Genome-wide association analysis to identify QTL for carcass traits in Hanwoo (Korean native cattle)

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ABSTRACT

A genome-wide association study (GWAS) was performed to investigate the genetic markers associated with carcass traits of Hanwoo (Bos taurus coreanae) steer in the Gangwon region of Korea. Hanwoo steer (139) from the Gangwon region were genotyped with Bovine SNPS50K BeadChip, and 35,769 SNPs were analyzed for five specific carcass traits after applying several filters. A total of seven quantitative trait loci were detected, of which four, one, and 2 SNPs were detected on various B. taurus autosomal chromosomes (BTA) by the respective model. The four significant SNPs associated with backfat thickness were ARS-BFGL-NGS–14775 on BTA 5, ARS-BFGL-NGS-36359 on BTA 19, ARS-BFGL-NGS-56813 on BTA 22, and Hapmap25048-BTA-138242 on BTA 25. Among the detected SNPs, one and two SNPs were associated with marbling score (ARS-BFGL-NGS–110066 on BTA 23) and meat colour (BTB-0192039 on BTA 15 and ARS-BFGL-NGS-24934 on BTA 18). In this GWAS, we identified three positional candidate genes for carcass traits, backfat thickness (Fibulin-2, FBLN2; Sorting nexin 29, SNX29) and meat colour (WW domain containing oxidoreductase, WWOX). Our results suggest that the candidate SNP markers do affect the genomic selection of associated carcass traits for Hanwoo in the Gangwon region.

Key words: Gangwon, Genome-wide association study (GWAS), Hanwoo, SNP markers

The Korean native cattle Hanwoo (Bos taurus coreanae) is a crossbreed between Bos indicus and Bos primigenius (Lee et al. 2013a), and is vastly present in the Korean peninsula. Hanwoo was predominantly used for farming purpose in the early days of Korea; however, with industrial development it was taken as beef cattle in the Korean peninsula. Resultant to that, the Hanwoo selection was primarily on progeny test-based breeding programs in order to increase meat production and quality, and to meet the growing demands for beef in Korea (Rhee et al. 2002, Lee et al. 2013a, 2014; Venkata et al. 2013b). Korean consumers prefer Hanwoo meat for its palatability and chewiness (Cho and Ko 1998). Therefore, estimated breeding values for carcass weight (CW), backfat thickness (BFT), eye muscle area (EMA), marbling score (MS), and meat colour (MC) were commonly used as selection criteria in an attempt to increase meat yield and quality, which determined further the profitability of the Korean beef industry (Lee et al. 2013b).

The use of genomic selection (GS) using genetic markers may be substantially greater than that achieved by selection based upon traditional breeding for economic traits that are being determined post-mortem, that occur late in life, have a low heritability, or are difficult and/or expensive to accurately measure (Davis and DeNise 1998). Thus, in order to identify cattle with superior genetic merits, researchers have identified quantitative trait loci (QTL) for numerous economically important traits using various methods such as restriction fragment length polymorphism and microsatellite typing (Ashwell et al. 1997, Boichard et al. 2003, Park et al. 2012, Nishimaki et al. 2013, Kwon et al. 2016).

Genome-wide association studies (GWAS) allow to search the genome for associated disease or economic traits by researching variations in genetic frequencies in the whole genome. In addition, SNP chips have facilitated GWAS or GS, which allows for the identification of causative SNPs for economic traits and improves the reliability of breeding value predictions (VanRaden et al. 2009). A recently established bovine SNP panel enabled QTL mapping and the prediction of genetic merits without the use of phenotype and pedigree records (Goddard and Hayes 2009). This approach has been used for precise QTL mapping of milk traits in dairy cattle (Na et al. 1985, Daetwyler et al. 2008, Kolbehdari et al. 2008); in beef cattle, GWAS with SNP array research associated with feed intake traits or carcass traits have been conducted (Barendse et al. 2007, Hulett et al. 2007, Karim et al. 2011).
In Korea, a number of studies on several QTL mapping methods that exploited a linkage disequilibrium between molecular markers and QTL have been suggested and applied (Kim et al. 2011, Lee et al. 2012b, Li and Kim 2015, Li et al. 2017). In addition, a few researchers have reported chromosomal regions, genes, and specific alleles on the B. taurus autosomal chromosome (BTA) 6 that was associated with carcass traits in Hanwoo (Li et al. 2011, Lee et al. 2012a, 2013b). However, most studies on Hanwoo have used the populations (proven bulls, steers) from governmental institutions such as the Korean Cattle Improvement Center of the National Agricultural Cooperative Federation or the National Institute of Animal Science. Although the breeding and improvement of Hanwoo is managed at the national level in Korea, it is expected that there will be differences locally and nationally. Especially, the Gangwon region of Korea has more forests and a more mountainous terrain than other provinces resulting in Gangwon Hanwoo with a larger body physique and a stronger constitution than those in other provinces. They are also relatively good at forming marbling due to their ability to adjust to wide daily temperature range. The aim of this study was to identify candidate genes that affect carcass traits and how SNP distributions impact the traits using GWAS in Hanwoo from the Gangwon region, Korea.

MATERIALS AND METHODS

Resource population and phenotypic measurements: Hanwoo steers (139) sired by 18 bulls that were raised at livestock farms in Hongcheon-gun, Gangwon province, Korea, were used. The steers were born between May 2010 and January 2012 and they were shipped to slaughterhouses from April 2013 to June 2013. Phenotypic and carcass trait data were obtained from the Korea Animal Improvement Cooperative Federation or the National Institute of Animal Improvement Center of the National Agricultural Cooperative Federation or the National Institute of Animal Science. Although the breeding and improvement of Hanwoo is managed at the national level in Korea, it is expected that there will be differences locally and nationally. Especially, the Gangwon region of Korea has more forests and a more mountainous terrain than other provinces resulting in Gangwon Hanwoo with a larger body physique and a stronger constitution than those in other provinces. They are also relatively good at forming marbling due to their ability to adjust to wide daily temperature range. The aim of this study was to identify candidate genes that affect carcass traits and how SNP distributions impact the traits using GWAS in Hanwoo from the Gangwon region, Korea.

Genotyping: Tissue samples were collected from Hanwoo steers in Hongcheon-gun of Gangwon province, Korea. DNA was isolated from the tissue using G-spin™ Total DNA Extraction Mini Kit (Intron Biotechnology Inc., Seongnam, Korea). Genotyping was conducted with the Bovine SNP50K BeadChip (Illumina Inc., San Diego, CA), which resulted in 54,609 SNPs. Then, the detected non-biallelic SNPs of over than 2 proceed allele flip. Of the 54,609 SNPs genotyped, 201 SNPs failed to produce genotypes for all the samples in this process. SNP selection criteria were

- 1,647 sex chromosomes that were unmapped to the bovine genome (Bos_taurus_UMD_3.0),
- 1,898 SNPs that had a <98% call rate
- 4,756 SNPs that had a significant departure from the Hardy Weinberg equilibrium (P<0.001), and
- 10,338 SNPs that had a minor allele frequency (MAF) P<0.05 in Hanwoo. As a result, 35,769 SNPs were used to identify functional loci (Table 2).

This quality control procedure was conducted using the PLINK program (Purcell et al. 2007).

Statistical analysis: A total of 35,769 autosomal SNP markers remained for GWAS. A linear mixed-model association analysis between five carcass traits and these SNP markers using the genome-wide complex trait analysis (GCTA) program was also performed (Yang et al. 2011).
All association analyses were performed using the *mlma* option in GCTA, which applies a linear mixed-effects model that includes the candidate variant.

\[ y = Xb + S_1a + S_2u + e \]

where, \( y \) is the vector of each carcass trait; \( b \) is the fixed effects vector of the candidate variant to be tested for an association; a represents SNP marker effects; \( u \sim N(0, \sigma^2_u) \) is the random polygenic effect vector where \( G \) is the genomic relationship matrix (GRM) that was constructed using the 35,769 SNP markers and \( \sigma^2_G \) is the polygenic variance; and \( e \sim N(0, \sigma^2_e) \) is the random residual effect vector where \( I \) is the identity matrix and \( \sigma^2_e \) is the residual variance. \( S_1 \) is the incidence matrix for a while \( X \) and \( S_2 \) are the incidence matrices for \( b \) and \( u \), respectively. A Bonferroni correction was employed to account for multiple testing. P-values for associations with significant SNPs were also estimated by dominance and recessive genotypic models.

**RESULTS AND DISCUSSION**

In this study, 139 Hanwoo steers were used to identify QTL for carcass traits, viz. BFT, CW, EMA, MS, and MC. The carcass traits were adjusted to 918 days (the average age at slaughter). The carcass traits were generally higher in Gangwon Hanwoo (Table 1) compared with other studies. The carcass traits were adjusted to 918 days (the average age at slaughter). The carcass traits were generally higher in Gangwon Hanwoo (Table 1) compared with other studies. The carcass traits were generally higher in Gangwon Hanwoo (Table 1) compared with other studies.

Table 3. Identification of SNP associated with carcass traits in Hanwoo genotyped with Bovine SNP50K

| SNP                      | Chr | Position   | Allele | MAF  | b     | SE   | p-value  | Gene   |
|--------------------------|-----|------------|--------|------|-------|------|----------|--------|
| Backfat thickness (cm)    |     |            |        |      |       |      |          |        |
| ARS-BFGL-NGS-41475       | 5   | 14,556,289 | G/A    | 0.22 | 2.802 | 0.648| 1.52E-05 | None   |
| ARS-BFGL-NGS-36359       | 19  | 62,694,734 | A/G    | 0.19 | 2.495 | 0.584| 1.97E-05 | None   |
| ARS-BFGL-NGS-56813       | 22  | 58,998,502 | A/G    | 0.13 | 3.248 | 0.771| 2.54E-05 | FBLN2  |
| Hasmap25048-BTA-138242    | 25  | 10,818,342 | G/A    | 0.41 | 2.203 | 0.506| 1.33E-05 | SNX29  |
| Marbling score* (1–9)    |     |            |        |      |       |      |          |        |
| ARS-BFGL-NGS-110066      | 23  | 961,672    | G/A    | 0.43 | 0.979 | 0.233| 2.72E-05 | None   |
| Meat color† (1–7)        |     |            |        |      |       |      |          |        |
| BTB-01920239             | 15  | 79,637,627 | A/G    | 0.31 | -0.366 | 0.082| 6.97E-06 | None   |
| ARS-BFGL-NGS-24934       | 18  | 5,545,560  | G/A    | 0.13 | -0.545 | 0.125| 1.38E-05 | WWOX   |

*The score ranged from 1 (trace) to 9 (very abundant); †The score ranged from 1 (very light cherry red) to 7 (very dark red)
the current study using Hanwoo steers from a single region has completely different results compared to the others. In addition, Hong et al. (2017) reported that the LD value of Gangwon Hanwoo was higher than that of the national average. This could be attributed to the effect of Hanwoo inbreeding within the area, and it suggest that the results of current study could have shown as these reasons. Therefore, further studies that identify specific chromosomes might be needed for Hanwoo.

As shown in Table 3, Fibulin-2 (FBLN2) and Sorting nexin 29 (SNX29) had shown possibility as positional and functional candidate genes that the BFT trait related SNPs detected in these genes on chromosomes 22, 25 in Hanwoo, respectively. The SNP for MC was positioned on BTA 18, which is one of the gene WW domains containing oxidoreductase (WWOX). FBLN2 functions associated with BFT in cattle were previously unknown, but the gene is now known to be associated with extracellular matrix-related factors in bovine oviduct epithelial cells (Tanaka et al. 2016). Raghunath et al. (1999) also described that FBLN2 was associated with fibrillin-containing microfibrils, except for the part immediately adjacent to the dermo-epidermal junction in a normal dermis. In humans, the SNX29 gene is associated with abdominal fat and there is significant evidence that it is located on chromosome 16 (Fox et al. 2012). Sung et al. (2016) found it to be associated with subcutaneous and visceral adipose tissue in women. In the case of cattle, Flury et al. (2014) reported that significant SNPs for the rear udder height trait were positioned on the SNX29 gene. Although the two genes, FBLN2 and SNX29 were not directly related to the BFT trait in cattle, it was suggested that they might be sufficient candidate gene markers of BFT in Gangwon Hanwoo. Fernandes et al. (2016) identified a significant association between the WWOX gene and BFT in Nellore cattle; however, we found that the gene was associated with the MC trait. Overall, our results were significantly different compared to those of previous studies that used Hanwoo cattle (Kim et al. 2011, Lee et al. 2012a, Lee et al. 2013b, Li and Kim 2015). The present study is a good example of how results for a population can differ based on area.

It is very important to develop excellent economic carcass traits in Hanwoo. In the past, the performance test was the only test that considered the phenotype-like progeny test, which normally takes long time to perform, and results could not be predicted. The discovery of genetic markers attributable to a specific economic trait has made it possible to genetically improve livestock, and this is a key factor for decoding various traits or diseases in the future. Recently, many studies that reported an association of SNP markers in Hanwoo on a large-scale SNP panel (50K, 700K, and HD, etc.) have been commercialized. However, Hanwoo were managed for breeding improvement using artificial insemination from a few sires at the national scale, and as a consequence, the coefficient of inbreeding for all Korean Hanwoo have increased (Dang et al. 2011). According to a study on the genetic distance estimation
between a domestic Hanwoo population using microsatellite markers, Hanwoo populations were difficult to maintain by region (Oh et al. 2008). However, we identified different candidate SNP markers associated with carcass traits and compared them with other studies that investigated the association of carcass traits in Hanwoo. Even though the genetic distance is not far between the domestic Hanwoo and Gangwon Hanwoo populations, it has been suggested that Gangwon Hanwoo have inherent characteristics due to the influence of a wide daily temperature range and a mountainous territory compared to other provinces. Therefore, the results from present study might be used as future genetic markers for Gangwon Hanwoo. It is necessary to carry out further studies using a larger number of Hanwoo because of the small number of Hanwoo analyzed in this study. Also, our results suggest that these data could be used as a basic data for the genetic improvement of Hanwoo in the Gangwon region.

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