI) and Carcass Length (CL) ($p<0.01$). In addition, the *FBP1* gene was assigned on BTA8 by a hybrid radiation (RH) mapping method. (**Key Words** : Different Expression, Localization, *FBP1*, mRNA, Cattle)

INTRODUCTION

Fructose-1.6-bisphosphatase (EC 3.1.3.11) (FBPase) is a key regulatory enzyme of gluconeogenesis that catalyzes the hydrolysis of fructose-1,6-bisphosphate to generate fructose-6-phosphate and inorganic phosphate. The enzyme has been shown to occur in a variety of species, including bacteria, fungi, plants and animals (Tillmann et al., 2000; Stein et al., 2001). There are two genes, *FBP1* (liver FBPase) and *FBP2* (muscle FBPase) that code for FBPases in mammals. *FBP1* is expressed predominantly in liver, kidney, lung and monocytes (El-Maghrabi et al., 1991, 1995), while *FBP2* is expressed predominantly in muscle (Tillmann et al., 2000). Patients with defective *FBP1*, leading to impaired liver gluconeogenesis, have been identified and in several cases the specific mutations causing the disease could be traced (Kikawa et al., 1994; Herzog et al., 1999). Besides the human *FBP1* gene (Solomon et al., 1988, El-Maghrabi et al., 1993, 1995), the gene orthologs have been characterized in several other species, including mouse (Stein et al., 2001), pig (Marcus et al., 1982), rat (El-Maghrabi et al., 1988, 1991), and zebra fish (Woods et al., 2005). The human, mouse and pig *FBP1* gene encode a polypeptide of 338 amino acids (aa) (Marcus et al., 1982; Skalecki et al., 1999; Stein et al., 2001), whereas zebra fish and rat (El-Maghrabi et al., 1991) *FBP1* genes encode polypeptides of 337 aa and 363 aa, respectively. In humans, a report found that a hereditary lack of liver FBPase leads to FBPase deficiency, which is an autosomal recessive condition associated with hypoglycemia and metabolic acidosis (Stein et al., 2001). Molecular genetic analyses have revealed several mutations from the coding region in *FBP1* as possible causes for the problem of hypoglycemia and metabolic acidosis (Kikawa et al., 1997; Herzog et al., 1999). In bovine industry, the metabolic acidosis and hypoglycemia (such as acetonemia) happens frequently and is usually resolved by dietary means

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Table 1. Primers used for the bovine *FBP1* and *GAPDH* genes

Gene name	Primer names	Primer sequences	Tm (°C)	Size (bp)	Binding region
FBP1[1]	F	5'-AGGCTATGCGAAGGACTTTG-3'	62	1,157	exon5
	R	5'-ATAGACTAGGGTGCGGTGTA-3'			exon6
	F1	5'-TCATCTCTCCTTCCACCTCT-3'	59	507	intron5
	3'-RACE	5'-GCCCAGCCCACACCAAATGGAATTG-3'	68	154	3'-UTR
	5'-RACE	5'-GTTGAGCAGTTGGGTCATCTCGCC-3'	68	181	exon1
	F2	5'-CGACACCAATATCGTCACCG-3'	60	869	exon1
	R2	5'-TAGCCATTCCTCCTGCCTTC-3'			exon7
	Real-time PL	5'-ATGGCAGTGCCACTATGTTG-3'	60	156	exon4
	Real-time PR	5'-AGTCCTTCGCATAGCCTTCA-3'			exon5
	Taqman probe	5'-CATGGCGAATGGAGTCAACTGCTT-3'			exon4
GAPDH	Real-time PL	5'-GATCAAGAAGGTGGTGAAGC-3'	60	156	exon10
	Real-time PR	5'-TTGACAAAGTGGTCGTTGAG-3'			exon11
	Taqman probe	5'-CCTCTCAAGGGCATTCTAGGCTACA-3'			exon10

[1] The primer pair F-R was used to isolate the 1,157 bp length genomic DNA fragment of the *FBP1*, the primer pair F1-R was used for RH mapping. The primer pair F2-R2 for isolating CDS. Real-time PL and Real-time PR were used for the gene expression patterns.

(Schonewille et al., 1999; McNeill et al., 2002). Understanding the molecular mechanism of acidosis and the genes involved in hypocalcaemia will provide the molecular basis for finding new solutions to this problem. In this paper, we report the full-length cDNA sequence of the bovine *FBP1* gene, its predicted aa sequence, genomic structure, expression patterns and single nucleotide polymorphisms (SNPs) as an initial step for identifying the relationship between *FBP1* and the acidosis-related performance in cattle.

MATERIALS AND METHODS

Source of animals and tissues

A total of 51 crossbred steers (Simmental crossbred with indigenous female yellow cattle in China) (405±50.5 kg; 30±2 months of age) were selected randomly and housed in a concrete-floored cowshed (in a single pan for each animal). The pre-trial period (for adaptation to treatment) was 15 days, and the test period was 180 days. Steers were fed according to the NY/T 815-2004 feeding standard of beef cattle (Agricultural Department of People's Republic of China, 2004), and had free access to water and feed during the entire 195 days of the experimental period. All animals were weighed on days 1, 75 and 195 at 8:00 AM, with prior removal of feed and water (12 h). The remainders of feed each day were recorded at 10:00 PM each day. Immediately after the trial; steers were harvested at a processing facility (KeErQing Beef cattle Co., Ltd. P. R. China). Carcass traits were measured according to the criterion GB/T 17238-1998 Cutting Standard of fresh and chilled beef in China (China Standard Publishing House). A total of 51 crossbred steers were used to investigate associations of gene variant with traits. In addition, a total of 82 animals including Simmental (n = 28), Angus (n = 25), Hereford (n = 29) randomly selected from commercial populations were used to analyze the *FBP1* allelic frequencies.

Approximately eleven different tissues (heart, liver, lung, spleen, intestine, kidney, loin-muscle, rumen, testis, lymph, and thymus) were collected from mature Simmental cattle (Inner Mongolia Province, China) at slaughter.

Statistical analyses

The following traits, Backfat Thickness (BF), Carcass Weight (CW), Meat Percent (MP), Average Daily Gain (ADG), Average Daily Feed Intake (ADFI), Ratio of Feed-to-Meat (RFM), Beginning Average Daily Gain (BADG), Finishing Average Daily Gain (FADG), Loin-Muscle Area (LMA) and Carcass Length (CL) were measured or calculated. The collected data were analyzed using a GLM procedure of SAS (SAS Institute Inc. Cary, NC, USA) according to the following model:

$$Y_{ij} = \mu + G_i + b_{ij}w_{ij} + \varepsilon_{ij}$$

Where Y_{ij} stands for observed value; μ, population mean; G_i, ith genotype; b_{ij}: regression coefficient; w_{ij}: weight, for growth traits the w_{ij} stands for starting weight, for meat traits, slaughter weight.; ε_{ij}, random error.

DNA extraction and RNA extraction

Blood samples were collected. The routine phenol chloroform extraction method was used to isolate the genomic DNA that was diluted to 50 ng/μl for PCR. Total RNA was extracted from eleven tissues using the TRIzol reagent kit (Life Technologies, Grand Island, NE, USA).

Primer design

The mRNA sequence for the human *FBP1* (NM_000507) gene was obtained from NCBI, and was

Table 2. Allele frequencies of the bovine *FBP1* gene in different breeds

Breeds	No. of animals	Allele frequency	
		A	B
Simmental	28	0.91	0.09
Angus	25	0.76	0.24
Hereford	29	0.71	0.29
Cross simmental	51	0.78	0.22

employed to search for bovine expressed sequence tags (ESTs) in the EST-others database through standard BLAST (http://www.ncbi.nlm.nih.gov/blast/) algorithm. The bovine ESTs that have more than 80% similarity with the corresponding human mRNA were candidates, assembled into a contig and used to design the gene-specific primers.

The bovine ESTs were selected for *FBP1* (Acc. CN789074, CF767326, CK940889, CN786320, CK969850, CK962726, BF773830, CN786689) from the BLAST analysis and primer pairs were designed using the primer design software Primer 5.0 (PREMIER Biosoft International. Palo Alto, CA. USA) (Table 1) and synthesized by Shanghai Bioasia Biotechnology Co. Ltd in China. PCR was performed in a PTC-200 Peltier Thermal Cycler (MJ Research Inc. Hercules, CA. USA).

Obtaining mRNA by RT-PCR and RACE

Rapid amplification of cDNA ends (RACE) and RT-PCR methods were used to clone the full-length mRNA of the bovine *FBP1* gene from liver tissue (Guan et al., 2006). Primers on the basis of the contig sequence are given in Table 1. The RT-PCR was carried out as described by Pan et al. (2003). RACE was performed according to the instructions of the SMART™ RACE cDNA Amplification Kit (Clontech Inc, Palo Alto. CA. USA). The PCR products of RACE were purified with Wizard PCR Preps DNA Purification System (Promega. Madison. WI. USA) and cloned into the pGEM T-easy vector (Promega. Madison. WI. USA), and then several random clones were selected and sequenced commercially. ORFs were found and the amino acid sequences were deduced with the program Seqman (DNA Star, Madison, WI, USA)

Taqman real-time PCR analysis of gene expression patterns

The syntheses of the first-strand cDNA was followed by PCR using gene-specific primers and Taqman probes (Table 1). Each real-time PCR (in 20 μl) contained 1×PCR buffer (TaKaRa). 3.0 mM $MgCl_2$. 100 μM each dNTP. 0.3 μM primers. 0.1 μM probe, and 2 U *Taq* DNA polymerase (TaKaRa) plus 2 μl template cDNA. The cycling conditions consisted of an initial, single cycle for 5 min at 95°C followed by 35 cycles of two-temperature cycling consisting of 15 s at 95°C (for denaturation) and 1 min at 60°C (for annealing and polymerization). Relative mRNA quantification was performed using standard curves generated with heart tissue total RNA. PCRs were performed in triplicate and gene expression levels were quantified relative to the expression of *GAPDH* using Gene Expression Macro software (Bio-Rad) employing the comparative C_t ($\Delta\Delta C_t$) value method. Expression levels were considered not detectable when the C_t value of the targeted gene exceeded 35 in the sample tissue.

Isolation and sequencing of the gene fragment

The amplified products were obtained for *FBP1* in a 20 μl reaction volume consisting of 50 ng of bovine genomic DNA. 1×PCR buffer, 0.3 μM of each primer, 75 μM of each dNTP. 1.5 mM $MgCl_2$ and 1 U Taq DNA polymerase (Promega, Madison, WI, USA). The PCR conditions were: 5 min at 95°C followed by 30 s at 94°C, 30 s at the annealing temperature (Table 1) and 30 s at 72°C for 34 cycles and a final extension of 72°C for 10 min. The PCR products were analyzed on a 1.5% agarose gel stained with 0.5 μg/ml ethidium bromide. To verify their identity, the products were purified with the Wizard Prep PCR Purification Kit (Shanghai Bioasia Biotechnology Co., Ltd. P. R. China), cloned into the pGEM-T Easy Vector Kit (Promega, Madison, WI, USA), and sequenced (Applied Biosystems 3730xl DNA Analyzer. Foster city, CA. USA). The sequences of the fragments were then used in BLASTn searches at the NCBI to ensure that they were the expected bovine sequences.

SNP identification and allele frequency analysis

A PCR-RFLP approach was applied to detect polymorphisms in the bovine *FBP1*. The PCR products for *FBP1* were digested with *Msp I* at 37°C for 4 hrs. Restriction fragments were scored on 1.5% agarose gels. A total of 133 unrelated cattle from four breeds (Simmental, Angus, Hereford, crossbreed) were genotyped and allele frequencies were determined (Table 2). Sequence comparison among the 10 individuals revealed a SNP (A/G transition) at the position of nt 822 within the fragment, which located within the 5th intron of the gene. Digestion of the PCR fragments with *Msp I* revealed two alleles: Allele A (A-base)-two fragments of 712 and 445 bp, and allele B (G-base)-three fragments of 712, 336 and 109 bp observed on the agarose gels (Figure 4). Using the primer pair F-R that flanks the 5th intron of the *FBP1* gene (Table 1), we amplified a fragment of 1.157 bp from all animals studied. Sequencing the fragment (DQ166528) confirmed the origin of the bovine *FBP1*.

Chromosomal location

A 7000-rad whole-genome cattle-hamster RH panel

Table 3. Nucleotide sequence of exon-intron junction of the bovine *FBP1* gene

Exon number	Intron number	Start-end position in the cDNA	Start-end position in the genomic DNA[a]	Size (bp)
1		1-233	364084-364316	233
	1		364317-376965	12,649
2		234-396	376966-377128	163
	2		377129-380087	2,959
3		397-489	380088-380180	93
	3		380181-388435	8,255
4		490-630	388436-388576	141
	4		388577-391584	3,008
5		631-768	391585-391722	138
	5		391723-392743	1,021
6		769-888	392744-392863	120
	6		392864-394429	1,566
7		889-1,080	394430-394620	191

[a]The Bos taurus chromosome Un genomic contig (NW-969386, BtUn-WGA4471-2).

```
   1 ccgcttcccaccgctcccgccgcccgcacctgtcgcccagcgagccgcaaccgggcccc 60
  61 agcatgacggaccaggcagccctcgacacccaatatcgtcaccgtgacccgccccgtcatg 120
       M  T  D  Q  A  A  F  D  T  N  I  V  T  V  T  R  F  V  M
 121 gaagagggcaggaaggcccgtggcacgggcgagatgacccaactgctcaactcgctttgc 180
       E  E  G  R  K  A  R  G  T  G  E  M  T  Q  L  L  N  S  L  C
 181 accgcggtcaaagccatctccacggccgtgcgcaaggcgggcatcgcgcacctctatgga 240
       T  A  V  K  A  I  S  T  A  V  R  K  A  G  I  A  H  L  Y  G
 241 attgctggcactaccaatgtgacgggagatcaagtgaagaagctggatgtcctctccaat 300
       I  A  G  T  T  N  V  T  G  D  Q  V  K  K  L  D  V  L  S  N
 301 gacctggttgttaatgtgttaaagtcatcttttgctacctgtgttcttgtgtcagaagaa 360
       D  L  V  V  N  V  L  K  S  S  F  A  T  C  V  L  V  S  E  E
 361 gatgaacatgccataatagtagaacctgagaaaaggggtaaatatgtggtctgttttgat 420
       D  E  H  A  I  I  V  E  P  E  K  R  G  K  Y  V  V  C  F  D
 421 ccccttgatggatccatccaacatccgactgccttgtgtccattggaaccatccctggcatc 480
       P  L  D  G  S  S  N  I  D  C  L  V  S  I  G  T  I  F  G  I
 481 tacaaaaagatttcgaaagatgacccttctgagaaggacgctctgcagccaggccggaac 540
       Y  K  K  I  S  K  D  D  P  S  E  K  D  A  L  Q  P  G  R  N
 541 ctggtggctgccggctatgcgccctatggcagtgccactatgttggtccctggccatgggcg 600
       L  V  A  A  G  Y  A  L  Y  G  S  A  T  M  L  V  L  A  M  A
 601 aatggagtcaactgcttcatgctggacccggccattggagagttccatttcggtgggacagg 660
       N  G  V  N  C  F  M  L  D  P  A  I  G  E  F  I  L  V  D  R
 661 gatgtgaagatcaaaaagaaagggagctctatagccttcaatgaaggctatgcgaaggac 720
       D  V  K  I  K  K  K  G  S  I  Y  S  L  N  E  G  Y  A  K  D
 721 tttgatcctgccctcaccgagtatgtccagaggaagaagttcccccagacaactcagcc 780
       F  D  P  A  L  T  E  Y  V  Q  R  K  K  F  P  P  D  N  S  A
 781 ccctatggcgccaggtacgtgggctccatggtagccgatgtacaccgcaccctagtctat 840
       P  Y  G  A  R  Y  V  G  S  M  V  A  D  V  H  R  T  L  V  Y
 841 ggagggatctttatgtatccagctaacaagaaaagccccagtggaaagctgagactactg 900
       G  G  I  F  M  Y  P  A  N  K  K  S  P  S  G  K  L  R  L  L
 901 tatgaatgtaaccccatggcctatgtcatagagagaaggcaggaggaatggctaccaccggg 960
       Y  E  C  N  P  M  A  Y  V  I  E  K  A  G  G  M  A  T  T  G
 961 aaggaaactgtgctggacattgttcccattgacatccatcagaagtcgcccatcatcttg 1020
       K  E  T  V  L  D  I  V  P  I  D  I  H  Q  K  S  P  I  I  L
1021 gggtctcctgaagatgtgactgagttcctggagatatcaagaagcatgctgccaaatga 1080
       G  S  P  E  D  V  T  E  F  L  E  I  Y  K  K  H  A  A  K  *
1081 agagtgggcccagcccacaccaaatggaattgccttttatctggaccctccatcccacat 1140
1141 agtgttaccacatcccgactgttccaccatacattcctagtcatagaaataaaaagtatgg 1200
1201 ctgttttccccaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa                  1241
```

Figure 1. Full-length cDNA and deduced amino acid (aa) sequences of FBP1.

(SUNbRH) consisting of 92 hybrids was used to map the *FBP1* gene with the primer pair F1-R (Table 1). The PCR typing, vector scoring and RH mapping followed the methods described exactly in Guo et al. (2006) and Liu et al. (2005).

RESULTS AND DISCUSSION

Sequence analysis of the bovine *FBP1* gene

A BLAST search against the bovine EST database (http://www.ncbi.nlm.nih.gov/blast/) with the human *FBP1* mRNA (NM_000507) resulted in eight ESTs for the bovine *FBP1* gene. These ESTs were assembled into a contig that was used for PCR primer design (Table 1). Using a combination of RT-PCR and 5'- and 3'-RACE techniques, we obtained a full-length bovine *FBP1* cDNA of 1241 nucleotides (DQ520945). This cDNA contains a single possible open reading frame (ORF) (nt 64-1080), a 63 bp 5' untranslated region (UTR) and 131 bp 3' UTR, and a poly (A) tail of 30 bp (Figure 1). The deduced protein sequence of the bovine *FBP1* gene contains 338 amino acids (aa) (Figure 1) with a calculated molecular mass of 36.75 kDa. Sequence comparison revealed that the bovine *FBP1* gene is 89%, 85%, 82%, 82% and 74% identical to the orthologs of the pig (NM_213979), human (NM_000507), rat (NM_012558), mouse (NM_019395) and zebra fish (NM_213132) at mRNA level, and 97%, 96%, 93%, 94%, and 91% identical to the corresponding peptides at the protein level, respectively (Figure 2).

Searching the recently released bovine genome sequence (Build 2.1) (http://www.ncbi.nlm.nih.gov/genome/seq/BtaBlast.html) with the bovine *FBP1* cDNA resulted in a *Bos Taurus* un-mapped genomic contig (NW_969386, BtUn-WGA4471-2) with an E-value of $2e^{-167}$. Further analysis indicated that this genomic contig contains the entire *FBP1* gene. There are 7 exons and 6 introns present in the bovine *FBP1* gene (Table 3). The size of exons range from 93 to 233 bp, while the size of introns from 1,566 to 12,649 bp (Table 3). The gene spans 30.8 kb in the bovine genome. These results demonstrate that the gene structure of bovine *FBP1* is very similar to that of the human as the human *FBP1* also contains the same number of exons and introns and spans ~37 kb in the genome (NT_008470).

Expression pattern of the bovine *FBP1* gene

Eleven different tissues including heart, liver, lung, spleen, small intestine, kidney, loin-muscle, rumen, testis, lymph, and thymus were collected from a mature

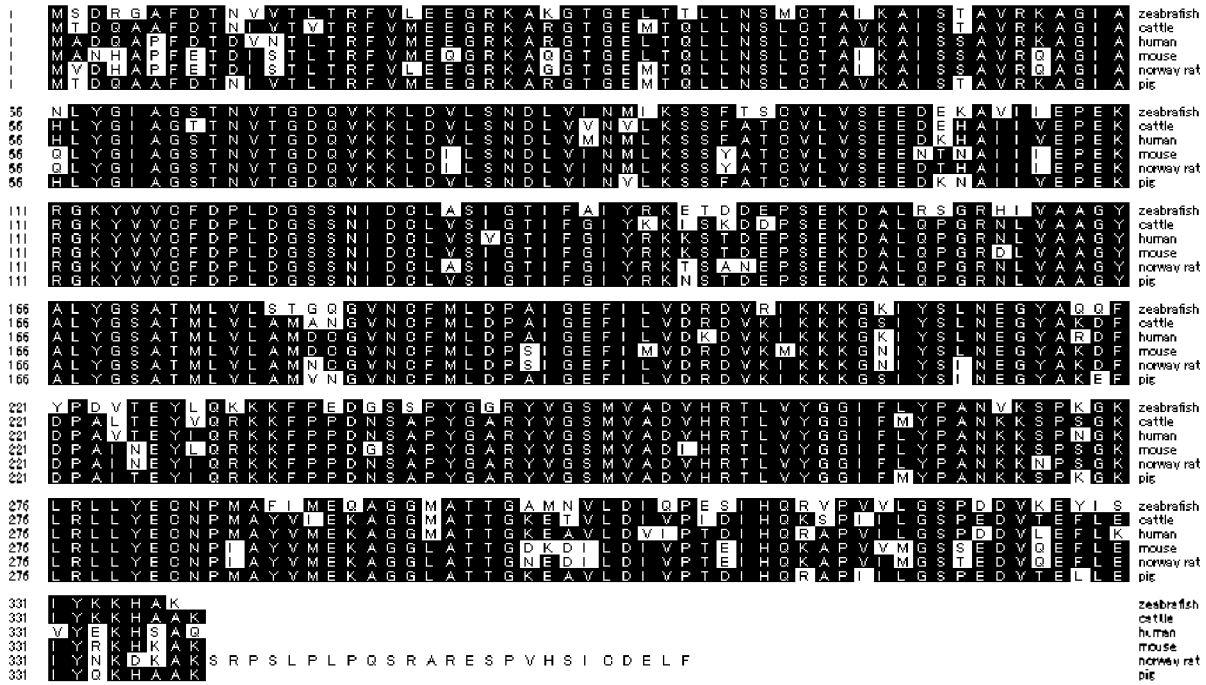

Figure 2. Multiple sequence alignment of the deduced aa sequence of FBP1 with the sequences of pig (NP_999144), human (NP_000498), rat (NP_036690), mouse (NP_062268) and zebra fish (NP_998297).

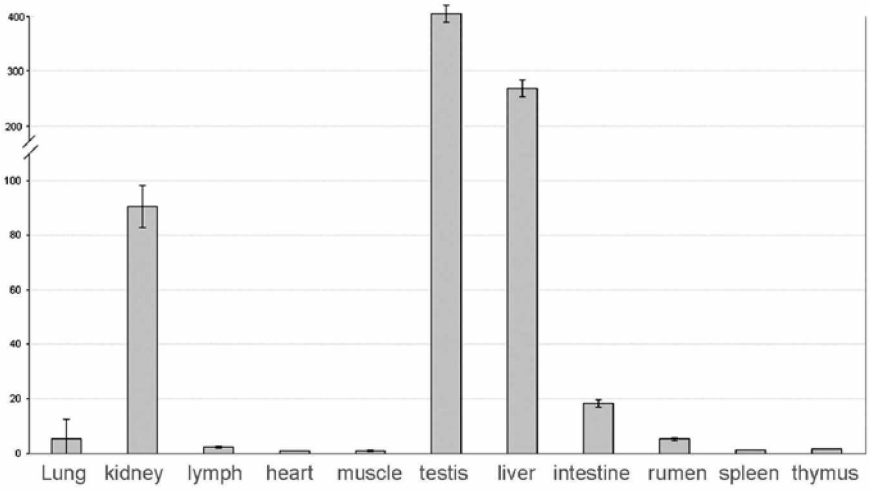

Figure 3. Taqman real-time PCR analysis of *FBP1* gene expression patterns.

Simmental bull (Inner Mongolia province, China) for a gene expression study of *FBP1*. Taqman analysis was performed to determine the relative mRNA level of *FBP1* in various tissues. The housekeeping gene glyceraldeyhyde-3-phosphate dehydrogenase (*GAPDH*) was used for endogenous control (Hye et al., 2006). Expression data (Figure 3) demonstrate that the bovine *FBP1* gene is expressed predominantly in testis, liver and kidney, relatively low in intestine, rumen and lung, and very low in the remaining tissues including spleen, thymus, lymph, heart and muscle.

Characterization of a SNP in the bovine *FBP1* gene and association of SNP with traits

Using the primer pair F-R that flanks the 5th intron of the *FBP1* gene (Table 1), we amplified a fragment of 1,157 bp from all animals studied. Sequencing the fragment (DQ166528) confirmed the origin of the bovine *FBP1*. By careful comparison of the sequences from different animals, we found a SNP (A/G transition) at the position of nt 822 within the fragment, which located within the 5th intron of the gene. Digestion of the PCR fragments with *Msp I* revealed two alleles: Allele A (A-base)-two fragments of

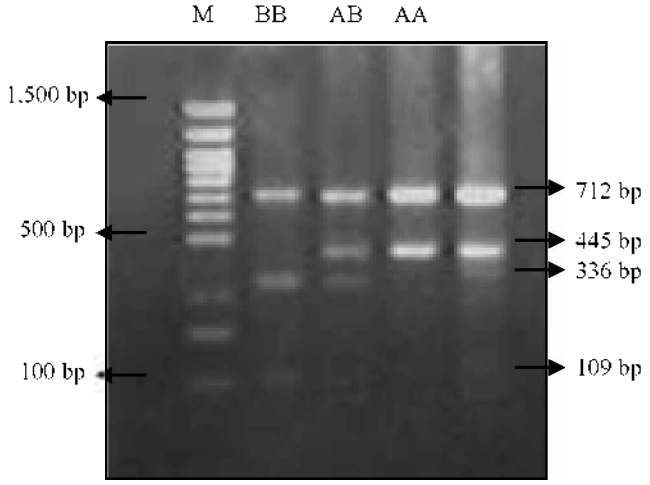

Figure 4. Agrose gel (2.0%) showing different PCR-RFLP (*Msp I*) genotypes of bovine *FBP1* . M:100-1,500 bp ladder.

712 and 445 bp, and allele B (G-base)-three fragments of 712, 336 and 109 bp observed on the agarose gels (Figure 4).

Allele frequency was investigated in a total of 133 animals from four different beef populations and the results were listed in Table 2. The average frequency of the allele A was 0.7897 ranging from 0.7069 to 0.9107, and allele B 0.2103, ranging from 0.0893 to 0.2931. A preliminary association study of this intronic SNP with 10 traits, including Backfat Thickness (BF), Carcass Weight (CW), Meat Percent (MP), Average Daily Gain (ADG), Average Daily Feed Intake (ADFI), Ratio of Feed-to-Meat (RFM), Beginning Average Daily Gain (BADG), Finishing Average Daily Gain (FADG), Loin-Muscle Area (LMA) and Carcass Length (CL), indicated that there is a significant association between the SNP and two traits: ADFI and CL ($p<0.01$). Animals with an AA genotype ($6.29^{a}\pm0.45$) had a significant increase in ADFI (~26.81% and ~3.97%) in comparison to the AB ($6.05^{b}\pm0.63$) and BB ($4.96^{c}\pm0.64$) genotypes. For CL, AA ($146.76^{a}\pm0.80$) and AB ($147.27^{a}\pm1.32$) genotypes had a significant increase (~7.02%) over BB ($136.67^{b}\pm6.17$) genotypes (Table 4).

Mapping the bovine FBP1 on BTA8 by RH mapping

By typing on the 7000-rad SUNbRH panel and analyzing the vector together with 3216 framework markers (http://www.animalgenome.org/cattle/maps) (Itoh et al., 2005), we found that the bovine *FBP1* gene is closely linked to a framework marker *BM3412* at a 2-point LOD score of 29.1, and maps between *BM3412* and *BMS2196* with a distance of 7.04 cR to *BM3412*, and 7.46 cR to *BMS2196*. Therefore, the *FBP1* gene was assigned to the bovine chromosome (BTA) 8. Comparative mapping has demonstrated that BTA8 is partly conserved with the human chromosome (HSA) 9 on which the human *FBP1* maps (Itoh et al., 2005), supporting the mapping of the bovine *FBP1* gene on BTA8 in this work.

In the present study, we cloned and characterized the cDNA of the bovine *FBP1* gene. We found that the deduced protein sequence is 338 aa long (Figure 1). Comparison of the bovine cDNA sequence to the genomic sequence (BtUn-WGA4471) indicated that the bovine gene is composed of 7 exons and 6 introns (Table 3), which is very similar to the human gene in terms of the gene structure. The genomic contig (BtUn-WGA4471) that contains the bovine *FBP1* gene has not been associated with any bovine chromosomes in the current bovine genome sequence (Build 2.1). Mapping the *FBP1* gene to BTA 8 in this work provides direct evidence to place this genomic contig on the bovine chromosome 8 in future bovine sequence assemblies.

In human and mouse, *FBP1* occurs at a high level in liver and low in some nongluconeogenic tissues, such as skeletal muscle and brain (Horecker et al., 1975; Majumder et al., 1977; El-Mghrabi et al., 1988). Our Taqman real-time PCR data (Figure 3) indicated that the bovine *FBP1* mRNA was most abundant in testis and liver, and was very low in heart and muscle, in agreement with the human and mouse gene expression data in general. However, the finding of the high expression level in testis from this work is new and is of interest as it may indicate an association between the gene and the hormonal regulation and the possibly subsequent growth performance in cattle.

In human, several mutations of the coding region in *FBP1* was associated with FBPase deficiencies among patients (Kikawa et al., 1997; Herzog et al., 1999), the molecular basis for this deficiency, however, remains undetermined. Although we have no measurement data of FBPase activity in the bovine population used in the experiment, a significant association between the intronic

Table 4. Effect of *FBP1* genotype on feed intake, growth and partial carcass in the cattle (51)

Genotypes	Traits[1] (mean±SE)[2]									
	ADG	CW	LMA	MP	BF	BADG	FADG	RFM	ADFI	CL
AA	0.75±0.03	$274.43^{ab}\pm4.51$	62.40±2.09	50.43±0.70	$1.10^{b}\pm0.06$	0.63±0.10	0.63±0.03	9.10±0.90	$6.29^{a}\pm0.45$	$146.76^{a}\pm0.80$
AB	0.80±0.05	$282.54^{a}\pm8.70$	69.47±2.04	49.36±0.51	$1.11^{a}\pm0.06$	0.76±0.09	0.67±0.05	8.26±1.25	$6.05^{b}\pm0.63$	$147.27^{a}\pm1.32$
BB	0.64±0.03	$245.93^{b}\pm14.28$	59.00±1.15	50.33±0.45	$1.62^{a}\pm0.17$	0.95±0.22	0.46±0.04	7.76±1.16	$4.96^{c}\pm0.64$	$136.67^{b}\pm6.17$

[1] Average daily gain (ADG), carcass weight (CW), loin-muscle area (LMA), meat percent (MP), backfat thickness (BF), beginning average daily gain (BADG; average daily gain in the test period from day 1 to day 75), finishing average daily gain (FADG: average daily gain in the test period from day 75 to day 180), ratio of feed-to-meat (RFM), average daily feed intake (ADFI) and carcass length (CL).

[2] Mean values in the same column with different superscripts differ significantly (a vs. b, $p<0.01$).

SNP and ADFI, and CL was observed in the experimental population. It is reasonable to predict that animals with a higher ADFI should have a faster growth, and yield a relatively longer carcass length (CL). However, as the frequency of the recessive allele B is rather low (~0.2) in the four beef populations investigated, the proportion of animals with BB genotype is ~4% according to the Hardy Weinberg Law. The feasibility of using this SNP for marker assisted selection (MAS) needs to be further studied in a larger population of different beef breeds. Furthermore, it is important to determine, in future investigations, whether this intronic mutation has really affected some biological function, such as the FBPase activity, that may directly or indirectly affect the traits in cattle.

Note: The sequences of the 1.157 bp fragment (Accession No. DQ166528) and the 1.241 bp fragment (Accession No. DQ520945) have been submitted to GenBank.

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