Identification of whole-genome significant single nucleotide polymorphisms in candidate genes associated with body conformation traits in Chinese Holstein cattle

Zhengui Yan, Zhonghua Wang, Qin Zhang, Shujian Yue, Bin Yin, Yunliang Jiang and Kerong Shi

College of Animal Science and Technology, Shandong Key Laboratory of Animal Bioengineering and Disease Prevention, Shandong Agricultural University, Taian, Shandong 271018, China

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GWAS has been proven to be a powerful tool for detecting genetic variants associated with economically important traits such as production, body conformation and reproduction traits, and diseases. The objective of this study was to identify SNPs with significant association effects on body conformation traits in Chinese Holstein through the GWAS approach.

The experimental population consisted of 445 Chinese Holstein cows, with parity no. 2, raised at four farms in Shandong province of China. Hair follicle samples were collected individually within four days in the same season. In total, 29 body conformation traits were analyzed by GWAS. There were 23 phenotypes measured individually on 1–9 scores, which were body height, height at front end, body depth, chest width, stature, angularity, rump angle (RA), pin width, loin strength, foot angle, heel depth, bone quality, rear legs – side view, rear legs – rear view, udder depth, udder texture, median suspensory, fore udder attachment, front teat placement, attachment height, attachment width (AW), rear teat placement and teat length. The remaining six functional body conformation traits, measured on 1–100 scores, were stature score, rump system score, feet and legs score, udder system score, mammary system score and conformation final score (FS). The definitions of these conformation traits for dairy cattle are available on the website of Canadian Dairy Network (https://www.cdn.ca/articles.php). The summary statistics of these phenotypes are listed in Table S1.

Each experimental animal was genotyped by the GGP BovineLD V3 SNP chip, containing 26 151 public SNPs. Because all of the animals in these study populations were female, the SNPs from the X chromosome were also counted. The data quality control procedure excluded individuals with more than 10% missing genotypes and SNPs with a call rate of less than 90%, MAF less than 3% or P-value of the Hardy–Weinberg Equilibrium test less than 1.0E – 6. Finally, 421 animals with 20 632 SNP genotypes were retained for the subsequent GWAS analysis. Physical map length, the number of SNPs and SNP density on each chromosome based on reference genome UMD3.1, before and after the data cleaning procedure, are shown in Table S2. Results of pair-wise LD analysis of the 421 animals from four different farms showed high similarity of LD patterns among the whole population, indicating that similar breeding histories were shared by the four subpopulations (data not provided).

A fixed-effects linear regression model was used to carry out the GWAS according to a previous method. Bonferroni correction for the genome-wide significance and suggestive thresholds were computed to be 2.42E – 06 (= 0.05/20 632) and 4.85E – 05 (= 1/20 632), respectively.

The GWAS based on the mixed-effect linear regression model identified 79 SNPs with genome-wide significant (P < 0.05) association effects on 14 body conformation traits (Table 1). Of these SNPs, 28 SNPs were found to be associated with RA, and 14 SNPs associated with FS (Table 1, Fig. S1). We also found six single SNPs that were associated with multiple body conformation traits of dairy cow. These SNPs were BovineHD0600000461, BovineHD1900007686, ARS-BFGL-NGS-41612, BovineHD1700010514, BovineHD3000037672 and ARS-BFGL-NGS-109467 (their detailed information is provided in Table S4). In this study, the DNA region from 44.6 to 65.4 Mb of chromosome 18 was identified to be associated with four different body conformation traits (AW, FS, mammary system score and RA) of dairy cows (Table 1, Table S3). This coincided with previously reported QTL or DNA regions associated with calving performance and udder traits of dairy cattle. Among these SNPs, Hapmap57004-rs29011610, located near MON1B (MON1 secretory trafficking family member B) gene, were also found to be associated with length of productive life in Holstein cows. ARS-BFGL-NGS-116541, which was located within the LIG1 (Ligase I) gene region, was reported to be associated with body weight at birth. Similarly, DNA regions on chromosomes 21, 25 and X were also identified to be significantly associated with multiple body conformation traits (Table 2, Table S3). In particular, six SNPs in the small region from 57.55 to 57.62 Mb on the chromosome 21 were found to be associated with AW and FS in the study, and these SNPs were all located within or near the gene region of SLC24A4 (Table S5). Interestingly, SLC24A5 and SLC19A2, members of solute carrier family proteins with SLC24A4, were also found to be associated with RA and FS, respectively (Table 1). Additionally, UA-IFASA-6670, located within the region of GABARAPL1 [GABA (A) receptor-associated protein like 1] gene, were found to be significantly associated with median suspensory in this study. It was reported previously to affect udder attachment and height.

Also, BovineHD2200013812 was identified to have genetic effects on RA. This SNP was located within the CACNA1D (calcium channel alpha 1D subunit) gene region. Coincidentally, this SNP was also reported to be
| Category                  | Trait               | Chromosome | Position | SNP name               | P-Value     | Nearest gene | Distance | Reference | Alteration | MAF     |
|---------------------------|---------------------|------------|----------|------------------------|-------------|--------------|----------|-----------|------------|---------|
| Dairy strength            | Angularity          | 30         | 11022328 | BTA-116883-no-rs       | 1.56E-06    | LOC786124    | D:27320  | G         | A          | 0.2404  |
|                           |                      | 30         | 11627304 | BovineHD3000032546     | 6.55E-07    | LOC537655    | Within   | C         | A          | 0.3061  |
|                           |                      | 30         | 13270521 | BovineHD3000037672     | 5.10E-08    | LOC786725    | U:54284  | T         | C          | 0.449   |
|                           | Stature             | 10         | 90090600 | Hapmap39512-BTA-79353  | 5.11E-07    |              |          | T         | G          | 0.2744  |
|                           |                      | 19         | 26049717 | BovineHD1900007686     | 2.15E-06    |              |          | T         | C          | 0.0362  |
|                           |                      | 17         | 38504014 | BovineHD1700010514     | 1.34E-07    | LOC512119    | D:176780 | T         | C          | 0.0954  |
|                           |                      | 11         | 80730546 | ARS-BFGL-NGS-41612     | 4.93E-07    | KCNS3        | U:112015 | G         | A          | 0.1485  |
|                           |                      | 19         | 26049717 | BovineHD1700010514     | 1.34E-07    | LOC512119    | D:176780 | T         | C          | 0.0954  |
|                           |                      | 17         | 38504014 | BovineHD1700010514     | 2.42E-08    | LOC512119    | D:176780 | T         | C          | 0.0954  |
| Mammary system            | Attachment width    | 9          | 20356212 | BTA-85319-no-rs        | 7.16E-07    | BCKDHB       | D:21051  | T         | C          | 0.2614  |
|                           |                      | 18         | 65402237 | BovineHD1800009409     | 4.75E-07    | LOC789374    | Within   | C         | T          | 0.2143  |
|                           |                      | 19         | 24263948 | BovineHD1900006183     | 4.26E-07    | LOC524240    | Within   | G         | A          | 0.3515  |
|                           |                      | 23         | 39248351 | BovineHD2300011340     | 2.39E-06    | NHLR1        | D:7508   | T         | C          | 0.4341  |
|                           |                      | 27         | 39335460 | Hapmap38550-BTA-98603  | 1.66E-06    | LRRC3B       | D:101345 | C         | A          | 0.3497  |
|                           |                      | 10         | 45053776 | BovineHD1000013564     | 9.37E-07    | NID2         | D:57117  | G         | T          | 0.2324  |
|                           |                      | 17         | 38504014 | BovineHD1700010514     | 2.42E-08    | LOC512119    | D:176780 | T         | C          | 0.0954  |
|                           | Median suspensory   | 5          | 10020614 | UA-IFASA-6670          | 6.37E-07    | GABARAPL1    | Within   | C         | T          | 0.398   |
|                           |                      | 9          | 93370896 | BovineHD0900026424     | 5.03E-07    | NOX3         | U:2759   | T         | C          | 0.325   |
|                           |                      | 17         | 73901259 | BovineHD1700021616     | 9.77E-07    | LOC531271    | Within   | A         | G          | 0.2381  |
|                           |                      | 30         | 13830453 | BovineHD3000039710     | 5.31E-07    | LOC782196    | U:18403  | T         | C          | 0.213   |
|                           | Mammary system score| 13         | 28331553 | ARS-BFGL-NGS-109467    | 5.49E-07    | SEPHS1       | D:16954  | A         | G          | 0.4388  |
|                           |                      | 15         | 43538866 | ARS-BFGL-NGS-115625    | 2.03E-06    | SWAP70       | Within   | G         | A          | 0.4467  |
|                           |                      | 18         | 55956772 | ARS-BFGL-NGS-60829     | 6.86E-07    | NUCB1        | D:1160   | C         | T          | 0.4172  |
|                           |                      | 30         | 13270521 | BovineHD3000037672     | 7.03E-08    | LOC786725    | U:54284  | T         | C          | 0.449   |
|                           | Rear teat placement | 9          | 25458692 | BTA-83107-no-rs        | 1.10E-06    | MIR2284O     | U:14492  | A         | G          | 0.3307  |
|                           | Udder depth         | 25         | 35623801 | ARS-BFGL-NGS-28167     | 2.16E-06    | CUX1         | D:12155  | A         | G          | 0.4023  |
|                           |                      | 25         | 36266951 | BovineHD2500010029     | 1.27E-07    | LOC100298352 | Within   | T         | C          | 0.356   |
|                           | Udder system score  | 6          | 1770665  | BovineHD060000461      | 1.03E-07    | 1-Mar        | Within   | G         | A          | 0.39    |
### Table 1 (Continued)

| Category          | Trait                | Chromosome | Position | SNP name                          | P-Value | Nearest gene | Distance | Reference | Alteration | MAF  |
|-------------------|----------------------|------------|----------|-----------------------------------|---------|--------------|----------|-----------|------------|------|
| **Rump structure**| Loin strength        | 5          | 61620118 | BovineHD050001.7277               | 2.90E-07 | NEDD1        | D:121565 | T         | G          | 0.4853 |
|                   |                      | 7          | 53932886 | ARS-BFGL-NGS-20-197               | 5.71E-07 | PCDH86       | U:892    | T         | C          | 0.4966 |
|                   |                      | 28         | 46248750 | BovineHD280001.3502               | 4.71E-07 | LOC1001411022| D:32551  | T         | C          | 0.2523 |
| **Rump angle**    |                      | 1          | 5519845  | B7-0003652                        | 1.76E-06 | GRK1         | Within   | C         | T          | 0.25  |
|                   |                      | 1          | 68909418 | BovineHD100001.9488               | 4.88E-07 | CCDC14       | Within   | A         | G          | 0.4182 |
|                   |                      | 1          | 12385156 | BovineHD100003.4972               | 1.97E-06 |               | T         | C         | 0.2011    |
|                   |                      | 1          | 14296716 | BovineHD10004.1062                | 2.03E-06 | BACE2        | Within   | G         | A          | 0.4354 |
|                   |                      | 2          | 12756675 | BovineHD20003.7025                | 6.11E-07 | PDK1L        | Within   | G         | A          | 0.3417 |
|                   |                      | 4          | 10148342 | Hapmap35652                        | 4.57E-07 | LOC100295705 | D:88944  | C         | T          | 0.2268 |
|                   |                      |            |          |                                   |         |              |          |           |            |      |
|                   |                      | 6          | 83512619 | BTA-107087-no-rs                  | 2.18E-06 | LOC100298985 | U:19994  | G         | A          | 0.3243 |
|                   |                      | 6          | 87715723 | Hapmap38371-BTA-105598             | 1.58E-06 | AMBN         | D:9991   | C         | A          | 0.3356 |
|                   |                      | 7          | 83260664 | BovineHD700002.4393               | 4.32E-09 | MS43         | D:33418  | G         | A          | 0.1746 |
|                   |                      | 7          | 83757564 | BovineHD70002.4587                | 1.14E-07 | SSBP2        | Within   | C         | A          | 0.11  |
|                   |                      | 7          | 91507089 | ARS-BFGL-NGS-118534               | 2.83E-07 |               | G         | A         | 0.2761    |
|                   |                      | 8          | 10166481 | BovineHD80003.0195                | 2.25E-06 | SVEP1        | Within   | G         | A          | 0.1236 |
|                   |                      | 9          | 81329823 | BTA-106078-no-rs                  | 9.84E-07 | HVEP2        | D:62953  | A         | G          | 0.4592 |
|                   |                      | 10         | 43438784 | BovineHD100001.3067               | 8.09E-08 | MAP4K5       | Within   | G         | A          | 0.3246 |
|                   |                      | 10         | 62563388 | BovineHD100001.8043               | 7.73E-07 | SLC24A5      | D:73734  | T         | C          | 0.4376 |
|                   |                      | 10         | 73979984 | Hapmap49737-BTA-75278             | 6.00E-07 | PRKCH        | D:52860  | A         | G          | 0.04762|
|                   |                      | 18         | 55399510 | ARS-BFGL-NGS-116541               | 2.37E-06 | UG1          | Within   | C         | A          | 0.4487 |
|                   |                      | 18         | 55514759 | BovineHD180001.6250               | 7.28E-08 | SYNGM        | Within   | T         | C          | 0.2494 |
|                   |                      | 18         | 55621823 | ARS-BFGL-NGS-31529                | 2.12E-06 | LMTK2        | Within   | T         | G          | 0.2426 |
|                   |                      | 22         | 47989704 | BovineHD20003.3812                | 1.72E-06 | CACNA1D      | Within   | T         | C          | 0.2727 |
|                   |                      | 22         | 48408579 | BovineHD20001.3926                | 5.34E-07 | RFT1         | Within   | G         | A          | 0.0839 |
|                   |                      | 24         | 29554807 | BovineHD200008.0307               | 2.70E-07 | LOC782418    | D:63567  | G         | A          | 0.2323 |
|                   |                      | 25         | 31147780 | Hapmap2474-BTC-028427             | 1.82E-08 | LOC100301342 | Within   | T         | C          | 0.3898 |
|                   |                      | 25         | 42364359 | ARS-BFGL-NGS-101981               | 1.32E-06 | ADAP1        | D:1441   | T         | C          | 0.2823 |
|                   |                      | 26         | 16504170 | BovineHD260004.1345               | 1.32E-06 | LOC522146    | Within   | A         | G          | 0.1927 |
|                   |                      | 29         | 72164110 | BovineHD29000002021               | 5.13E-07 | LOC10033656 | Within   | G         | A          | 0.1926 |
|                   |                      | 30         | 2037499  | BovineHD3000000680                | 2.28E-06 | KLHL13       | Within   | C         | A          | 0.03855|
|                   |                      | 30         | 141936249 | BTA-21001-no-rs                | 2.16E-06 | MSL3         | U:105193 | C         | T          | 0.1939 |
| Category          | Trait          | Chromosome | Position | SNP name                  | P-Value | Nearest gene | Distance | Reference | Alteration | MAF    |
|------------------|----------------|------------|----------|---------------------------|---------|--------------|----------|-----------|------------|--------|
| Final conformation score | Final score    | 6          | 1770665  | BovineHD0600000461        | 1.75E-06| 1-Mar        | Within   | G         | A          | 0.39   |
|                  |                | 11         | 80730546 | ARS-BFGL-NGS-41612        | 4.61E-07| KCNS3        |          | U:112015  | G          | 0.1485 |
|                  |                | 13         | 28331553 | ARS-BFGL-NGS-109467       | 1.80E-06| SEPHS1       | D:16954  | A         | G          | 0.4388 |
|                  |                | 16         | 37787772 | ARS-BFGL-NGS-34764        | 2.01E-06| NME7         | Within   | G         | A          | 0.4887 |
|                  |                | 16         | 37904090 | BTB-00637941               | 1.17E-06| SLC19A2      | Within   | C         | T          | 0.4863 |
|                  |                | 16         | 41384258 | BovineHD1600011634        | 2.18E-06| LOC614226    | U:123143 | T         | C          | 0.4558 |
|                  |                | 18         | 4463083  | Hapmap57004-rs29011610     | 1.17E-06| MONT1        | D:5928   | G         | A          | 0.3129 |
|                  |                | 18         | 65405023 | BovineHD1800019051        | 2.04E-06| LOC789374    | Within   | A         | G          | 0.1179 |
|                  |                | 19         | 26049717 | BovineHD1900007686        | 7.31E-07| AIPL1        | D:7711   | T         | C          | 0.03628|
|                  |                | 21         | 57552082 | BovineHD2100016535        | 8.46E-07| SL2CA4A4     | U:44433  | A         | G          | 0.234  |
|                  |                | 25         | 39558290 | BovineHD2500011031        | 2.24E-06| WIP12        | U:7420   | T         | G          | 0.09524|
|                  |                | 25         | 40192570 | BovineHD4100017518        | 1.07E-06| SDK1         | Within   | T         | C          | 0.03061|
|                  |                | 26         | 44324248 | BovineHD2600012439        | 1.82E-06| OAT          | U:53908  | C         | A          | 0.3379 |
|                  |                | 30         | 12103258 | BovineHD3000003945        | 8.38E-07| ACTRT1       | U:55118  | G         | A          | 0.1497 |

1Chromosome 30 refers to X chromosome.
2The distance from the SNP locus to the gene (unit: bp); D and U indicate that the SNP site is located downstream and upstream of the gene, respectively; ‘Within’ indicates that the SNP locus is located within the gene.
associated with the length of productive life and udder cleh.\textsuperscript{14}

In summary, a GWAS using linear statistical model was conducted on 29 body conformation traits in a Chinese Holstein cattle population, and 79 SNPs were found to have genome-wide-significant ($P < 0.05$) association effects on 14 body conformation traits. Among these significant SNPs, 74 of them are newly detected in this study, five have been reported in previous literature and 26 are located in genes and are worth further investigation to potentially identify the causative mutations underlying the QTL.

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Conflict of interest
The authors declare that there is no conflict of interest.

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Correspondence: K. R. Shi (krshi@sdau.edu.cn)

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Genome-wide plots of \(-\log_{10}(P\text{-value})\) SNP association effects on body conformation traits of rump angle (RA, a) and final score (FS, b) obtained by mixed-effect linear regression model.

Table S1 Descriptive statistics of the 29 conformation traits used in the GWAS.
Table S2 Distribution of SNP markers by chromosomes before and after quality control.
Table S3 The chromosomal distribution of significant SNPs associated with body conformation traits.
Table S4 The SNPs identified associated with multiple body conformation traits of dairy cows.
Table S5 Multiple SNPs located in the SLC24A4 gene region were significantly associated with body conformation traits of dairy cows.

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Processed pseudogene confounding the identification of a putative lethal recessive deletion in the bovine 60S ribosomal protein L11 gene (uL5)

Xuying Zhang, Christin Wacker, Ekkehard Schütz and Bertram Brenig

Institute of Veterinary Medicine, University of Goettingen, Burchhardtweg 2, 37077, Göttingen, Germany

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Background: In higher eukaryotes, impaired ribosome biogenesis and function can result in specific phenotypes, the so-called ribosomopathies.1 In humans at least six ribosomopathies have been described, i.e. Diamond-Blackfan anemia (DBA), 5q-syndrome, Shwachman-Diamond syndrome, X-linked dyskeratosis congenita, Treacher Collins syndrome and cartilage hair hypoplasia.1,2 DBA belongs to a rare group of disorders known as inherited bone marrow failure syndromes.3 DBA7 is caused by variants of the 60S ribosomal protein L11 gene (uL5, formerly RPL11).4–6 So far naturally occurring uL5 defects have only been described in humans. Owing to the role of uL5 in ribosome biogenesis and its association with fatal ribosomopathies, we addressed the question whether lethal uL5 variants exist in cattle. Several deleterious variants have been identified, including a 2 bp deletion resulting in a frameshift and premature stop (ENSBTAG00000020905; g.129,195,922_129,195,923del; ARS-UCD1.2: rs381576999). To monitor this variant a probe located on BTA2 between positions 129,195,924 and 129,195,973 (ARS-UCD1.2) has been included as an expert-selected marker in the custom add-on part of the BovineLD BeadChip (Fig. 1).

Variant detection: The presence of the deletion was tested in 370,527 cattle, including British Angus, Charolais, Brownvieh, Belted Galloway, Simmental, Dexter, German Black Pied cattle, Gelbvieh, Hereford, Limousin, Red Holstein, German Red, Holstein, Shorthorn, Uckermärker, Wagyu, Welsh Black and Belgian Blue. We found 299,218 homozygous wt and 71,249 apparently heterozygous cattle but no homozygous carriers.7 A complete cluster separation and high GC scores of the uL5 SNP excluded any technical bias by the chip-based genotyping method. However, we did not detect the putative deletion by Sanger sequencing of PCR-amplified uL5 gene segments in 10 randomly chosen heterozygous cattle (primer sequences are listed in Table S1). In addition, the BeadChip probe matches almost perfectly (49/50 nucleotides) to a processed uL5 pseudogene on BTA18 (LOC112442347; position 54,982,088–54,982,687). Sequencing of a PCR fragment of this pseudogene (primer sequences are listed in Table S1) from an individual scored as heterozygote revealed the 2 bp deletion of rs381576999 (Fig. 1). To verify the indel within uL5 and its processed pseudogene, 1323 (uL5) and 346 (pseudogene) random cattle samples were genotyped using FRET8 (primer sequences are listed in Table S1). None of the 1323 individuals analyzed carried the deletion in uL5, whereas all 346 cattle were homozygous carriers of the deletion in the pseudogene. In order to further prove the suspected genotyping error by the pseudogene, we interrogated the genomic region for the presence of a haplotype with correlation to the chip data. A total of 82,014 samples were used, where 54 SNPs around uL5 (+1.5 Mb up- and downstream) were phased using BEAGLE (version 3.32)9 omitting position rs381576999. No haplotype with a significant correlation to the rs381576999 chip-genotype nor any suspected lethal haplotype could be established (Fig. S1).

Comments: We conclude that there is currently no evidence for the existence of the rs381576999 indel in the functional uL5 gene or in its pseudogene on BTA18. Instead, our results suggest that the deletion has been fixed in the