RESEARCH ARTICLE

Genome-wide association studies identified multiple genetic loci for body size at four growth stages in Chinese Holstein cattle

Xu Zhang¹,², Qin Chu³, Gang Guo⁴, Ganghui Dong⁴, Xizhi Li⁴, Qin Zhang¹, Shengli Zhang¹, Zhiwu Zhang²*, Yachun Wang¹*

¹ Key Laboratory of Agricultural Animal Genetics and Breeding, National Engineering Laboratory for Animal Breeding, College of Animal Science and Technology, China Agricultural University, Beijing, P.R. China, ² Department of Crop and Soil Sciences, Washington State University, Pullman, Washington, United States of America, ³ Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences, Beijing, P.R. China, ⁴ Beijing Sunlon Livestock Development Co. Ltd, Beijing, P.R. China

* wangyachun@cau.edu.cn (YW); zhiwu.zhang@wsu.edu (ZZ)

Abstract

The growth and maturity of cattle body size affect not only feed efficiency, but also productivity and longevity. Dissecting the genetic architecture of body size is critical for cattle breeding to improve both efficiency and productivity. The volume and weight of body size are indicated by several measurements. Among them, Heart Girth (HG) and Hip Height (HH) are the most important traits. They are widely used as predictors of body weight (BW). Few association studies have been conducted for HG and HH in cattle focusing on single growth stage. In this study, we extended the Genome-wide association studies to a full spectrum of four growth stages (6-, 12-, 18-, and 24-months after birth) in Chinese Holstein heifers. The whole genomic single nucleotide polymorphisms (SNPs) were obtained from the Illumina BovineSNP50 v2 BeadChip genotyped on 3,325 individuals. Estimated breeding values (EBVs) were derived for both HG and HH at the four different ages and analyzed separately for GWAS by using the Fixed and random model Circuitous Probability Unification (Farm-CPU) method. In total, 27 SNPs were identified to be significantly associated with HG and HH at different growth stages. We found 66 candidate genes located nearby the associated SNPs, including nine genes that were known as highly related to development and skeletal and muscular growth. In addition, biological function analysis was performed by Ingenuity Pathway Analysis and an interaction network related to development was obtained, which contained 16 genes out of the 66 candidates. The set of putative genes provided valuable resources and can help elucidate the genomic architecture and mechanisms underlying growth traits in dairy cattle.

Introduction

Early development of heifer is important to the subsequent performance of a dairy herd because well-grown heifers probably calve and begin producing milk sooner, which do
increase profit[1]. For dairy farms, heifer growth is routinely monitored for breeding selection [2] and management. Body weight (BW) has been reported as one of the measurements to determine the early growth in dairy farm[3]. The process of collecting routine BW measurements is labor intensive. Alternatively, measuring heifer body size was frequently selected by dairy farm managers to monitor growth and track the developmental progress of each animal throughout the rearing period[2].

Heart girth (HG) and hip height (HH) are the two major characteristics of body size in cattle. It is much easier to measure HG and HH than measuring BW. Both HG and HH are high heritable and have high phenotypic and genetic correlations with BW[4]. The prediction of BW based on HG and HH is regarded as highly reliable and accurate[5]. In addition, HG and HH are known having a relationship with important economic traits. For example, good depth of HG in cattle is a sign of good forage convertibility and good feet and leg conformation[6]. Heifers with higher HH will subsequently produce more milk[7]. It is widely accepted that HG and HH are quantitative traits, which are probably controlled by multiple genes[8]. Previous studies using marker association analysis[9–11], have reported some genes related to HG and HH in cattle. Recently, genome-wide association (GWAS) have revealed many important findings associated with production traits[12–14], growth traits[15,16], fertility traits[17,18] etc. in cattle. For HG and HH, many studies focus on only one growth stage, such as post-weaning height[19], yearling height[20], and stature in adulthood[13,21]. However, to date, no GWAS has been performed to evaluate the genetic architecture of HG and HH through the whole growing period in cattle.

In Chinese Holstein cattle, HG and HH from birth to first calving have become routine measurements during the past 20 years, which provides valuable resources to study the complete growing period. Thus, the purpose of our study was to provide new information of genetic markers related to HG and HH through the entire growth and development process by using the GWAS approach in dairy cattle.

Materials and methods

Blood samples used in this study were collected along with the regular quarantine inspection in dairy farms, so no ethical approval was required.

Experimental animals

In this study, 3 datasets were involved (Table 1). First, HG and HH phenotypic data were collected on 38,602 Chinese Holstein cows born between 2002 and 2011, and raised on 27 dairy farms in Beijing, China. Second, to obtain reliable breeding value estimates, the available pedigree information was traced back to the 1960s. All estimated breeding values (EBVs) of 73,806 cattle in the pedigree, including the 38,602 cows, were calculated. At last, a total of 3,325 individuals out of the 73,806 cattle in pedigree, consisting of 2,942 cows and 383 bulls, were genotyped.

Table 1. Structures of the three types of datasets used in this study.

| Dataset          | No. of bulls | No. of cows | Total  | Interval of birth |
|------------------|--------------|-------------|--------|-------------------|
| Phenotypic data  | 0            | 38,602      | 38,602 | 2002–2011         |
| Pedigree data    | 2,006        | 71,800      | 73,806 | 1965–2011         |
| Genotypic data   | 383          | 2,942       | 3,325  | 1965–2011         |

https://doi.org/10.1371/journal.pone.0175971.t001
Phenotypic data
For each cow, HG and HH were measured simultaneously. Totally, 90,664 HG and 90,664 HH records from 38,602 cows (Table 1) were obtained, with an average of 3 records per cow. These measurements were collected roughly around 6, 12, 18 and 24 months, but not exactly across all the individuals, as shown in S1 Fig (RAW). Therefore, an adjustment was implemented firstly, using the Gompertz curve[22] modeled as follows:

\[
HG_{\text{age}} = 222.8 \exp(-0.8839 \exp(-0.002932 \text{age})) \quad (r^2 = 0.973) \tag{1}
\]

\[
HH_{\text{age}} = 144.1 \exp(-0.5856 \exp(-0.003522 \text{age})) \quad (r^2 = 0.890) \tag{2}
\]

Then, the adjusted coefficients were calculated to adjust the HG and HH to four age categories: 6, 12, 18, and 24 months, which corresponded to the measurements in 2–10, 8–16, 14–22, 20–28 months old, respectively. If two or more measurements from the same individual were contained in the same category, only the one closest to the adjusted month was kept. The basic statistics of the adjusted HG and HH were listed in Table 1. In this study, HG6, HH6, HG12, HH12, HG18, HH18, HG24, and HH24, were treated as eight traits for the subsequent analysis, as shown in S1 Fig (ADJUSTED).

Estimated breeding values
Using the adjusted age-trait phenotypes, we calculated the EBVs of HG and HH for all 73,806 cattle in the pedigree (Table 1). We used a multiple-trait animal model in the genetic evaluation, as follows:

\[
y_{ijk} = \mu + \text{Herd}_i + a_{jk} + e_{ijk}
\]

where \(y_{ijk}\) is the phenotype for the \(j^{th}\) cow from the \(i^{th}\) herd for the \(k^{th}\) trait (age-adjusted HG or HH); \(\mu\) is the overall mean; \(\text{Herd}_i\) is the effect of the \(i^{th}\) herd; \(a_{jk}\) is the additive genetic effect of the \(j^{th}\) cow and the \(k^{th}\) trait; and \(e_{ijk}\) is the vector of residual effects.

The DMU software[23] was used to estimate the EBVs. The Average Information Restricted Maximum Likelihood (AI-REML)[24] was applied to the variance components estimation. Furthermore, the EBVs of each trait were tested for normality of distribution by the Shapiro-Wilk Normality Test[25].

Genotypic data
Blood samples of 2,942 cows and semen samples of 383 bulls (Table 1) were used to extract the DNA by routine procedures. After that, the DNAs were quantified and genotyped using the BovineSNP50 v2 (Illumina, Inc., San Diego CA). The rate of missing genotypes was lower than 10% in all individuals. The SNPs were removed from dataset if it exhibited: 1) a call rate less than 90%; 2) minor allele frequency (MAF) less than 5%; 3) Fisher’s exact test \(p\)-value for Hardy-Weinberg Equilibrium (HWE) greater than or equal to \(1 \times 10^{-6}\); and 4) chromosome and position were unknown.

Of the total 54,609 SNPs, 42,307 on the 30 bovine chromosomes satisfied these selection criteria. The number of SNPs varied among chromosomes, with Bos Taurus autosome 1(BTA1) having the largest number of SNPs (2,711) and BTA 27 and BTA 28 having the fewest number of SNPs (778). The distribution of the minor allele frequency (MAF), heterozygous rate, linkage disequilibrium (LD) changed by marker distance and the average marker distance were shown in Fig 1.
Association analysis

The Fixed and random model Circuitous Probability Unification (FarmCPU) model [26] was used to perform the single-SNP analysis for the markers. Initially, a permutation test was applied to determine the significant threshold for the first iteration in FarmCPU. Subsequently, Bonferroni-corrected threshold probability of 0.05/N was implemented to verify the significance levels for the GWAS results, where N was the number of trait-SNP combinations tested.

Identification and annotation of candidate genes

Gene identification information was downloaded from the UC Santa Cruz genome annotation database (http://hgdownload.soe.ucsc.edu/goldenPath/bosTau6/database/) for the Nov. 2009 (Bos_taurus_UMD_3.1/bosTau6). Ingenuity Pathway Analysis (IPA) was performed to identify the potential pathway, network and functional annotation of candidate genes.

Results

Genetic parameters of the traits

Using the multiple-trait model, the variance components and the genetic parameters for each HG and HH trait were estimated (Tables 2 and 3). Obviously, both HH and HG are highly...
heritable, with similar heritability estimates. The heritabilities for HG varied from 0.33 ± 0.019 to 0.40 ± 0.018, while those for HH ranged from 0.35 ± 0.020 to 0.40 ± 0.036.

It is obvious that the phenotypic correlations were commonly higher between the same traits at different ages compared with the correlations between different traits. The phenotypic correlations between HG traits ranged from 0.35 ± 0.013 to 0.75 ± 0.005, while those between HH traits varied from 0.43 ± 0.013 to 0.71 ± 0.05. By contrast, the phenotypic correlations between HG and HH traits were lower, ranging from 0.24 ± 0.007 to 0.47 ± 0.006.

The genetic correlations have a similar trend as the phenotypic correlations. The genetic correlations between HG traits ranged from 0.35 ± 0.004 to 0.81 ± 0.001, and those between HH traits ranged from 0.54 ± 0.002 to 0.90 ± 0.001. The genetic correlations between HG and HH traits ranged from 0.30 ± 0.002 to 0.65 ± 0.001. The phenotypic and genetic correlations between HG12 and HG18 were 0.75 and 0.81, respectively, which were the highest among HG between different age categories. Interestingly, for HH, the highest phenotypic and genetic correlations were also from the correlation between HH12 and HH18, with the estimates of 0.71 and 0.90, respectively.

The number neighboring the trait indicates the adjusted-age of measurement, e.g., HG6 = heart girth at 6 months.

Heritabilities (on diagonal), genetic correlations (below the diagonal) and phenotypic correlations (above the diagonal) were derived using a multiple-trait animal model.

Estimates in parenthesis were the standard errors.

Table 2. Statistics for adjusted measurements of 38,602 individuals at 6, 12, 18, and 24 months after birth.

| Trait          | Age | N       | Mean   | S.D.  | $\sigma^2_a$ | $\sigma^2_p$ |
|----------------|-----|---------|--------|-------|-------------|-------------|
| Heart girth (cm) | 6   | 24,951  | 133.61 | 7.520 | 14.87 (0.948) | 45.13 (1.213) |
|                | 12  | 28,667  | 165.04 | 8.192 | 17.05 (0.876) | 42.85 (1.103) |
|                | 18  | 22,651  | 181.69 | 8.610 | 16.09 (1.014) | 45.46 (1.291) |
|                | 24  | 8,341   | 203.58 | 8.312 | 16.42 (1.815) | 46.93 (2.338) |
| Hip height (cm) | 6   | 24,951  | 105.94 | 4.305 | 4.81 (0.306)  | 13.79 (0.390) |
|                | 12  | 28,667  | 122.15 | 4.201 | 4.69 (0.252)  | 12.25 (0.318) |
|                | 18  | 22,651  | 131.89 | 4.286 | 4.86 (0.291)  | 12.93 (0.369) |
|                | 24  | 8,341   | 138.02 | 4.301 | 5.18 (0.514)  | 12.95 (0.657) |

Age: age of month
N: number of adjusted-records
S.D.: standard deviation
$\sigma^2_a$: the additive variance, with their standard errors (in parenthesis)
$\sigma^2_p$: the phenotypic variance, with their standard errors (in parenthesis)

https://doi.org/10.1371/journal.pone.0175971.t002

Table 3. Heritabilities, genetic and phenotypic correlations for Heart Girth (HG) and Hip Height (HH) in Chinese Holstein.

| Trait     | HH6   | HG6   | HH12  | HG12  | HH18  | HG18  | HH24  | HG24  |
|-----------|-------|-------|-------|-------|-------|-------|-------|-------|
| HG6       | 0.64 (0.001) | 0.33 (0.019) | 0.24 (0.007) | 0.43 (0.007) | 0.25 (0.009) | 0.42 (0.008) | 0.24 (0.014) | 0.35 (0.013) |
| HH12     | 0.56 (0.001) | 0.36 (0.002) | 0.38 (0.018) | 0.46 (0.005) | 0.71 (0.005) | 0.35 (0.006) | 0.50 (0.011) | 0.29 (0.013) |
| HG12     | 0.34 (0.002) | 0.43 (0.002) | 0.65 (0.001) | 0.40 (0.018) | 0.41 (0.006) | 0.75 (0.005) | 0.34 (0.012) | 0.50 (0.011) |
| HH18     | 0.54 (0.002) | 0.36 (0.002) | 0.90 (0.001) | 0.57 (0.001) | 0.38 (0.020) | 0.44 (0.006) | 0.57 (0.012) | 0.33 (0.013) |
| HG18     | 0.30 (0.002) | 0.39 (0.002) | 0.58 (0.001) | 0.81 (0.001) | 0.57 (0.001) | 0.35 (0.020) | 0.34 (0.013) | 0.51 (0.012) |
| HH24     | 0.62 (0.003) | 0.45 (0.003) | 0.67 (0.002) | 0.43 (0.003) | 0.63 (0.003) | 0.43 (0.004) | 0.40 (0.036) | 0.44 (0.010) |
| HG24     | 0.40 (0.004) | 0.35 (0.004) | 0.35 (0.004) | 0.53 (0.003) | 0.36 (0.004) | 0.56 (0.004) | 0.60 (0.003) | 0.35 (0.035) |

The number neighboring the trait indicates the adjusted-age of measurement, e.g., HG6 = heart girth at 6 months.

Heritabilities (on diagonal), genetic correlations (below the diagonal) and phenotypic correlations (above the diagonal) were derived using a multiple-trait animal model.

Estimates in parenthesis were the standard errors.

https://doi.org/10.1371/journal.pone.0175971.t003
GWAS

In our study, EBVs for HH and HG at 6, 12, 18, and 24 months old of the 3,325 cattle all passed the Shapiro-Wilk normality test and were used as eight traits in the GWAS. The GWAS results for the eight HG and HH traits were illustrated by Manhattan plots and Quantile-Quantile (Q-Q) plots in Fig 2. The Q-Q plots revealed that the population stratification was well controlled ($\lambda$ close to 1). In Manhattan plots, a total of 27 SNPs achieved genome-wide significance ($p < 2.38 \times 10^{-7}$) associated with at least one of the eight traits, with the $p$-value ranging from $2.14 \times 10^{-7}$ (BTB-00553140 for HH12) to $5.67 \times 10^{-13}$ (ARS-BFGL-NGS-83478 for HG24), and the MAF ranging from 0.08 (ARS-BFGL-NGS-36823) to 0.47 (BTB-01040075).

The 27 significant SNPs (listed in Table 4) were located on 14 chromosomes, that is, BTA 1, 3, 6, 8, 10, 11, 12, 14, 15, 19, 22, 25, 29 and X chromosome. On BTA6, four SNPs were detected to be significant, three of which were associated with traits HG12, HG18, HG24, respectively, and the other one (ARS-BFGL-NGS-79814) was related to both HH6 and HH18. Meanwhile, 4 SNPs on X chromosome were found significantly associated with HG6, HG12, HH18 and HH24, respectively.

Of these 27 SNPs, 7 were associated with HG and 21 were associated with HH, with one SNP Hapmap47137-BTA-95505 on BTA25 associated with both HG and HH at the age of 18 months. According to the four growth stages, 7 SNPs, 8 SNPs, 10 SNPs and 10 SNPs were found to be associated with body size at 6 months, 12 months, 18 months and 24 months, respectively. For each trait, at least 2 SNPs showed a significant association, especially HH18, which had 8 significantly relevant SNPs. Three HG-significant SNPs (ARS-BFGL-NGS-79814, ARS-BFGL-NGS-83478, and ARS-BFGL-NGS-14625) and three HH-significant SNPS (ARS-BFGL-NGS-76122, ARS-BFGL-NGS-111370, and ARS-BFGL-NGS-13985) were associated with more than one age category, especially ARS-BFGL-NGS-83478, which was the most significant SNP for all four HG traits.

On BTA3, three significant SNPs were detected. Two of them (ARS-BFGL-NGS-36823 and ARS-BFGL-NGS-111370) were only 144 kb away from each other but were not in a strong LD ($r^2 = 0.01$). The two SNPs were associated with HG (HH12) and HH (both HH6 and HH18), respectively. The other faraway SNP was associated with HH at 18 months old.

Identification and annotation of candidate genes

Candidate regions were defined as a ±200-kb distance from the significant SNPs identified by GWAS in current study. Then, 62 genes locating within or overlapping the candidate regions of 23 SNPs were discovered. Nevertheless, none known gene within candidate regions of the rest 4 SNPs were found, for which the nearest genes along the chromosome were listed instead. In total, 66 candidate genes were determined and presented in Table 5. Among these candidates, 22 genes were associated with HG, mainly distributed in 6 chromosomes, that is, BTA3, 6, 12, 22, 25 and X chromosome. Besides, 46 candidate genes were related to HH, locating on 12 chromosomes, including BTA1, 3, 6, 8, 10, 11, 14, 15, 19, 25, 29 and X chromosome. Two genes, ATPase Na+/K+ transporting subunit alpha 1 (ATP1A1) on BTA3 and calcium-regulated heat stable protein 1 (CARHSP1, also called CRHSP24) on BTA25, were associated with both HG and HH.

Using these 66 putative genes, the potential pathway and gene-to-gene interaction network were constructed by IPA. Totally, 25 significant pathways were identified ($p < 0.05$) (S2 Fig), most of which were obvious pathways related to development and growth factors, such as G12/13 alpha signaling, p70S6K signaling, PDGF signaling.

Seven relevant networks were constructed from these candidate genes for both HH and HG (S1 Table), and one interaction network of them containing 16 candidate genes had the high IPA score above 30 (IPA score = 34), which was related to developmental disorder as well as...
connective tissue development and function, with the v-myc avian myelocytomatosis viral oncogene homolog (MYC) gene at the center, including Jun proto-oncogene, AP-1 transcription factor subunit (JUN), dual specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A), etc. (Fig 3).

https://doi.org/10.1371/journal.pone.0175971.g002

Fig 2. Results of the genome-wide association studies. The strengths of genome-wide association studies (GWAS) are illustrated by the Manhattan plots on the left panel. The deviations of the signals from null hypothesis are illustrated as the Quantile-Quantile (QQ) plots on the right panel. The negative logarithms of the observed (y axis) and the expected (x axis) P values are plotted for each SNP (dot). GWAS were performed on HG and HH at age of 6, 12, 18, and 24 months separately. Each analysis is labeled as trait (HG or HH) and month on the far right. The number neighboring each trait indicates the age of measurement (e.g., HG6 = Heart Girth at 6 months). The dependent variables were the estimated breeding values (EBVs) calculated from 3,325 individuals, which were genotyped with 42,307 SNPs. The horizontal red lines on the Manhattan plots are the Bonferroni multiple test threshold corresponding type I error of 1%. The red lines on the QQ plots indicate the null hypothesis of no true association. Deviation from the expected P-value distribution is evident only in the tail area for each trait, suggesting that population stratification was adequately controlled. In total, these GWAS identified 27 SNPs significantly associated with HG and HH. The genes listed at the top of the Manhattan plots are reported as known genes which are highly related to growth or body size traits.
Functional annotation results revealed more than 500 functions belonging to 255 categories from IPA \((p < 0.05)\), out which 139 functions were annotated by two or more candidate genes. Developmental disorder and skeletal and muscular disorders were the two major function categories relating to body size, which contained 21 and 28 functions consisting of 16 and 18 genes, respectively (S2 and S3 Tables). Among these genes, 13 were both related to developmental disorder and skeletal and muscular disorders.

### Discussion

**Estimates of genetic parameters**

Heritabilities of HG and HH at four growth stages obtained in this study were moderated to high estimates, which were in the range of previous estimates reported in the literature. For example, the heritability of HG was reported 0.22–0.38 in Holstein\([4,28–31]\), and 0.35–0.47 in other breeds\([29,32]\); while the heritability of HH was 0.36–0.69 in Holstein\([4,28,29,31,33]\), and 0.35–0.74 in other breeds\([29,34]\). 

---

**Table 4. Significant SNPs identified for Heart Girth (HG) and Hip Height (HH) in Chinese Holstein.**

| SNP*          | Chr | Position (bp) | MAF  | HG  |   | HH  |   |
|---------------|-----|---------------|------|-----|---|-----|---|
|               |     |               | MAF  | HG  | 6 | 12  | 18 | 24 |
|               |     |               | MAF  | HG  | 6 | 12  | 18 | 24 |
| ARS-BFGL-NGS-76122 | 1   | 151590335     | 0.38 | 1.04E-09 | 8.06E-08 |
| ARS-BFGL-NGS-36823 | 3   | 26971936      | 0.08 | 4.51E-08 |
| ARS-BFGL-NGS-111370 | 3   | 27115580      | 0.19 | 4.24E-11 | 5.02E-08 |
| ARS-BFGL-NGS-110127 | 3   | 87809919      | 0.38 | 3.58E-08 |
| BTA-77986-no-rs  | 6   | 18898455      | 0.08 | 3.30E-08 |
| BTA-00262834   | 6   | 73008129      | 0.14 | 1.61E-07 |
| ARS-BFGL-NGS-82167 | 6   | 98952769      | 0.41 | 3.32E-09 |
| ARS-BFGL-NGS-79814 | 6   | 108635821     | 0.30 | 2.09E-07 | 5.26E-08 |
| BTA-92628-no-rs | 8   | 4546756       | 0.27 | 1.34E-07 |
| ARS-BFGL-NGS-39319 | 8   | 31409233      | 0.38 | 6.60E-08 |
| ARS-BFGL-NGS-8362 | 10  | 7943252       | 0.20 | 1.61E-07 |
| ARS-BFGL-NGS-33225 | 11  | 12478437      | 0.60 | 1.07E-07 |
| Hapmap8235-BTA-17587 | 12  | 23340411      | 0.49 | 7.99E-09 |
| ARS-BFGL-NGS-70821 | 14  | 3078843       | 0.24 | 1.46E-08 |
| BTA-00553140   | 14  | 13051936      | 0.12 | 2.14E-07 |
| BTA-01912619   | 15  | 20712027      | 0.44 | 9.85E-08 |
| BTA-08811-rs29022367 | 15  | 36997905      | 0.27 | 1.27E-07 |
| ARS-BFGL-NGS-88748 | 19  | 62087156      | 0.22 | 5.15E-08 |
| ARS-BFGL-NGS-83478 | 22  | 9271442       | 0.15 | 6.71E-09 | 1.48E-10 | 1.38E-10 | 5.67E-13 |
| Hapmap47137-BTA-95505 | 25  | 8060061      | 0.45 | 1.25E-09 |
| ARS-BFGL-NGS-13985 | 25  | 35071379      | 0.23 | 1.70E-07 | 2.42E-08 |
| ARS-BFGL-NGS-1643 | 25  | 37783875      | 0.40 | 8.11E-08 |
| ARS-BFGL-NGS-38901 | 29  | 400533        | 0.24 | 1.40E-07 |
| BTA-01040075   | X   | 5644107       | 0.47 | 5.67E-09 |
| ARS-BFGL-NGS-14625 | X   | 13611851      | 0.21 | 1.30E-08 | 1.26E-07 |
| ARS-BFGL-NGS-118669 | X   | 105267785     | 0.19 | 3.05E-08 |
| Hapmap58680-rs29019274 | X   | 112742618     | 0.36 | 6.73E-10 |

* All the SNPs listed were genome-wide significant in at least one trait (Bonferroni cutoff = 2.38E-7).

The numbers in the column headings below the trait names indicate the age of measurement, e.g., HG6 = heart girth at 6 months.

[https://doi.org/10.1371/journal.pone.0175971.t004](https://doi.org/10.1371/journal.pone.0175971.t004)
Table 5. List of candidate genes associated with Heart Girth (HG) and Hip Height (HH) in Chinese Holstein.

| Gene   | Chr | Pos            | Related SNPs                  | Associated traits |
|--------|-----|----------------|------------------------------|------------------|
| DYRK1A | 1   | 151320007−15141420 | ARS-BFGL-NGS-76122           | HH18, HH24       |
| ATP1A1 | 3   | 27002624−27035616  | ARS-BFGL-NGS-36823, ARS-BFGL-NGS-111370 | HG12, H6, H18   |
| IGSF3  | 3   | 26691950−26817441  | ARS-BFGL-NGS-36823           | H12              |
| MYSM1  | 3   | 87924698−87966272  | ARS-BFGL-NGS-110127          | H24              |
| JUN    | 3   | 87841042−87843089  | ARS-BFGL-NGS-110127          | H24              |
| PAPSS1 | 6   | 18740229−18854670  | BTA-77968-no-rs              | H6               |
| CEP135 | 6   | 73021614−73095955  | BTA-00262834                 | H18              |
| EXOC1  | 6   | 72512587−72989476  | BTA-00262834                 | H18              |
| HNRNPDL| 6   | 9893517−98990087   | ARS-BFGL-NGS-82167           | H24              |
| HNRNPD | 6   | 98916663−98933786  | ARS-BFGL-NGS-82167           | H24              |
| TMEM150C| 6   | 99050255−99068749  | ARS-BFGL-NGS-82167           | H24              |
| ENOPH1 | 6   | 98990766−99029323  | ARS-BFGL-NGS-82167           | H24              |
| HAUS3  | 6   | 10850717−10852817  | ARS-BFGL-NGS-79814           | H12, H18         |
| PIGG   | 8   | 108729415−108758212| ARS-BFGL-NGS-79814           | H12, H18         |
| FGGFRL1| 15  | 7956084−7965803   | ARS-BFGL-NGS-8362            | H12              |
| F2R    | 15  | 7992986−7995677   | ARS-BFGL-NGS-8362            | H12              |
| MRPL23 | 15  | 8139772−8140292   | ARS-BFGL-NGS-8362            | H12              |
| F2RL1  | 15  | 8038070−8052119   | ARS-BFGL-NGS-8362            | H12              |
| S100Z  | 15  | 7777598−7788321   | ARS-BFGL-NGS-8362            | H12              |
| AGGF1  | 15  | 8110496−8156758   | ARS-BFGL-NGS-8362            | H12              |
| CYP26B1| 15  | 12371180−12387041 | ARS-BFGL-NGS-33225           | H24              |
| STOML3 | 15  | 23280345−23308336 | Hapmap48235-BTA-17587        | H24              |
| NLRC3  | 15  | 23232200−23241391 | Hapmap48235-BTA-17587        | H24              |
| TSNARE1| 15  | 3054763−3171547   | ARS-BFGL-NGS-70821           | H18              |
| MYC*   | 17  | 13769242−13774438 | BTA-00553140                 | H12              |
| ARHGPAPA20 | 15 | 20907087−21120246 | BTA-01912619                 | H18              |
| RDX    | 15  | 20589670−20657445 | BTA-01912619                 | H18              |
| FOX1   | 15  | 20772619−20808021 | BTA-01912619                 | H18              |
| SOX6   | 15  | 36000420−37082361 | BTA-08811-rs29022367         | H12              |
| ABCA9  | 16  | 62099290−62151581 | ARS-BFGL-NGS-88748           | H6               |
| MGCI4105| 19  | 62207474−62223857 | ARS-BFGL-NGS-88748           | H6               |
| ARPP21*| 22  | 9674589−9681188   | ARS-BFGL-NGS-83478           | HG6, HG12, HG18, HG24|
| CARHSP1*| 25  | 7734585−7746003   | Hapmap47137-BTA-95505        | H18, H18         |
| YWHAG  | 25  | 34884279−34906639 | ARS-BFGL-NGS-13985           | H18, H24         |
| LRWD1  | 25  | 35096009−35102381 | ARS-BFGL-NGS-13985           | H18, H24         |
| POLR2J | 25  | 35091302−35095680 | ARS-BFGL-NGS-13985           | H18, H24         |
| UPK3B  | 25  | 35018574−35023605 | ARS-BFGL-NGS-13985           | H18, H24         |
| UPK3BL | 25  | 35034017−35039183 | ARS-BFGL-NGS-13985           | H18, H24         |
| DTX2   | 25  | 34979329−35015686 | ARS-BFGL-NGS-13985           | H18, H24         |
| ORAI2  | 25  | 35114124−35126594 | ARS-BFGL-NGS-13985           | H18, H24         |

(Continued)
It was well documented that the HG and HH had a medium correlation both genetically and phenotypically in bovine at adult stage[4,29,35,36]. Very few studies targeted on relationships between HG and HH at a younger age in cattle. A recent study reported a phenotypic (0.50) and a genetic (0.58) correlation between HG & HH at birth in Chinese Sanhe cattle[37], which fell into similar range with that in adulthood. In the Chinese Holstein, the phenotypic correlation between HG24 and HH24 was estimated to be 0.44, which was very close to an earlier report in adult Holstein in Netherlands(0.46)[4], and in adult Swiss Braunvieh (0.48) and Simmental (0.55)[35], as well as in adult Chinese Sanhe cattle (0.48). The genetic correlation between HG and HH in this study was 0.30–0.65, comparable with the reported range in dairy cattle (0.34–0.54)[4,29] and dual purpose cattle (0.47–0.58)[35–37].

### Candidate genes associated with HG and HH

In the present study, 66 genes nearby 27 significant SNPs were considered to be potential genes associated with body size traits. Among these genes, 22 locating on 6 chromosomes were associated with HG and 46 distributing on 12 chromosomes were associated with HH. Since these eight traits were not independent, it is expected that certain genes may be associated with two or more related body traits. 26 genes out of the 66 candidates were found regulating two or more traits, with two genes, *ATP1A1* and *CARHSP1*, being associated with not only HG but also HH. It is obvious that candidate genes discovered in this study relating to HH were even twice of those to HG.

All 66 candidate genes were used for pathway analysis and gene-to-gene interaction network construction. Totally 65 genes, except for *MGC134105*, which was failed to be annotated.

#### Table 5. (Continued)

| Gene  | Chr | Pos            | Related SNPs               | Associated traits |
|-------|-----|----------------|---------------------------|-------------------|
| ZP3   | 25  | 34960637–34968378 | ARS-BFGL-NGS-13985        | HH18, HH24        |
| PRKRP1| 25  | 35147760–35173861 | ARS-BFGL-NGS-13985        | HH18, HH24        |
| SSC4D | 25  | 34930217–34940479 | ARS-BFGL-NGS-13985        | HH18, HH24        |
| ALKBH4| 25  | 35102455–35109899 | ARS-BFGL-NGS-13985        | HH18, HH24        |
| TRRAP | 25  | 37807383–37895047 | ARS-BFGL-NGS-1643         | H12              |
| TMEM130| 25 | 37902689–37918785 | ARS-BFGL-NGS-1643         | H12              |
| KPNA7 | 25  | 37664542–37691692 | ARS-BFGL-NGS-1643         | H12              |
| ARPC1A| 25  | 37564390–37587556 | ARS-BFGL-NGS-1643         | H12              |
| PANX1 | 29  | 590732–644582    | ARS-BFGL-NGS-38901        | H12              |
| ZBTB33*| X   | 5043333–5050947  | BTF-01040075              | HH18              |
| SASH3 | X   | 13681411–13695775 | ARS-BFGL-NGS-14625        | HG6, HG12         |
| XPNPEP2| X  | 13645603–13672116 | ARS-BFGL-NGS-14625        | HG6, HG12         |
| UTP14A| X   | 13790537–13807201 | ARS-BFGL-NGS-14625        | HG6, HG12         |
| OCR1  | X   | 13452405–13501005 | ARS-BFGL-NGS-14625        | HG6, HG12         |
| ZDHHC9| X   | 13704736–13734121 | ARS-BFGL-NGS-14625        | HG6, HG12         |
| APLN  | X   | 13559123–13564931 | ARS-BFGL-NGS-14625        | HG6, HG12         |
| SMARCA1| X  | 13358542–13433876 | ARS-BFGL-NGS-14625        | HG6, HG12         |
| MAOB  | X   | 105235855–105359155 | ARS-BFGL-NGS-118669       | H12              |
| MAGEB16| X  | 112749378–112760963| Hapmap58680-rs29019274    | H12              |

Chr: Chromosome  
Pos: Position  
* The nearest gene but not locating within the candidate region of the SNP.

https://doi.org/10.1371/journal.pone.0175971.1005
in IPA database, were enriched in functions related to developmental disorder and skeletal abnormalities and were constructed to seven relevant networks (S1 Table). Furthermore, we compared candidate genes detected in our study with previously revealed genes and genomic related information and found nine out of these 66 candidate genes were already reported relevant to body size, growth and development in cattle, human, mice, and/or other animal species, which would be discussed following.
Three genes were related to HH12. The first one, transmembrane protein 130 (TMEM130) on BTA25 was also detected by Wu et al[38], that was associated with animal size and capacity in Chinese Holstein. Meanwhile, this gene is within a QTL region affecting calf size reported in Danish Holstein cattle[39]. In 2014, Zhao et al[40] has identified a selective signature in seven dairy and beef cattle breeds. For TMEM130 is one of the poorly characterized genes[41], the function of this gene was still unknown. The second one was MYC on BTA14. MYC was suggested as a crucial mediator of signals that determine organ and body size in mammals[42]. And as it was also shown in Fig 3, MYC cooperated with most of the genes in the significant network. In human, it was also reported that a gene implicated in c-myc regulation, FUBP3, was associated with height[43]. The third gene SOX6 (SRY-box 6) on BTA 15, was known to be important for cartilage formation[44] and skeletal development[45]. Deng et al. showed that SOX6 is a direct target of Yap1 for regulating chondrocyte differentiation in both skeletal development, postnatal growth, and bone repair[46]. And in human, SNPs in SOX6 were demonstrated to be significantly associated with the bone mass index and hip bone mineral density[47]. In chicken, a selective signature was detected maybe due to its essential roles in growth, development and reproduction[48,49].

Four genes were associated with HH24. The first one was DYRK1A on BTA 1, which was simultaneously related to HH18, was thought to be a causative factor in Down syndrome in human[50,51]. Point mutations in DYRK1A have been shown to be responsible for lower height. It was also documented in mice that mutations in DYRK1A could cause a decrease in body length and weight from birth to adulthood[52]. Furthermore, a selection signature was detected on this gene in European and African cattle populations, which explained the differences in the body size[53]. The second gene JUN, locating less than 32 kb from the significant SNP ARS-BFGL-NGS-110127 on BTA3 was reported to be required for axial skeletogenesis by regulating notochord survival and intervertebral disc formation in mice[54]. The third gene was Centrosomal protein 135 (CEP135) on BTA6, which is known as a centrosomal component and plays an important role in promoting centriole assembly and stability[55]. It was reported that CEP135 was one of the MCPH (autosomal recessive primary microcephaly; MIM251200) genes, and pathogenic mutations in this gene caused autosomal recessive primary microcephaly and reduced the growth rate in human, characterized by reduced brain and skull size with sloping forehead and short stature[56,57]. The fourth CYP26B1 (cytochrome P450, family 26, subfamily B, polypeptide 1) on BTA11, is one of the three CYP26 gene isoforms[58]. CYP26B1 was revealed to be responsible for the normal function of the growth plate and growing bones in zebrafish[59,60], mice[61] and human[62].

Furthermore, fibroblast growth factor receptor-like 1 (FGFRL1) on BTA6 was detected associated with HG12 and HG18. FGFRL1 is the most recently discovered member of the FGFR family[63], which plays important roles in cell adhesion[64], embryonic development of slow muscle fibers[65], and bone formation[66]. In mice, this gene was preferentially expressed in skeletal tissues and skeletal malformations, including axial and appendicular skeletal anomalies[67], or a slight reduction in the overall size of the skeleton[68] were observed in the targeted FGFRL1 knock-out mice. In human, Matoso et al. also reported that FGFRL1 gene was associated with overgrowth[69]. Besides, ATP1A1 on BTA3, as we aforementioned was associated with HG12, HH6 and HH18. ATP1A1 encodes the α1 isoform, the major isoform of the α subunit of Na+/K+-ATPase[70]. Polymorphisms in ATP1A1 could be potentially contributing to thermal tolerance[71], which further affected growth[72]. Additionally, Na+/K +-ATPase was proposed as a potential factor causing the unconventional secretion of Fibroblast Growth Factor 2 (FGF2)[70,73–75], which was known having profound effects on skeletal myoblasts proliferation in various animal systems. In chicken, selection for body size could significantly change the expression of FGF2[76,77]. And the abnormal of FGF2 would cause a
variety of skeletal malformations, including shortening and flattening of long bones and moderate macrocephaly[78] in mice.

Conclusions
In total, 27 SNPs targeting to 66 candidate genes were identified to be associated with HG and HH at 6, 12, 18 and 24 months. Since many complex traits have a similar architecture across different species[21], we have made an attempt to compare some of our GWAS candidate genes with the previous reports about the same genes and their association with growth. Our findings confirmed nine known genes involving in development or associated with body size in cattle, human and mice studies. We also identified new genes through network and function analyses, including ARPP21, AGGF, ZDHHC9, PDE6B, PIGG, MPDZ, OCRL, F2R, APLN, F2RL1, TRRAP. Further investigations were required to identify or confirm the functions of these candidate genes, especially in relation to growth in dairy cattle.

Supporting information
S1 Fig. Scatter plots and fitting curves of growth traits. The growth traits are measured as hear girth (HG) displayed on the top panel and hip height (HH) on the bottom panel. The left panel illustrates the raw measurements over actual age that the measurements are taken. The dots in blue are accepted for the adjustment on age. The dots in yellow are removed for the adjustment. The distributions of adjusted measurements on age are displayed as blue dots on the right panel. The fitting curves (red curves) are derived from all the raw measurements, including both the blue and yellow dots.

S2 Fig. Pathways identified in Ingenuity Pathway Analysis (IPA). The significances of the pathways are indicated by the bars as the negative log p values calculated by Fischer’s test. In total, 25 pathways were identified at threshold of p < 0.05 indicated by the yellow straight horizontal line. Most of the pathways are related to development and growth factors, such as G12/13 alpha signaling, p70S6K signaling, and PDGF signaling. The bars are shaded into gray for pathways where predictions are not currently possible in IPA. The yellow line with squares presents the ratio of the candidate genes found by current study to the total number of genes within each pathway. The scale of the ratio is indicated by the secondary vertical axis on the right.

S1 Table. Gene interaction networks constructed by IPA based on the candidate gene list.

S2 Table. The 21 developmental disorder related diseases and bio functions identified by IPA.

S3 Table. Skeletal and muscular disorders related 28 diseases and bio functions identified by IPA.

Acknowledgments
The authors thank Linda R. Klein for writing advice and editing the manuscript. Grateful acknowledgments are also made to Xiangyu Guo, Aoxing Liu for correcting the pedigree data.
The authors would like to thank Yali Hou from Beijing Institute of Genomics, Chinese Academy of Sciences, for her help on data analysis.

**Author Contributions**

**Conceptualization:** YW XZ QC GG XL SZ QZ ZZ.

**Data curation:** XZ GD GG SZ QZ XL.

**Formal analysis:** XZ QC YW ZZ.

**Funding acquisition:** YW XZ.

**Investigation:** XZ QC GD GG XL SZ.

**Methodology:** YW ZZ QC QZ.

**Project administration:** YW SZ QZ ZZ.

**Resources:** YW SZ QZ ZZ XL.

**Software:** XZ ZZ.

**Supervision:** YW ZZ.

**Visualization:** XZ ZZ.

**Writing – original draft:** XZ QC YW ZZ.

**Writing – review & editing:** YW XZ QC GD GG XL SZ QZ ZZ.

**References**

1. VanRaden PM. Invited review: selection on net merit to improve lifetime profit. J Dairy Sci. 2004; 87: 3125–3131. https://doi.org/10.3168/jds.S0022-0302(04)73447-5 PMID: 15377590

2. Heinrichs AJ, Losinger WC. Growth of Holstein Dairy Heifers in the United States. J Anim Sci. 1998; 76: 1254–1260. PMID: 9621930

3. Heinrichs AJ, Erb RN, Rogers GW, Cooper JB, Jones CM. Variability in Holstein heifer heart-girth measurements and comparison of prediction equations for live weight. Prev Vet Med. 2007; 78: 333–338. https://doi.org/10.1016/j.prevvetmed.2006.11.002 PMID: 17157398

4. Koenen EP, Groen AF. Genetic evaluation of body weight of lactating Holstein heifers using body measurements and conformation traits. J Dairy Sci. 1998; 81: 1709–1713. https://doi.org/10.3168/jds.S0022-0302(98)75738-8 PMID: 9684178

5. Lesosky M, Dumas S, Conradie I, Handel IG, Jennings A, Thumbi S, et al. A live weight-heart girth relationship for accurate dosing of east African shorthorn zebu cattle. Trop Anim Health Prod. 2012; 45: 311–6. https://doi.org/10.1007/s11250-012-0220-3 PMID: 22923040

6. Dechow CD, Rogers GW, Klei L, Lawlor TJ. Heritabilities and correlations among body condition score, dairy form and selected linear type traits. J Dairy Sci. 2003; 86: 2236–2242. https://doi.org/10.3168/jds.S0022-0302(03)73814-4 PMID: 1283661

7. Bardakcioglu HE, Sekkin S, Toplu HDO. Relationship between some teat and body measurements of Holstein cows and sub-clinical mastitis and milk yield. J Anim Vet Adv. 2011; 10: 1735–1737.

8. Dekkers JC. Commercial application of marker- and gene-assisted selection in livestock: strategies and lessons. Journal of Animal Science. 2004. pp. 313–328.

9. Liu YF, Zan LS, Cui WT, Xin YP, Jiao Y, Li K. Molecular cloning, characterization and association analysis of the promoter region of the bovine CDK6 gene. Genet Mol Res. 2011; 10: 1777–86. https://doi.org/10.4238/vol10-3gmr1466 PMID: 21948744

10. Yang YQ, Hui YT, Liu RY, Jiao RG, Gong Y, Zhang YY, et al. Molecular cloning, polymorphisms, and association analysis of the promoter region of the STAM2 gene in Wuchuan Black cattle. Genet Mol Res. 2013; 12: 3651–3661. https://doi.org/10.4238/2013.September.13.9 PMID: 24085429
11. Clempson AM, Pollott GE, Brickell JS, Bourne NE, Munce N, Wathes DC. Evidence that leptin genotype is associated with fertility, growth, and milk production in Holstein cows. J Dairy Sci. 2011; 94: 3618–3628. https://doi.org/10.3168/jds.2010-3626 PMID: 21700051

12. Meredith BK, Kearney FJ, Finlay EK, Bradley DG, Fahey AG, Berry DP, et al. Genome-wide associations for milk production and somatic cell score in Holstein-Friesian cattle in Ireland. BMC Genet. 2012; 13: 21. https://doi.org/10.1186/1471-2156-13-21 PMID: 2249276

13. Cole JB, Wiggans GR, Ma L, Sonstegard TS, Lawlor TJ, Crooker BA, et al. Genome-wide association analysis of thirty one production, health, reproduction and body conformation traits in contemporary U. S. Holstein cows. BMC Genomics. 2011; 12: 408. https://doi.org/10.1186/1471-2164-12-408 PMID: 21831322

14. Jiang L, Liu J, Sun D, Ma P, Ding X, Yu Y, et al. Genome-wide association studies for milk production traits in Chinese Holstein population. PLoS One. 2010; 5: e13661. https://doi.org/10.1371/journal.pone.0013661 PMID: 21048968

15. Buzanskas ME, Grossi DA, Ventura RV, Schenkel FS, Sargolzaei M, Meirelles SLC, et al. Genome-wide association for growth traits in Canchim beef cattle. PLoS One. 2014; 9: e94802. https://doi.org/10.1371/journal.pone.0094802 PMID: 24733441

16. Lu D, Miller S, Sargolzaei M, Kelly M, Vander Voort G, Caldwell T, et al. Genome-wide association analyses for growth and feed efficiency traits in beef cattle. J Anim Sci. 2014; 91: 3612–3633.

17. Huang W, Kirkpatrick BW, Rosa GJM, Khatib H. A genome-wide association study using selective DNA pooling identifies candidate markers for fertility in Holstein cattle. Anim Genet. 2010; 41: 570–8. https://doi.org/10.1111/j.1365-2052.2010.02046.x PMID: 20394602

18. Sahana G, Guldbrandtsen B, Bendixen C, Lund MS. Genome-wide association mapping for female fertility traits in Danish and Swedish Holstein cattle. Anim Genet. 2010; 41: 579–588. https://doi.org/10.1111/j.1365-2052.2010.02064.x PMID: 20477799

19. Bolormaa S, Goddard ME. Genome-wide association studies for net feed intake, body weight and hip height in beef cattle. Proceedings of the 18th Conference of the Association for the Advancement of Animal Breeding and Genetics. Barossa Valley, South Australia; 2009. pp. 125–128.

20. Jahuey-Martínez FJ, Parra-Bracamonte GM, Sifuentes-Rincón AM, Martínez-González JC, Gondro C, García-Pérez CA, et al. Genomewide association analysis of growth traits in Charolais beef cattle. J Anim Sci. 2016; 4570–4582. https://doi.org/10.2527/jas.2016-0359 PMID: 27898967

21. Pryce JE, Hayes BJ, Bolormaa S, Goddard ME. Polymorphic regions affecting human height also control stature in cattle. Genetics. 2011; 187: 981–984. https://doi.org/10.1534/genetics.110.123943 PMID: 21212230

22. Winsor CP. The Gompertz Curve as a Growth Curve. Proc Natl Acad Sci U S A. 1932; 18: 1–8. PMID: 16577417

23. Madsen P, Jensen J. A User’s Guide to DMU, A Package for Analysing Multivariate Mixed Models. Version 6, release 5.2. 2013;

24. Gilmour AR, Thompson R, Cullis BR. Average Information REML: An Efficient Algorithm for Variance Parameter Estimation in Linear Mixed Models. Biometrics. 1995; 51: 1440–1450.

25. Shapiro SS, Wilk MB. An Analysis of Variance Test for Normality (Complete Samples). Biometrika. 1965; 52: 591–611.

26. Liu X, Huang M, Fan B, Buckler ES, Zhang Z. Iterative Usage of Fixed and Random Effect Models for Powerful and Efficient Genome-Wide Association Studies. PLoS Genet. 2016; 12: e1005957. https://doi.org/10.1371/journal.pgen.1005957 PMID: 26991120

27. R Development Core Team. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing Vienna Austria. 2013.

28. Kadarmideen HN, Wegmann S. Genetic parameters for body condition score and its relationship with type and production traits in Swiss Holsteins. J Dairy Sci. 2003; 86: 3685–93. https://doi.org/10.3168/jds.S0022-0302(03)73974-5 PMID: 14672199

29. De Haas Y, Janss LLG, Kadarmideen HN. Genetic and phenotypic parameters for conformation and yield traits in three Swiss dairy cattle breeds. J Anim Breed Genet. 2007; 124: 12–19.

30. Gallo L, Carrier P, Cassandro M, Dal Zotto R, Bittante G. Test-day genetic analysis of condition score and heart girth in Holstein Friesian cows. J Dairy Sci. 2001; 84: 2321–6. https://doi.org/10.3168/jds.S0022-0302(01)74680-2 PMID: 11699465

31. Haile-Marium M, Nieuwhof GJ, Beard KT, Konstantinov K V., Hayes BJ. Comparison of heritabilities of dairy traits in Australian Holstein-Friesian cattle from genomic and pedigree data and implications for genomic evaluations. J Anim Breed Genet. 2013; 130: 20–31. https://doi.org/10.1111/j.1439-0388.2013.01001.x PMID: 23317062
32. Munim T, Oikawa T, Ibi T, Kunieda T. Genetic relationship of body measurement traits at early age with carcass traits in Japanese black cattle. Anim Sci J. 2013; 84: 206–212. https://doi.org/10.1111/asj.12005 PMID: 23480700
33. Bilal G, Cue R, Hayes J. Genetic and phenotypic associations of type traits and body condition score with dry matter intake, milk yield and number of breedings in first lactation Canadian Holstein cows. Can J Anim Sci. NRC Research Press; 2016; 3: 434–447.
34. Santana JL, Eler JP, Cucco DC, Bignardi AB, Ferraz JBS. Genetic associations between hip height, body conformation scores, and pregnancy probability at 14 months in Nelore cattle. Livest Sci. 2013; 154: 13–18.
35. Hagger C, Hofer A. Phenotypic and genetic relationships between wither height, heart girth and milk yield in the Swiss Braunvieh and Simmental breeds. Livest Prod Sci. 1991; 28: 265–271.
36. Yu Y, Li J, Gao H, Shangzong X. Estimation of Genetic Parameters for Partial Economic Traits in Sanhe Cattle. China Anim Husb Vet Med. 2010; 37: 106–108.
37. Wu X, Fang M, Liu L, Wang J, Ding X, et al. Genome wide association studies for body conformation traits in the Chinese Holstein cattle population. BMC Genomics. 2010; 3: 897. https://doi.org/10.1186/1471-2164-14-897 PMID: 26089079
38. Thomasen JR, Guldbrandtsen B, Sørensen P, Thomsen B, Lund MS. Quantitative trait loci affecting calving traits in Danish Holstein cattle. J Dairy Sci. 2008; 91: 2098–2105. https://doi.org/10.3168/jds.2007-0602 PMID: 18420641
39. Zhao F, McParland S, Kearney F, Du L, Berry DP. Detection of selection signatures in dairy and beef cattle using high-density genomic information. Genet Sel Evol. 2015; 47: 49. https://doi.org/10.1186/s12711-015-0127-3 PMID: 26582928
40. Lettre G, Jackson AU, Gieger C, Schumacher FR, Berndt SI, Sanna S, et al. Identification of ten loci associated with height highlights new biological pathways in human growth. Nat Genet. 2008; 40: 584–591. https://doi.org/10.1038/ng.125 PMID: 18391950
41. Smits P, Li P, Mandel J, Zhang Z, Deng JM, Behringer RR, et al. The transcription factors L-Sox5 and Sox6 are essential for cartilage formation. Dev Cell. 2001; 1: 277–290. PMID: 11702786
42. Liu YZ, Pei YF, Liu JF, Yang F, Guo Y, Zhang L, et al. Powerful bivariate Genome-wide association analyses suggest the SOX6 gene influencing both obesity and osteoporosis phenotypes in males. PLoS One. 2009; 4.
43. Fu W, Lee WR, Abasht B. Detection of genomic signatures of recent selection in commercial broiler chickens. BMC Genet. 2016; 17: 122. https://doi.org/10.1186/s12863-016-0430-1 PMID: 27565946
44. Liu Z, Sun C, Qu L, Wang K, Yang N. Genome-wide detection of selective signatures in chicken through high density SNPs. PLoS One. 2016; 11: e0166146. https://doi.org/10.1371/journal.pone.0166146 PMID: 27820849
45. Luco SM, Pohl D, Sell E, Wagner JD, Dyment DA, Daoud H, et al. Case report of novel DYRK1A mutations in 2 individuals with syndromic intellectual disability and a review of the literature. BMC Med Genet. 2016; 17: 15. https://doi.org/10.1186/s12881-016-0276-4 PMID: 26922654
46. Kopf T. Importance of gene dosage in controlling dendritic arbor formation during development. European Journal of Neuroscience. 2015. pp. 2234–2249. https://doi.org/10.1111/ejn.13002 PMID: 26108333
47. Fotaki V, Dierssen M, Alcántara S, Martinez S, Martí E, Casas C, et al. Dyrk1A haploinsufficiency affects viability and causes developmental delay and abnormal brain morphology in mice. Mol Cell Biol. 2002; 22: 6636–47. https://doi.org/10.1128/MCB.22.18.6636-6647.2002 PMID: 12192061
53. Randhawa IA, Khatkar M, Thomson P, Raadsma H. Composite selection signals can localize the trait specific genomic regions in multi-breed populations of cattle and sheep. BMC Genet. 2014; 15: 34. https://doi.org/10.1186/1471-2156-15-34 PMID: 24636660

54. Behrens A, Haigh J, Mechta-Grigoriou F, Nagy A, Yaniv M, Wagner EF. Impaired intervertebral disc formation in the absence of Jun. Development. 2003; 130: 103–109. PMID: 12441295

55. Kraatz S, Guichard P, Obbieni JM, Olieric N, Hatzopoulos GN, Hilbert M, et al. The Human Centriolar Protein CEP135 Contains a Two-Stranded Coiled-Coil Domain Critical for Microtubule Binding. Structure. 2016; 24: 1358–1371.

56. Hussain MS, Baig SM, Neumann S, Nürnberg G, Farooq M, Ahmad I, et al. A Truncating Mutation of CEP135 Causes Primary Microcephaly and Disturbed Centrosomal Function. Am J Hum Genet. 2012; 90: 871–878. https://doi.org/10.1016/j.ajhg.2012.03.016 PMID: 22521416

57. Farooq M, Fatima A, Mang Y, Hansen L, Kjaer KW, Baig SM, et al. A novel splice site mutation in CEP135 is associated with primary microcephaly in a Pakistani family. J Hum Genet. 2015; 61: 271–273. https://doi.org/10.1038/jhg.2015.138 PMID: 26657937

58. Ross AC, Zolfagharri R. Cytochrome P450s in the regulation of cellular retinoic acid metabolism. Annu Rev Nutr. 2011; 31: 65–87. https://doi.org/10.1146/annurev-nutr-072610-145127 PMID: 21529158

59. Ross AC, Zolfagharri R. Cytochrome P450s in the regulation of cellular retinoic acid metabolism. Annu Rev Nutr. 2011; 31: 65–87. https://doi.org/10.1146/annurev-nutr-072610-145127 PMID: 21529158

60. Reijntjes S, Rodaway A, Maden M. The retinoic acid metabolism gene, CYP26B1, patterns the cartilaginous cranial neural crest in zebrafish. Int J Dev Biol. 2003; 51: 351–360.

61. Minegishi Y, Sakai Y, Yahara Y, Akiyama H, Yoshikawa H, Hosokawa K, et al. Cyp26b1 within the growth plate regulates bone growth in juvenile mice. Biochem Biophys Res Commun. 2014; 454: 12–18. https://doi.org/10.1016/j.bbrc.2014.10.001 PMID: 25305491

62. Wen J, Lopes F, Soares G, Farrell SA, Nelson C, Qiao Y, et al. Phenotypic and functional consequences of haploinsufficiency of genes from exocyst and retinoic acid pathway due to a recurrent microdeletion of 2p13. 2. Orphanet J Rare Dis. 2013; 8: 100. https://doi.org/10.1186/1750-1172-8-100 PMID: 23837398

63. Trueb B. Biology of FGFRL1, the fifth fibroblast growth factor receptor. Cell Mol Life Sci. 2011; 68: 951–964. https://doi.org/10.1007/s00018-010-0576-3 PMID: 21080029

64. Yang X, Steinberg F, Zhuang L, Bessey R, Trueb B. Receptor FGFRL1 does not promote cell proliferation but induces cell adhesion. Int J Mol Med. 1899; 38: 30–38.

65. Amann R, Wyder S, Slavotinek AM, Trueb B. The FgfrL1 receptor is required for development of slow muscle fibers. Dev Biol. 2014; 394: 228–241. https://doi.org/10.1016/j.ydbio.2014.08.016 PMID: 25172430

66. Niu T, Liu N, Zhao M, Xie G, Zhang L, Li J, et al. Identification of a novel FGFRL1 MicroRNA target site polymorphism for bone mineral density in meta-analyses of genome-wide association studies. Hum Mol Genet. 2015; 24: 4710–4727. https://doi.org/10.1093/hmg/ddv144 PMID: 25941324

67. Catela C, Bilbao-Cortes D, Sbonimsky E, Krasios P, Rosenthal N, te Welscher P. Multiple congenital malformations of Wolf-Hirschhorn syndrome are recapitulated in Fgfrl1 null mice. Dis Model Mech. 2009; 2: 283–294. https://doi.org/10.1242/dmm.002287 PMID: 19383940

68. Bluteau G, Zhuang L, Amann R, Trueb B. Targeted disruption of the intracellular domain of receptor Fgfrl1 in mice. PLoS One. 2014; 9: e105210. https://doi.org/10.1371/journal.pone.0105210 PMID: 25126760

69. Matoso E, Ramos F, Ferrão J, Pires LM, Mascarenhas A, Melo JB, et al. Interstitial 287 kb deletion of 4p16. 3 including FGFRL1 gene associated with language impairment and overgrowth. Mol Genet. 2014; 7: 7. https://doi.org/10.1186/s13039-014-0087-2 PMID: 25506393

70. Dahl JP, Binda A, Canfield VA, Levenson R. Participation of Na,K-ATPase in FGF-2 secretion: Rescue of ouabain-inhibitable FGF-2 secretion by ouabain-resistant Na,K-ATPase alpha subunits. Biochemistry. 2000; 39: 14877–14883. PMID: 11101303

71. Kashyap N, Kumar P, Deshmukh B, Bhat S, Kumar A, Chauhan A, et al. Association of ATP1A1 gene polymorphism with thermotolerance in Tharparkar and Vrindavani cattle. Vet world. Veterinary World; 2015; 8: 892. https://doi.org/10.14202/vetworld.2015.892-897 PMID: 27047171

72. Tao S, Monteiro APA, Thompson IM, Hayen MJ, Dahl GE. Effect of late-gestation maternal heat stress on growth and immune function of dairy calves. J Dairy Sci. Elsevier; 2012; 95: 7128–7136.

73. Florkiewicz RZ, Anchin J, Baird A. The inhibition of fibroblast growth factor-2 export by cardenolides implies a novel function for the catalytic subunit of Na+,K+-ATPase. J Biol Chem. 1998; 273: 544–551. PMID: 9417114
74. Engling A, Backhaus R, Stegmayr C, Zehe C, Seelenmeyer C, Kehlenbach A, et al. Biosynthetic FGF-2 is targeted to non-lipid raft microdomains following translocation to the extracellular surface of CHO cells. J Cell Sci. 2002; 115: 3619–3631. PMID: 12186948

75. Zacherl S, La Venuta G, Müller H-M, Wegehingel S, Dimou E, Sehr P, et al. A direct role for ATP1A1 in unconventional secretion of fibroblast growth factor 2. J Biol Chem. 2015; 290: 3654–3665. https://doi.org/10.1074/jbc.M114.590067 PMID: 25533462

76. Lu Y, Chen S, Yang N. Expression and methylation of FGF2, TGF-β and their downstream mediators during different developmental stages of leg muscles in chicken. PLoS One. 2013; 8: e79495. https://doi.org/10.1371/journal.pone.0079495 PMID: 24260234

77. Lu Y, Bradley JS, Siegel PB, Yang N, Johnson SE. Selection for divergent body size alters rates of embryonic skeletal muscle formation and muscle gene expression patterns. Dev Growth Differ. 2015; 57: 614–624. https://doi.org/10.1111/dgd.12250 PMID: 26660844

78. Coffin JD, Florkiewicz RZ, Neumann J, Mort-Hopkins T, Dorn GW, Lightfoot P, et al. Abnormal bone growth and selective translational regulation in basic fibroblast growth factor (FGF-2) transgenic mice. Mol Biol Cell. 1995; 6: 1861–1873. PMID: 8590811