Genome-Wide Association Study Reveals the *PLAG1* Gene for Knuckle, Biceps and Shank Weight in Simmental Beef Cattle

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Abstract

Carcass traits of beef cattle have been genetically improved to increase yield of high quality meat. Genome-wide association study (GWAS) is a powerful method to identify genetic variants associated with carcass traits. For the 770K genotyped SNPs from 1141 Chinese Simmental cattle, we used the compressed mixed linear model (CMLM) to perform a genome-wide association study for knuckle, biceps and shank of beef carcass traits. Seventeen significantly associated SNPs were found, which are located on BTA6, BTA14 and BTA15. Interestingly, one pleiotropic quantitative trait nucleotide (QTN), named *BovineHD1400007259* ($p < 10^{-8}$) within the well-known gene region *PLAG1-CHCHD7* on BTA14, was found to govern variation of the knuckle, biceps and shank traits. The QTN accounted for 8.6% of phenotypic variance for biceps. In addition, 16 more SNPs distributed on BTA14 were detected as being associated with the carcass traits.

Introduction

Genome-wide association studies (GWAS) have enabled us to detect many genetic variants associated with quantitative traits in human [1] and farm animals [2]. The huge number of markers genotyped by the BovineSNP50K Bead Chip [3] provide an ample opportunity for us to perform GWAS in beef cattle. In the past few decades, numerous studies were carried out to map quantitative trait loci (QTLs) responsible for milk production, disease resistance [3, 4], reproduction [5], growth [5–8], meat quality [9–12] and carcass traits [12–15] in cattle. For carcass traits, previous studies had mapped several QTLs for carcass weights on BTA 6, 8 and 14, where one QTN was detected on BTA 6 and related to the encoding of *NCAPG Ile442Met* and the others were identified within gene *PLAG1-CHCHD7* in the region on BTA14 [16]. The association of gene *PLAG1* with carcass weight was found in Hanwoo steers [17]. Gene *NCAPG-L-CORL* was found to be associated with carcass traits in multi-breed beef cattle [18]. Both *PLAG1* and *NCAPG* were found to segregate in Japanese Black steers [6]. Meanwhile, *NCAPG* also plays a role in decreasing the subcutaneous fat thickness and increasing carcass yield [6]. Previous studies revealed that five SNPs within or near four genes, including *HS3ST1*, *DVL1*, *PRKCQ*

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and *HIVEP3*, were significantly associated with eye muscle area, backfat thickness and marbling score [19]. Also, several SNPs in genes *NCAPG* and *LCORL* on BTA6 were found to control hot carcass weight, ribeye area and lower adjusted fat thickness [18]. Several GWAS have been carried out for body compositions in Simmental beef cattle. For instance, the majority of significant SNPs within several haplotype blocks were found to be strongly associated with fore shank weight and triglyceride levels. Moreover, 36 SNPs with high linkage disequilibrium (LD) were detected to be associated with these traits in the *GNAQ* gene [11]. In addition, based on gene set analysis, the gamma aminobutyric acid ergic synapse pathway related to feed intake, weight gain and the non-alcoholic fatty liver disease pathway were found to be responsible for live weight and longissimus muscle area [20].

Statistical power of SNP detection in GWAS can be improved by increasing sample sizes, increasing marker density and choosing advanced statistical methods. In recent years, Bovine 10K, 35K and 77K, Bovine 50K chip had been widely applied for GWAS in cattle [6–10, 13, 16, 18, 19, 21–27]. Since 2014, high throughput Bovine 770K array has been used for GWAS to detect genetic variants associated with intramuscular fat deposition and composition in Nellore cattle [28] and with shank weight and triglyceride levels in Simmental beef cattle [11]. In terms of statistical methods, since PLINK [29] does not consider the confounding effects caused by relatedness among individuals on the power to detect QTL, EMMAX [30] and GAPIT [31] based on mixed linear models have been introduced as standard methods of GWAS for carcass traits [11, 16].

Carcass can be dissected into various body compositions, following the standard of slaughter practice. Body compositions consist of tissue pieces and organs. The separated pieces of meat or tissue not only differ in body part and edible quality, but their qualities are also determined by body morphological structure and construction. The knuckle, biceps and shank are important body compositions of carcass. In this study, we carried out GWAS on separated meat tissue pieces using BovineHD 770k in 1242 Simmental cattle. The objective of this study was to search for QTNs controlling knuckle, shank and biceps in Simmental cattle using the GAPIT software.

Materials and Methods

Ethics statement

All procedures strictly followed the guidelines developed by the China Council on Animal Care, and all protocols were approved by the Science Research Department of the Institute of Animal Science, Chinese Academy of Agricultural Sciences (CAAS) (Beijing, China). Samples were collected during regular quarantine inspections on the farms. All farm owners agreed the use of the animals and provided fields for this study.

Animal resource and phenotypes

The experimental population consisted of 1242 young Simmental cattle born in 2009–2014. This population was derived from the Simmental cattle resource population established in Ulgai, Xilingol league, Inner Mongolia of China (45˚N,118˚E). After weaning, cattle were moved to Beijing Jinweifuren fattening farm for feedlot finishing under the same feeding and management system. Cattles were well rested for 24 hours before slaughtering to relieve from fear and then made bloodletting after electrical stunning. All measurements of traits were in strict compliance with the Institutional Meat Purchase Specifications for fresh beef guidelines and GB/T 27643–2011. Experimental individuals were slaughtered at 16–18 months of age and at roughly the same time, after which beef carcasses were separated and carcass traits were measured.

For the three carcass traits of interest, knuckle is the quadriceps femoris mucle of beef cattle. Biceps is a piece of bovine fore leg muscle, which is composed of two short-fibred heads separated longitudinally by a thick internal tendon which stretches from the origin on the supraglenoid tubercle to the insertion on the medial radial tuberosity. Shank is meat part along the tibia to the femur in the back of hind leg. During slaughtering, the three traits were dissected following their definitions.

Sample genotyping and quality control

Collection of blood samples were operated along with regular quarantine inspection on the farms. Genomic DNA was extracted from blood samples using the TIANamp Blood DNA Kit (Tiangen Biotech Company limited, Beijing, China), DNAs with an A260/280 ratio ranging between 1.8 and 2.0 were subjected to further analysis. The IlluminaBovineHDBeadChip with 774,660 SNPs were chosen for individual genotyping. The SNPs were uniformly distributed on the whole bovine genome with a mean inter-marker space of 3.43 kb. SNP chips were scanned and analyzed using the GenomeStudio software package.

For the quality control, individual cattle and their SNP genotypes were handled by the PLINK software package (v1.9) [29]: First, all individuals with missing SNP genotypes $\geq 10\%$ or Mendelian error rate more than 2% were excluded. All SNPs with either less than 90% call rates, minor allele frequencies (MAF) of less than 5%, less than 10% genotype appearances or Hardy-Weinberg equilibrium test p-value $< 10^{-7}$ were removed from the data. Part of the data are uploaded to Dryad, Digital Repository: doi: 10.5061/dryad.4qc06.

Statistical analysis

Phenotypic correction. Prior to GWAS, phenotypes were corrected for some systemic environmental factors using the following linear model,

$$y = \mu + b_1 \cdot ew + b_2 \cdot fd + b_3 \cdot sy + b_4 \cdot cs + e$$

where y is a vector of phenotypic values of the trait under consideration, μ is the population mean, ew is the live weight of calf entering fattening farm and b_1 is effect of live weight, fd is the number of fattening days, b_2 is effect of fattening days, sy is slaughtering year, b_3 is the slaughtering year effect, cs is calving seasons including the three levels (November to April, May to August and September to October), b_4 is the effect of cs, and e is a vector of the residuals. The estimated residuals, denoted by y^*, were eventually used as the adjusted phenotypic values in the subsequent association study.

Association test. To take into account the effects of population stratification and unequal relatedness among individuals, the "Q+K" model of Yu et al. (2011) was used to detect the ith QTNs for $i = 1, \ldots, N$, where N is the number of markers in the genome. The "Q + K" model is

$$y^* = Xb_i + Qv + Zu + e$$

where y^* is the vector of phenotypic values adjusted by non-genetic effects as described early, b_i is additive genetic effect of the ith SNP, X is a vector of genotype indicator variables whose value is defined as 0, 1, or 2 for the three possible genotypes, G_{11}, G_{12} and G_{22}, respectively, v is vector of population structure effects, Q is the principal component matrix calculated from part of the SNPs, u is a vector of polygenetic effects assumed to be $N(0, K\sigma_\alpha^2)$ distributed with K being the genomic relationship matrix from all genotyped SNPs and σ_α^2 the additive variance, Z is a design matrix for the random effects (identity matrix in this case), and e is the vector of residual errors following a $N(0, I\sigma_e^2)$ distribution with an unknown error variance σ_e^2.

Essentially, the "Q+K" model is a mixed linear model (MLM), where population stratification and SNP effects are treated as fixed effects and the polygenic effects are considered random. GWAS with this model in its original form is computationally very expensive because the mixed model needs to be solved for each SNP tested. By compressing the genomic relationship matrix, MLM has been transformed into a "sire model" that contains only a few effects of individual groups and such a sire model can greatly improve the computational efficiency. The GAPIT software package [31] implemented the "sire model" GWAS using the REML algorithm.

After SNP effects were estimated and their standard deviations calculated by GAPIT, a student-t test statistic was calculated using

$$t_i = \frac{|\hat{b}_i|}{S_{b_i}}$$

for the ith SNP, where $\hat{b}_i$ is the estimated SNP effect and S_{b_i} is the standard error. Bonferroni correction for multiple tests was adopted to draw the critical value of the p-value. A SNP was claimed to be significant at the 0.05 genome Type 1 error level if the p-value for the ith SNP was smaller than $0.05/N$, where N is the total number of SNPs. The positive false discovery rate (FDR) across association tests between a SNP and the trait of interest was estimated using the Benjamini-Hochberg method [31]. Finally, the UMD3.1 genome assembly was used to search for genes in the database (http://www.animalgenome.org).

Results

Phenotype and quality control

Means (standard deviations), coefficients of variations, heritabilities and pairwise phenotypic correlations between traits (knuckle, biceps and shank) are presented in Table 1. These traits have intermediate heritabilities. These traits are highly correlated with the highest correlation occurring between knuckle and shank. After quality control, seven individuals were removed because they had more than 10% missing genotypes and 94 additional individuals were deleted due to missing phenotypes of the analyzed traits. A total of 59,621, 14,366 and 9,142 SNPs were deleted with call rates $< 90\%$, MAF $< 5\%$ and HWE $< 10^{-7}$, respectively. As a result, only 588,150 SNPs on 1141 individuals were used for the GWAS analysis.

Population stratification assessment

Based on the kinship matrix calculated from 50,000 randomly sampled SNPs, the principal component analysis (PCA) showed that the first two principal components (PC1 and PC2) contributed more than 85% of the marker variation. The population was clustered into four groups, as displayed in Fig 1, and hence there was significant population stratification. The first two PCs were considered as covariates and incorporated into the "Q+K" mixed model in our study.

Table 1. Means (standard deviations), coefficients of variations, heritabilities and pairwise phenotypic correlations between traits knuckle, biceps and shank

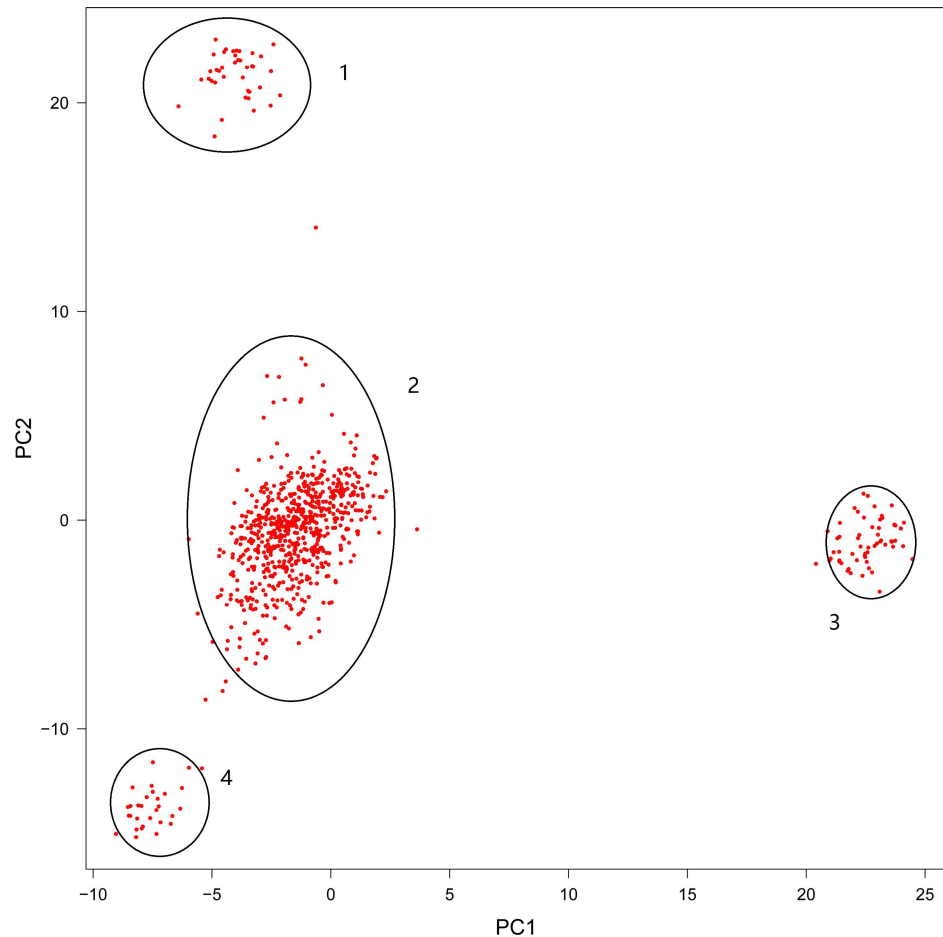| Trait	N	Mean (kg)	Coefficient of variation	Heritability	Knuckle	Biceps	Shank
Knuckle	1141	9.26(1.47)	6.30	0.44	1		
Biceps	1136	1.07(0.30)	3.57	0.39	0.44	1	
Shank	1141	8.07(1.19)	6.78	0.51	0.87	0.45	1

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Fig 1. Principal component (PC) plot. The second principal component (PC2) is plotted against the first principal component (PC1). The four separated groups are marked by circles with number 1,2,3 and 4.

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GWAS analysis

With 588,150 markers, the threshold for the p-value was $p = 0.05/588150 = 8.50\times10^{-8}$ and $-\log10$ $(p) = 7.07$ after Bonferroni correction. Fig 1 shows the Manhattan plots of $-\log10(p)$ against genome locations of the SNP markers. Significant QTNs were claimed at the peaks that passed over the critical value in the Manhattan plot. Based on this criterion, a total of 9 SNPs were significantly associated with the three traits, of which one for knuckle, six for biceps and two for shank. The GWAS results are listed in Table 2. Additionally, 8 SNPs at high peaks whose p-values were close to the threshold p-value were also considered as candidate QTNs. The FDRs of these QTNs were all $<$ 0.05, except for four QTNs occupying the following four positions: *BovineHD0600009630*, *BTB-00622352*, *BovineHD1400007161* and *BovineHD1400007161*.

Knuckle. A total of 4 QTNs were mapped at *Hapmap26308-BTC-057761* and *BovineHD0600009630* on BTA6, at *BovineHD1400007259* on BTA14 and at *BTB-00622352* on BTA15. Only *BovineHD1400007259* was identified as a QTN that is 25 Mb away from the initial marker on BTA14, because the p-value exceeded the corrected threshold. The most significant QTN resides within gene *PLAG1*. The two SNPs on BTA6 are candidate QTNs belonging to genes *LAP3* and *CCSER1*, respectively. *BTB-00622352* is located in the region where genes *LOC100848982* and *LOC514367* overlapp. The largest QTN at *BovineHD1400007259* only explained 2.9% phenotypic variation.

Table 2. SNPs identified for Knuckle, biceps and shank.

Trait	Chromosome	QTN	p-value	FDR adjust p-value	Located gene
Knuckle	14	BovineHD1400007259	4.73E-08	0.0317	PLAG1
	6	Hapmap26308-BTC-057761	2.36E-07	0.0790	LAP3
	6	BovineHD0600009630	2.36E-07	0.1242	CCSER1
	15	BTB-00622352	7.52E-07	0.1259	-
Biceps	14	BovineHD1400007259	2.76E-08	0.0071	PLAG1
	6	BovineHD0600010952	4.85E-08	0.0071	-
	6	BovineHD0600010950	5.07E-08	0.0071	-
	6	BovineHD0600010951	5.40E-08	0.0071	-
	6	BovineHD0600010953	6.26E-08	0.0071	-
	6	BovineHD0600010956	7.06E-08	0.0071	-
	6	BovineHD0600011178	5.45E-07	0.0367	-
	6	Hapmap30134-BTC-034283	1.90E-07	0.0165	-
	6	BovineHD4100004565	4.71E-07	0.0357	-
	6	BTA-75902-no-rs	7.27E-07	0.0441	KCNIP4
Shank	14	BovineHD1400007259	9.48E-09	0.0064	PLAG1
	14	BovineHD4100011330	7.82E-08	0.0262	-
	14	BovineHD1400007161	6.88E-07	0.1537	-

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Biceps. A total of 10 SNPs were associated with biceps, representing the most SNPs associated with a single trait. Five SNPs on BTA6 and one SNP on BTA14 were identified as the QTNs, while four SNPs as QTN candidates on BTA6. As displayed in the middle panel of Fig 2, five QTNs were mapped at *BovineHD0600010952, BovineHD0600010950, BovineHD0600010951, BovineHD0600010953* and *BovineHD0600010956*. The last one overlaps with a peak on BTA6. Notably, we found *BovineHD1400007259* and *BTA-75902-no-rs* overlapped with genes *PLAG1* and *KCNIP4*. The phenotypic variance explained by the 10 detected SNPs ranged from 0.009 to 0.086.

Shank. A QTN mapped at *BovineHD1400007259* resides within gene *PLAG1*. Two other QTNs are 24 Mb and 26 Mb away from the initial marker on BTA 14 and very close to gene *PLAG1*.

In summary, a QTN at *BovineHD1400007259* was found to be simultaneously associated with knuckle, biceps and shank, showing a typical pleiotropic effect of of the QTN in gene *PLAG1*. All detected QTNs contributed relatively low proportions of the phenotypic variation, but QTN BovineHD1400007259 explained over 8.61% of the phenotypic variance for biceps.

Discussion

In this study, we performed a GWAS for body compositions including knuckle, biceps and shank using high density Bovine HD BeadChip in Chinese Simmental cattle. Previous studies using PLINK [29] have located some QTLs for carcass weight, eye muscle area, back fat thickness and marbling score [19]. Hot carcass weight, adjusted fat thickness, marbling, rib eye area and slice shear force have also been analyzed with PLINK to obtain estimated breeding values (EBV) in genetic evaluation using multivariate animal models [18]. Similarly, polygene effects have also been incorporated into the animal models to control the polygenic background for detection of single SNPs and the interaction between two SNPs to map cold carcass weight, longissimus muscle area, rib thickness, subcutaneous fat thickness, beef marbling standard and carcass yield [6]. The methods adopted above did not consider the effects of the SNPs outside the test SNP on the statistical power of QTL detection. The EMMAX software [30] was
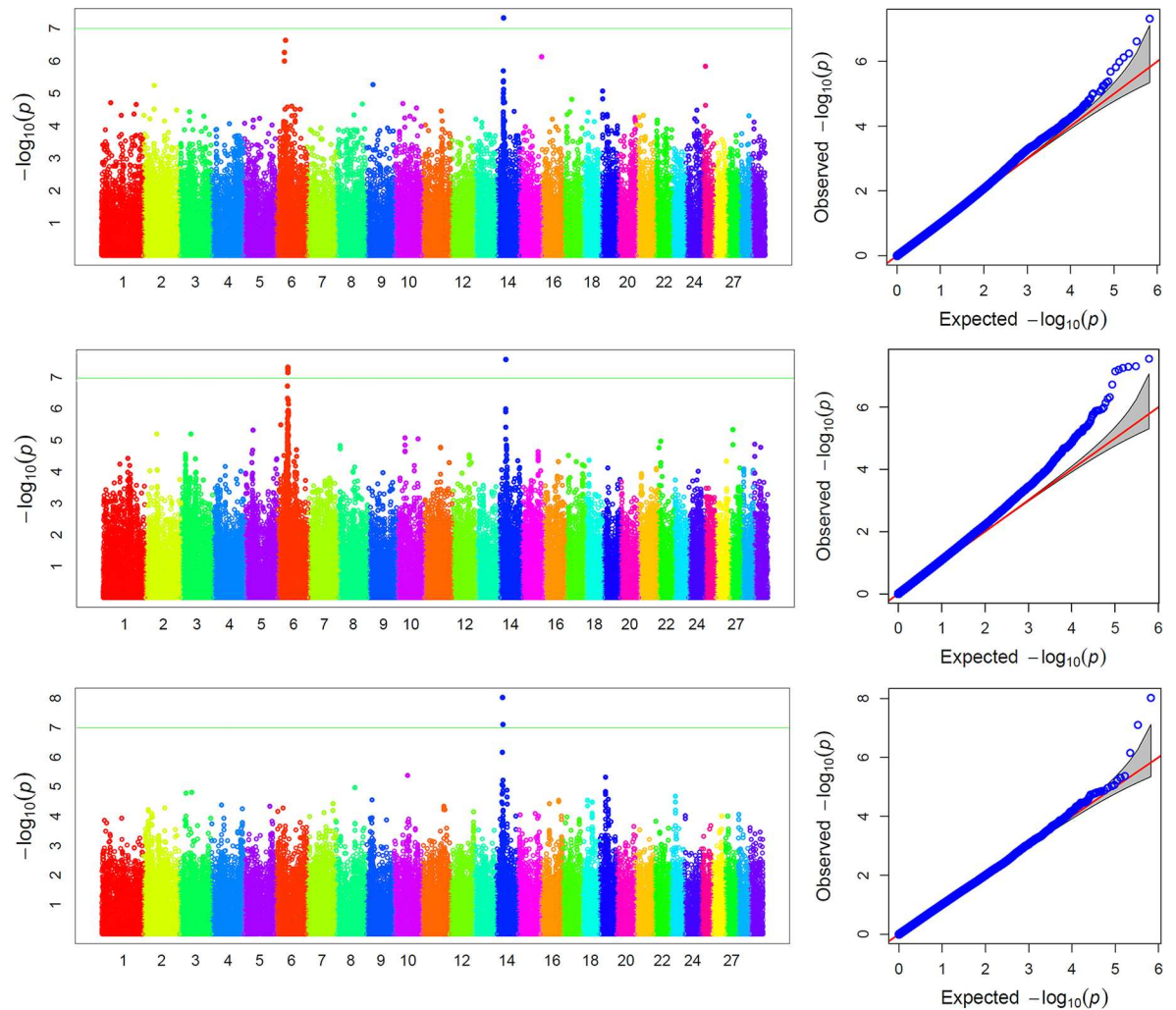
Fig 2. Manhattan plot of -log10 (p) from the beef cattle GWAS for three carcass traits (knuckle, biceps and shank). Chromosomes 1–29 are color coded separately. The horizontal reference line indicated the genome-wise significance levels (–log$_{10}$(p) = 7.07).

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therefore adopted to analyze carcass weight [16] by modifying PLINK with genomic best linear unbiased prediction. Notably, causal variants or QTNs associated with knuckle, biceps and shank in Chinese Simmental cattle have been efficiently detected here using the compressed MLM implemented in GAPIT [31].

Importantly, the most significant SNP for knuckle, biceps and shank weights is located within the *PLAG1* locus. Actually, several QTNs have been found to be located in the well-known *PLAG1-CHCHD7* region [5, 6, 13, 16, 17] on BAT14. In human, *PLAG1* is related to the pleiomorphic adenoma gene [32–34] and regulates human height [35]. Additionally, the *PLAG1* knock-out mice are 30% smaller than their wild-type litter mates at birth [36]. Also in cattle, Karim et al.[37] reported two QTNs influencing on bovine stature and mapped them to the *PLAG1-CHCHD7* intergenic region. Carcass weights have been proven to be associated with gene *PLAG1* by GWAS in Japanese Black cattle [16] and Hanwoo steers [17]. In addition to QTNs related to *PLAG1*, some QTNs were found to be responsible for changes in knuckle, biceps and shank in Chinese Simmental cattle, which carry gene *LAP3*, *CCSER1* and *KCNIP4*.

As an aminopeptidase that catalyzes the exclusion of amino acids from intracellular proteins and peptides, *LAP3* (*leucine aminopeptidase 3*) is highly articulated in the bovine pineal gland, kidney, skin, intestine, mammary, and adipose tissues (UGID: 1959690, UniGene Bt.56962). *LAP3* has been confirmed to be also associated with milk production and body size in Charolais [37–39]. For the knuckle, biceps and shank traits in Chinese Simmental Cattle, no significant SNPs that harbor the two genes *LCORL* and *NCAPG* have been detected, but two QTN candidates at *Hapmap30134-BTC-034283* and *BovineHD4100004565* for biceps are in high LD with some SNPs within gene *NCAPG*.

Supporting Information

S1 File. GWAS result of knuckle. The GWAS result of the SNPs that are nominally significant (P<0.05) for biceps, including: SNP names, chromosome, position, p-value and FDR. (CSV)

S2 File. GWAS result of biceps. The GWAS result of the SNPs that are nominally significant (P<0.05) for biceps, including: SNP names, chromosome, position, p-value and FDR. (CSV)

S3 File. GWAS result of shank. The GWAS result of the SNPs that are nominally significant (P<0.05) for biceps, including: SNP names, chromosome, position, p-value and FDR. (CSV)

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Writing – original draft: YS LX YC LZ HG BZ HN WZ JX.

Writing – review & editing: YS XG JL YC LZ.

References

1. Cordell HJ. Detecting gene-gene interactions that underlie human diseases. Nature reviews Genetics. 2009; 10(6):392–404. doi: 10.1038/nrg2579 PMID: 19434077

2. Goddard ME, Hayes BJ. Mapping genes for complex traits in domestic animals and their use in breeding programmes. Nature reviews Genetics. 2009; 10(6):381–91. doi: 10.1038/nrg2575 PMID: 19448663

3. Matukumalli LK, Lawley CT, Schnabel RD, Taylor JF, Allan MF, Heaton MP, et al. Development and characterization of a high density SNP genotyping assay for cattle. PLoS One. 2009; 4(4):e5350. doi: 10.1371/journal.pone.0005350 PMID: 19390634

4. Pant SD, Schenkel FS, Verschoor CP, You Q, Kelton DF, Moore SS, et al. A principal component regression based genome wide analysis approach reveals the presence of a novel QTL on BTA7 for MAP resistance in holstein cattle. Genomics. 2010; 95(3):176–82. doi: 10.1016/j.ygeno.2010.01.001 PMID: 20060464

5. Crispim AC, Kelly MJ, Guimaraes SE, Fonseca e Silva F, Fortes MR, Wenceslau RR, et al. Multi-Trait GWAS and New Candidate Genes Annotation for Growth Curve Parameters in Brahman Cattle. PLoS One. 2015; 10(10):e0139906. doi: 10.1371/journal.pone.0139906 PMID: 26445451

6. Hoshiba H, Setoguchi K, Watanabe T, Kinoshita A, Mizoshita K, Sugimoto Y, et al. Comparison of the effects explained by variations in the bovine PLAG1 and NCAPG genes on daily body weight gain, linear skeletal measurements and carcass traits in Japanese Black steers from a progeny testing program. Anim Sci J. 2013; 84(7):529–34. doi: 10.1111/asj.12033 PMID: 23607392

7. Snelling WM, Allan MF, Keele JW, Kuehn LA, McDaneld T, Smith TP, et al. Genome-wide association study of growth in crossbred beef cattle. J Anim Sci. 2010; 88(3):837–48. doi: 10.2527/jas.2009-2257 PMID: 19966163

8. Bolormaa S, Hayes BJ, Savin K, Hawken R, Barendse W, Arthur PF, et al. Genome-wide association studies for feedlot and growth traits in cattle. J Anim Sci. 2011; 89(6):1684–97. doi: 10.2527/jas.2010-3079 PMID: 21239664

9. Barendse W. Haplotype analysis improved evidence for candidate genes for intramuscular fat percentage from a genome wide association study of cattle. PLoS One. 2011; 6(12):e29601. doi: 10.1371/journal.pone.0029601 PMID: 22216329

10. Dang CG, Cho SH, Sharma A, Kim HC, Jeon GJ, Yeon SH, et al. Genome-wide Association Study for Warner-Bratzler Shear Force and Sensory Traits in Hanwoo (Korean Cattle). Asian-Australas J Anim Sci. 2014; 27(9):1328–35. doi: 10.5713/ajas.2013.13690 PMID: 25178377

11. Wu Y, Fan H, Wang Y, Zhang L, Gao X, Chen Y, et al. Genome-wide association studies using haplotypes and individual SNPs in Simmental cattle. PLoS One. 2014; 9(10):e109330. doi: 10.1371/journal.pone.0109330 PMID: 25330174

12. Weng ZQ, Su HL, Saatchi M, Lee J, Thomas MG, Dunkelberger JR, et al. Genome-wide association study of growth and body composition traits in Brangus beef cattle. Livestock Science. 2016; 183:4–11.

13. Littlejohn M, Grala T, Sanders K, Walker C, Waghorn G, Macdonald K, et al. Genetic variation in PLAG1 associates with early life body weight and peripubertal weight and growth in Bos taurus. Anim Genet. 2012; 43(5):591–4. doi: 10.1111/j.1365-2052.2011.02293.x PMID: 22497486

14. Bolormaa S, Pryce JE, Kemper K, Savin K, Hayes BJ, Barendse W, et al. Accuracy of prediction of genomic breeding values for residual feed intake and carcass and meat quality traits in Bos taurus, Bos indicus, and composite beef cattle. J Anim Sci. 2013; 91(7):3088–104. doi: 10.2527/jas.2012-5827 PMID: 23658330

15. Sasaki S, Ibi T, Matsuhashi T, Takeda K, Ikeda S, Sugimoto M, et al. Genetic variants in the upstream region of activin receptor IIA are associated with female fertility in Japanese Black cattle. BMC Genet. 2015; 16:123. doi: 10.1186/s12863-015-0282-0 PMID: 26486459

16. Nishimura S, Watanabe T, Mizoshita K, Tatsuda K, Fujita T, Watanabe N, et al. Genome-wide association study identified three major QTL for carcass weight including the PLAG1-CHCHD7 QTN for stature in Japanese Black cattle. BMC Genet. 2012; 13:40. doi: 10.1186/1471-2156-13-40 PMID: 22607022

17. Seung Hwan Lee. BHC, Dajeong Lim, Cedric Gondro, Young Min Cho, Chang Gwon Dang, Aditi Sharma, Gul Won Jang, Kyung Tai Lee, Duhak Yoon, Hak Kyo Lee, Seong Heum Yeon, Boh Suk Yang, Hee Seol Kang, Seong Koo Hong. Genome-Wide Association Study Identifies Major Loci for Carcass Weight on BTA14 in Hanwoo (Korean Cattle). PLoS ONE. 2013.

18. Lindholm-Perry AK, Sexten AK, Kuehn LA, Smith TP, King DA, Shackelford SD, et al. Association, effects and validation of polymorphisms within the NCAPG—LCORL locus located on BTA6 with feed intake, gain, meat and carcass traits in beef cattle. BMC Genet. 2011; 12:103. doi: 10.1186/1471-2156-12-103 PMID: 22168586

19. Kim Y, Ryu J, Woo J, Kim JB, Kim CY, Lee C. Genome-wide association study reveals five nucleotide sequence variants for carcass traits in beef cattle. Anim Genet. 2011; 42(4):361–5. doi: 10.1111/j.1365-2052.2010.02156.x PMID: 21749418

20. Fan H, Wu Y, Zhou X, Xia J, Zhang W, Song Y, et al. Pathway-Based Genome-Wide Association Studies for Two Meat Production Traits in Simmental Cattle. Scientific reports. 2015; 5:18389. doi: 10.1038/srep18389 PMID: 26672757

21. Browning SR, Browning BL. Rapid and accurate haplotype phasing and missing-data inference for whole-genome association studies by use of localized haplotype clustering. American journal of human genetics. 2007; 81(5):1084–97. doi: 10.1086/521987 PMID: 17924348

22. 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(10):e13661. doi: 10.1371/journal.pone.0013661 PMID: 21048968

23. Pryce JE, Hayes B, Bolormaa S, Goddard ME. Polymorphic Regions Affecting Human Height Also Control Stature in Cattle. Genetics. 2011; 187(3):981–4. doi: 10.1534/genetics.110.123943 PMID: 21212230

24. Wu X, Fang M, Liu L, Wang S, Liu J, Ding X, et al. Genome wide association studies for body conformation traits in the Chinese Holstein cattle population. BMC Genomics. 2013; 14:897. doi: 10.1186/1471-2164-14-897 PMID: 24341352

25. Lee SH, Choi BH, Lim D, Gondro C, Cho YM, Dang CG, et al. Genome-wide association study identifies major loci for carcass weight on BTA14 in Hanwoo (Korean cattle). PLoS One. 2013; 8(10):e74677. doi: 10.1371/journal.pone.0074677 PMID: 24116007

26. Bolormaa S, Neto LR, Zhang YD, Bunch RJ, Harrison BE, Goddard ME, et al. A genome-wide association study of meat and carcass traits in Australian cattle. J Anim Sci. 2011; 89(8):2297–309. doi: 10.2527/jas.2010-3138 PMID: 21421834

27. Wang H, Jiang L, Wang W, Zhang S, Yin Z, Zhang Q, et al. Associations between variants of the HAL gene and milk production traits in Chinese Holstein cows. BMC Genet. 2014; 15:125. doi: 10.1186/s12863-014-0125-4 PMID: 25421803

28. Cesar AS, Regitano LC, Mourao GB, Tullio RR, Lanna DP, Nassu RT, et al. Genome-wide association study for intramuscular fat deposition and composition in Nellore cattle. BMC Genet. 2014; 15:39. doi: 10.1186/1471-2156-15-39 PMID: 24666668

29. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. American journal of human genetics. 2007; 81(3):559–75. doi: 10.1086/519795 PMID: 17701901

30. Zhou X, Stephens M. Genome-wide efficient mixed-model analysis for association studies. Nature genetics. 2012; 44(7):821–4. doi: 10.1038/ng.2310 PMID: 22706312

31. Lipka AE, Tian F, Wang Q, Peiffer J, Li M, Bradbury PJ, et al. GAPIT: genome association and prediction integrated tool. Bioinformatics. 2012; 28(18):2397–9. doi: 10.1093/bioinformatics/bts444 PMID: 22796960

32. Hibbard MK, Kozakewich HP, Dal Cin P, Sciot R, Tan XL, Xiao S, et al. PLAG1 fusion oncogenes in lipoblastoma. Cancer Res. 2000; 60(17):4869–72. PMID: 10987300

33. Hensen K, Braem C, Declercq J, Van Dyck F, Dewerchin M, Fiette L, et al. Targeted disruption of the murine Plag1 proto-oncogene causes growth retardation and reduced fertility. Dev Growth Differ. 2004; 46(5):459–70. doi: 10.1111/j.1440-169x.2004.00762.x PMID: 15606491

34. Martins C, Fonseca I, Roque L, Pereira T, Ribeiro C, Bullerdiek J, et al. PLAG1 gene alterations in salivary gland pleomorphic adenoma and carcinoma ex-pleomorphic adenoma: a combined study using chromosome banding, in situ hybridization and immunocytochemistry. Modern pathology: an official journal of the United States and Canadian Academy of Pathology, Inc. 2005; 18(8):1048–55.

35. Gudbjartsson DF, Walters GB, Thorleifsson G, Stefansson H, Halldorsson BV, Zusmanovich P, et al. Many sequence variants affecting diversity of adult human height. Nature genetics. 2008; 40(5):609–15. doi: 10.1038/ng.122 PMID: 18391951

36. Hayes BJ, Pryce J, Chamberlain AJ, Bowman PJ, Goddard ME. Genetic architecture of complex traits and accuracy of genomic prediction: coat colour, milk-fat percentage, and type in Holstein cattle as contrasting model traits. Plos Genet. 2010; 6(9):e1001139. doi: 10.1371/journal.pgen.1001139 PMID: 20927186

37. Karim L, Takeda H, Lin L, Druet T, Arias JA, Baurain D, et al. Variants modulating the expression of a chromosome domain encompassing PLAG1 influence bovine stature. Nature genetics. 2011; 43(5):405–13. doi: 10.1038/ng.814 PMID: 21516082

38. Gautier M, Flori L, Riebler A, Jaffrezic F, Laloe D, Gut I, et al. A whole genome Bayesian scan for adaptive genetic divergence in West African cattle. BMC Genomics. 2009; 10:550. doi: 10.1186/1471-2164-10-550 PMID: 19930592

39. Xu L, Bickhart DM, Cole JB, Schroeder SG, Song J, Tassell CP, et al. Genomic signatures reveal new evidences for selection of important traits in domestic cattle. Molecular biology and evolution. 2015; 32(3):711–25. doi: 10.1093/molbev/msu333 PMID: 25431480