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# Validation of Single Nucleotide Polymorphisms Associated with Carcass Traits in a Commercial Hanwoo Population

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**ABSTRACT:** Four carcass traits, namely carcass weight (CW), eye muscle area (EMA), back fat thickness (BF), and marbling score (MS), are the main price decision parameters used for purchasing Hanwoo beef. The development of DNA markers for these carcass traits for use in a beef management system could result in substantial profit for beef producers in Korea. The objective of this study was to validate the association of highly significant single nucleotide polymorphisms (SNPs) identified in a previous genome-wide association study (GWAS) with the four carcass traits in a commercial Hanwoo population. We genotyped 83 SNPs distributed across all 29 autosomes in 867 steers from a Korean Hanwoo feedlot. Six SNPs, namely ARS-BFGL-NGS-22774 (Chr4, Pos:4889229), ARS-BFGL-NGS-100046 (Chr6, Pos:61917424), ARS-BFGL-NGS-39006 (Chr27, Pos:38059196), ARS-BFGL-NGS-18790 (Chr10, Pos:26489109), ARS-BFGL-NGS-43879 (Chr9, Pos:39964297), and BTB-00775794 (Chr20, Pos:20476265), were found to be associated with CW, EMA, BF, and MS. The ARS-BFGL-NGS-22774, BTB-00775794, and ARS-BFGL-NGS-39006 markers accounted for 1.80%, 1.72%, and 1.35% ($p<0.01$), respectively, of the phenotypic variance in the commercial Hanwoo population. Many genes located in close proximity to the significant SNPs identified in this study were previously reported to have roles in carcass traits. The results of this study could be useful for marker-assisted selection programs. (**Key Words:** Validation, Single Nucleotide Polymorphism, Carcass Traits, Hanwoo)

## INTRODUCTION

Meat quality and yield traits such as carcass weight (CW), eye muscle area (EMA), back fat thickness (BF), and marbling score (MS) are key traits used to determine the grade and market price of Hanwoo beef. Thus, knowing the genetic potential of an animal for these traits at an early age becomes an important decision-making factor for the farmer. The ability to perform an early genetic assessment of these traits would provide significant benefits to the Korean beef industry.

Many genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) that are significantly associated with carcass traits in different cattle breeds (Nishimura et al., 2012; Lee et al., 2013b; Lu et al., 2013). In a previous GWAS study performed by Lee et al. (2013b), statistically significant SNPs were identified on *Bos taurus* autosome (BTA) 14 for carcass traits in a progeny-tested Hanwoo population. Before any SNP marker is delivered to the beef industry, a validation study that accurately estimates the additive genetic marker effect in a population different from the discovery population should be done. Moreover, a validation population should be considered for different management practices, environment, and genetic structure (Barendse et al., 2007; Van Eenennaam et al., 2007). Validation of the effects of genetic markers in

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independent populations would determine the utility of the genetic testing technology and support the process of incorporating the DNA tests into nationwide cattle evaluation programs. Validated markers could then be used to estimate the genomic breeding value of female animals sired by national bulls on small Hanwoo farms.

Further identification of genes with large effects on quantitative traits would improve the efficiency of animal selection and increase our understanding of the underlying biology of quantitative traits. Given the importance of validating markers, we genotyped 83 SNP markers, which were significantly identified in a previous GWAS performed by Lee et al. (2013b), and validated the effects of those markers in a commercial Hanwoo population in this study.

## MATERIALS AND METHODS

### Animals, trait measurements, and genotyping

DNA was extracted from blood samples of 867 Hanwoo steers belonging to six feedlot groups comprising animals derived from 75 KPNs (Hanwoo proven bull). Blood samples were collected in the central abattoir of Korea (Eum-Sung Abattoir, National Agricultural Cooperative Federation, Eum-Sung, Korea). Management information was obtained from the Korea Animal Improvement Association and Traceability Records for Animal Products. Approval was obtained from the Animal Care and Use Committee for this study based on the animal health and welfare guidelines of the National Institute of Animal Science, Rural Development Administration, Korea. A genome-wide SNP validation analysis was performed using 83 markers (Supplementary Tables S1 to S4), which were genotyped using the Illumina GoldenGate and Infinium genotyping assays to detect the association of SNPs with four phenotypes: CW, EMA, BF, and MS. The SNPs used in this study were selected from the previous GWAS results of Lee et al. (2013b). Phenotypic data were provided by the abattoir (Table 1) and were recorded using standard measurement methods, i.e., CW (kg) was the weight after slaughter at 32 months of age, and EMA ($cm^2$), BF (mm), and MS were measured at the twelfth and thirteenth rib junctions after 24 hours of chilling. MS was assessed on a scale of 1 to 9 points following the beef grading system, which has been standardized by the Korea Institute for Animal Products Quality Evaluation (http://www.ekape.or.kr/ view/eng/system/beef.asp).

### Statistical analysis

In a previous GWAS (Lee et al., 2013b), highly significant SNPs were identified for CW (22 SNPs), EMA (29 SNPs), BF (9 SNPs), and MS (23 SNPs). A multiple linear regression model was implemented to identify the best SNPs and their effects. To validate significant SNPs from the GWAS analysis, we fitted all significant SNPs as fixed effects in a multiple regression model with the validation dataset (n = 867). The additive effect of each SNP on carcass traits (CW, EMA, BF, and MS) was calculated by multiple regression analysis, with values of the covariate coded as 0, 1, or 2 copies of the variant allele. The model was described according to the equation:

$$Y_{ijk} = \mu + CG_i + b_1 Sday_j + \sum_{k=1}^{nSNP} SNP_k + e_{ijk}$$

Where, $Y_{ijk}$ is a vector of carcass traits, μ is a vector of the overall mean for carcass traits, $CG_i$ is a vector of the contemporary group for birth year and season, $b_1$ is a regression coefficient, $Sday_j$ is a vector of the day of slaughter as a covariate effect, *nSNP* indicates the number of SNPs to be tested in a multiple regression model, $SNP_k$ is a single-locus SNP genotype coded as 0, 1, or 2 as a covariate effect, and $e$ is a vector of random residual ~N(0, $I\sigma_e^2$).

### Proportion of SNP variance and bioinformatics analysis

The phenotype data were analyzed with a basic linear model using the R programming language (R Core Team, 2015) to determine the effect of markers and their significance in the studied animals. We generated four model equations indicating four traits and their candidate SNPs from previous GWAS results (Lee et al., 2013b). Genotype was the effect of interest in the model. The additive effect was fitted as a regression of phenotype on allele count. The results of the regression analyses of carcass phenotypes on genotypes are tabulated in Supplementary Tables S1 to S4. The percentage of the phenotypic variance explained by each significant SNP was calculated using the equation:

$$\%\mathrm{Vp_i} = 100 \times \frac{2p_i q_i a_i^2}{\sigma_\mathrm{p}^2}$$

Where, $p_i$ and $q_i$ are the allele frequencies for the $i^{th}$ SNP estimated for Hanwoo, $a_i$ is the estimated additive effect of

**Table 1.** Observed phenotypic data from a commercial Hanwoo population

| Trait | N | Mean | SD | Min. | Max. | $\sigma^2_p$ |
|---|---|---|---|---|---|---|
| Carcass weight (kg) | 867 | 418.26 | 42.40 | 302 | 555 | 1,797.39 |
| Eye muscle area ($cm^2$) | 867 | 87.85 | 10.55 | 53 | 132 | 111.39 |
| Back fat thickness (mm) | 867 | 12.62 | 4.55 | 3 | 33 | 20.73 |
| Marbling score (1 to 9) | 867 | 4.45 | 2.12 | 1 | 9 | 4.50 |

N, number of animal; SD, standard deviation; Min., minimum value in the traits; Max., maximum value in the traits; $\sigma^2_p$, phenotypic variation.

the $i^{th}$ SNP on the phenotype in the equation, and $\sigma_p^2$ is a phenotypic variance for meat quality traits in Hanwoo. SNPs were considered significant at p≤5%. Identified genes that were colocated with or located near the significant SNPs were annotated based on information from GeneCards (Rebhan et al., 1998) and AmiGO (Carbon et al., 2009).

## RESULTS AND DISCUSSION

The results of this validation study, including allele frequencies, are summarized in Supplementary Tables S1 to S4. In a panel of 83 SNPs distributed across 29 autosomes, six markers were significantly associated (p≤0.05) with carcass traits in the commercial Hanwoo population (Table 2). ARS-BFGL-NGS-22774 (Chr4, Pos:4889229) was associated with CW; ARS-BFGL-NGS-100046 (Chr6, Pos:61917424) and ARS-BFGL-NGS-39006 (Chr27, Pos:38059196) were associated with EMA; ARS-BFGL-NGS-18790 (Chr10, Pos:26489109) was associated with BF; and ARS-BFGL-NGS-43879 (Chr9, Pos:39964297) and BTB-00775794 (Chr20; Pos: 20476265) were associated with MS. Two SNPs, BTB-00775794 and ARS-BFGL-NGS-39006, were the most significant markers (p<0.01), for MS and EMA, respectively. These two markers, along with ARS-BFGL-NGS-18790, exhibited negative effects on MS, EMA, and BF, respectively. Furthermore, the BTB-00775794 and ARS-BFGL-NGS-39006 markers in combination with ARS-BFGL-NGS-22774 moderately explained phenotypic variation (1.72%, 1.35%, and 1.80%, respectively) with respect to the total phenotypic variation in the carcass traits. Genes located in the close proximity to the significant SNPs are compiled in Table 3.

The BTB-00775794 marker on BTA20 exhibited an allele substitution effect of –0.40 on MS. BTA20 was previously reported to harbor quantitative trait loci (QTLs) for carcass traits including beef marbling (Han et al., 2009; Garcia et al., 2010). Genes located in close proximity to the significant marker included cyclic adenine monophosphate (cAMP)-specific phosphodiesterase 4D (PDE4D) and Ras-related protein Rab-3C (RAB3C). PDE4D plays a role in phosphodiesterase activity in skeletal muscle (Fleming-Waddell et al., 2009; Yu, 2013). PDE4D regulates β2-adrenergic receptor, which is found mostly in adipose tissue. If β2-adrenergic regulated, the size and proportion of myofibers will increase and the body fat content, which is important to develop marblingness, will decrease. This mechanism was connected to the activity of *RAB3C* gene, a member of the Ras-related guanine triphosphate (GTP) binding proteins family. GTP was capable to bind the G protein, which is used to compose the β2-adrenergic signal and activate cAMP (Talton, 2006).

The ARS-BFGL-NGS-39006 (BTA27) marker had a negative effect (–1.77 $cm^2$) on EMA. Nalaila et al. (2011) previously reported a QTL at approximately 40 cM on BTA27 to be associated with carcass traits including rib eye area in beef cattle. The zinc finger matrin type-4 (ZMAT4) gene was found to be located close to the identified SNP (~38 cM). Downregulation of ZMAT4 and β2-adrenergic receptor genes affects insulin production (Jost et al., 2002), which promotes muscle development. Cattle that are more muscular and have larger EMA values accumulate high levels of muscle glycogen and subcutaneous fat (McGilchrist et al., 2012).

The ARS-BFGL-NGS-18790 (BTA10) marker exhibited

**Table 2.** Results for six significant SNPs associated with meat quality traits in a commercial Hanwoo population

| Marker | NCBI reference | SNP[1] | BTA | Position[2] | GWAS results[3] | | | | | | | | Validation results | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Allele frequency[4] | | | | | p-value (F-value) | %Vg[5] | Trait | Allele frequency | | | | | Effect[6] | p-value (F-value) | %Vp[7] |
| | | | | | N | n0 | n1 | n2 | MAF | | | | N | n0 | n1 | n2 | MAF | | | |
| ARS-BFGL-NGS-22774 | rs109952763 | G | 4 | 4889229 | 1,090 | 790 | 278 | 22 | 0.15 | 0.0004893 (12.2) | 2.83 | CW | 857 | 673 | 171 | 13 | 0.11 | 12.61 | 0.0356 (4.45) | 1.80 |
| ARS-BFGL-NGS-100046 | rs110527834 | G | 6 | 61917424 | 1,089 | 847 | 228 | 14 | 0.12 | 0.0002482 (13.53) | 3.01 | EMA | 865 | 678 | 181 | 6 | 0.11 | 1.98 | 0.0314 (4.65) | 0.70 |
| ARS-BFGL-NGS-43879 | rs109783827 | A | 9 | 39964297 | 1,089 | 557 | 442 | 90 | 0.29 | 0.0002597 (13.44) | 1.51 | MS | 864 | 496 | 320 | 48 | 0.24 | 0.30 | 0.0149 (5.96) | 0.71 |
| ARS-BFGL-NGS-18790 | rs110384732 | A | 10 | 26489109 | 1,085 | 362 | 559 | 164 | 0.41 | 0.0005577 (8.87) | 2.32 | BF | 839 | 279 | 413 | 147 | 0.42 | –0.45 | 0.0520 (3.79) | 0.48 |
| BTB-00775794 | rs41937398 | G | 20 | 20476265 | 1,091 | 364 | 546 | 181 | 0.42 | 0.0001272 (14.8) | 1.71 | MS | 853 | 288 | 420 | 145 | 0.42 | –0.40 | 0.0005 (12.20) | 1.72 |
| ARS-BFGL-NGS-39006 | rs109741381 | C | 27 | 38059196 | 1,090 | 442 | 510 | 138 | 0.36 | 0.0004045 (12.6) | 2.60 | EMA | 864 | 307 | 414 | 143 | 0.41 | –1.77 | 0.0086 (6.96) | 1.35 |

SNPs, single nucleotide polymorphisms; NCBI, National Center for Biotechnology Information; BTA, *Bos taurus* autosome; GWAS, genome-wide association study; MAF, minor allele frequency; CW, carcass weight; EMA, eye muscle area; BF, back fat thickness; MS, marbling score.

[1] SNP constitute to minor allele.

[2] Marker positions are based on the Btau 4.1 assembly of the bovine genome sequence.

[3] Genome-wide association study of meat quality traits in Hanwoo cattle performed by Lee et al. (2013b).

[4] n0, major homozygous alleles; n1, heterozygous alleles; and n2, homozygous least common alleles.

[5] Proportion of SNP variation.

[6] Estimated effect of the minor allele derived from regression analyses denominated based on the traits: CW (kg), EMA ($cm^2$), BF (mm), MS (1–9).

[7] Percentage of phenotypic variation calculated from the equation presented in the Materials and Methods section.

**Table 3.** Genes located in close proximity to the significant SNPs associated with meat quality traits in a commercial Hanwoo population

| Trait | Marker name | BTA | Position[1] | Effect[2] | Gene information | |
|---|---|---|---|---|---|---|
| | | | | | Name | Description |
| Carcass weight (CW) | ARS-BFGL-NGS-22774 | 4 | 4889229 | 12.61 | COBL | Member of the G/F-actin binding protein family |
| Eye muscle area (EMA) | ARS-BFGL-NGS-100046 | 6 | 61917424 | 1.98 | RBM47 | An RNA-binding motif protein |
| | ARS-BFGL-NGS-39006 | 27 | 38059196 | –1.77 | ZMAT4 | Zinc ion binding |
| Back fat thickness (BF) | ARS-BFGL-NGS-18790 | 10 | 26489109 | –0.45 | OR11G2 | Olfactory receptor (*OR*) genes |
| | | | | | OR11H6-like | |
| | | | | | OR11H12 | |
| Marbling score (MS) | ARS-BFGL-NGS-43879 | 9 | 39964297 | 0.30 | LAMA4 | Intramuscular extracellular matrix (ECM) protein family |
| | BTB-00775794 | 20 | 20476265 | –0.40 | PDE4D | Phosphodiesterase activator and regulates β2-adrenergic receptor |
| | | | | | RAB3C | Guanine triphosphate (GTP) binding protein family |

SNP, single nucleotide polymorphism; BTA, *Bos taurus* autosome.
[1] Marker positions are based on the Btau 4.1 assembly of the bovine genome sequence.
[2] Estimated effect of the minor allele derived from regression analyses denominated based on the traits: CW (kg), EMA ($cm^2$), BF (mm), MS (1 to 9).

a negative effect (–0.45 mm) on BF. Many olfactory receptor (*OR*) genes were identified near this SNP including the OR11G2 (LOC784260), OR11H6-like (LOC784302), and OR11H12 (LOC784332) genes. In Hanwoo cattle, a large number of *OR* genes (1,423) were identified across chromosomes. The highest number was on BTA15 (303), and 80 *OR* genes were observed on BTA10 (Lee et al., 2013a). OR proteins detect G protein and promote its binding to GTP. G protein is a component of the β2-adrenergic receptor (Talton, 2006), which plays a role in accumulation of muscle glycogen and subcutaneous fat.

The ARS-BFGL-NGS-22774 (BTA4) marker exhibited a significant association with CW, with an estimated effect of 12.61 kg. Another marker in the same vicinity (4.5 cM) was also identified previously to be associated with carcass traits in Hanwoo cattle (Li and Kim, 2015). A cordon-bleu WH2 repeat protein (*COBL*) gene was found in close proximity to the significant SNPs. COBL is a member of the G/F-actin binding protein family, which act as actin nucleators (Renault et al., 2008; Campellone and Welch, 2010; Wayt and Bretscher, 2014). G- and F-actin are constituents of myofibrils, which are small membraneless parallel fibers in muscle (Murray, 1995). An increase in myofibrils promotes hyperplasia, which involves muscle growth and is a biological basis for meat tenderness (Koohmaraie et al., 2002) and mass (Owens et al., 1993).

The ARS-BFGL-NGS-43879 (BTA9) marker exhibited a significant association with MS. The effect of this marker on MS was 0.3. The laminin alpha 4 (*LAMA4*) gene was located near this marker. LAMA4 is a member of the intramuscular extracellular matrix (ECM) protein family and plays a role in myogenesis (Okazaki et al., 2002; Miner and Yurchenco, 2004). ECM signaling pathway has been found to be up regulated and play critical functions in metabolic network for intramuscular adipose tissue deposition of Hanwoo cattle (Lee et al., 2014). It was already known that the degree of intramuscular adipose deposition in the 13th rib interface of the longissimus dorsi muscle tissue will determines the level of MS.

The ARS-BFGL-NGS-100046 (BTA6) marker exhibited a significant association with EMA, with an allele substitution effect of 1.98 $cm^2$. An RNA-binding motif protein 47 (*RBM47*) gene was located close to this marker (61.9 cM). A similar region and candidate gene were shown to be associated with milk production traits in Chinese Holstein dairy cattle (Hu et al., 2010). RBM47 in vertebrates tend to interact with Wing and Int (Wnt)/β-catenin signaling pathway and act as tissue-specific regulators (Guan et al., 2013; Vanharanta et al., 2014). In ruminant, Wnt will promote myogenesis if it regulated (Du et al., 2010). As studied by Jeong et al. (2013) in the Hanwoo steer, this mechanism contributes to increase intramuscular fat deposition in the longissimus dorsi.

Major loci on BTA14 that have been found significantly in the previous GWAS study are not included in the present study because these effects have already validated from many studies (Sharma et al., 2014; Li and Kim, 2015). The amount of effect for SNPs that were found significant in GWAS study might vary in the other population. The SNP effect could be influenced by the environmental conditions and different management practices of the animals used in the validation study. Furthermore, the other possible reason is the fact that many selection evidences have remained unrevealed in the association study due to the possible bias (Qanbari and Simianer, 2014), so that different significant SNPs could be found.

In conclusion, this validation study confirmed that six SNPs are associated with the four carcass traits in a commercial Hanwoo population. Many genes that lie in close proximity to the significant markers identified in this study have been previously been reported to have roles in carcass traits. The outcome of this study could be exploited for marker-assisted selection programs.

## CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the

manuscript.

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