Mol. Cells *30*, 393-401, November 30, 2010
DOI/10.1007/s10059-010-0129-5

Molecules and Cells

Molecular Cloning, Expression and Characterization of Bovine UQCC and Its Association with Body Measurement Traits

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Ubiquinol-cytochrome c reductase complex chaperone (*UQCC*) involved in the development and maintenance of bone and cartilage is an important candidate gene for body measurement traits selection through marker-assisted selection (MAS). The expression of *UQCC* is upregulated in many human and animal models of height as well as other stature indexes. We have cloned the cDNA sequence coding *UQCC* gene in bovine. Genomic structural analysis indicated that bovine *UQCC* shares a high similarity with human *UQCC*. Furthermore, Real-Time PCR analysis showed that the expression of bovine *UQCC* is remarkably different in diverse tissues, including high level expression in the spleen, heart and windpipe, and relatively low expression in other tissues. We also analyzed allele frequencies in different cattle breeds and an association study on the selected SNPs. SNP DraI A2691T in intron 1 and SNP Bsh1236I A3150G in intron 8 are significantly associated with Body Length (BL), Rump Length (RL), Chest Depth (CD) and Pin Bone Width (PBW). For the A2691T SNP marker, there are significant effects on the RL ($p = 0.0001$), CD ($p = 0.0059$) and PBW ($p < 0.0001$) in 679 individuals; with A3150G SNP marker, there are significant effects on the BL ($p = 0.0047$) and CD ($p = 0.0454$). Regarding association analysis of combination of the two SNPs, there are significant effects on the BL ($p = 0.0215$), CD ($p = 0.0282$) and PBW ($p = 0.0329$) in the total population. The results suggest that the *UQCC* gene is a candidate gene of body measurement traits in bovine reproduction and breeding, and provide data for establishing an animal model using cattle to study big animal body type.

INTRODUCTION

Ubiquinol-cytochrome c reductase complex chaperone (*UQCC*, also known as *BFZB* or *C20orf44*) encodes a zinc-binding protein repressed by basic fibroblast growth factor (*bFGF*, also known as *FGF2*). It is also involved in the network of *FGF2*-regulated growth (Sanna et al., 2008; Vetter and Wurst, 2001). *UQCC* is present in differentiating chondrocytes (Imabayashi et al., 2003), and is first expressed at early stages of mesenchymal cell proliferation in mouse (Goldring et al., 2006). In mouse embryonic stem cells, *UQCC* is downregulated after addition of *FGF2* (Vetter and Wurst, 2001), which functions in concert with bone morphogenic proteins and several homeobox genes products to initiate and promote morphogenesis and growth of the skeleton. *UQCC* has been selected as an important candidate gene in a genome-wide search for regions in chondrocyte differentiation, bone growth or development (Voight et al., 2006). To our knowledge, no information exists about the genomic structure, tissue expression patterns and polymorphisms of bovine *UQCC* gene.

Based on the importance of *UQCC* in chondrogenesis, morphogenesis and growth of the skeleton from determinations in mouse and human, *UQCC* could be an attractive candidate gene for body measurement traits in bovine. The objective was therefore to analyze molecular characterization and detect SNPs in bovine *UQCC* gene; and to explore their possible association with body measurement traits in *Bos taurus*.

MATERIALS AND METHODS

Animal

Animal procedures were performed according to protocols approved by Biological Studies Animal Care and Use Committee PR China. Nine tissues including bladder, testis, fat, heart, large intestine, kidney, muscle, spleen and trachea were collected from one purebred Qinchuan cattle, 3 years old. Total RNAs were extracted for spatial expression analysis of bovine *UQCC* gene. For the gene variants that identified, allele frequencies were estimated on a restricted population composed of unrelated, randomly selected purebred and crossbred individuals representing 8 breeds including Qinchuan (QC, n = 105, Shaanxi province of China), Qinchuan improvement steers (QI,

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Received December 24, 2009; revised July 20, 2010; accepted July 26, 2010; published online August 27, 2010

Keywords: association analysis, body measurement, cattle, genomic structure, SNP polymorphism, Ubiquinol-cytochrome c reductase complex chaperone (UQCC)

n = 137, Shaanxi province of China), Nanyang (NY, n = 51, Henan province of China), Jiaxian red (JR, n = 75, Henan province of China), Xia'nan (XN, n = 70, Henan province of China), Luxi (LX, n = 66, Shandong province of China), Simmental and Luxi crossbred steers (SL, n = 54, Shandong province of China), Xuelong (Angus crossed with descendant of male Japanese Black cattle and female Fuzhou cattle)(XL, n = 121, Liaoning province of China). Meanwhile, the following traits, Body length (BL), Withers height (WH), Hip height (HH), Rump length (RL), Hip width (HW), Chest depth (CD), Heart girth (HG) and Pin bone width (PBW) were measured (Gilbert et al., 1993). For each of the body measurement traits, we measured the same one trait with the same people to minimize error.

DNA samples were extracted from leukocytes and tissue samples using standard phenol-chloroform protocol (Mullenbach et al., 2003).

SYBR Green RT-PCR analysis of the expression patterns of bovine *UQCC*

Tissue distribution of bovine *UQCC* was analyzed by RT-PCR. The primer pair (qPCR-F, qPCR-R, Table 1) was designed. Total RNAs were extracted from 9 different tissues of mature Qinchuan cattle (bladder, testis, fat, heart, large intestine, kidney, muscle, spleen and windpipe); the RNA concentration from each sample was adjusted to the same level.

RT- PCR in 20 μl reaction mixture contained SYBR® Green Real-time PCR Master Mix (Applied Biosystems, USA), gene-specific primers and template cDNA. The cycling conditions consisted of an initial, single cycle for 5 min at 95°C followed by 40 cycles of 15 s at 95°C and 60 s at 61°C. PCRs were performed in triplicate and gene expression levels were quantitated relative to the expression of β-action using 7500 System SDS Software V 1.4.0 (Applied Biosystems, USA), employing the comparative Ct ($^{\triangle\triangle}$Ct) value method, in which β-action was used as an internal control to correct the differences in the mRNA quantities.

cDNA clone and sequence analysis

Human mRNA sequences of *UQCC* (GenBank accession no.NM_018244.3) was compared with all sequences available for *Bos taurus* in the express tag (EST) databases using the Blast algorithm (http://www.ncbi.nlm.nih.gov/BLAST). Bovine ESTs were selected that shared > 80% sequence identity with the corresponding human cDNA in order to assemble the bovine gene using the SeqMan (DNASTAR, Inc., USA). Partial 5′-utr, CDS and partial 3′-utr fragment were amplified by 2 primer pairs (CDS-F and CDS-R, 3utr-F and 3utr-R, Table 1) using the cDNA of Qinchuan cattle. The cDNA sequences of bovine UQCC were analyzed using the BLAT Search Genome (http://www.genome.ucsc.edu/cgi-bin/hgBlat). To verify and clone the cDNA sequence of bovine UQCC, RNA extraction and sequencing were performed as previously described (Huang et al., 2009; Wang et al., 2007).

Furthermore, the different species *UQCC* gene amino acid sequences were acquired from the National Center for Biotechnology Information (NCBI) server (http://www.ncbi.nlm.nih.gov), including *Homo sapiens* (basic FGF-repressed Zinc binding protein isoform a, NP_060714.3), *Homo sapiens* (basic FGF-repressed Zinc binding protein isoform b, NP_955781.1), *Mus musculus* (basic FGF-repressed Zinc binding protein, NP_061376.2), *Gallus gallus* (ubiquinol-cytochrome c reductase complex chaperone, NP_001006285.1), *Pongo abelii* (ubiquinol-cytochrome c reductase complex chaperone, NP_001125945.1). The deduced amino acid sequences of bovine *UQCC* gene were compared with the corresponding protein of other species. Bioinformatics domain searching analysis was performed using PSORT II (http://psort.nibb.ac.jp) and PROSITE (http://au.expasy.org). The translated polypeptide sequences were aligned using ClustalX software with the default parameters. Phylogenetic trees were constructed using the MEGA program (http://www.megasoftware.net/).

Polymorphism identification, allele frequencies and association analysis

Genetic polymorphisms in the *UQCC* were identified by sequencing PCR products from eight breeds, and compared with each other using SeqMan (DNASTAR, Inc., USA). Several single nucleotide polymorphisms (SNPs), among them, one SNP 2691 (A/T) in intron 1 and one SNP 3150 (A/G) in intron 8 were chosen for further analysis. Using SNP primers (SNP1-F and SNP1-R, SNP 8-F and SNP 8-R, Table 1), PCR was performed on samples from 8 breeds. PCR were done in 20 μl reaction mixture containing 50 ng mixed DNA template (DNA template was mixed 8 DNA samples from 8 different breeds with the same volume, respectively), 10 pM of each primer, 0.20 mM dNTP, 2.5 mM $MgCl_2$ and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). The PCR protocol was 95°C for 5 min followed by 32 cycles of 94°C for 30 s, 63°C or 58°C (SNP 1-63°C and SNP 8-58°C) annealing for 30 s, and 72°C for 30 s or 40 s (SNP 1-30 s and SNP 8-40 s), and a final ex-

Table 1. Primers information in experiments

Primer name	Primer sequence (5′-3′)	Binding region	PCR (Tm) (°C)	Size (bp)
CDS-F	TCACCCGGAAATGGTTGTCG	5′-utr	63	1096
CDS-R	CCCTATCAGACTCCTGGGCGT	3′-utr		
3utr-F	TACGCCCAGGAGTCTGATAGG	3′-utr	58	1113
3utr-R	CTGGCTCGGCTCTGGTTTAT	3′-utr		
SNP1-F	CTCCACATCTTTGCTGACAT	Intron 1	64	288
SNP1-R	TCCAATACTTTAGCCACCTG	Intron 1		
SNP8-F	TGTCCCTCAGTCACCCTTGC	Intron 8	58	976
SNP8-R	CCTCACACTGGAGTCTGGGAAC	Intron 9		
qPCR-F	GGATGCAGCCGATTGCTACTTG	Exon 2	60	140
qPCR-R	TTCCTCCTCAGCTCAATGTCTACTCC	Exon 3		
β-actin-F	TGGCACCCAGCACAATGAA		60	107
β-actin-R	ACATCTGCTGGAAGGTGGACAG			

Table 2. Genomic structure information of the bovine *UQCC* gene. Exon sequences are in uppercase, intron sequences are in lowercase.

Coding exons	Exon length (bp)	5′ splice donor	Intron	Intron length (bp)	3′ splice donor
1	24	ATGGCG/gtgagt	1	12610	ctctag/GTCCTT
2	105	AGGAAC/gtaaga	2	9913	ctccag/TTCCAG
3	87	ATGAGC/gtgaga	3	1315	aaatag/AGTAAG
4	108	CTTTCT/gtaaag	4	7880	gtctag/AAATGG
5	73	AAAATT/gtaagt	5	6003	ttctag/TTCTAA
6	58	GGTGTC/gtaagt	6	14509	acccag/TGTCTG
7	109	GATGTG/gtaagt	7	34713	ctgtag/ATGGGG
8	78	GTTAAT/gtaaga	8	3226	ttccag/GATGAG
9	114	GGGATC/gtaagc	9	3176	ttgcag/AAACAG
10	135	ATACAG/			/CTTTGA

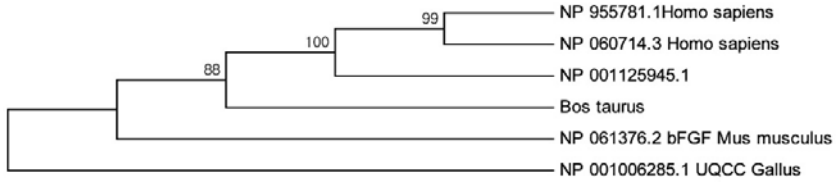

Fig. 1. Phylogenetic tree of UQCC gene in different species. The bootstrap confidence values are shown at the nodes of the tree. The horizontal branch lengths are proportional to the estimated divergence of the sequence from the branch point.

tension at 72°C for 10 min. The products were purified with a Wizard Prep PCR purification kit (Shanghai Bioasia Biotechnology, China) and sequenced (Beijing Aolaibo Biotechnology, PR China; Applied Biosystems 3730xl DNA sequencer, USA). Allele frequencies were analyzed by genotyping the site among 679 individuals. Association analysis of the relationship between genotypes and body measurement traits were carried out on our resource population. The PCR restriction fragment length polymorphism (PCR-RFLP) method was employed to genotype the polymorphic sites, 2 restriction enzymes, Dra I and Bsh1236 I (MBI, Fermentas) were used to digest the corresponding PCR products containing the SNPs site, and the products run on agarose gels and stained with ethidium bromide to assess size and quality.

Statistic analysis

The following items were statistically analyzed according to the previous approaches (Liu et al., 2010; Nei and Li, 1979; Nei and Roychoudhury, 1974), including genotypic frequencies, allelic frequencies, Hardy-Weinberg equilibriums, gene homozygosity, gene heterozygosity, effective allele numbers and polymorphism information content (PIC). The linkage disequilibrium and haplotype analysis was performed by SHEsis software (Mateescu et al., 2005; Shi and He, 2005). The association between SNP marker genotypes of the *UQCC* gene and records of body measurement traits (BL, WH, HH, RL, HW, CD, HG and PBW) was analyzed by the least-squares method as applied in the GLM procedure of SAS (SAS Institute Inc., USA), and according to the following statistical linear model:

$$Y_{ijkl} = \mu + G_i + S_j + BF_k + Ma_l + \varepsilon_{ijkl}$$

Where Y_{ijkl} is the observed body measurement trait, μ itheoverall mean for each trait, G_i the genotype effect, S_j the fixed effect of sex, BF_k the fixed effect of breed and farm, Ma_l the regression variable for measure age, and ε_{ijkl} the random environment effect.

RESULTS

Molecular cloning and sequence analysis of bovine *UQCC*

In order to define the actual intron-exon arrangements, a BLAT Search Genome (http://www.genome.ucsc.edu/cgi-bin/hgBlat) using the amplified mRNA sequences was undertaken. The results indicated that the bovine *UQCC* gene is composed of 10 exons and 9 introns (Table 2), spanning 10.6 kb of the genome. All exon/intron junctions were in accordance with universal RNA splice criteria (GT-AG rule). The sequence provides a good source for SNP search and association analysis. Using the ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html), sequence analysis showed that bovine *UQCC* cDNA contained an ORF (Open Reading Frame) of 891 nucleotides (*nt*), encoding a protein of 296 amino acids with a calculated molecular mass of 34.02 kDa and an isoelectric point (pI) of 8.99.

Sequence alignments and inferring phylogenetic tree

A GenBank database search using BLAST revealed that the predicted bovine *UQCC* amino acid sequence shared high similarity with other mammalian *UQCC* protein sequences, with 89% identity to human and 84% identity to mouse, respectively. The phylogenic relationship among all the hitherto characterized members of the *UQCC* gene was illustrated according to the phylogenetic distance calculated by the MEGA4.0.1 program (Fig. 1). The phylogenetic tree analysis was employed to find the positions of bovine *UQCC* in relation to a selection of other animals; *UQCC* of bovine, human and gorilla fell into one evolutionarily related group, except for mouse and chicken.

The transmembrane helix in bovine *UQCC* predicted by TMHMM (http://www.cbs.dtu.dk/services/TMHMM-2.0) gives all 1-296 amino acid residues are being outside. Analyses of their amino acid sequences detected transmembrane regions signal peptides site, mitochondrial presequence, center position of membrane topology and phosphorylation and N-glycosylation sites but no protein-binding motifs, or transmembrane regions common to any other known protein family by either PSORT or EXPASY. Like their orthologous genes in human, mouse, go-

Fig. 2. Multiple amino acid sequence alignments of *UQCC* gene in different species. The deduced amino acid sequence of the bovine *UQCC* was aligned to that from *Homo sapiens BFZBa* (GenBank Accession No. NP_060714.3), *Homo sapiens BFZBb* (GenBank Accession No. NP_955781.1), *Mus musculus BFZB* (GenBank Accession No. NP_061376.2), *Gallus gallus UQCC* (Gen-Bank Accession No. NP_001006285.1), *Pongo abelii UQCC* (GenBank Accession No. NP_ 001125945.1). Conserved amino acid residues among different species are presented with asterisks.

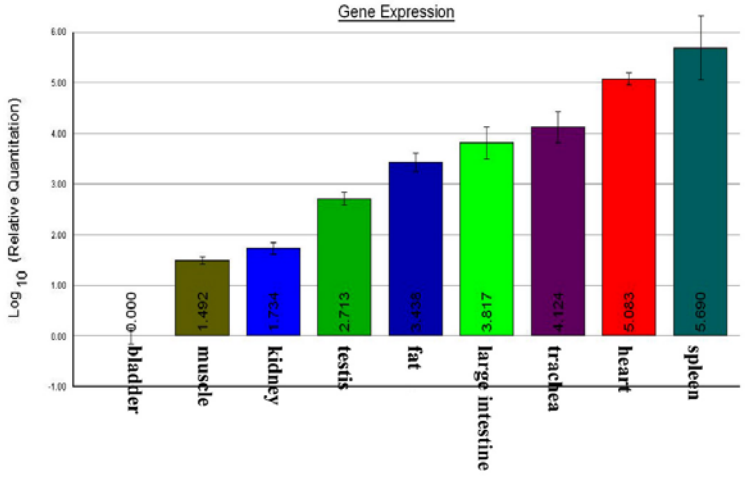

Fig. 3. The tissue distribution of bovine *UQCC* mRNA assessed by real-time PCR. Error bars indicate the SD (n = 3) of relative mRNA expression levels of *UQCC* to β-actin. The values were normalized to endogenous β-actin expression and the value of *UQCC* in bladder was arbitrarily set to 0.

rilla and chicken, bovine *UQCC* gene shows the highest homologies in their amino and carboxyl termini (asterisks in Fig. 2), with the intervening amino acids being less well conserved.

Tissue expression patterns of bovine *UQCC*

The expression of *UQCC* in a number of bovine organs was performed and analyzed (see Fig. 3). RT-PCR analysis showed that the bovine *UQCC* mRNA expression was markedly different in diverse tissues, high level expression occurring in the spleen, heart and windpipe, moderate levels in the large intestine, fat and testis, and low levels in kidney, muscle and bladder.

Polymorphism identification, genetic variation identification in different breeds and association analysis

The direct sequencing of several parts of bovine *UQCC* genome allowed for the detection of potential polymorphisms by cloning of these PCR products into the T-easy vector; several polymorphisms were detected by this method (data not shown). Among them, one SNP 2691 (A/T) in intron 1 (12610 bp) and one SNP 3150 (A/G) in intron 8 (3226 bp) were chosen for further analysis by using the restriction fragment length polymorphism (RFLP) method (Fig. 4). The SNP 2691 (A/T) in exon 1 creates a site for the restriction enzyme Dra I (TTT^AAA). 288 bp PCR product amplified with primer pair (SNP1-F, SNP1-R, Table 1) was digested according to the manufacturer's instructions. For the A2691T SNP in the populations, we analyzed the localization of migration bands of the restriction fragments and found 3 genotypes of "mutation A > T" (Fig. 4A). The genotype AA represented the occurrence of one band of 288 bp, genotype AT represented 3 restriction fragment bands of 288, 243 and 45 bp, and genotype TT represented two bands of 243 and 45 bp (Fig. 4B). Moreover, The SNP 3150(A/G) in exon 8 creates a site for the restriction enzyme Bsh1236 I (CG^CG). Subsequently, 976 bp PCR product amplified with primer pair (SNP8-F, SNP8-R, Table 1) was digested according to the manufacturer's instructions. For the A3150G SNP in the populations, we analyzed the localization of migration bands of the restriction fragments and found 3 genotypes of "mutation A >

Table 3. Genotypes frequencies (%) at the *UQCC* gene for the A2691T SNP in bovine populations

Breeds	Observed genotypes (number)			Total	Allelic frequencies		χ^2 (HW*)	*P* value (HW*)
	AA	AT	TT		A	T		
QC	0.2476(26)	0.5333(56)	0.2190(23)	105	0.5143	0.4857	0.48	0.7870
QI	0.2336(32)	0.3869(53)	0.3796(52)	137	0.4270	0.5730	6.01	0.0496
NY	0.0588(3)	0.2941(15)	0.6471(33)	51	0.2059	0.7941	0.52	0.7728
JR	0.2933(22)	0.4533(34)	0.2533(19)	75	0.5200	0.4800	0.63	0.7286
XN	0.1714(12)	0.3857(27)	0.4429(31)	70	0.3643	0.6357	1.96	0.3758
LX	0.0909(6)	0.8333(55)	0.0758(5)	66	0.5076	0.4924	29.37	0.0000
SL	0.2037(11)	0.5926(32)	0.2037(11)	54	0.5000	0.5000	1.85	0.3962
XL	0.5455(66)	0.4380(53)	0.0165(2)	121	0.7645	0.2355	5.66	0.0590
Total	0.2622(178)	0.4786(325)	0.2592(176)	679	0.5015	0.4985	1.24	0.5385

HW, Hardy-Weinberg equilibrium

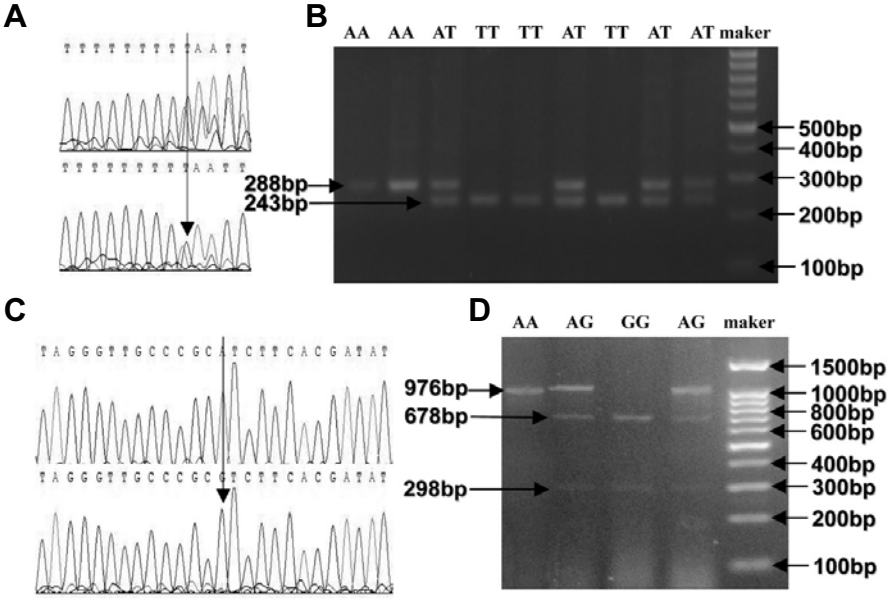

Fig. 4. PCR-RFLP genotyping information. (A) DNA sequencing maps from mixed DNA template in both directions at bovine *UQCC* A2691T locus. (B) 2.5% agarose gel electrophoresis showing the 3 different DraI PCR-RFLP genotypes of bovine *UQCC* A2691T SNP. AA genotype demonstrates one fragment (288bp); AT genotype shows 3 fragments (288, 243, and 45 bp) and TT genotype shows 2 fragments (288 and 45 bp). Although 45 bp fragment is not long enough to be visible in 2.5% agarose gel electrophoresis, 288 and 243 bp fragments can exactly classify the different genotypes. (C) Sequencing maps from several DNA templates at bovine *UQCC* A3150G locus. (D) 2.0% agarose gel electrophoresis showing the 3 different Bsh1236I PCR-RFLP genotypes of bovine *UQCC* A3150G SNP. The genotypes (AA AG GG) are shown at the top.

G" (Fig. 4C). The genotype AA represented the occurrence of one band of 976 bp, genotype AG represented 3 restriction fragment bands of 976, 678 and 298 bp, and genotype GG represented two bands of 678 and 298 bp (Fig. 4D).

Moreover, we further analyzed the genotype of unrelated animals from 8 different bovine breeds, including (Qinchuan, Qinchuan improvement steers, Nanyang, Jiaxian red, Xia'nan, Luxi, Simmental and Luxi crossbred steers and Xuelong). Genotypic frequencies, allelic frequencies and Hardy-Weinberg equilibriums of the SNPs in different populations were shown (see Tables 3 and 4), respectively. The data demonstrate that the range of frequencies of A allele of A2691T and A3150G alleles were from 0.2059 to 0.7645 and 0.3788 to 0.7521 among 8 different subpopulations, respectively. Furthermore, frequencies of A2691T and A3150G alleles were 0.5015/0.4985 and 0.6171/0.3829 in total population, respectively. Except for the QI and LX subpopulations at A2691T SNP, all other subpopulations at the 2 SNPs were in Hardy-Weinberg equilibrium ($p > 0.05$). Genotypic frequencies of combination of the SNPs (Table 5) show that genotypic frequencies were 0.1281, 0.1134, 0.0221, 0.1826, 0.2342, 0.0604, 0.0677, 0.1296 and 0.0619; the range of genotypic frequencies were from 0 to 0.3306, 0.0392 to 0.1818, 0 to 0.0413, 0.0588 to 0.333, 0.1570 to 0.4394, 0.0146 to 0.3030, 0 to 0.1961, 0.0083 to 0.3137 and 0.0083 to 0.1429 among 8 different subpopulations at AAAA, AAAG, AAGG, ATAA, ATAG, ATGG, TTAA, TTAG and TTGG genotypes, respectively. The data (see Tables 6 and 7) revealed that the following items, gene heterozygosity, effective allele numbers, polymorphism information (PIC) content of A2691T SNP and A3150G SNP in 8 different subpopulations, varied from 0.3270 to 0.5000 and 0.3729 to 0.4974, 1.4859 to 2.000 and 1.5947 and 1.9898, 0.2735 to 0.3750 and 0.3034 to 0.3737, respectively. Haplotype analysis showed frequencies of 4 haplotypes AA, AG, TA and TG were 0.358, 0.144, 0.259 and 0.239 by SHEsis software, respectively (data not shown). The linkage disequilibrium values D′ and r^2 obtained by SHEsis software between T1532C SNP and C1899T SNP in the population were 0.276 and 0.044, respectively (data not shown).

Furthermore, 8 body measurement traits were analyzed by comparison of the genotypes of 679 individuals and their phenotypic data. The results of association analysis of the gene-

Table 4. Genotype frequencies (%) at the *UQCC* gene for the A3150G SNP in bovine populations

Breeds	Observed genotypes (number)			Total	Allelic frequencies		χ^2 (HW*)	*P* value (HW*)
	AA	AG	GG		A	G		
QC	0.4476(47)	0.4381(46)	0.1143(12)	105	0.6667	0.3333	0.02	0.9893
QI	0.4015(55)	0.5182(71)	0.0803(11)	137	0.6606	0.3394	3.32	0.1900
NY	0.2549(13)	0.5686(29)	0.1765(9)	51	0.5392	0.4608	1.06	0.5881
JR	0.3200(24)	0.5333(40)	0.1467(11)	75	0.5867	0.4133	0.75	0.6888
XN	0.1857(13)	0.5571(39)	0.2571(18)	70	0.4643	0.5357	1.01	0.6041
LX	0.1061(7)	0.5455(36)	0.3485(23)	66	0.3788	0.6212	1.67	0.4341
SL	0.5185(28)	0.3889(21)	0.0926(5)	54	0.7130	0.2870	0.13	0.9351
XL	0.5785(70)	0.3471(42)	0.0744(9)	121	0.7521	0.2479	0.58	0.7483
Total	0.3785(257)	0.4772(324)	0.1443(98)	679	0.6171	0.3829	0.06	0.9685

HW, Hardy-Weinberg equilibrium

Table 5. Genotype frequencies (%) at the *UQCC* gene for the A2691T and A3150G SNP in bovine populations

Breeds	Observed genotypes (number)									Total
	AAAA	AAAG	AAGG	ATAA	ATAG	ATGG	TTAA	TTAG	TTGG	
QC	0.1238(13)	0.0952(10)	0.0286(3)	0.2381(25)	0.2762(29)	0.0190(2)	0.0857(9)	0.0667(7)	0.0667(7)	105
QI	0.0949(13)	0.1168(16)	0.0219(3)	0.1606(22)	0.2117(29)	0.0146(2)	0.1460(20)	0.1898(26)	0.0438(6)	137
NY	0.0000(0)	0.0392(2)	0.0196(1)	0.0588(3)	0.2157(11)	0.0196(1)	0.1961(10)	0.3137(16)	0.1373(7)	51
JR	0.1467(11)	0.1333(10)	0.0133(1)	0.1467(11)	0.2667(20)	0.0400(3)	0.0267(2)	0.1333(10)	0.0933(7)	75
XN	0.0571(4)	0.1000(7)	0.0143(1)	0.1286(9)	0.1714(12)	0.1000(7)	0.0000(0)	0.2857(20)	0.1429(10)	70
LX	0.0152(1)	0.0606(4)	0.0152(1)	0.0909(6)	0.4394(29)	0.3030(20)	0.0000(0)	0.0455(3)	0.0303(2)	66
SL	0.0926(5)	0.1111(6)	0.0000(0)	0.3333(18)	0.2037(11)	0.0556(3)	0.0926(5)	0.0741(4)	0.0370(2)	54
XL	0.3306(40)	0.1818(22)	0.0413(5)	0.2479(30)	0.1570(19)	0.0248(3)	0.0000(0)	0.0083(1)	0.0083(1)	121
Total	0.1281(87)	0.1134(77)	0.0221(15)	0.1826(124)	0.2342(159)	0.0604(41)	0.0677(46)	0.1296(88)	0.0619(42)	679

Table 6. Population genetic indexes at the *UQCC* A2691T locus in bovine populations

Breeds	Gene homozygosity	Gene heterozygosity	Effective allele numbers	PIC
QC	0.5004	0.4996	1.9984	0.3748
QI	0.5107	0.4893	1.9583	0.3696
NY	0.6730	0.3270	1.4859	0.2735
JR	0.5008	0.4992	1.9968	0.3746
XN	0.5368	0.4632	1.8628	0.3559
LX	0.5001	0.4999	1.9995	0.3749
SL	0.5000	0.5000	2.0000	0.3750
XL	0.6399	0.3601	1.5628	0.2953
Total	0.5000	0.5000	2.0000	0.3750

Table 7. Population genetic indexes at the *UQCC* A3150G locus in bovine populations

Breeds	Gene homozygosity	Gene heterozygosity	Effective allele numbers	PIC
QC	0.5556	0.4444	1.8000	0.3457
QI	0.5516	0.4484	1.8130	0.3479
NY	0.5031	0.4969	1.9878	0.3735
JR	0.5150	0.4850	1.9471	0.3674
XN	0.5026	0.4974	1.9898	0.3737
LX	0.5294	0.4706	1.8890	0.3599
SL	0.5907	0.4093	1.6929	0.3255
XL	0.6271	0.3729	1.5947	0.3034
Total	0.5274	0.4726	1.8960	0.3609

specific SNPs markers were shown (see Table 8). At the A2691T SNP marker, there are significant effects on the RL ($p = 0.0001$), CD ($p = 0.0059$) and PBW ($p < 0.0001$) in the total population. Animals with the genotype AA and AT had higher mean values for RL than those with the TT genotype ($p < 0.01$), individuals with the genotype AA had significantly higher means values for CD than those with the TT genotype ($p < 0.05$), ones with the genotype AT had significantly higher means values for PBW than those with the TT genotype ($p < 0.01$), and ones with the genotype AA and AT had significantly lower means values for PBW than those with the TT genotype ($p < 0.01$). Meanwhile, at the A3150G SNP marker, there were significant effects on BL ($p = 0.0047$) and CD ($p = 0.0454$) in the total population. Animals with the genotype AA and AG had significantly lower mean values for BL than those with the GG genotype ($p < 0.01$), and ones with the genotype AA and AG had significantly lower means values for CD than those with the GG genotype ($p < 0.05$). We also carried out association analysis of the combination of A2691T and A3150G SNP genotypes with body measurement traits at bovine UQCC gene (Table 9). There were

Table 8. Associations analysis of A2691T and A3150G SNP genotypes with body measurement traits at bovine *UQCC* gene

SNP	Genotypes	Traits (cm, Mean ± SE)							
		BL	WH	HH	RL	HW	CD	HG	PBW
A2691T	AA	149.78 ± 1.04	138.87 ± 0.79	151.35 ± 9.43	45.17 ± 0.34^{A}	49.67 ± 0.56	73.16 ± 0.59^{a}	210.23 ± 13.83	25.23 ± 0.38^{A}
	AT	150.78 ± 0.74	150.17 ± 5.03	147.99 ± 0.55	44.94 ± 0.33^{A}	49.35 ± 0.38	73.47 ± 0.44^{A}	194.71 ± 1.42	25.12 ± 0.30^{A}
	TT	152.06 ± 1.07	146.09 ± 0.77	148.41 ± 0.72	42.99 ± 0.45^{B}	48.49 ± 0.52	71.23 ± 0.63^{B}	199.70 ± 1.66	27.18 ± 0.41^{B}
	P value	0.2010	0.1517	0.8608	0.0001	0.2407	0.0059	0.2595	0.0000
A3150G	AA	149.81 ± 0.92^{A}	139.84 ± 0.67	150.16 ± 6.11	44.68 ± 0.31	49.17 ± 0.47	72.65 ± 0.50^{a}	204.27 ± 9.02	25.19 ± 0.31
	AG	150.63 ± 0.67^{A}	149.81 ± 5.22	148.06 ± 0.53	44.78 ± 0.33	49.44 ± 0.37	73.13 ± 0.45^{a}	198.49 ± 1.37	25.66 ± 0.30
	GG	154.51 ± 1.49^{B}	148.34 ± 1.01	152.38 ± 1.13	45.28 ± 0.64	49.79 ± 0.68	75.00 ± 0.81^{b}	199.36 ± 2.94	25.04 ± 0.64
	P value	0.0047	0.1338	0.8516	0.6693	0.7268	0.0454	0.7742	0.4140

^{a,b}means with different superscripts were significantly different (P < 0.05).
$^{A, B}$means with different superscripts were significantly different (P < 0.01).

Table 9. Associations analysis of combination of A2691T and A3150G SNP genotypes with body measurement traits at bovine *UQCC* gene

Genotypes	BL	WH	HH	RL	HW	CD	HG	PBW
AAAA	147.29 ± 1.62^{ab}	128.34 ± 1.23	148.59 ± 15.40	45.88 ± 0.44	47.28 ± 0.81	70.12 ± 0.82^{a}	207.83 ± 22.57	24.92 ± 0.50^{a}
AAAG	144.30 ± 1.39^{a}	135.51 ± 1.15	139.11 ± 1.08	45.41 ± 0.48	47.47 ± 0.72	69.92 ± 0.85^{abc}	188.18 ± 2.90	24.85 ± 0.54^{a}
AAGG	149.52 ± 2.82^{ab}	143.11 ± 2.05	146.63 ± 2.21	46.86 ± 1.27	48.70 ± 1.36	72.52 ± 1.47^{a}	190.94 ± 6.89	24.33 ± 1.37^{a}
ATAA	147.46 ± 1.17^{b}	137.22 ± 0.90	141.03 ± 0.82	45.61 ± 0.44	47.65 ± 0.58	70.53 ± 0.62^{a}	186.37 ± 2.19	25.55 ± 0.56^{abc}
ATAG	147.85 ± 0.93^{b}	147.16 ± 7.85	142.20 ± 0.73	45.70 ± 0.45	47.30 ± 0.45	71.05 ± 0.56^{ac}	186.94 ± 1.62	24.96 ± 0.35^{a}
ATGG	148.31 ± 2.11^{b}	140.04 ± 1.27	145.21 ± 1.05	45.93 ± 0.73	46.71 ± 0.93	71.49 ± 0.99^{a}	184.08 ± 3.22	23.72 ± 0.58^{ad}
TTAA	144.55 ± 2.08^{a}	137.32 ± 1.51	139.82 ± 1.35	43.58 ± 0.72	45.15 ± 0.90	67.61 ± 1.13^{b}	186.49 ± 2.48	25.65 ± 0.69^{abc}
TTAG	149.65 ± 1.36^{b}	139.3 ± 0.99	141.76 ± 0.86	44.83 ± 0.55	47.05 ± 0.69	69.34 ± 0.75^{ab}	192.52 ± 2.29	26.64 ± 0.50^{b}
TTGG	149.95 ± 2.08^{b}	140.73 ± 1.52	144.57 ± 1.42	44.68 ± 0.83	46.75 ± 0.81	71.01 ± 0.99^{a}	187.28 ± 2.84	25.66 ± 0.79^{abc}
P value	0.0215	0.3072	0.9791	0.0943	0.3226	0.0282	0.7085	0.0329

^{a,b,c,d}means with different superscripts were significantly different (P < 0.05).

significant effects on the BL (p = 0.0215), CD (p = 0.0282) and PBW (p = 0.0329) in the total population. Animals with the genotype AAAG and TTAA had significantly lower mean values for BL than those with the genotype ATAA, ATAG, ATGG, TTAG and TTGG (p < 0.05), and ones with the genotype AAAA, AAGG, ATAA, ATAG, ATGG and TTGG had significantly higher means values for CD than those with the TTAA genotype (p < 0.05), as well as ones with the genotype AAAA, AAAG, AAGG, ATAG, ATAG, ATGG and TTGG had significantly lower means values for PBW than those with the TTAG genotype (p < 0.05). On the whole, animals with combined genotypes TTAA had significantly lower body length and significantly higher chest depth, while animals with combined genotypes TTAG had significantly higher pin bone width.

DISCUSSION

Our results provide the complete fundamental structure of the bovine *UQCC* CDS domain. Comparison with the genome structure of human *UQCC* demonstrates a remarkably high similarity between the two species. Namely, human and bovine *UQCC* contained 10 exons and 9 introns, and with the exception of the third exon, all the other exons share the same size. The CDS region and the deduced amino acid sequence of bovine *UQCC* share 91 and 89% identity to that of human, respectively. Phylogenetic trees analysis also indicated *Bos Taurus, Homo sapiens* and *Pongo abelii UQCC* protein sequences share higher homology than other species. The regulation of *UQCC* activity involves in several mechanisms, including gene expression, proenzyme activation, and inhibition of enzyme by tissue inhibitor. In the bovine sequence, these sites are identical to those in the human sequence in our study, and may therefore play a similar regulatory role in bovines as in humans.

RT-PCR analysis was employed to determine the relative mRNA expression of UQCC in various cattle tissues. The date in Fig. 3 show that the tissue expression patterns in bovine UQCC are ranked in ascended order: spleen, heart, trachea, large intestine, fat, testis, kidney, muscle and bladder. We compared our data with the human UQCC and mouse UQCC microarray expression data shown in Gene Sorter (http://genome.ucsc.edu/). Although UQCC gene expression patterns in bovine are different to that in human, they are consistent to the expression of the corresponding gene in mouse. Functionally, this gene encodes a transmembrane protein that is structurally similar to the mouse basic fibroblast growth factor repressed ZIC-binding protein. Therefore, this protein may be involved in fibroblast growth factor regulated growth control in mouse. Together, these results indicate the expression of UQCC in the bovine's different organs could play an important role on the regulation of growth and development. The further studies of this gene function will be investigated in the future.

If $r^2 > 0.33$, the linkage disequilibrium was considered strong (Ardlie et al., 2002). Linkage disequilibrium between the 2 SNPs in the population estimated that they were less linked. Thus, this region cannot be inherited as a unit. We also ana-

lyzed the 2 separated SNPs and the combination effects of the 2 SNPs. The frequencies of 2 alleles of each of SNPs were very approximate. Moreover, the gene heterozygosity and effective allele numbers were also medius. These results clearly indicates that the heredity of these population and the sites controlling body measurement traits were all relatively stable. Furthermore, the occurrence in QI and LX populations were not in Hardy-Weinberg equilibrium due to gene random drift. Generally, PIC was classified into the following 3 types: low polymorphism (PIC value < 0.25), median polymorphism (0.25 < PIC value < 0.5), and high polymorphism (PIC value > 0.5) (Mateescu et al., 2005). According to this classification of PIC, all the population at the 2 SNPs belonged to the median polymorphism level. Therefore, our data indicated that the high frequency of A2691T and A3150G alleles at the bovine *UQCC* locus could be used to characterize cattle breeds.

The body measurement traits are affected by many factors, such as genotype, sex, age, breed, herd, location and other random environment factors. But, we have established the new statistical model in which the three factors (breed, herd, and location) were involved and then we have employed the least-squares method in GLM procedure of SAS software to do the related analysis. However, we did not find the significant difference ($p > 0.05$) (data not shown).

For the A2691T SNP marker in the intron 1 at bovine *UQCC* gene, there are significant effects on the RL, CD and PBW (Table 8); for the A3150G SNP marker in the intron 8 at bovine UQCC gene, there are significant effects on the BL and CD in 679 individuals (Table 8). Moreover, for combination of the 2 SNPs marker in bovine *UQCC* gene, there are significant effects on the BL, CD and PBW in 679 individuals (Table 9). A number of studies have recently demonstrated that the functional SNPs in *UQCC* gene have a plausible biological role in height and other stature indexes in human (Gudbjartsson et al., 2008; Lettre et al., 2008; Sanna et al., 2008; Soranzo et al., 2009; Sovio et al., 2009; Weedon et al., 2008). Using data from GWA studies, Sanna et al. first identified a common variants at the *UQCC* locus (rs6060369, $p = 1.9 \times 10^{-10}$), which robustly associated with stature in adult humans not selected for tall or short stature. The *UQCC* effects were analyzed using large datasets (6669 individuals) to identify stature variance; some of the SNPs reported here fall in or near strong candidate height genes, such as the recently described associations with *HMGA2* and *UQCC*, whereas others identify previously unsuspected loci (Lettre et al., 2008). Together, these associations highlight the biological pathways that are important in regulating human growth (Lettre et al., 2008). A SNP (rs6060373) in *UQCC* gene showed an association ($p < 0.05$) with peak height velocity in infancy (PHV1), and was also associated with adult height in their study (Sovio et al., 2009). A study also confirmed *UQCC* (rs6088813, $p = 9.8 \times 10^{-14}$) as the loci with the strongest overall association with height, a SNP significantly associated with hip axis length on the strongest statistical associations at *UQCC* (rs4911494, $p = 1.9 \times 10^{-4}$) (Soranzo et al., 2009). Above all, although many studies focus on association of *UQCC* gene variants with body measurement traits in human, none have been reported for bovine and other livestock. Therefore, based on these results of the genome-wide approach in human and according to the conformity of the conservation of biological evolution in different organism, we applied the research results from human *UQCC* in analyzing polymorphism and genetic effect in cattle *UQCC* gene locus. The new findings is that the A2691T and A3150G SNPs of *bovine UQCC* were significantly associated with Body length, Rump length, Chest depth and Pin bone width (Tables 8 and 9), which are consistent with the human data.

In summary, we obtained the genomic sequence contained the entire coding exons and introns sequence of bovine *UQCC* gene and uncovered its genomic structure. We also analyzed its tissue expression differences by RT-PCR in cattle. Genotyping analysis and association analysis performed on the A2691T SNP Dra I and A3150G SNP Bsh1236 I, demonstrated that these SNPs were significantly associated with body measurement traits in bovine. The associations may serve as genetic markers for molecular-assisted selection (MAS) during animal breeding for growth and development; however, further analysis is needed to validate our results within other breeds and larger populations.

ACKNOWLEDGMENTS

We thank Dr. Baoli Hu of Dana-Farber Cancer Institute, Boston MA, USA, for critically reading and discussing this manuscript. This work was supported by the China National “863” Program (2006AA10Z1A1), the National Eleventh “Five-Year” Science and Technology Support Project (2006BAD01A10-3), the “13115” Scientific and Technological Innovation Program of Shaanxi Province (2007ZDCY-01) and National 973 Program (2006CB102105). Moreover, the bovine populations were supported by Excellent research team of livestock gene resource and breed improvement of Chinese academy of agricultural sciences, Qinchuan beef cattle breeding center of Shaanxi province, Nanyang, Jiaxian and Xianan cattle breeding center of Henan province, Luxi cattle breeding center of Shandong province, and Snow dragon beef cattle breeding center of Liaoning province (People’s Republic of China).

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