Multiple association analysis of loci and candidate genes that regulate body size at three growth stages in Simmental beef cattle

Bingxing An
Chinese Academy of Agricultural Sciences Institute of Animal Science
Lei Xu
Chiese Academy of Agricultural Sciences Institute of Animal Science
Jiangwei Xia
Westlake Institute for Advanced Study Insititute of Basic Medical Sciences
Xiaoqiao Wang
Chinese Academy of Agricultural Sciences Institute of Animal Science
Jian Miao
Chinese Academy of Agriculture Science Institute of Animal Sciences
Tianpeng Chang
Chinese Academy of Agricultural Sciences Institute of Animal Science
Meihua Song
Zhuang Yuan Veterinaria Station of Qixia city
JunQing Ni
Heibei Live Breeding Workstation
Lingyang Xu
Chinese Academy of Agricultural Sciences Institute of Animal Science
Lupei Zhang
Chinese Academy of Agricultural Sciences Institute of Animal Science
Junya Li
Chinese Academy of Agricultural Sciences Institute of Animal Science
Huijiang Gao (gaohuijiang@caas.cn)
Chinese Academy of Agricultural Sciences Institute of Animal Sciences https://orcid.org/0000-0002-5502-2528

Research article

Keywords: Simmental Beef cattle; Genome-wide association studies; Body size; Candidate genes; Bovine HD 770K SNP

Posted Date: March 5th, 2020

DOI: https://doi.org/10.21203/rs.2.13744/v5

Version of Record: A version of this preprint was published at BMC Genetics on March 14th, 2020. See the published version at https://doi.org/10.1186/s12863-020-0837-6.

Abstract

Background: Body size traits as one of the main breeding selection criteria was widely used to monitor cattle growth and to evaluate the selection response. In this study, body size was defined as body height (BH), body length (BL), hip height (HH), heart size (HS), abdominal size (AS), and cannon bone size (CS). We performed genome-wide association studies (GWAS) of these traits over the course of three growth stages (6, 12 and 18 months after birth) using three statistical models, single-trait GWAS, multi-trait GWAS and LONG-GWAS. The Illumina Bovine HD 770K BeadChip was used to identify genomic single nucleotide polymorphisms (SNPs) in 1217 individuals. Results: In total, 19, 29, and 10 significant SNPs were identified by the three models, respectively. Among these, 21 genes were promising candidate genes, including SOX2, SNRPD1, RASGEF1B, EFNA5, PTBP1, SNX9, SV2C, PKDCC, SYNDIG1, AKR1E2, and PRIM2 identified by single-trait analysis; SLC37A1, LAP3, PCDH7, MANEA, and LHCGR identified by multi-trait analysis; and P2RY1, MPZL1, LINGO2, CMIP, and WSCD1 identified by LONG-GWAS. Conclusions: Multiple association analysis was performed for six growth traits at each growth stage. These findings offer valuable insights for the further investigation of potential genetic mechanism of growth traits in Simmental beef cattle.

Background

In China, the production of beef cattle is a very important agribusiness, and the Simmental breed accounts for more than 70% of beef-producing herds. Beef producers use body size to monitor the growth of each animal throughout the fattening period [1, 2], as this trait is an indicator of cattle [3] and longevity [4]. Monitoring the development of each animal can help to increase profits by enhancing the efficiency of feed and management [5–7]. Besides in human, additive genetic effect explains 81% of the variation in height [8], and the heritability for both hip height (HH) and height size (HS) is 0.33-0.4 in cattle [9]. Bouwman et al. reported that the lead variants in significant regions explained at most 13.8% of the phenotypic variance in their meta-analysis of 58,265 cattle from 17 populations [10]. In addition, daily body linear measurements, specifically body height (BH) and HH, two highly reliable and accurate indicators for body weight, are easier to obtained than body weight [11]. Furthermore, good depth of HS in cattle is a sign of good feet and leg conformation [12], and dairy cows with higher HH will subsequently have better milk performance [13]. However, there is little information on the molecular mechanisms of body size traits in Chinese Simmental beef cattle.

Genome-wide association studies (GWAS) are robust statistical tools are that broadly identify candidate genes with significant SNPs involved in production traits [14–16], growth traits [17, 18], carcass quality traits and fertility traits [19, 20]. In beef cattle, various SNPs, genes, and haplotype blocks have been found to associate with growth, however the current GWAS-based studies focus mainly on only one growth parameter [21], such as the weaning size [22], yearling weight or stature upon slaughter [23]. Furthermore, loci controlling growth traits may be variable in different growth stages, and some loci may control traits throughout the lifetime of the animal [24]. Therefore, it is more reasonable to perform GWAS on growth traits on each stage separately. Multi-trait methods have been developed to increase statistical power and to identify pleiotropic loci in GWAS [25]. The longitudinal GWAS consider all time points when assessing whether significant SNPs associate with trait development over time [26], and this method is powerful for identifying these time-dependent and consistent loci [27]. We performed multiple trait GWAS (multi-trait GWAS) and longitudinal-GWAS (LONG-GWAS) based on single-trait analysis. Multi-trait GWAS and LONG-GWAS were not replacements for the single-trait GWAS; instead, they complemented single-trait GWAS. Thus, understanding the genetic mechanisms involved in inter-individual variations in body size may provide new insights that can help to manipulate cattle growth and production.

In this study, six body size traits were routine measured from the time cattle entering farm to slaughter, which provides valuable resources to study the complete growing period. The aim of our study was to comprehensively analyze of candidate genes and QTL regions associated with growth traits by conducting three GWAS approaches in Simmental beef cattle. Our findings offer valuable insights for the further investigation of the potential mechanism of growth traits in Simmental beef cattle.

Results

Population stratification assessment

Figure 1 shows that the population stratification of the Simmental population based on the PCA was divided into five separate clusters, demonstrating an obvious stratification in the reference population. The population stratification caused by different genetic influences and breeding conditions, as potential confounders, was corrected by significance testing. We summarized the genome-wide significant and suggestive SNP regions for these traits in Figure 2. The Manhattan plots and quantile-quantile (Q-Q) plots are shown in Figure S1 and Figure S2, while Q-Q plots suggested that there was no inflation or systematic bias in this research. Most points were near diagonal line because the GWAS model sufficiently considered the population structure and only a few SNPs were associated with the target traits. Meanwhile, the genomic inflation factors (λ) at each trait ranged from 1.03 to 1.10, indicating consistent consequence with PCA.

Summary of significant loci identified by three approaches

Briefly, we found 45, 66 and 19 SNPs significantly were associated with six body size traits by single-trait GWAS, multi-trait GWAS and LONG-GWAS, respectively. There were no significant loci for single-trait BH6 (single-trait GWAS for BH at 6 months after birth, and so forth), single-trait AS6, single-trait CS6, and LONG-AS. In addition, ten SNPs were associated with at least one of the six traits and eight SNPs were strongly associated with these traits in at least one of the three models. While according to their biological functions, 21 suggestive genes were selected as candidate genes and some details of them, including their positions in the genome, the nearest reported genes, the minor allele frequencies (MAF) and the p values are listed in Table 2.

SNPs identified by single-trait GWAS

A total of 45 SNPs achieved genome-wide significance associated with at least one of the six traits, with the p-value ranging from 9.99×10^{-6} (BovineHD0700018941 for BL18) to 2.11×10^{-8} (BovineHD0200014365 for HS18), and the MAF ranging from 0.003 (BovineHD0700034055) to 0.497 (BovineHD2600012755). Among these, two SNPs near *SOX2* (SRY-Box 2) on BTA1 were also identified in the liver and stomach [28]. On BTA2, two loci in the 0.69 Kb region were significantly associated with single-trait HS18 and one of them (BovineHD0200014365) was also associated with multi-trait18. On BTA6, three SNPs in the 0.46 Mb region were located near *RASGEF1B* (RasGEF Domain Family Member 1B). On BTA7, one SNP (BovineHD0700034055) was associated with single-trait HS12 and single-trait AS12, namely *EFNA5* and another SNP (BovineHD0700012966) was associated with single-trait HH6 and multi-trait6, namely *PTBP1*. On BTA10, one SNP (BovineHD1000002378) was associated with single-trait BH6 and multi-trait6, namely *SV2C*. While on BTA11, four loci in 0.04 Mb region were associated with the single-trait HS12 and one of them (BovineHD1100007368) was also associated with multi-trait12, all of which were near *PKDCC*. On BTA13, SNP BovineHD1300012489 and BovineHD1300012894 were associated with single-trait BH18 and single-trait AS18, respectively. These two SNPs also strongly associated with multi-trait18. On BTA23, two SNPs were found within *PRIM2*.

SNPs identified by multi-trait GWAS

Multi-trait GWAS identified 66 SNPs within or near 36 genes that were distributed on 21 chromosomes, including 8 loci that were also identified by single-trait GWAS, which indicated that these loci suggestively regulate the development of the body growth (Table 2). Among these, two promising loci within the 11.4 Kb region were detected, namely *SLC37A1*. On BTA6, two suggestive loci were detected, one near *LAP3* that associated with multi-trait12 and another near *PCDH7*. Two genome-wide loci were identified within the 0.02 Mb region of *MANEA* on BTA9. Furthermore, four promising loci were detected within in 0.04 Mb region of *LHCGR* on BTA11, and 13 loci were identified within the 0.03Mb region of *AKR1E2* on BTA13, which were also detected by single-trait analysis.

SNPs identified by LONG-GWAS

Nineteen loci were identified by LONG-GWAS, including three significant loci on 12 chromosomes (Table 2). Among them, two suggestive loci in the 8.3Kb region near *P2RY1* were detected, whereas the other (BovineHD0100032742) was also associated with LONG-CS, LONG-BH and LONG-HH. Another two loci in the 4.2 Kb region near *MPZL1* were identified, and the latter SNP (BTA-68271-no-rs) was also associated with LONG-HH and LONG-BH. Furthermore, one suggestive SNP near *LINGO2* on BTA8 was associated with LONG-HS. In addition, four promising loci in the 0.03 Kb region were associated with LONG-HH, namely *CMIP*, a key gene in the T-cell signaling pathway. On BTA19, a suggestive locus near *WSCD1* was associated with LONG-CS. No loci were associated with AS in our research.

Discussion

We performed single-trait GWAS, multi-trait GWAS, and LONG-GWAS for six body size traits on three growth stages in Simmental beef cattle. However, the three methods yielded different results with few shared loci. The reason for this discrepancy was likely due to the restricted dataset in single-trait GWAS and LONG-GWAS analyses. One universal phenomenon that cannot be ignored is that growth traits are controlled by multiple genes [29], and each method had its specific advantages in the identification of distinct loci. For example, single-trait GWAS is robust in detecting trait-specific QTLs and multi-trait GWAS is efficient for mapping pleiotropic QTLs [30], whereas LONG-GWAS can improve the detection power for time-dependent and consistent loci [31]. Thus, combining these three GWAS methods was expected to markedly improve the analysis the genetic mechanism of the body traits of beef cattle. In addition, since many complex traits have a similar architecture across diverse species [7], which prompted us to compare some of our significant genes with the previous reports that investigate the same genes and their involvements in growth. As a result, 21 suggestive genes were considered as candidate genes that were involved in the growth of cattle, swine, mice, and humans.

Candidate Genes Identified by Single-trait GWAS

On BTA1, two SNPs were near *SOX2* (SRY-Box 2), which encodes a transcription factor involved in the regulation of embryonic development [32, 33]. The paralog of this gene is *SOX17*, which positively affects the growth traits of cattle, and the conserved regions of this gene in human genome is closely related to body development [34]. On BTA2, two SNPs were near *SNRPD1* (small nuclear ribonucleoprotein D1 polypeptide), which is a member of the ghrelin receptor family, and the encoded protein is involved in zinc-dependent signaling in epithelial tissue [35]. On BTA6, variations near *RASGEF1B* (RasGEF domain family member 1B) were associated with body height [36]. In addition, body height was positively correlated with calcium absorption, which is an important determinant of calcium balance [37]. On BTA7, a SNP near *EFNA5* (ephrin A5) was associated with two traits (HS and AS) at the same stage. It was also identified as a candidate gene for growth traits in broiler chicken [38]. Another SNP near *PTBP1* (polypyrimidine tract binding protein 1) was found to show genome-wide association with growth traits at 6 months by both single-trait and multi-trait GWAS. Its expression level determined the release of insulin, thereby affecting development [39]. On BTA9, a SNP (BovineHD0900027283) located in *SNX9* (sorting nexin 9), as olfactory receptor, was associated with growth traits in Yorkshire pigs [40]. The SNP near *SV2C* (synaptic vesicle glycoprotein 2C) was associated with BH by both single-trait and multi-trait GWAS. This gene was reported to modulate dopamine release in neural and endocrine cells [41]. On BTA11, *PKDCC* (protein kinase domain containing, cytoplasmic) was associated with HS in both single-trait and multi-trait analyses. This gene was involved in the maintenance of bone density in humans [42]. On BTA13, *SYNDIG1* (synapse differentiation inducing 1) has been reported as a factor influencing the final weight and backfat thickness of Landrace pigs [43], whereas the *AKR1E2* (aldo-keto reductase family 1 member E2) variant was associated with body length and girth in cattle [44]. On BTA23, the *PRIM2* (DNA primase subunit 2) was associated with body weight and trait changes in pigs [45, 46].

Single-trait GWAS versus multi-trait GWAS

Multiple-trait analysis of linkage experiments has been reported to significantly enhance the power to detect common SNPs across traits [47, 48]. Therefore, we used multi-trait analysis to complement single-trait GWAS rather than to replace it. In the single-trait GWAS, the minimum *p* values for the three stages were 3.90E-07, 9.92E-07 and 2.11E-08 respectively. These three values decreased to 8.23E-13, 1.73E-09 and 3.84E-10 in the multi-trait GWAS, respectively. We also identified several critical loci as follows. On BTA1, the *SLC37A1* (solute carrier family 37, member A1) gene, which encodes a glucose-6-phosphate transporter that is involved in the homeostasis of blood glucose [49], was found to be the best candidate gene for modifying milk production traits [50]. On BTA6, *LAP3* (leucine aminopeptidase 3) was reported to play critical roles in the regulation of hormone levels and protein maturation. Another study demonstrated that putative regulatory elements in the *PCDH7* (protocadherin 7) gene may have roles in residual feed intake in Nelore cattle [51]. In addition, *MANEA* (mannosidase endo-alpha), which has roles in proteolysis, was associated with the birth weight of Canchim beef cattle [52]. On BTA11, a mutation in the *LHCGR* (luteinizing hormone/choriogonadotropin receptor) gene was as the cause of empty follicle syndrome [53].

Single-trait GWAS versus LONG-GWAS

We used LONG-GWAS, which involved multiple phenotype measurements for each individual [24]. One disadvantage of this method was that incorporating all data may have overwhelmed significant signal, that is, if QTL effects varied during the different stages [54]. In this study, these time-specific expressed QTLs identified by the single-trait and multi-trait GWAS were not detected by LONG-GWAS. However LONG-GWAS also detected some significant functional loci as follows. On BTA1, *P2RY1* (purinergic receptor P2Y1), a candidate gene that affects the serum Ca^{2+}, encoded for a member of the family of G protein-coupled receptor family that works as receptor for extracellular ATP and ADP [55]. On BTA3, the *MPZL1* (myelin protein zero like 1) gene could significantly enhance the migratory and metastatic potential of hepatocellular carcinoma cells by phosphorylating and activating the pro-metastatic protein [56]. Besides on BTA8, *LINGO2* (leucine rich repeat and Ig domain containing 2), which is expressed in the central nervous system of mouse embryos, has been reported to associate with the body mass in a cohort of elderly Swedes [57]. On BTA18, *CMIP* (C-Maf inducing protein), a candidate gene for reading-related traits, was also associated with plasma lipoprotein levels [58]. Moreover, *WSCD1* (WSC domain containing 1), which encodes a protein with sulfotransferase activity that participates in the metabolism of glucose, was a candidate gene for feed efficiency and feeding behaviors in the White Duroc × Erhualian F2 population [59].

Conclusions

In conclusion, a total of 58 SNPs corresponding to 21 genes were found to be associated with six body size traits at 6, 12 and 18 months. Future studies characterizing the functions of these candidate genes may uncover the genetic architecture underlying the body size traits in Simmental beef cattle.

Methods

(see Methods in the Supplementary Files)

Abbreviations

GWAS: Genome-wide association study; BH: body height; BL: body length;

HH: hip height; HS: heart size; AS: abdominal size; CS: cannon bone size;

SNP: single nucleotide polymorphism; QTL: quantitative trait loci;

PCA: principal components analysis; CMLM: compressed mixed linear model;

GAPIT: Genome Association and Prediction Integrated Tool; BTA: Bos Taurus chromosome

LONG-GWAS: longitudinal GWAS

Declarations

Acknowledgements

Not applicable.

Authors' contributions

BA wrote, and JL and HG revised the paper. LX1 and JX performed experiments. MS, JN, and LZ collected the GWAS data. XW, JM, TC, and LX2 interpreted the data. All authors reviewed and approved the final manuscript.

Funding

This work was supported by funds from the National Natural Science Foundations of China (NSFC 31872975, 31472079 and 31702084) and Cattle Breeding Innovative Research Team (cxgc-ias-03). NSFC funded the collection, analysis, and interpretation of data. Cattle Breeding Innovative Research Team supported statistical analysis and writing of paper.

Availability of data and materials

We confirm that all raw data underlying our findings are publicly available without restriction. Data is available from the Dryad Digital Repository: doi:10.5061/dryad.4qc06.

Ethics approval and consent to participate

All animals used in the study were treated following the guidelines established by the Council of China Animal Welfare. Protocols of the experiments were approved by the Science Research Department of the Institute of Animal Sciences, Chinese Academy of Agricultural Sciences (CAAS), Beijing, China (approval number: RNL09/07). The use of animals and private land in this study was approved in written by their respective legal owners.

Consent for publication

Not applicable.

Competing interests

There are no competing interests.

Author details

1Institute of Animal Science, Chinese Academy of Agricultural Science, Beijing, 100193, China.

2Institute of Basic Medical Sciences, Westlake Institute for Advanced Study, Hangzhou, 310000, China.

3Zhuang Yuan Veterinary Station of Qixia city, Yantai, 265300, China

4Heibei Livestock Breeding Workstation, Shijiazhuang, 050061, China.

References

1. Heinrichs AJ, Losinger WC. Growth of Holstein Dairy Heifers in the United States. J Anim Sci. 1998;76:1254–60.
2. Heinrichs AJ, Erb HN, 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;78:333–8.
3. Lund T, Miglior F, Dekkers JCM, Burnside EB. Genetic relationships between clinical mastitis, somatic cell count, and udder conformation in Danish Holsteins. Livest Prod Sci. 1994;39:243–51.
4. Vollema AR, Van Der Beek S, Harbers AGF, De Jong G. Genetic Evaluation for Longevity of Dutch Dairy Bulls. J Dairy Sci. 2000;83:2629–39.
5. 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.
6. Xia J, Qi X, Wu Y, Zhu B, Xu L, Zhang L, et al. Genome-wide association study identifies loci and candidate genes for meat quality traits in Simmental beef cattle. Mamm Genome. 2016;27:246–55.
7. Pryce JE, Hayes BJ, Bolormaa S, Goddard ME. Polymorphic regions affecting human height also control stature in cattle. Genetics. 2011;187:981–4.
8. Silventoinen K, Magnusson PKE, Tynelius P, Kaprio J, Rasmussen F. Heritability of body size and muscle strength in young adulthood: A study of one million Swedish men. Genet Epidemiol. 2008;32:341–9.

9. Zhang X, Chu Q, Guo G, Dong G, Li X, Zhang Q, et al. Genome-wide association studies identified multiple genetic loci for body size at four growth stages in Chinese Holstein cattle. PLoS One. 2017;12:e0175971.
10. Bouwman AC, Daetwyler HD, Chamberlain AJ, Ponce CH, Sargolzaei M, Schenkel FS, et al. Meta-analysis of genome-wide association studies for cattle stature identifies common genes that regulate body size in mammals. Nat Genet. 2018.
11. 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.
12. 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. 2010;86:2236–42.
13. 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;10:1735–7.
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.
15. 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.
16. 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.
17. Buzanskas ME, Grossi DA, Ventura R V., Schenkel FS, Sargolzaei M, Meirelles SLC, et al. Genome-wide association for growth traits in canchim beef cattle. PLoS One. 2014;9.
18. Jin B, Bao WJ, Wu ZQ, Xia XH. In situ monitoring of protein adsorption on a nanoparticulated gold film by attenuated total reflection surface-enhanced infrared absorption spectroscopy. Langmuir. 2012;28:9460–5.
19. 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.
20. 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;41:579–88.
21. Sorbolini S, Bongiorni S, Cellesi M, Gaspa G, Dimauro C, Valentini A, et al. Genome wide association study on beef production traits in Marchigiana cattle breed. J Anim Breed Genet. 2017;134:43–8.
22. Santana MHA, Utsunomiya YT, Neves HHR, Gomes RC, Garcia JF, Fukumasu H, et al. Genome-wide association analysis of feed intake and residual feed intake in Nellore cattle. BMC Genet. 2014;15.
23. 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;94:4570–82.

24. Furlotte NA, Eskin E, Eyheramendy S. Genome-Wide Association Mapping With Longitudinal Data. Genet Epidemiol. 2012;36:463–71.

25. Porter HF, O'Reilly PF. Multivariate simulation framework reveals performance of multi-trait GWAS methods. Sci Rep. 2017.

26. Das K, Li J, Wang Z, Tong C, Fu G, Li Y, et al. A dynamic model for genome-wide association studies. Hum Genet. 2011;129:629–39.

27. Kim S, Xing EP. Statistical estimation of correlated genome associations to a quantitative trait network. PLoS Genet. 2009;5.

28. Arnold K, Sarkar A, Yram MA, Polo JM, Bronson R, Sengupta S, et al. Sox2 + adult stem and progenitor cells are important for tissue regeneration and survival of mice. Cell Stem Cell. 2011;9:317–29.

29. Dekkers JCM. Commercial application of marker- and gene-assisted selection in livestock: Strategies and lessons1,2. J Anim Sci. 2004;82 suppl_13:E313–28. doi:10.2527/2004.8213_supplE313x.

30. Guo Y, Huang Y, Hou L, Ma J, Chen C, Ai H, et al. Genome-wide detection of genetic markers associated with growth and fatness in four pig populations using four approaches. Genet Sel Evol. 2017;49.

31. Yi G, Shen M, Yuan J, Sun C, Duan Z, Qu L, et al. Genome-wide association study dissects genetic architecture underlying longitudinal egg weights in chickens. BMC Genomics. 2015;16.

32. Chen S, Li X, Lu D, Xu Y, Mou W, Wang L, et al. SOX2 regulates apoptosis through MAP4K4-Survivin signaling pathway in human lung cancer cells. Carcinogenesis. 2014;35:613–23.

33. Tani Y, Akiyama Y, Fukamachi H, Yanagihara K, Yuasa Y. Transcription factor SOX2 up-regulates stomach-specific pepsinogen A gene expression. J Cancer Res Clin Oncol. 2007;133:263–9.

34. Pausch H, Flisikowski K, Jung S, Emmerling R, Edel C, Götz KU, et al. Genome-wide association study identifies two major loci affecting calving ease and growth-related traits in cattle. Genetics. 2011;187:289–97.

35. Luechtefeld R. A general-purpose collaborative system: Theory and design of the dialogic web. 2017 IEEE Technol Eng Manag Soc Conf TEMSCON 2017. 2017;:455–9.

36. He M, Xu M, Zhang B, Liang J, Chen P, Lee JY, et al. Meta-analysis of genome-wide association studies of adult height in East Asians identifies 17 novel loci. Hum Mol Genet. 2015;24:1791–800.

37. Lundeen EA, Stein AD, Adair LS, Behrman JR, Bhargava SK, Dearden KA, et al. Height-for-age z scores increase despite increasing height deficits among children in 5 developing countries. Am J Clin Nutr. 2014;100:821–5.

38. Liu R, Sun Y, Zhao G, Wang H, Zheng M, Li P, et al. Identification of loci and genes for growth related traits from a genome-wide association study in a slow- × fast-growing broiler chicken cross. Genes and Genomics. 2015;37:829–36.

39. Hansen TH, Vestergaard H, Jørgensen T, Jørgensen ME ik., Lauritzen T, Brandslund I, et al. Impact of PTBP1 rs11085226 on glucose-stimulated insulin release in adult Danes. BMC Med Genet.

2015;16:17.

40. Meng Q, Wang K, Liu X, Zhou H, Xu L, Wang Z, et al. Identification of growth trait related genes in a Yorkshire purebred pig population by genome-wide association studies. Asian-Australasian J Anim Sci. 2017;30:462–9.
41. Dunn AR, Stout KA, Ozawa M, Lohr KM, Hoffman CA, Bernstein AI, et al. Synaptic vesicle glycoprotein 2C (SV2C) modulates dopamine release and is disrupted in Parkinson disease. Proc Natl Acad Sci U S A. 2017;114:E2253–62.
42. Zhou H, Mori S, Ishizaki T, Takahashi A, Matsuda K, Koretsune Y, et al. Genetic risk score based on the prevalence of vertebral fracture in Japanese women with osteoporosis. Bone Reports. 2016;5:168–72. doi:10.1016/j.bonr.2016.07.001.
43. Lee YS, Shin D, Song KD. Dominance effects of ion transport and ion transport regulator genes on the final weight and backfat thickness of Landrace pigs by dominance deviation analysis. Genes and Genomics. 2018;40:1331–8.
44. Du M, Auer PL, Jiao S, Haessler J, Altshuler D, Boerwinkle E, et al. Whole-exome imputation of sequence variants identified two novel alleles associated with adult body height in African Americans. Hum Mol Genet. 2014;23:6607–15.
45. Borowska A, Reyer H, Wimmers K, Varley PF, Szwaczkowski T. Detection of pig genome regions determining production traits using an information theory approach. Livest Sci. 2017;205:31–5.
46. Wang L, Zhang L, Yan H, Liu X, Li N, Liang J, et al. Genome-wide association studies identify the loci for 5 exterior traits in a large white x Minzhu pig population. PLoS One. 2014;9.
47. Klomp HM, Steyerberg EW, Ubbink DT. Letter: Systematic review and meta-analysis of controlled trials assessing spinal cord stimulation for inoperable critical leg ischaemia (Br J Surg 2004; 91: 948-955) [2] (multiple letters). Br J Surg. 2005;92:120–1.
48. Andersson L, Haley CS, Ellegren H, Knott SA, Johansson M, Andersson K, et al. Genetic mapping of quantitative trait loci for growth and fatness in pigs. Science (80-). 1994;263:1771–4.
49. Andersson L, Haley CS, Ellegren H, Knott SA, Johansson M, Andersson K, et al. Genetic mapping of quantitative trait loci for growth and fatness in pigs. Science (80-). 1994;263:1771–4.
50. Kemper KE, Littlejohn MD, Lopdell T, Hayes BJ, Bennett LE, Williams RP, et al. Leveraging genetically simple traits to identify small-effect variants for complex phenotypes. BMC Genomics. 2016;17.
51. Jung EJ, Park HB, Lee JB, Yoo CK, Kim BM, Kim HI, et al. Genome-wide association study identifies quantitative trait loci affecting hematological traits in an F2intercross between Landrace and Korean native pigs. Anim Genet. 2014;45:534–41.
52. Buzanskas ME, Grossi DA, Ventura R V., Schenkel FS, Sargolzaei M, Meirelles SLC, et al. Genome-wide association for growth traits in canchim beef cattle. PLoS One. 2014;9.
53. Yariz KO, Walsh T, Uzak A, Spiliopoulos M, Duman D, Onalan G, et al. Inherited mutation of the luteinizing hormone/choriogonadotropin receptor (LHCGR) in empty follicle syndrome. Fertil Steril. 2011;96:e125–30.

54. Zhang Z, Hong Y, Gao J, Xiao S, Ma J, Zhang W, et al. Genome-Wide Association Study Reveals Constant and Specific Loci for Hematological Traits at Three Time Stages in a White Duroc × Erhualian F 2 Resource Population. PLoS One. 2013;8.
55. Nishi H, Arai H, Momiyama T. NCI-H295R, a Human Adrenal Cortex-Derived Cell Line, Expresses Purinergic Receptors Linked to Ca2+-Mobilization/Influx and Cortisol Secretion. PLoS One. 2013;8.
56. Jia D, Jing Y, Zhang Z, Liu L, Ding J, Zhao F, et al. Amplification of MPZL1/PZR promotes tumor cell migration through Src-mediated phosphorylation of cortactin in hepatocellular carcinoma. Cell Res. 2014;24:204–17.
57. Rask-Andersen M, Almén MS ällma., Lind L, Schiöth HB. Association of the LINGO2-related SNP rs10968576 with body mass in a cohort of elderly Swedes. Mol Genet Genomics. 2015;290:1485–91.
58. Bryant EK, Dressen AS, Bunker CH, Hokanson JE, Hamman RF, Kamboh MI, et al. A Multiethnic Replication Study of Plasma Lipoprotein Levels-Associated SNPs Identified in Recent GWAS. PLoS One. 2013;8.
59. Guo YM, Zhang ZY, Ma JW, Ai HS, Ren J, Huang LS. A genomewide association study of feed efficiency and feeding behaviors at two fattening stages in a white duroc × erhualian F2 population. J Anim Sci. 2015;93:1481–9.
60. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. Am J Hum Genet. 2007;81:559–75. doi:10.1086/519795.
61. Wang SB, Feng JY, Ren WL, Huang B, Zhou L, Wen YJ, et al. Improving power and accuracy of genome-wide association studies via a multi-locus mixed linear model methodology. Sci Rep. 2016;6.
62. Zhang Z, Ersoz E, Lai CQ, Todhunter RJ, Tiwari HK, Gore MA, et al. Mixed linear model approach adapted for genome-wide association studies. Nat Genet. 2010;42:355–60.
63. Lipka AE, Tian F, Wang Q, Peiffer J, Li M, Bradbury PJ, et al. GAPIT: Genome association and prediction integrated tool. Bioinformatics. 2012;28:2397–9.
64. VanRaden PM. Efficient Methods to Compute Genomic Predictions. J Dairy Sci. 2008;91:4414–23.
65. Nakagawa S. A farewell to Bonferroni: The problems of low statistical power and publication bias. Behavioral Ecology. 2004;15:1044–5.
66. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Stat Soc Ser B. 1995;57:289–300.
67. Bolormaa S, Porto 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;89:2297–309.
68. Furlotte NA, Eskin E, Eyheramendy S. Genome-Wide Association Mapping With Longitudinal Data. Genet Epidemiol. 2012;36:463–71.
69. Zhou X, Stephens M. Efficient multivariate linear mixed model algorithms for genome-wide association studies. Nat Methods. 2014;11:407–9.

Tables

Table 1 Descriptive statistics of body size traits at 6, 12, and 18 months after birth.

Month	Trait (cm)	N[a]	Mean	Min.	Max.	SD	(SE)
6	BH	218	100.44	80	127	9.803	0.490.04
	HH	121	105.01	85	136	10.42	0.510.06
	BL	214	105.31	72	138	10.65	0.520.05
	HS	213	125.44	89	170	15.61	0.510.06
	AS	211	140.87	97	188	17.54	0.510.07
	CS	119	16.377	12	21.5	2.323	0.620.04
12	BH	457	116.69	97	135	7.274	0.290.08
	HH	453	123.69	105	142	7.415	0.270.08
	BL	453	130.18	104	157	9.541	0.530.07
	HS	454	168.24	129	202	13.09	0.330.05
	AS	454	198.53	155	238	15.24	0.300.07
	CS	436	18.069	15	21.5	1.221	0.290.06
18	BH	516	126.46	105	139	4.577	0.280.06
	HH	267	132.61	109	147	5.453	0.410.04
	BL	514	144.19	123	169	7.843	0.280.07
	HS	513	188.11	160	214	8.133	0.300.08
	AS	512	219.01	193	244	8.909	0.140.06
	CS	381	20.155	17	23	1.506	0.540.07

heritability, SE standard error, BH body height, BL body length, HH hip height, HS heart size, AS abdominal size, CS cannon bone size

[a]Number of animal with phenotypes

Table 2 List of suggestive candidate genes associated with six body size traits in Simmental beef cattle.

Genes	Related SNPs	BTA	Position (bp)	MAF	Distance (bp)	p value	Associated traits
P2RY1	BovineHD0100032742	1	115755082	0.40	79694	9.51E-06	LONG-BH
	BovineHD0100032742	1	115755082	0.40	79694	1.54E-06	LONG-HH
	BovineHD0100032742	1	115755082	0.40	79694	9.75E-06	LONG-CS
	BovineHD0100032745	1	115763334	0.40	71442	1.99E-06	LONG-HH
SLC37A1	BovineHD0100041625	1	144414936	0.24	within	7.32E-08	multi-trait18
	BovineHD0100041630	1	144426364	0.24	within	7.32E-08	multi-trait18
SOX2	BTB-00037008	1	86134439	0.21	200052	8.34E-06	single-trait CS18
	Hapmap44647-BTA-38494	1	86087819	0.19	153432	3.58E-06	single-trait CS18
SNRPD1	BovineHD0200014364	2	49864811	0.20	128412	9.22E-06	single-trait HS18
	BovineHD0200014365	2	49865504	0.25	129105	2.11E-08	single-trait HS18
	BovineHD0200014365	2	49865504	0.24	129105	4.06E-08	multi-trait18
MPZL1	BovineHD0300000230	3	966159	0.38	within	7.60E-06	LONG-BL
	BTA-68271-no-rs	3	970386	0.42	within	8.93E-07	LONG-BH
	BTA-68271-no-rs	3	970386	0.42	within	6.64E-06	LONG-HH
PCDH7	BovineHD0600014096	6	51063986	0.00	472877	4.90E-08	multi-trait6
RASGEF1B	BovineHD0600027173	6	97864370	0.15	125438	8.09E-06	single-trait HS12
	BovineHD0600027180	6	97871595	0.15	118213	4.49E-06	single-trait HS12
	BovineHD0600027330	6	98320692	0.10	306357	9.24E-06	single-trait HH6
LAP3	Hapmap26308-BTC-057761	6	38576012	0.39	within	1.94E-08	multi-trait12

PTBP1	BovineHD0700012966	7	45003982	0.02	12339	3.90E-07	single-trait HH6
	BovineHD0700012966	7	45003982	0.02	12339	7.42E-06	single-trait BH6
	BovineHD0700012966	7	45003982	0.02	12339	8.23E-13	multi-trait6
EFNA5	BovineHD0700034055	7	107333030	0.00	1716560	1.84E-06	single-trait HS12
	BovineHD0700034055	7	107333030	0.00	1716560	7.68E-06	single-trait AS12
LINGO2	BTB-01533287	8	15773921	0.31	495444	7.98E-06	LONH-HS
MANEA	BovineHD0900015135	9	55157981	0.01	within	5.01E-08	multi-trait6
	BovineHD0900015139	9	55177601	0.01	within	5.01E-08	multi-trait6
SNX9	BovineHD0900027283	9	95932814	0.23	within	5.18E-06	single-trait BL12
SV2C	BovineHD1000002378	10	7434970	0.35	within	4.16E-07	single-trait BH6
	BovineHD1000002378	10	7434970	0.35	within	2.99E-09	multi-trait6
	BovineHD1000002381	10	7437227	0.14	within	5.96E-08	multi-trait6
PKDCC	BovineHD1100007360	11	24440415	0.30	89224	2.65E-06	single-trait HS12
	BovineHD1100007363	11	24458336	0.30	71303	4.35E-06	single-trait HS12
	BovineHD1100007368	11	24473651	0.49	55988	3.84E-06	single-trait HS12
	BovineHD1100007368	11	24473651	0.50	55988	2.88E-08	multi-trait12
	BovineHD4100008641	11	24432416	0.29	97223	4.62E-06	single-trait HS12
LHCGR	BovineHD1100009199	11	30836078	0.42	within	2.57E-08	multi-trait12
	BovineHD1100009205	11	30849406	0.43	within	4.07E-08	multi-trait12
	BovineHD1100009208	11	30866881	0.43	within	4.62E-08	multi-trait12

	BovineHD1100009211	11	30880359	0.43	within	4.07E-08	multi-trait12
SYNDIG1	BovineHD1300012489	13	42903665	0.43	10485	1.06E-06	single-trait BH18
	BovineHD1300012489	13	42903665	0.43	10485	6.31E-08	multi-trait18
AKR1E2	ARS-BFGL-NGS-5872	13	44206849	0.49	within	4.17E-09	multi-trait18
	BovineHD1300012890	13	44187504	0.50	16132	3.20E-09	multi-trait18
	BovineHD1300012891	13	44188743	0.50	14893	4.89E-09	multi-trait18
	BovineHD1300012894	13	44201625	0.46	2011	8.08E-06	single-trait AS18
	BovineHD1300012894	13	44201625	0.47	2011	3.84E-10	multi-trait18
	BovineHD1300012895	13	44202958	0.50	678	4.65E-08	multi-trait18
	BovineHD1300012896	13	44203607	0.50	29	6.37E-09	multi-trait18
	BovineHD1300012899	13	44205509	0.50	within	2.75E-09	multi-trait18
	BovineHD1300012900	13	44206016	0.49	within	4.18E-09	multi-trait18
	BovineHD1300012903	13	44209748	0.50	within	2.75E-09	multi-trait18
	BovineHD1300012905	13	44211339	0.49	within	2.62E-08	multi-trait18
	BovineHD1300012906	13	44212364	0.49	within	3.11E-09	multi-trait18
	BovineHD1300012907	13	44213101	0.48	within	5.66E-09	multi-trait18
	BovineHD1300012909	13	44217192	0.48	2915	2.63E-08	multi-trait18
CMIP	BovineHD1800002818	18	8171554	0.48	39261	5.92E-06	LONG-HH
	BovineHD1800002831	18	8197531	0.43	13284	6.66E-06	LONG-HH
	BovineHD1800002834	18	8200906	0.43	9909	5.72E-06	LONG-HH

	BovineHD1800002837	18	8203446	0.43	7369	4.87E-06	LONG-HH
WSCD1	BovineHD1900007802	19	26316747	0.37	31164	5.47E-06	LONG-CS
PRIM2	ARS-BFGL-NGS-59707	23	2570211	0.36	within	6.65E-06	single-trait HH18
	BovineHD2300000488	23	2589039	0.36	within	8.16E-06	single-trait HH18

Name of trait: BH, body height; BL, body length; HH, hip height; HS, heart size; AS, abdominal size; CS, cannon bone size.

Name of SNPs: Single nucleotide polymorphism name in the Bovine HD panel.

BTA: Bos Taurus autosome.

MAF: minor allele frequency.

Position: Position (bp) on UMD3.1.

Distance: distance between SNP and the nearest gene.

P value: p-values calculated from the mixed linear model analysis.

Supplemental File Legends

Figure S1. Figure S2. 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 six body size traits months 6, 12 and 18 after birth separately. Each analysis is labeled as trait (BH or HH) and month on the far right. The number neighboring each trait indicates the age of measurement (e.g., BH6 = Body Height at 6 months). The 29 chromosomes are color coded.

Figures

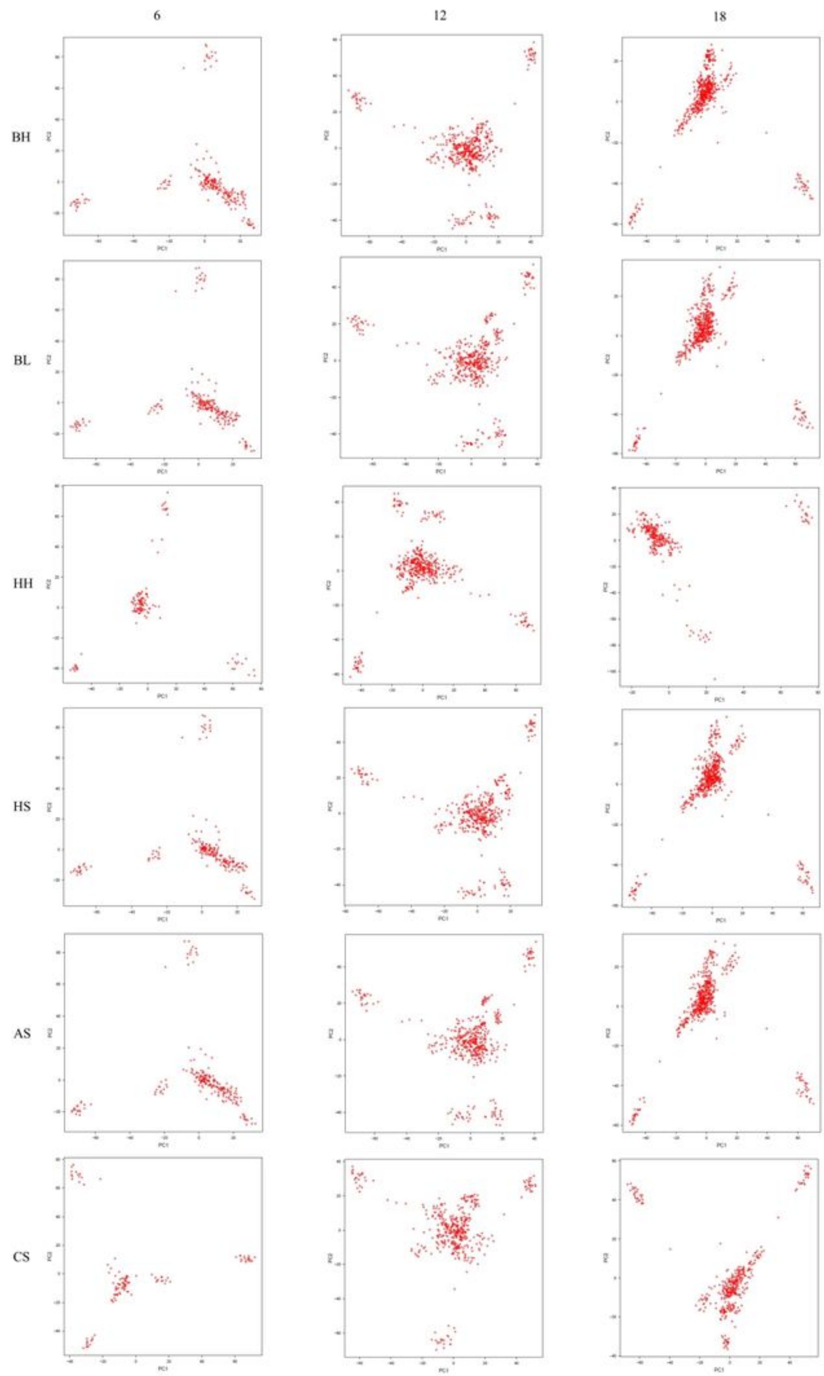

Figure 1

Principal components (PC) plot drawn from the second principal component (PC2) against the first principal component (PC1).

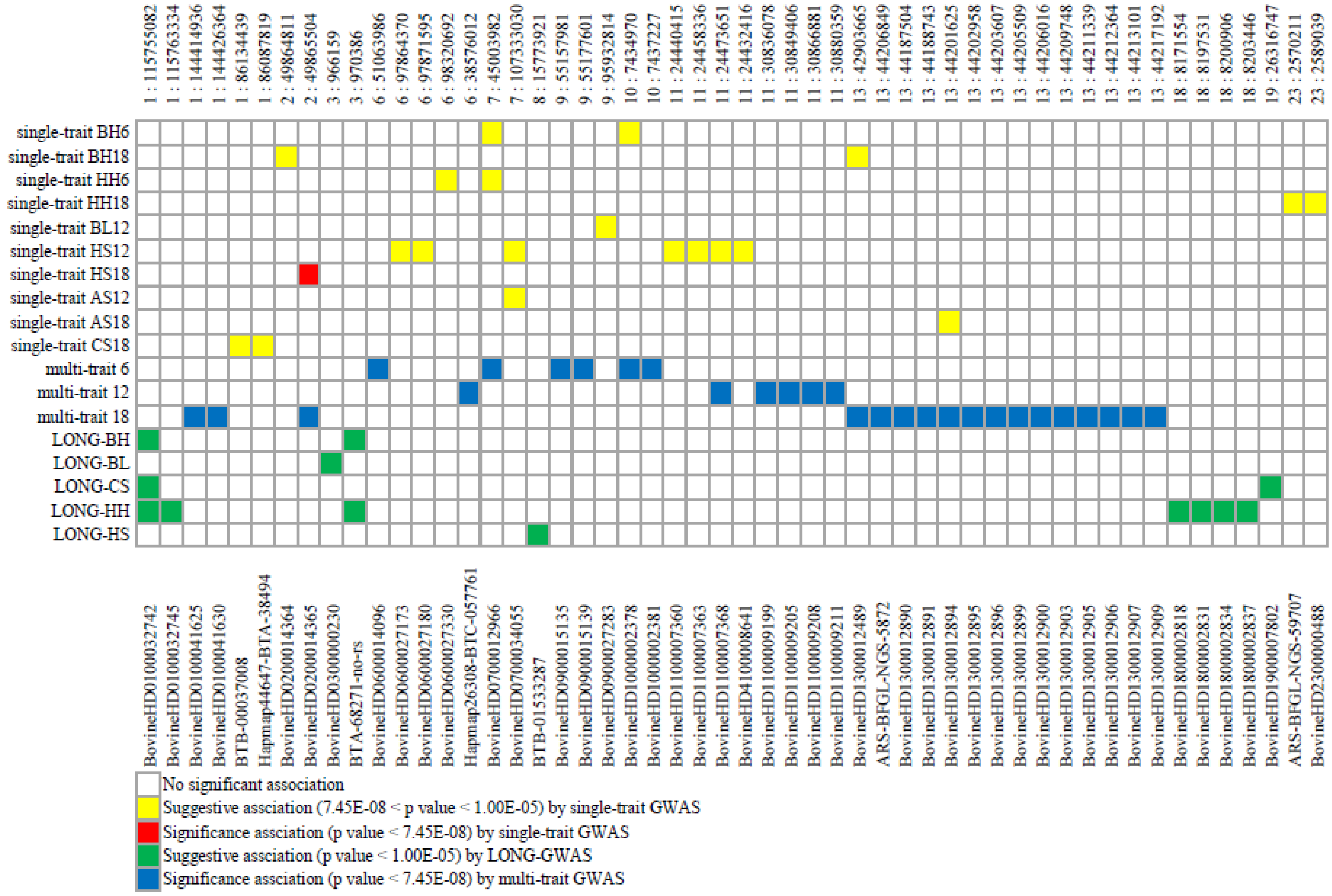

Figure 2

Summary of six body size traits associations across genomic regions (SNPs) with three association strategies. Each row represents a trait, and each column, a genomic region containing SNPs that are genome-wide suggestively or significantly associated with a trait. Only traits with at least one associated SNP and SNPs associated with at least one trait are shown.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Methods.pdf
- TableS1.docx
- figureS1.Tiff
- figureS2.Tiff