Detection of genetic association and functional polymorphisms of UGDH affecting milk production trait in Chinese Holstein cattle

Qing Xu²†, Gui Mei³†, Dongxiao Sun¹*, Qin Zhang¹, Yuan Zhang¹, Cengceng Yin¹, Huiyong Chen¹, Xiangdong Ding¹ and Jianfeng Liu¹

Abstract

Background: We previously localized a quantitative trait locus (QTL) on bovine chromosome 6 affecting milk production traits to a 1.5-Mb region between BMS483 and MNB-209 via genome scanning followed by fine mapping.

Results: Totally 15 genes were mapped within such linkage region through bioinformatic analysis of the cattle-human comparative map and bovine genome assembly. Of them, the UDP-glucose dehydrogenase (UGDH) was suggested as a potential positional candidate gene for milk production traits based on its corresponding physiological and biochemical functions and genetic effects. By sequencing all the coding exons and the untranslated regions in UGDH with pooled DNA of 8 sires represented the separated families detected in our previous studies, a total of ten SNPs were identified and genotyped in 1417 Holstein cows of 8 separation families. Individual SNP-based association analysis revealed 4 significant associations of SNP Ex1-1, SNP Int3-1, SNP Int5-1, and SNP Ex12-3 with milk yield (P < 0.05), and 2 significant associations of SNP Ex1-1 and SNP Ex12-3 with protein yield (P < 0.05). Furthermore, our haplotype-based association analyses indicated that haplotypes G-C-C, formed by SNP Ex12-2-SNP Int11-1-SNP Ex11-1, T-G, formed by SNP Int9-3-SNP Int9-2, and C-C, formed by SNP Int5-1-SNP Int3-1, are significantly associated with protein percentage (F=4.15; P=0.0418) and fat percentage (F=5.18~7.25; P=0.0072~0.0231). Finally, by using an in vitro expression assay, we demonstrated that the A allele of SNP Ex1-1 and T allele of SNP Ex11-1 of UGDH significantly decreases the expression of UGDH by 68.0% at the RNA, and 50.1% at the protein level, suggesting that SNP Ex1-1 and Ex11-1 represent two functional polymorphisms affecting expression of UGDH and may partly contributed to the observed association of the gene with milk production traits in our samples.

Conclusions: Taken together, our findings strongly indicate that UGDH gene could be involved in genetic variation underlying the QTL for milk production traits.

Keywords: Dairy cattle, BTA6, Positional candidate, Milk traits, UGDH, Function validation

* Correspondence: sundx@cau.edu.cn
† Equal contributors
1 Department of Animal Genetics and Breeding, College of Animal Science and Technology, Key Laboratory of Animal Genetics and Breeding of Ministry of Agriculture, National Engineering Laboratory for Animal Breeding, China Agricultural University, Beijing 100193, China
2 Full list of author information is available at the end of the article

© 2012 Xu et al; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Background

Both theoretical and simulation studies agree that application of gene-assisted selection has the potential to increase the rate of genetic gain by pre-selecting young candidate bulls prior to progeny testing in dairy cattle [1]. This kind of selection is based on the identification of genes that may affect the traits of interest. In dairy cattle, since the first report on quantitative trait locus (QTL) mapping by Georges et al. [2], extensive QTL mapping has been implemented to detect QTLs with major effect on the milk production traits. So far, a total number of 1,651 QTLs for milk yield and composition traits have been reported via genome scan based on marker-QTL linkage analysis (http://www.animalgenome.org/QTLdb/cattle.html, April 29, 2012). With the completion of genome sequencing of cattle and development of comparative map of human/bovine, it is feasible to identify the genes or other functional elements, which are responsible for a quantitative trait with comparative candidate positional cloning strategy [3]. In recent years, based on fine mapping results and positional cloning tools, several positional candidate SNPs have been confirmed to be true QTL, including the AA to GC dinucleotide substitution (K232A) in the exon 8 of GHR confirmed to be true QTL, including the AA to GC tools, several positional candidate SNPs have been required for this study.

Animals and phenotypic data

A total of 1417 Chinese Holstein cows were selected from 8 sire families with 67–540 daughters in each family. Such 8 sires represent all the segregating families detected in our previous QTL mapping studies [8,9,14]. Estimated breeding values (EBV) for five milk production traits (i.e., milk yield, fat yield, protein yield, fat percentage, and protein percentage over 305 days) were provided by the Dairy Data Processing Center, Dairy Association of China (DAC) which was calculated with a random regression multiple traits test-day model based on 6,980,000 test-day records of 585,121 Holstein cows collected from 1993 to 2008 in China.

Genomic DNA was extracted from whole blood samples of cows and frozen semen of the 8 bulls by a standard phenol-chloroform method and stored at −20°C.

Positional candidate cloning, SNP discovery and genotyping

Within the 1.5-Mb region between microsatellites BMS483 and MNB-209 on BTA 6 where harbored a QTL for milk production traits [8,9,14], positional candidate cloning was implemented to mine the known functional genes by bioinformatic analysis of a high-resolution whole-genome cattle-human comparative map [15-17] and the bovine genome assembly Btau 4.0 (http://www.ncbi.nlm.nih.gov), a total of 15 known genes have been mapped within such narrow linkage region. Out of them, we herein focused on UGDH as the most plausible comparative functional candidate gene affecting milk production traits. The enzyme UGDH can convert UDP-glucose to UDP-glucuronic acid, a critical component of the glycosaminoglycans, hyaluronan, chondroitin sulfate, and heparan sulfate, thus promotes normal cellular growth, embryogenesis and adult organism physiology [18,19]. UGDH is also implicated in the progression of epithelial cancers such as colon, breast, and prostate [20-22]. The purpose of the study was to determine the genetic effect of the UGDH gene on milk yield and component traits in dairy cows and regulation by functional polymorphisms.
Twelve pairs of primers for amplification of all the 12 exons and partial introns of UGDH were presented in Table 1. Pooled DNA from the 8 bulls was amplified at each exon followed by directly sequencing the PCR products to search SNPs. DNAMAN 6.0 (Lynnon Biosoft, USA) and Chromas 2.0 (Technelysium, Australia) were run for alignment between sequences of the 8 bulls and the reference sequences in NCBI. Individual genotyping of the discovered SNPs was performed for all of the 1417 cows with SNPlex assay (ABI, USA) or TaqMan probe (ABI, USA). For the 8 bulls, SNP genotyping was performed by direct sequencing.

**Association analysis**

**Single SNP analysis**

Associations between individual SNP and the five milk production traits were estimated using the MIXED procedure of the SAS 8.02 software based on the model

\[ y = \mu + G + a + e \]

where \( y \) is the EBV of cows, \( \mu \) is the general mean, \( G \) is the SNP genotype effect, \( a \) is the random polygenic effect with distribution of \( N(0, \sigma_a^2) \) (\( A \) is the additive genetic relationship matrix among all individuals, \( \sigma_a^2 \) is the additive genetic variance), and \( e \) is a random residual with distribution of \( N(0, W \sigma_e^2) \) (\( W \) is a diagonal matrix with the diagonal elements equal to \( 1/REL_{ij} \), \( REL_{ij} \) is the reliability of the EBV of daughter j in family i, and \( \sigma_e^2 \) is the residual error variance).

**Haplotype analysis**

Pair-wise linkage disequilibrium (LD) between all SNP pairs and haplotype blocks were estimated using the program Haploview4.0 [23]. Then, haplotype reconstruction of each cow was carried out within the blocks via expectation maximization (EM) algorithm [24]. Association between each haplotype and the five milk production traits was performed based on the model

\[ y_{ij} = \mu + s_i + bh_{ij} + e_{ij} \]

where \( s_i \) is the fixed effect of sire \( i \), \( b \) is the regression coefficient, \( h_{ij} \) is an indicator variable with a value 0, 1 or 2 to indicate the copy number of the haplotype carried by the individual.

Both analyses (e.g. Single SNP analysis and Haplotype analysis) were corrected for multiple testing using false discndocvery rate (FDR) methods. We declared a significant SNP or haplotype if the corresponding FDR value < 0.05.

**cDNA synthesis, plasmid construction and mutagenesis**

On the basis of association analysis results and the nucleotide location of each SNP within the gene, we investigated three SNPs with two located in exon 1 (SNPs exon1-1 and exon1-2), one in exon 11 (SNP exon11-1). The complete coding region of UGDH was synthesized based on the sequence of cattle full-length cDNA at NCBI (GenBank Accession No. NM: 174211). To make cloning step easier, we added a restriction endonuclease enzyme digestion site to the 5’ and 3’ end of the synthesized gene, respectively. The synthesized DNA fragment was double-digested with restriction enzymes BamH I and Xho I and then cloned into the digested pcDNA3.1/myc-HisA expression vector (Clontech, Mountain View, CA) with the same enzymes; this yielded the first plasmid construct, named as pcDNA3-GAC (g-a-c). The other seven plasmid constructs (i.e., a-a-c, g-c-c, g-a-t, a-c-c, g-c-t, a-a-t, and a-c-t) were generated from the g-a-c plasmid using a site-directed mutagenesis kit purchased from Stratagene Inc. (La Jolla, CA). All eight plasmid constructs were verified by automated DNA sequencing in both directions.

**Cell culture and transfection**

COS-7 cells (an African Green Monkey SV40-transf’d kidney fibroblast cell line) were purchased from the American Tissue Cell Culture Inc. (Manassas, VA), and

### Table 1 PCR primers for amplification and identification of single nucleotide polymorphisms in UGDH

| Amplicon number | Amplicon content | Forward primer sequence | Reverse primer sequence | Amplicon size (bp) |
|-----------------|------------------|-------------------------|-------------------------|-------------------|
| 1               | Exon 1           | GAA TGA ATC CTC CGC CT  | TTCCCTG TGG TGA TGT CGC| 437               |
| 2               | Exon 2           | CTTCCCTATACCTGGTACC     | ACTGCTCATCTCAGTCAA     | 508               |
| 3               | Exon 3           | GTTCAGTTGACCTGGCCTGTC   | CTAAGTGGCTCAAGTGGCTC   | 443               |
| 4               | Exon 4           | GGG CTA CTG ATG ATG TT  | CTC TGT GTG GTT TCT CT | 494               |
| 5               | Exon 5           | AGC AGA AAG TAT TCG TCG| CAG CTG AGC AGC AGG TaG| 450               |
| 6               | Exon 6           | ACC AAC CCA CCC ATT GCC T| CCG ACA CCA CTG AGC TAC T | 669               |
| 7               | Exon 7           | AGT TGA TTA GTT TCT TGC| TAA TTA TTG AGT TGT CCG| 744               |
| 8               | Exon 8           | TTT GGA GAA GTT GGC TAA GG| TGC TAT GCT TCT GAA TGG C | 707               |
| 9               | Exon 9           | CGT TCA TAT CCT CAC AAG A| TGG GGA AAT CCG TAC AG A | 522               |
| 10              | Exon 10          | TTC AAC CAG ACC ATT CCA CT| TGC GTT CAT AAT CCA GTT CC | 339               |
| 11              | Exon 11          | TAA GGT GAA GTG ACG GAA GC| GGA GCA ACT AAG CCT GTG TG | 473               |
| 12              | Exon 12          | GGG AATGTA GAC TGA TCT GCT ACT CAT CCA ATC ACT | 1801               |
cultured in RPMI-1640 supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) at 37°C in 5% CO₂. The culture medium was changed every other day. The cells were transfected by Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. Briefly, a day prior to transfection, the cells were trypsinized and plated at 4 × 10⁴ cells per well on a 48-well plate. Subsequently, 0.4 μg of plasmid DNA containing the appropriate structure was diluted in 20 μl of Opti-MEM I (Invitrogen, Carlsbad, CA) Reduced Serum Medium and mixed with an equal volume of Opti-MEM I Reduced Serum Medium containing 1 μl of Lipofectamine 2000. The mixture was incubated for 20 min at room temperature and added directly to the cells in a 48-well plate. The cells were cultured for an additional 48 hours prior to harvesting for extraction of RNA and protein for real-time RT-PCR and Western blotting, respectively.

RNA isolation and real-time quantitative RT-PCR
To evaluate the regulatory effect of SNPs exon1-1 (G/A), exon1-2 (A/C) and exon11-1 (C/T) on the expression of UGDH, eight plasmids were constructed corresponding to different allele combinations of exon1-1 (G/A), exon1-2 (A/C) or exon11-1 (C/T). Total RNA from cell transfected by eight plasmids respectively and control cells was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA). One microgram of total RNA was reverse transcribed in a final volume of 20 ul containing 4 ul of 5 first-strand buffer (250 mM Tris–HCl, pH 8.3; 375 mM KCl; 15 mM MgCl₂), 10 mM DTT, 0.5 mM each dNTP, 40 U RNaseOUT™, 1 ul of 50 nM random hexamers, and 200 U of Superscript II RNase H− reverse transcriptase (Invitrogen). The TaqMan® specific probes and primers used for real-time PCR were designed according to the above synthesized UGDH fragment and myc sequences in pcDNA3.1/myc-HisA expression vector were: forward primer 5'-CTCCTTCTGTTGAAATTTCAAGT-3', reverse primer 5'-TCTAGACTCGAGTACTCTGGGTTCCTT-3', and the probe 5'-AGTCCTTCAGGATATGTCCCAA-3'. Amplification of 2 ul of cDNA was carried out in a total volume of 20 ul according to the manual of TaqMan® Gene Expression Assays (Applied Biosystems). The mRNA level of each gene was determined using a calibration method [25] and normalized to that of 18S rRNA in each sample. In addition, the RNA of transfected cells was treated with RNA-free DNase I (Ambion Inc, Foster City, CA) to remove any potential remaining plasmid DNA from the RNA sample prior to reverse transcription. All the primers and probe were synthesized by Applied Biosystems Inc. (Foster, CA).

Western blot
Total protein was extracted from individual transfected cell from an independent experiment by homogenization in RIPA buffer (50 mM Tris–HCl, pH 8.0; 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, and 0.1% SDS), and the protein concentration was determined by the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA). Ten micrograms of total protein was separated by 10% SDS-PAGE followed by transfer to nitrocellulose membranes (0.45 um) at 25V overnight at 4°C. The membrane was first incubated in a blocking buffer (5% nonfat milk and 0.2% Tween 20) for 1.5h at room temperature and then 1.5h at room temperature in the blocking buffer containing cattle anti-His antibody (dilution 1:5000; Abcam Inc, Cambridge, MA). After three washes in TBST (10 mM Tris–HCl, pH 8.0; 0.15 M NaCl; 0.2% Tween 20) for 10 min each, the membranes were exposed to horseradish peroxidase-conjugated secondary antibody at room temperature for 1.5h, and then exposed to X-ray film. After hybridization with the antibody of interest, membranes were stripped and re-probed with antibody to β-Actin (dilution 1:5000; Abcam Inc, Cambridge, MA), which was used for normalization of the protein content of each sample. Then, the films were scanned for quantitative analysis with ImageQuant 5.2 (Molecular Dynamics, Sunnyvale, CA).

Results
Positional candidate cloning
With bioinformatic analysis of whole-genome cattle-human comparative map and the bovine genome assembly Btau 4.0, totally 13 annotated genes and 2 putative genes were mapped within the previously identified 1.5Mb linkage region on BTA6 (Mei et al., 2009), which were compose of TBC1D1, KLF3, TLR10, TLR1, TLR6, LOCS151583, TMEM156, LOC528668, WDR19, LOC514842, LOC514925, RPL9, LIAS and UGDH. Of them, five genes including KLF3, KLHL5, RFC1, WDR19 and UGDH, were suggested as candidates for milk production traits based on their corresponding physiological and biochemical functions in human, so that the entire coding region of each gene was screened to identify any potential polymorphisms by direct sequencing of pooled DNA from 8 segregating sire families. As a result, a total of 64 SNPs were revealed and their associations with five milk yield and milk composition traits were determined (data of KLF3, KLHL5, RFC1 and WDR19 were not shown). We herein considered UGDH as the most plausible comparative functional candidate gene affecting milk production traits.

SNPs identification and selection
As for UGDH gene, a total of 22 SNPs, including 12 in exons and 10 in introns, were found from the pooled DNA of the 8 unrelated bulls. 10 SNPs out of them were selected for the association analysis according to their heterozygosities among the 8 bulls and locations (polymorphisms in exons were preferred, Figure 1). The
locations and allele frequencies of the 10 SNPs are shown in Table 2.

### Association between individual SNP and milk production traits

Single-locus association analysis (Table 3) showed that three SNPs (SNP Ex1-1, SNP Int3-1 and SNP Ex12-3) were significantly associated with protein yield with their raw P values < 0.05 through single SNP analysis, and 2 of them (SNP Ex1-1 and SNP Ex12-3) also associated with milk yield. However, only the association (SNP Ex1-1 with milk yield) remained significant after correction for multiple testing (P = 0.0003).

### Associations between haplotypes and milk production traits

Figure 2 shows pair-wise D’ values and predicted haplotype blocks for the ten SNPs. Three blocks were identified. The first block consisted of 3 SNPs which formed 7 haplotypes in the studied population. The other two blocks both consisted of 2 SNPs which formed 4 haplotypes (Table 4). As shown in Table 4, three major haplotypes showed a significant association with two milk percentage traits. The haplotypes G-C-C, formed by SNP Ex12-2, SNP Int11-1, and SNP Ex11-1, showed a significant association with protein percentage (F = 4.15; P = 0.0418). The second haplotype, T-G, formed by SNP Int9-3 and SNP Int9-2, was significantly associated with fat percentage (F = 5.18; P = 0.0231). The last one, C-C, formed by SNP Int5-1 and SNP Int3-1, exhibited a significant association with fat percentage also (F = 7.25; P = 0.0072) which remained significant after multiple testing correction of FDR (adjusted significance level was 0.0083 for four major haplotypes).

### Analysis of UGDH and its mutant Ex1-1 (G/A), Ex1-2 (A/C) and Ex11-1 (C/T)

To define the functional significance of SNPs Ex1-1 (G/A), Ex1-2 (A/C) and Ex11-1 (C/T) on the expression of UGDH, we created plasmid constructs for the eight possible combinations (i.e., g-a-c, a-a-c, g-c-c, g-a-t,...

---

**Table 2 Information for 10 SNPs in UGDH and allele frequencies in Chinese Holstein population**

| SNP number | SNP name | SNP location | Position (BTA 6) | Alleles | Frequency | Primer and probe sequence |
|------------|----------|--------------|-----------------|---------|-----------|--------------------------|
| 1          | SNP Ex12-3 | Exon 12      | 60966191        | A/T     | 0.40/0.60 | F: ATTTTCGTCCTCCCTGAATTGAG |
|            |           |              |                 |         |           | R: ACCTGGCCAGCATAGACAGAAAA |
|            |           |              |                 |         |           | P: AAGGCCACACACTCTG-MGB    |
| 2          | SNP Ex12-2 | Exon 12      | 60966411        | C/G     | 0.42/0.58 | F: GTACTTCTTGATGGGAAATCTACA |
|            |           |              |                 |         |           | R: CGCTAAGGTGGTCTGACTCTGTTA |
|            |           |              |                 |         |           | P: AAGGCCACACACTCTG-MGB    |
| 3          | SNP Int11-1 | Intron 11   | 60969505        | A/C     | 0.39/0.61 | F: CCTGATATGCCTCTAATCTTCTGTCTT |
| 4          | SNP Ex11-1 | Exon 11      | 60969627        | C/T     | 0.66/0.34 | F: CGTCAAGATAGAGCTGTCTT |
|            |           |              |                 |         |           | R: CATTCTTTATAGATAGCTCAT-MGB |

**Note:**
Genotyping platform: ABI7900, SNP 1, 2, 4; Mass Spec, SNPs 3, 5–10.
a-c-c, g-c-t, a-a-t, and a-c-t) by site-directed mutagenesis from a complete UGDH cDNA clone (g-a-c) and then transfected them into COS 7 cells. Following transfection, we measured the mRNA and protein expressions from each construct using real-time RT-PCR and Western blotting (Figure 3).

After normalization to the corresponding 18S rRNA of each sample, we found all mutant constructs except g-c-c showed significantly lower mRNA expression than the construct g-a-c (~52.0%; P < 0.05). Moreover, three mutant constructs (i.e., a-a-c, a-c-c, and a-c-t) were observed to be highly significant (P < 0.01), where UGDH mRNA was down-regulated by 63%, 62%, and 68%, respectively (Figure 3B). At the protein level, as shown in Figure 3D, the seven mutant constructs showed generally lower expression than the construct g-a-c (~15.8–~51.1%)

![Figure 2 Haplotype block of 10 identified SNPs in UGDH.](image)

Table 3 Significant UGDH SNPs associated with milk production traits in Chinese Holstein population (P<0.05)

| SNP number | SNP       | Genotype | Milk yield, kg LSMEAN ± SE | Protein yield, kg LSMEAN ± SE |
|------------|-----------|----------|---------------------------|-------------------------------|
| 1          | SNP Ex12-3| AA       | 168.55±230.51             | 3.99±6.33                    |
|            |           | AT       | 275.43±160.02             | 9.93±4.39                    |
|            |           | TT       | 514.41±172.42             | 15.11±4.73                   |
| 8          | SNP Int13_1| GG       | 157.24±319.55             | 5.97±8.80                    |
|            |           | TG       | 279.02±180.27             | 8.61±4.94                    |
|            |           | TT       | 380.43±180.47             | 14.62±4.95                   |
| 10         | SNP Ex1-1 | AA       | −692.88±745.73*           | −12.54±20.55                 |
|            |           | GA       | −4.3120±199.98*           | 2.01±5.49                    |
|            |           | GG       | 467.51±157.89*            | 13.08±4.33                   |

Note: * Significant at FDR<0.05.
Table 4 Associations of UGDH haplotypes with milk production traits in Chinese Holstein population

| 2 | 3 | 4 | 5 | 6 | 7 | 8 | Freq | Milk yield | P value | P value | Protein yield | P value | P value | Fat percentage | P value | P value | Protein percentage | P value | P value |
|---|---|---|---|---|---|---|------|-----------|---------|---------|--------------|---------|---------|---------------|---------|---------|------------------|---------|---------|
| G | A | C | 0.1993 | 0.07 | 0.7947 | 0.20 | 0.6540 | 2.11 | 0.1468 | 1.45 | 0.2294 |
| G | C | T | 0.3802 | 0.24 | 0.6226 | 0.14 | 0.7084 | 0.42 | 0.5183 | 0.24 | 0.6263 |
| G | C | C | 0.1253 | 2.7 | 0.106 | 2.33 | 0.1272 | 2.03 | 0.1543 | 4.15 | 0.0418 |
| T | G | 0.4282 | 0.77 | 0.3803 | 0.60 | 0.4404 | 5.18 | 0.0231 | 0.19 | 0.6601 |
| C | G | 0.1390 | 0.39 | 0.5324 | 0.00 | 0.9526 | 0.14 | 0.7060 | 1.71 | 0.1907 |
| C | A | 0.4299 | 0.03 | 0.8666 | 0.09 | 0.7590 | 0.24 | 0.6218 | 0.82 | 0.3659 |
| T | T | 0.7441 | 1.52 | 0.2185 | 1.77 | 0.1836 | 7.25 | 0.0072 | 0.05 | 0.8237 |
| T | G | 0.1620 | 0.33 | 0.5660 | 0.04 | 0.8373 | 0.00 | 0.9987 | 0.72 | 0.3954 |

Notes:
1) Only major haplotypes with a frequency >0.10 are shown.
2) * Significant at FDR<0.05.

and among them, constructs a-a-t, a-c-c, a-a-c, c-c-c, and a-t-t reached the significance level of 0.05, with their UGDH protein concentration being reduced by 37.8%, 51.1%, 34.7%, 26.5%, and 50.1%, respectively.

These results indicate that at mRNA level, the mutation of Ex1-2 has little impact on the expression of UGDH and that all differences observed among the eight constructs were attributable primarily to SNPs Ex1-1 and Ex11-1, for which the A allele of SNP Ex1-1 and T allele of SNP Ex11-1 produced significantly lower expression than the G and C allele respectively. But at protein level, the two alleles of SNP Ex1-1 play more important role in the expression of UGDH than those of SNPs Ex1-2 and Ex11-1, where the G allele of SNP Ex1-1 corresponds to higher protein concentration.

Discussion
In this study, we provide a strong evidence for the significant associations of UGDH with 2 milk production traits in a Chinese Holstein cattle population. Moreover, we demonstrated that the SNPs Ex1-1 and Ex11-1 in exonic regions of UGDH are two functional polymorphisms in which A allele of SNP Ex1-1 and T allele of SNP Ex11-1 cause lower expression than the G and C allele respectively. Together, our findings not only confirmed our early finding that the SNP Ex1-1 located in the 5` UTR (c.exon1-1 G>A) was significantly associated, even after multiple testing correction of FDR, with milk yield (P = 0.003). Moreover, haplotype analysis revealed two significant associations of two percentage traits with haplotypes formed by SNP Ex12-2- SNP Int11-1- SNP Ex11-1 and SNP Int5-1- SNP Int3-1, respectively. Given the aforementioned association results of SNP Ex1-1 and SNP Ex11-1 with milk production traits and their locations within the gene, we hypothesized that these two SNPs and the other SNP Ex1-2 (located in the 5` UTR of UGDH) might be QTN responsible for this QTL.

Previously, we mapped a QTL near BMS470 with effects on milk production traits with daughter design using 14 microsatellites on bovine chromosome 6 in a Chinese Holstein population involving 26 paternal half-sib families with 2356 daughters [8,9]. By increasing the marker density with 17 microsatellite markers spanning from BMS690 to BMS4528 near BMS470 and genotyping them for 918 daughters from 8 sires families which had been proved to contain segregating QTLs affecting milk production traits in the previous studies [8,9], we further fine mapped this QTL in region of BMS483 and MNB-209 by employing linkage disequilibrium (LD) mapping approaches [14]. On the base of these studies, the UGDH gene within this interval was selected as a potential positional candidate gene for further study by genetic association and functional analysis as reported in this study. Association analysis of individual SNPs indicated that the SNP Ex1-1 located in the 5` UTR (c.exon1-1 G>A) was significantly associated, even after multiple testing correction of FDR, with milk yield (P = 0.003). Moreover, haplotype analysis revealed two significant associations of two percentage traits with haplotypes formed by SNP Ex12-2- SNP Int11-1- SNP Ex11-1 and SNP Int5-1- SNP Int3-1, respectively. Given the aforementioned association results of SNP Ex1-1 and SNP Ex11-1 with milk production traits and their locations within the gene, we hypothesized that these two SNPs and the other SNP Ex1-2 (located in the 5` UTR of UGDH and near to the SNP Ex1-1) contribute to the observed association of UGDH with milk production traits. By expressing plasmid constructs containing different alleles for above three SNPs in COS 7 cells, we found that the plasmid construct containing the A allele of SNP Ex1-1 and T allele of SNP Ex11-1 produced significantly lower expression of mRNA than the G and C allele respectively. At protein level, the two alleles of SNP Ex1-1 play more important role on the expression of UGDH than those of SNPs Ex1-2 and Ex11-1, where the G allele of SNP Ex1-1 corresponds to higher protein concentration. Thus, we concluded that SNP Ex1-1 is involved in the regulation of the expression both at the transcription and protein level and SNP Ex11-1 is involved in the regulation of the expression of UGDH at the transcription level Although further study is required to determine how these two DNA polymorphisms modulate the expression of UGDH, they are most likely
Figure 3 (See legend on next page.)
Conclusions

Based on the previously identified 1.5-Mb region between markers BMS483 and MNB-209 on BTA6 including a QTL for milk production traits, the UGDH gene was identified and shown to be associated with milk yield and milk composition. Further in vitro expression assay demonstrated that the SNPs in exons1 and exon11 of UGDH represent two functional polymorphisms affecting expression of UGDH and might partly contributed to the observed association with milk production traits.

Abbreviations

ABCG2: ATP-binding cassette; BTA: Bos Taurus Automosome; DAC: Dairy association of China; DGAT1: AcylCoA: diacylglycerol acyltransferase; GHR: Growth hormone receptor; LD: Linkage disequilibrium; SNP: Single nucleotide polymorphism; OPN: Osteopontin; QTL: Quantitative trait locus; UGDH: UDP-glucose dehydrogenase.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

QX performed the cell related experiments and prepared the manuscript. GM contributed to the SNP discovery and genotyping and association analysis. DS conceived and designed the experiments and prepared the manuscript. CY and HC performed the QTL mapping. XD and JL participated in the data analysis. All authors read and approved the final interpretation. CY and HC performed the QTL mapping. XD and JL participated in the data analysis. All authors read and approved the final interpretation.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31072016), Beijing InnovationTeam of Technology System in the National Dairy Industry, Key Development of New Transgenic Breeds Program of China (2009ZX08009-156B), National “984” Project (2011-G2A), National Key Technologies R & D Program (2011BAD28B02, 102CJFNC02700) and Agricultural Science and Technology Achievement Transformation Project (20110409002).

References

1. Khatare MS, Thomson PC, Tammen I, Raadisma HW: Quantitative trait loci mapping in dairy cattle: review and meta-analysis. Genet Sel Evol 2004, 36:163–190.

2. Georges M, Nielsen D, Mackinnon M, Mishra A, Okimoto R, Pasquinio AT, Sargeant LS, Sorensen A, Steele MR, Zhao X: Mapping quantitative trait loci controlling milk production in dairy cattle by exploiting progeny testing. Genetics 1999, 139:907–920.

3. Watanabe M, Nikaido M, Tsuda TT, Kobayashi T, Mindell D, Cao Y, Okada N, Hasegawa M: New candidate species most closely related to penguins. Gene 2006, 378:65–73.

4. Grisart B, Farnir F, Karim L, Cambisano N, Kim JJ, Kvasz A, Mni M, Simon P, Frère JM, Coppetier W, Georges M: Genetic and functional confirmation of the causality of the DGAT1 K232A quantitative trait nucleotide in affecting milk yield and composition. Proc Natl Acad Sci USA 2004, 101:2398–2403.

5. Blott S, Kim JJ, Mosio S, Schmidt-Kuntei A, Cornet A, Berz P, Cambisano N, Ford C, Grisart B, Johnson D, Karim L, Simon P, Snell R, Spelman R, Wong J, Vilkiu J, Georges M, Farnir F, Coppetier W: Molecular dissection of a quantitative trait locus: a phenylalanine-to-tyrosine substitution in the transmembrane domain of the bovine growth hormone receptor is associated with a major effect on milk yield and composition. Genetics 2003, 163:253–266.

6. Schnabel RD, Kim JJ, Ashwell MS, Sonsteberg TS, Van Tassell CP, Connor EE, Taylor JF: Fine-mapping milk production quantitative trait loci on BTA6: analysis of the bovine osteopontin gene. Proc Natl Acad Sci USA 2005, 102:6896–6901.

7. Cohen-Zinder M, Seroussi E, Larkin DM, Loor JJ, Everts-van der Wind A, Lee JH, Drackley JK, Band MR, Hernandez AG, Shani M, Levin HA, Weller J, Ron M: Identification of a missense mutation in the bovine ARBG2 gene with a major effect on the QTL on chromosome 6 affecting milk yield and composition in Holstein cattle. Genome Res 2005, 15:936–944.

8. Chen HF, Zhang Q, Wang CK, Shu J, Mei G, Yin CC, Hu F, Xu J, Gong WJ, Li HJ, Qiu XT. Mapping QTLs on BTA6 affecting milk production traits in a Chinese Holstein population. Chin Sci Bull 2005, 50:1737–1742.

9. Chen HF, Zhang Q, Yin CC, Wang CK, Gong WJ, Mei G: Detection of quantitative trait loci affecting milk production traits on bovine chromosome 6 in a Chinese Holstein population by the daughter design. J Dairy Sci 2006, 89:782–790.

10. Zhang Q, Boichard D, Hoeschele I, Ernst C, Eggen A, murkbe B, Pfister-Genskow M, Witte LA, Gogrona FE, Ulmair P, Thaller G, Bishop MD: Mapping quantitative trait loci for milk production and health of dairy cattle in a large outbred pedigree. Genetics 1998, 149:1959–1973.
11. Velmala RJ, Vikki HJ, Elo KT, de Koning DJ, Maki-Tanila AV: A search for quantitative trait loci for milk production traits on chromosome 6 in Finnish Ayshire cattle. Anim Genet 1999, 30:136–143.
12. Szyda J, Liu Z, Reinhardt F, Reents R: Estimation of quantitative trait loci parameters for milk production traits in German Holstein dairy cattle population. J Dairy Sci 2005, 88:356–367.
13. Hardy B, Bennewitz J, Reinsch N, Thaller G, Thomsen H, Kuhn C, Schwerin M, Erhardt G, Förster M, Reinhardt F, Kalm E: Mapping of quantitative trait loci for lactation persistency traits in German Holstein dairy cattle. J Anim Breed Genet 2006, 123:89–96.
14. Mei G, Yin C, Ding X, Zhang Q: Fine mapping quantitative trait loci affecting milk production traits on bovine chromosome 6 in a Chinese Holstein population. J Genet Genomics 2009, 36:553–660.
15. Weikard R, Goldammer T, Laurent P, Womack JE, Kuehn C: A gene-based high-resolution comparative radiation hybrid map as a framework for genome sequence assembly of a bovine chromosome 6 region associated with QTL for growth, body composition, and milk performance traits. BMC Genomics 2006, 7:63.
16. Everts-van der Wind A, Kata SR, Band MR, Rebeiz M, Larkin DM, Everts RE, Green CA, Liu L, Natarajan S, Golddammer T, Lee JH, McKay S, Womack JE, Levin HA: A 1463 gene cattle-human comparative map with anchor points defined by human genome sequence coordinates. Genome Res 2004, 14:1424–1437.
17. Everts-van der Wind A, Larkin DM, Green CA, Elliott JS, Olmstead CA, Chiu B, Schein JE, Marra NA, Womack JE, Levin HA: A high-resolution whole-genome cattle-human comparative map reveals details of mammalian chromosome evolution. Proc Natl Acad Sci USA 2005, 102:18526–18531.
18. Trochon V, Mabilat C, Bertrand P, Legrand Y, Smadja-Joffe F, Soria C, Delpesch B, Lu H: Evidence of involvement of CD44 in endothelial cell proliferation, migration and angiogenesis in vitro. Int J Cancer 1996, 66: C664–668.
19. Iozzo RV, Murdoch AD: Proteoglycans of the extracellular environment: clues from the gene and protein side offer novel perspectives in molecular diversity and function. FASEB J 1996, 10:598–614.
20. Ropponen K, Tammi M, Parkkinen J, Tammi R, Lipponen P, Agren U, Alhava E, Kosma VM: Hyaluronan in peritumoral stroma and malignant cells associates with breast cancer spreading and predicts survival. Am J Pathol 2000, 156:529–536.
21. Aaltomaa S, Lipponen P, Tammi R, Tammi M, Alhava E, Kosma VM: Strong Stromal Hyaluronan Expression Is Associated with PSA Recurrence in Local Prostate Cancer. Urol Int 2002, 69:266–272.
22. Barrett JC, Fry B, Maller J, Daly MJ: Haplview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005, 21:263–265.
23. Ding K, Zhang Q, Flury C, Simineri H: Haplotype reconstruction and estimation of haplotype frequencies from nuclear families with only one parent available. Hum Hered 2006, 62:12–19.
24. Winer J, Jung CK, Shackell I, Williams PM: Development and validation of real-time quantitative reverse transcriptase-polymerase chain reaction for monitoring gene expression in cardiac myocytes in vitro. Anal Biochem 1999, 270:1–9.
25. Kanda A, Chen W, Othman M, Branham KE, Brooks M, Khanna R, He S, Lyons R, Abecasis GR, Swaroop A: A variant of mitochondrial protein LOC387715/ARMS2, not HTRA1, is strongly associated with age-related macular degeneration. Proc Natl Acad Sci USA 2007, 104:16227–16232.
26. Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, Gottesman MM: A “silent” polymorphism in the MDR1 gene changes substrate specificity. Science 2007, 315:525–528.
27. Nackley AG, Sabalalina SA, Tchivileva IE, Satterfield K, Korchnykiy O, Makarov SS, Maxiner W, Diatchenko L: Human catechol-O-methyltransferase haplotypes modulate protein expression by altering mRNA secondary structure. Science 2006, 314:1930–1933.