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Genome-wide association studies on collagen contents trait for meat quality in Hanwoo

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Competing interests
No potential conflict of interest relevant to this article was reported.

Abstract

Beef consumers valued meat quality traits such as texture, tenderness, juiciness, flavor, and meat color that determining consumers' purchasing decision. Most research on meat quality has focused on marbling, a key characteristic related to meat eating quality. However, other important traits such as meat texture, tenderness, and color have not much studied in cattle. Among these traits, meat tenderness and texture of cattle are among the most important factors affecting quality evaluation of consumers. Collagen is the main component of connective tissues.It greatly affects meat tenderness. The objective of this study was to determine significant variants and candidate genes associated with collagen contents trait (total collagen) through genome-wide association studies (GWAS). Phenotypic and genomic data from 135 Hanwoo were used. The BLUPF90 family program and GRAMMAR method for GWAS were applied in this study. A total of 73 potential single nucleotide polymorphisms (SNPs) showed significant associations with collagen content. They were located in or near 108 candidate genes. TMEM135 and ME3 genes were identified to have the most significant SNPs associated with collagen contents trait. Data indicated that these genes were related to collagen. Biological processes and pathways for the prediction of biological functions of candidate genes were confirmed. We found that candidate genes were involved in positive regulation of CREB transcription factor activity and actin cytoskeleton related to tenderness and texture of beef. Three genes (CRTC3, MYO1C and MYLK4) belonging to these biological functions were related to tenderness. These results provide a basis for improving genomic characteristics of Hanwoo for the production of tender beef. Furthermore, they could be used they could be used as an index to select desired traits for consumers.

Keywords: Genome-wide association studies (GWAS), Single nucleotide polymorphisms (SNPs), Collagen, Meat quality, Tenderness, Hanwoo

INTRODUCTION

Korean beef consumers prefer Hanwoo cattle meat because of its tenderness and excellent flavor [1,2].

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Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Hwang I, Oh JD.
Data curation: Hwang I, Lee HK.
Formal analysis: Won KH.
Methodology: Hwang I, Oh JD.
Software: Won KH.
Validation: Kim D, Hwang I.
Investigation: Kim D.
Writing - original draft: Won KH.
Writing - review & editing: Won KH, Kim D, Hwang I, Lee HK, Oh JD.

Ethics approval and consent to participate
All animal experiments were performed in accordance with national and university guidelines. The animal protocol reported in this study was approved by the Jeonbuk National University Animal Ethics Committee in accordance with the guidelines of the Korean Council on Animal Care (CBNU 2015-048 revised in 2015).

In addition, consumers have considered meat quality such as tenderness, texture, juiciness, flavor and meat color as important factors affecting their purchasing decisions. Recently, consumers prefer tender meat to fatty meat. Among various meat traits, tenderness and texture of cattle are among the most important factors affecting the quality evaluation of consumers [3–5].

Most beef researches have focused on increasing marbling related to fat, a key characteristic associated with meat eating quality. On the other hand, tenderness in cattle has not been much studied. Increasing meat quality by increasing fat consumes a lot of production costs due to long feeding periods and expensive feed costs. This causes production of a large amount of methane gas that contributes to global warming, which is also related to the environment. Therefore, it is necessary to produce tender beef with a short-term of breeding.

Collagen is an abundant connective tissue protein. It is a contributing factor to meat tenderness and texture. Collagen also plays important roles in quality of cooked meat. Collagen fibers shrink when heated, resulting in loss of fluid and less tender meat. They also serve to hold muscle fibers together after contraction. Post-mortem degradation of collagen and the use of collagenases appear to play a role in providing desired tenderness and texture by altering connective tissue structure. Collagen is very important for maintaining an acceptable texture [6–8].

Meat sensory characteristics such as tenderness, flavor, juiciness, and color are important meat quality parameters affected by biological characteristics and proteolytic activities of muscles. Biological characteristics of muscles such as collagen, fiber type, and intramuscular adipose tissue can regulate meat tenderness and flavor. They are known to be influenced by genetic and nurturing factors [9–12].

Advances in genotyping technologies have made it possible to identify many single nucleotide polymorphisms (SNPs) distributed throughout the whole genome. This further deepens the search for genomic insights into complex traits [13]. Genome-wide association studies (GWAS) enables the detection of specific markers, genomic regions, and candidate genes associated with economically important traits. They have been conducted in livestock using high-density panels to enable large-scale genotyping [14].

Thus, the objective of this study was to detect significant variants and candidate genes associated with collagen contents trait (total collagen) using GWAS. Furthermore, this study will ultimately contribute to the the production of tender beef with short feeding periods.

MATERIALS AND METHODS

Animals and phenotypic data

A total of 135 cattle of the Hanwoo (steers, n = 103; bull, n = 5; and cow, n = 27) were used in this study. Hanwoo were raised in the same feeding condition and slaughtered in Jeollabuk-do Province. After slaughter, their longissimus dorsi (LD) muscles were sampled and cut into 2.5 cm thick steaks. These muscle samples were vacuum-packed and stored at 4℃ until 41 days postmortem [15].

Total collagen contents in each the sample was determined using the colorimetric method of Kolar [16] with suitable modifications. Briefly, 2 g of each sample was hydrolyzed with 7N H_2SO_4 at 105℃ for 16 h. The hydrolysate was diluted with distilled water to 500 mL and filtered. Filter a part of the mixture into 100 mL Erlenmeyer flask, the filtrate is stable at least 2 weeks at 4℃. About 2 mL of diluted filtrate was taken and added with chloramine T solution into a tube and left at room temperature for 20 min. Thereafter, a 4-dimethylamino benzaldehyde solution was added and the mixture was heated at 60℃ for 15 min. Absorbance values of samples and hydroxyproline standard were measured at 558 nm using a spectrophotometer. A standard calibration curve was plotted for 4-hydroxyproline and a regression line was drawn. Collagen content was expressed in

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mg/100 g sample after converting hydroxyproline into collagen with a conversion factor of 7.14. For insoluble (or heat stable) collagen contents, homogenized samples in Ringer's solution [17] were heated at 77℃ for 70 min, followed by centrifugation. Residual fractions were hydrolyzed in 7N H_2SO_4 for 16 h at 105℃. The hydroxyproline content of the hydrolysate was determined after neutralization according to the procedure of Kolar. The soluble collagen content was calculated based on the difference between total and insoluble collagen contents [18].

Genotypic data and quality control

A total of 135 Hanwoo, consisting of steers (n = 103), bulls (n = 5), and cows (n = 27) were genotyped using a Bovine SNP50k chip (Illumina, San Diego, CA, USA). A total of 52,195 SNPs were collected. We performed the process of quality control (QC) based on the following criteria to ensure the quality of genotypic data obtained: i) removing individuals with identical genotype using identity by state (IBS) distance test (> 0.99), ii) eliminating individuals with call rates less than 90%, iii) removing SNPs with minor allele frequency less than 1%, iv) filtering out SNPs with call rates less than 90%, and v) removing SNPs with significant departure from Hardy Weinberg equilibrium ($p < 10^{-7}$). These procedures were implemented with PLINK v1.07 [19] (Fig. 1).

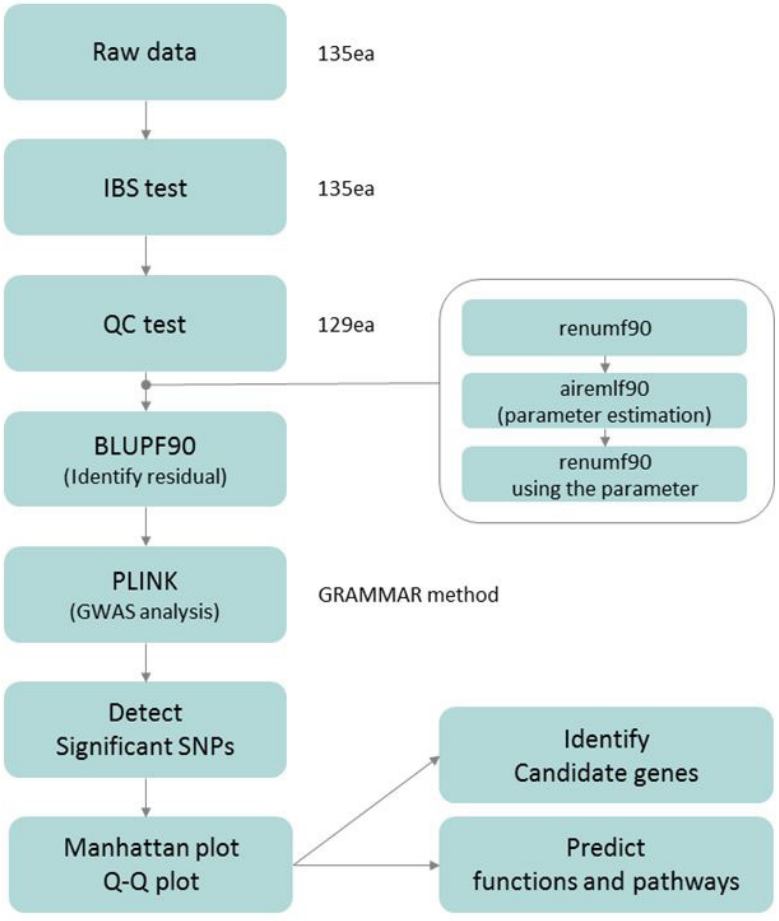

Fig. 1. Flow chart related to GWAS analysis using genomic and phenotypic data. IBS, identity by state; QC, quality control; GWAS, genome-wide association studies; GWAS, genome-wide association studies; SNPs, single nucleotide polymorphisms; QQ, quantile-quantile.

Statistical analysis

BLUPF90 is, a software that comprises a family of program in Fortran 90/95 for mixed model computations in animal breeding [20]. It was used to estimate variance components and genetic parameters of collagen contents trait and residuals that were difference between the actual phenotype value and the estimated value in order to identify only genetic effects. First, QC data were renumbered and variance was estimated using RENUMF90. Second, we estimated variance components and genetic parameter using AIREMLF90 [21]. Third, we performed RENUMF90 analysis one more time to improve the accuracy of analysis using the estimated variance components and genetic parameters. Finally, these various components were then used to identify residuals. These procedures were performed using a multiple-trait model. Using the multiple-trait model, we estimated the variance component and genetic parameters of the collagen contents traits. The equation was as follows:

$$Y = X\beta + Za + e;$$

Where Y was the vector of phenotypic observations for total collagen, heat insoluble collagen, or soluble collagen contents; β was the vector of fixed effects including contemporary group effects, time of slaughtering (year, month), time of age at slaughter (month, days, age) and sex (cow, bull, steer) as a linear covariate; a was the vector of direct additive effects; e was the vector of residual random effects; X was the incidence matrix relating the phenotypes to the fixed effects; and Z was the incidence matrix relating the animal to the phenotype [22].

Using genome-wide rapid association using mixed model and regression (GRAMMAR) [23] in PLINK and the residuals obtained, we estimated SNP effect that affected collagen contents trait in the meat of Hanwoo (Fig. 1).

Identification of significant SNPs and annotation of candidate genes

We obtained significant SNPs associated with the phenotype based on $p- < 0.001$. Candidate genes associated with significant SNPs were annotated within 500 kb downstream and upstream of detected SNPs based on the Bos taurus transfer format (GTF) (version ARS-UCD1.2.104) in Ensembl database.

Functional analysis

We performed Gene Ontology (GO) [24] and Kyoto Encyclopedia of Genes and Genomes (KEGG) [25] pathway analyses to investigate functions of candidate genes using the Database for Annotation, Visualization and Integrated Discovery (DAVID) [26].

RESULTS

Phenotypes and genotypes

We identified 129 individuals after removing six (ID: 002311515862, 002311652670, 002300057089, 002085007947, 002114973907 and 002112336318) out of 135 individuals by IBS distance test and outlier. We identified basic statistics for phenotypic data of 129 Hanwoo after QC. The estimates for collagen contents traits were on average 0.362, 0.250 and 0.114 respectively (Table 1).

Through the QC test, 5,715 SNPs out of 52,195 SNPs were removed and 46,480 SNPs associated with collagen contents trait were finally used for GWAS analysis. We confirmed the difference in the number of available SNPs per chromosome and the interval size (kb) of

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each autosome of autosuomes 1 to 29 before and after QC. We identified the number of SNPs considered useful for Hanwoo per chromosome, ranging from a minimum of 799 to a maximum of 2,909. About 89.1% of total SNPs were selected as available SNPs. The average distance of adjacent SNPs for each chromosome was 54.343 kb (Table 2).

Table 1. The basic statistics for phenotypic data

Traits	N	Min	Max	Mean	SD
Total collagen (TC)	129	0.140	1.500	0.362	0.216
Heat insoluble collagen (HC)	129	0.030	1.310	0.250	0.194
Soluble collagen (SC)	129	−0.020	0.310	0.114	0.069

Table 2. The number of available SNPs and average interval distance between adjacent SNPs in Bovine SNP50k chip

BTA	Number of SNPs		Remove frequency (%)	Mean of Interval SNP	
	Before QC	After QC		Before QC	After QC
1	3,225	2,909	0.902	49.037	54.365
2	2,756	2,444	0.887	49.614	55.94
3	2,582	2,280	0.883	46.937	53.157
4	2,479	2,214	0.893	48.678	54.507
5	2,156	1,912	0.887	56.185	63.359
6	3,158	2,820	0.893	37.698	42.218
7	2,481	2,250	0.907	45.316	49.971
8	2,246	2,011	0.895	50.339	56.224
9	2,077	1,858	0.895	50.802	56.793
10	2,357	2,085	0.885	44.216	49.987
11	2,181	1,915	0.878	49.164	55.997
12	1,651	1,427	0.864	55.118	63.776
13	1,684	1,521	0.903	49.829	55.173
14	2,274	1,965	0.864	36.583	42.338
15	1,681	1,486	0.884	50.437	57.06
16	1,599	1,420	0.888	50.947	57.374
17	1,567	1,386	0.884	47.84	54.07
18	1,303	1,167	0.896	50.231	55.883
19	1,380	1,243	0.901	46.078	51.16
20	1,571	1,388	0.884	45.602	51.618
21	1,398	1,255	0.898	50.893	56.697
22	1,212	1,085	0.895	50.55	56.472
23	1,124	1,019	0.907	46.505	51.175
24	1,230	1,108	0.901	50.745	56.137
25	939	847	0.902	45.633	50.596
26	1,032	922	0.893	49.511	55.324
27	918	823	0.897	49.435	55.149
28	905	799	0.883	51.126	57.873
29	1,029	921	0.895	50.084	55.546
Total	52,195	46,480	0.891	48.453	54.343

SNP, single nucleotide polymorphism; QC, quality control.

GWAS analysis

We identified residuals using BLUPF90 for GWAS analysis. These residuals were differences between observed and estimated phenotypic values. In the total collagen contents trait, the observed value was 0.180 and the estimated value was 0.594. Thus, the residual value was the lowest at −0.414 (ID: 002083966119). Observed and estimated values were 1.500 and 0.625, respectively. The residual value was the highest at 0.875 (ID: 002083963503) (Table 3).

The quantile-quantile (QQ) plot and inflation control (lambda) value were used to compare observed distributions of $-\log_{10}(p)$ to the expected distribution under the no association model for total collagen contents trait (Fig. 2). The QQ plot for trait showed that the mixed linear model fitted the data well. Estimates of SNP effects associated with total collagen contents trait were within a range of −0.273 to 0.438 (Fig. 3).

Identification of significant SNPs and annotation of candidate genes

We prepared a Manhattan plot of −log10(p) for genomic positions of SNP markers using *p*-value from the SNP effect result to confirm the significant SNP associated with total collagen contents trait. Based on $p < 0.001$, a total of 73 SNPs were significantly associated with the trait (Fig. 4

Table 3. The basic statistics for results of BLUPF90 analysis

Statistics	N	Min	Max	Range	Mean	Median	SE	VAR	SD	Coef.var
TC_observation	129	−0.209	1.5	1.709	0.359	0.31	0.019	0.049	0.221	0.615
TC_estimation	129	0.059	0.696	0.637	0.361	0.344	0.012	0.019	0.139	0.385
TC_residual	129	−0.414	0.875	1.289	0	−0.015	0.015	0.027	0.165	2.05E+17

VAR, variance; Coef.var, coefficient of variation.

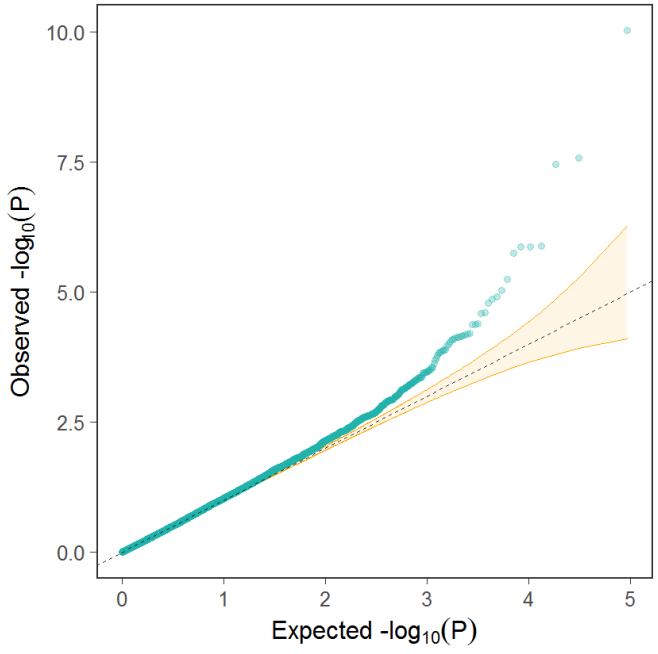

Fig. 2. The QQ-plot for the studied total collagen contents trait. The dotted line represents the 95% concentration band under the null hypothesis no association between trait and SNPs. The green dots represent the p-values. QQ, quantile-quantile; SNPs, single nucleotide polymorphisms.

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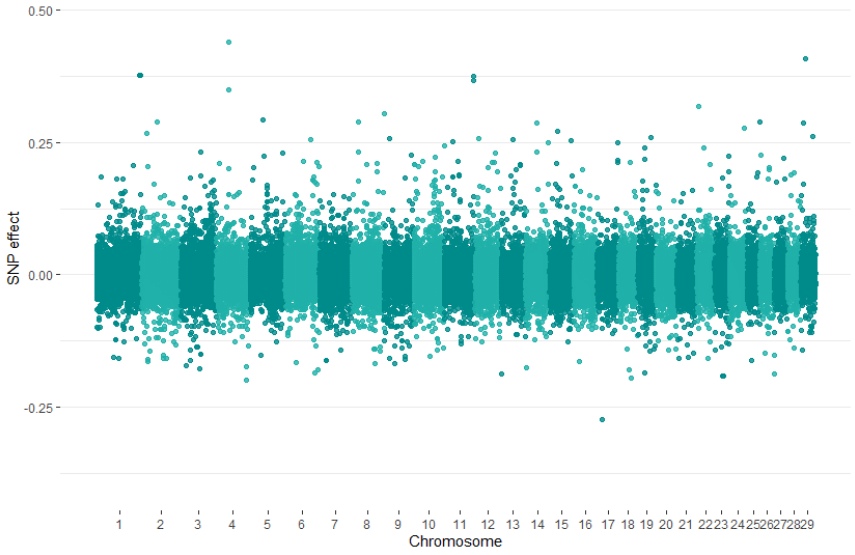

Fig. 3. The Manhattan plot of SNP effects for GWAS analysis using GRAMMAR methods in PLINK. SNP, single nucleotide polymorphism; GWAS, genome-wide association studies.

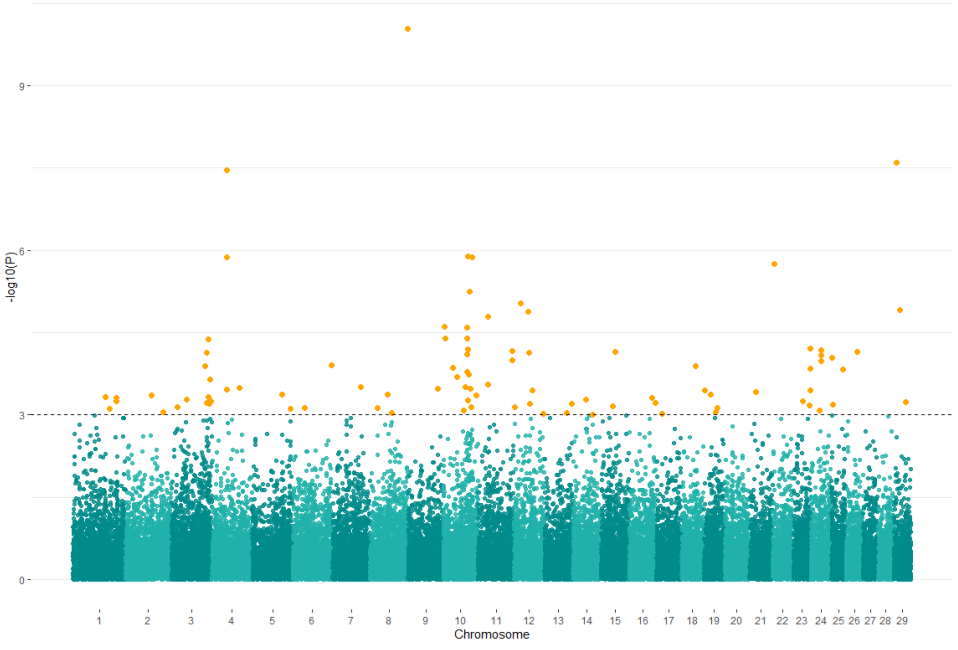

Fig. 4. The Manhattan plot of GWAS for total collagen contents trait with significance thresholds indicated at $-\log_{10}P > 1 \times 10^{-3}$. The orange dots represent significant SNPs associated with total collagen contents trait. GWAS, genome-wide association studies; SNPs, single nucleotide polymorphisms.

and Table 4). A total of 108 candidate genes associated with significant SNPs were annotated based on Bos taurus genome. The most significant SNP was confirmed to be UA-IFASA-8514 (chr29:8936383) in Transmembrane protein 135 (TMEM135) and Malic Enzyme 3 (ME3) genes.

Functional analysis

We identified functions of candidate genes associated with total collagen contents trait from GO

Table 4. Significant SNPs (total collagen)

SNP_id	SNP (MinorA/ MajorB)	Chr	Genomic position	Estimate	SE	*p*-value	−LogP	nAA	nAB	nBB	σ2 (SNP)/ σ2 (trait)	Gene_name
Hapmap38956-BTA-43309	[C/A]	1	97,366,225	0.1576	0.0440	0.0005	3.3186	1	10	119	0.0804	SLC7A14
ARS-BFGL-NGS-535	[G/A]	1	109,221,369	−0.0636	0.0185	0.0008	3.1119	27	52	51	0.0719	MFSD1, RSRC1
rs383007308	[A/G]	1	131,149,414	0.1204	0.0340	0.0006	3.2490	1	22	107	0.0893	ESYT3
ARS-BFGL-NGS-109828	[G/A]	2	77,033,601	0.1496	0.0415	0.0004	3.3557	1	13	116	0.0894	CNTNAP5
rs110414144	[A/C]	2	113,584,292	0.0834	0.0245	0.0009	3.0467	5	48	77	0.0886	DOCK10, NYAP2
3:101365765	[C/A]	3	101,365,765	0.1330	0.0337	0.0001	3.8871	1	22	107	0.1090	ZSWIM5, TMEM53
rs456169856	[A/G]	3	106,866,376	0.1053	0.0299	0.0006	3.2230	3	22	105	0.0783	MACF1
BTB-01834875	[G/A]	3	46,721,234	−0.0763	0.0215	0.0005	3.2758	11	47	72	0.0834	DPYD, PTBP2
ARS-BFGL-NGS-13158	[C/G]	3	19,337,220	0.0790	0.0228	0.0007	3.1439	8	48	74	0.0851	POGZ
BTB-00157833	[G/A]	3	111,842,741	0.0711	0.0198	0.0005	3.3249	19	57	54	0.0861	CSMD2
rs381136948	[A/C]	3	112,012,156	0.1779	0.0419	4.E-05	4.3832	1	11	118	0.1105	CSMD2, GIGYF2
Hapmap57254-rs29022776	[A/G]	3	113,433,557	0.0763	0.0218	0.0006	3.1947	12	62	56	0.0947	MROH2A
rs385230778	[A/G]	3	115,009,419	0.1587	0.0419	0.0002	3.6379	2	7	121	0.0750	AGAP1
ARS-BFGL-NGS-118221	[A/C]	5	90,684,739	0.0998	0.0276	0.0004	3.3663	2	40	88	0.1028	PLEKHA5
Hapmap41631-BTA-75177	[A/G]	5	114,405,063	0.0685	0.0199	0.0008	3.1054	16	45	69	0.0720	EFCAB6
ARS-BFGL-NGS-11339	[A/G]	4	45,128,142	0.3496	0.0689	1.E-06	5.8765	0	5	125	0.1695	RELN, LHFPL3
BTB-00182993	[C/A]	4	45,513,003	0.4383	0.0746	3.E-08	7.4631	0	4	126	0.2139	RELN, LHFPL3
BTA-70441-no-rs	[G/A]	4	45,602,047	0.2001	0.0544	0.0003	3.4599	0	9	121	0.0984	RELN, LHFPL3, ENSBTAG00000048818
ARS-BFGL-NGS-110196	[G/A]	4	81,109,721	−0.0970	0.0262	0.0003	3.4935	4	33	93	0.0919	SUGCT, POU6F2
Hapmap23877-BTA-143906	[A/C]	6	36,829,725	0.1503	0.0435	0.0007	3.1284	0	16	114	0.0959	HERC6, NCAPG
BTB-01265106	[A/C]	6	116,779,750	0.2034	0.0514	0.0001	3.8972	0	11	119	0.1232	ZFYVE28, FGFR3
BTB-00348139	[A/G]	8	52,645,428	0.1334	0.0369	0.0004	3.3626	0	22	108	0.1013	PCSK5
ARS-BFGL-NGS-66538	[A/G]	8	64,011,227	−0.1006	0.0297	0.0009	3.0301	2	29	99	0.0825	GABBR2, COL15A1
ARS-BFGL-NGS-34771	[A/G]	8	21,943,761	0.1775	0.0513	0.0007	3.1288	1	6	123	0.0691	ENSBTAG00000053368
BTB-00960162	[A/G]	7	83,915,853	0.1482	0.0400	0.0003	3.5092	0	18	112	0.1040	VCAN, EDIL3
rs443739156	[C/A]	11	30,840,950	0.1851	0.0495	0.0003	3.5511	0	11	119	0.1021	STON1, FSHR
ARS-BFGL-NGS-83866	[A/G]	11	30,851,124	0.2519	0.0562	2.E-05	4.7918	0	8	122	0.1391	STON1, FSHR
BTB-01944534	[A/G]	11	30,826,527	0.1851	0.0495	0.0003	3.5511	0	11	119	0.1021	FSHR
ARS-BFGL-NGS-2573	[A/G]	11	101,602,103	0.3742	0.0908	0.0001	4.1669	0	3	127	0.1174	ABL1, PRRC2B, MED27
ARS-BFGL-NGS-94862	[G/A]	11	103,534,103	0.3660	0.0911	0.0001	3.9978	0	3	127	0.1123	NACC2
BTA-62308-no-rs	[G/A]	10	29,176,267	0.2134	0.0542	0.0001	3.8626	0	9	120	0.1127	AVEN, FMN1
ARS-BFGL-NGS-3005	[C/A]	10	81,981,544	0.1212	0.0329	0.0003	3.4729	2	19	109	0.0871	SYNJ2BP
BTA-60292-no-rs	[A/G]	10	7,308,238	0.1575	0.0370	4.E-05	4.3893	1	16	113	0.1175	CERT1, IQGAP2
ARS-BFGL-NGS-118392	[G/A]	10	84,632,536	0.1263	0.0364	0.0007	3.1479	1	18	111	0.0833	DPF3, PSEN1, MIDEAS
Hapmap41972-BTA-79298	[A/G]	10	85,547,284	0.1577	0.0311	1.E-06	5.8690	3	15	112	0.1357	LIN52, YLPM1
Hapmap39952-BTA-86345	[C/A]	10	99,626,471	0.0730	0.0202	0.0004	3.3565	28	69	33	0.0978	SPATA7
Hapmap44561-BTA-72345	[G/A]	10	60,168,183	0.1684	0.0492	0.0008	3.0779	1	7	122	0.0697	TRPM7, ATP8B4
ARS-BFGL-NGS-110504	[A/C]	10	70,556,929	0.1485	0.0383	0.0002	3.7812	1	15	114	0.0991	PSMA3
ARS-BFGL-NGS-116012	[G/A]	10	71,266,946	0.1678	0.0411	0.0001	4.0971	1	12	115	0.1070	KIAA0586, JKAMP
BTB-00434096	[G/A]	10	71,082,204	0.1779	0.0419	4.E-05	4.3862	1	11	118	0.1105	DAAM1
ARS-BFGL-NGS-23163	[A/G]	10	71,835,260	0.1944	0.0445	3.E-05	4.5979	1	9	120	0.1126	RTN1, PCNX4
BTB-01203179	[A/G]	10	72,694,329	0.0909	0.0220	0.0001	4.2010	9	52	69	0.1195	LRRC9, SLC38A6
ARS-BFGL-NGS-69839	[G/A]	10	74,011,076	0.2210	0.0435	1.E-06	5.8897	1	9	120	0.1455	PRKCH, SYT16
rs378634523	[A/G]	10	76,078,820	0.0913	0.0237	0.0002	3.7395	6	45	79	0.1048	GPHB5, MTHFD1
ARS-BFGL-NGS-116025	[T/A]	10	79,496,312	0.1353	0.0286	6.E-06	5.2450	2	30	98	0.1530	GPHN, RAD51B
ARS-BFGL-NGS-101050	[G/A]	12	19,868,800	0.0917	0.0199	9.E-06	5.0292	21	67	42	0.1504	RNASEH2B
BTA-21437-no-rs	[C/A]	12	43,601,825	0.0932	0.0205	1.E-05	4.8732	14	51	60	0.1379	KLHL1
Hapmap45915-BTA-22734	[A/T]	12	46,867,626	0.0742	0.0212	0.0006	3.2022	12	48	70	0.0811	DACH1

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Table 4. Continued

SNP_id	SNP (MinorA/ MajorB)	Chr	Genomic position	Estimate	SE	*p*-value	−LogP	nAA	nAB	nBB	σ2 (SNP)/ σ2 (trait)	Gene_name
Hapmap51092-BTA-93283	[A/G]	12	3,172,154	−0.0684	0.0197	0.0007	3.1424	19	54	57	0.0786	DIAPH3
ARS-BFGL-NGS-43617	[A/G]	15	35,138,494	0.1610	0.0463	0.0007	3.1627	0	13	117	0.0905	SERGEF, PLEKHA7
ARS-BFGL-NGS-118358	[G/A]	15	42,960,757	0.1453	0.0353	0.0001	4.1554	0	24	105	0.1310	SBF2, SWAP70, DENND5A
Hapmap40712-BTA-33406	[A/G]	13	67,101,174	0.1238	0.0365	0.0009	3.0383	1	18	111	0.0800	KIAA1755, PPP1R16B
ARS-BFGL-NGS-115847	[A/G]	14	40,458,050	0.2866	0.0805	0.0005	3.2804	0	4	125	0.0922	ZFHX4
ARS-BFGL-NGS-116702	[A/G]	16	79,720,573	0.1447	0.0411	0.0006	3.2188	0	17	113	0.0941	IGFN1, PPP1R12B
BTA-40408-no-rs	[G/C]	17	17,408,409	−0.2725	0.0805	0.0010	3.0203	0	4	126	0.0827	RNF150, MAML3
ARS-BFGL-NGS-98331	[A/G]	21	22,057,989	0.1534	0.0421	0.0004	3.4121	0	16	114	0.0999	CRTC3, SLC28A1
Hapmap42198-BTA-39980	[G/A]	18	41,617,350	−0.0790	0.0200	0.0001	3.8824	30	68	32	0.1145	ZNF536
ARS-BFGL-NGS-111273	[A/G]	19	37,699,961	−0.0877	0.0258	0.0009	3.0501	4	40	86	0.0850	PHB, CALCOCO2, SKAP1
Hapmap60163-rs29015084	[G/A]	19	22,120,443	0.0910	0.0252	0.0004	3.3654	4	45	81	0.0988	MYO1C
ARS-BFGL-NGS-32460	[G/A]	19	40,716,234	0.0721	0.0209	0.0007	3.1266	14	56	60	0.0835	TNS4
BTB-00885986	[A/C]	24	31,423,445	−0.0754	0.0188	0.0001	3.9739	31	60	39	0.1040	ZNF521
BTB-00885964	[C/A]	24	31,457,933	−0.0771	0.0189	0.0001	4.0891	31	61	38	0.1088	ZNF521
Hapmap49083-BTA-21452	[A/G]	24	32,171,355	0.0820	0.0199	0.0001	4.1832	16	53	61	0.1088	ZNF521
BTB-00830153	[A/G]	22	4,534,118	0.3169	0.0633	2.E-06	5.7484	0	6	124	0.1665	RBMS3
ARS-BFGL-NGS-103489	[A/G]	23	49,135,538	0.1671	0.0479	0.0007	3.1708	0	13	117	0.0975	FARS2, CDYL
ARS-BFGL-NGS-27800	[A/G]	23	50,897,089	0.1354	0.0369	0.0004	3.4454	1	17	112	0.0913	SLC22A23, MYLK4, GMDS
ARS-BFGL-NGS-108494	[A/G]	23	51,241,585	0.1951	0.0471	0.0001	4.2057	0	12	118	0.1232	SERPINB6, GMDS
ARS-BFGL-NGS-13891	[A/G]	23	51,487,543	0.2238	0.0571	0.0001	3.8431	0	8	122	0.1098	GMDS
ARS-BFGL-NGS-27911	[A/G]	26	35,048,102	0.1982	0.0483	0.0001	4.1410	1	7	122	0.0965	ABLIM1, ATRNL1
UA-IFASA-8514	[C/A]	29	8,936,383	0.2864	0.0482	3.E-08	7.5884	0	11	119	0.2443	TMEM135, ME3
Hapmap27205-BTA-156398	[A/G]	29	18,203,037	0.4084	0.0897	1.E-05	4.9151	0	3	127	0.1399	GAB2, RSF1
ARS-BFGL-NGS-92926	[C/A]	25	4,131,112	0.1035	0.0296	0.0007	3.1795	3	23	104	0.0780	HMOX2, ENSBTAG00000026383
ARS-BFGL-BAC-42642	[A/G]	25	35,532,374	0.1930	0.0493	0.0001	3.8300	0	12	118	0.1206	CUX1

SNPs, single nucleotide polymorphisms; Chr, chromosome.

and KEGG analysis using DAVID (Tables 5 and 6). In biological process from GO, we confirmed eight gene ontologies: negative regulation of peptidyl-serine dephosphorylation (GO:1902309), negative regulation of ubiquitin-protein transferase activity (GO:0051444), receptor localization to synapse (GO:0097120), T cell receptor signaling pathway (GO:0050852), negative regulation of epidermal growth factor-activated receptor activity (GO:0007175), somite development (GO:0061053), positive regulation of CREB transcription factor activity (GO:0032793), and amino acid transmembrane transport (GO:0003333). In cellular component, we identified seven gene ontologies: cytosol (GO:0005829), nucleoplasm (GO:0005654), actin cytoskeleton (GO:0015629), perinuclear region of cytoplasm (GO:0048471), cytoplasm (GO:0005737), nuclear speck (GO:0016607), and cell projection (GO:0042995). Through KEGG analysis, we identified regulation of actin cytoskeleton (bta04810) pathway.

DISCUSSION

Meat tenderness of beef is one of the most important factors affecting meat quality evaluation of consumers [27]. The production of beef in consideration of a recent consumption pattern that favors tender meat with low fat is required. For this, it is necessary to produce tender meat with a short-term of breeding. This will make it possible to reduce production costs and protect the environment.

Table 5. The results of gene ontology (GO) analysis of candidate genes associated with total collagen contents trait from GWAS analysis

GO ID	Description	#Genes	Fold enrichment	*p*-value	Gene name
Biological process					
GO:1902309	Negative regulation of peptidyl-serine dephosphorylation	2	181.846	0.011	PPP1R16B, SWAP70
GO:0051444	Negative regulation of ubiquitin-protein transferase activity	2	51.956	0.037	ABL1, PSEN1
GO:0097120	Receptor localization to synapse	2	40.410	0.048	RELN, SYNJ2BP
GO:0050852	T cell receptor signaling pathway	3	7.577	0.059	ABL1, PSEN1, SKAP1
GO:0007175	Negative regulation of epidermal growth factor-activated receptor activity	2	30.308	0.063	ZFYVE28, PSEN1
GO:0061053	Somite development	2	25.978	0.074	RAD51B, MTHFD1
GO:0032793	Positive regulation of CREB transcription factor activity	2	24.246	0.079	CRTC3, RELN
GO:0003333	Amino acid transmembrane transport	2	20.205	0.094	SLC7A14, SLC38A6
Cellular component					
GO:0005829	Cytosol	25	1.881	0.002	CRTC3, CALCOCO2, SLC7A14, GPHN, SPATA7, GIGYF2, ZFYVE28, DOCK10, POGZ, ABL1, DENND5A, CERT1, HERC6, PRKCH, PLEKHA5, YLPM1, MED27, PLEKHA7, DAAM1, MTHFD1, DPYD, SERGEF, SLC28A1, SKAP1, RBMS3
GO:0005654	Nucleoplasm	20	2.037	0.003	RNASEH2B, CRTC3, SLC7A14, PLEKHA5, RSF1, EFCAB6, PSEN1, MED27, PLEKHA7, SPATA7, DOCK10, MYO1C, CUX1, POGZ, DPF3, MAML3, SERGEF, CERT1, SKAP1, HERC6
GO:0015629	Actin cytoskeleton	5	6.402	0.008	MACF1, ABLIM1, MYO1C, SWAP70, ABL1
GO:0048471	Perinuclear region of cytoplasm	7	3.399	0.016	PPP1R16B, CALCOCO2, ABL1, PSEN1, SYNJ2BP, CERT1, FGFR3
GO:0005737	Cytoplasm	27	1.547	0.017	MACF1, CRTC3, NCAPG, FMN1, IQGAP2, GPHN, FARS2, ABLIM1, DACH1, RELN, GMDS, RSRC1, RNF150, TNS4, GABBR2, PRKCH, SWAP70, KLHL1, GAB2, CDYL, SERPINB6, PSMA3, MYO1C, DPYD, PPP1R12B, SKAP1, GPHB5
GO:0016607	Nuclear speck	6	3.853	0.019	PPP1R16B, RSRC1, YLPM1, MAML3, CDYL, SLC28A1
GO:0042995	Cell projection	3	6.031	0.087	PPP1R16B, MACF1, STON1

GWAS, genome-wide association studies.

Table 6. The results of KEGG pathway analysis of candidate genes associated with total collagen contents trait from GWAS analysis

KEGG ID	Description	#Genes	Fold enrichment	*p*-value	Gene name
bta04810	regulation of actin cytoskeleton	5	5.610	0.011	DIAPH3, IQGAP2, PPP1R12B, FGFR3, MYLK4

KEGG, Kyoto Encyclopedia of Genes and Genomes; GWAS, genome-wide association studies.

We studied collagen contents that contribute to meat tenderness and texture for the production of tender meat. In particular, since collagen is greatly affected by genetics [28], it is thought to be suitable for use as a selection index to soft Hanwoo meat production through genomic analysis. Therefore, we conducted this study to detect significant SNPs and candidate genes associated with collagen contents trait using GWAS.

We identified a total of 73 significant SNPs and 108 candidate genes associated with total collagen contents trait. We identified TMEM135 and ME3 genes in which the most significant SNPs associated with collagen contents trait were located. TMEM135 gene was essential for

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collagen production and secretion in human cells [29]. In mouse hearts, forced overexpression TMEM135 can lead to collagen accumulation [30]. ME3 gene has beem found to be related to COL14A1, which plays a role in cross-linking collagen 1 and developing fibrous structure [31]. These collagen cross-links are regulated by myofibrillar protein and diverse gene expression related to muscle development. They determine meat tenderness [32]. Since we confirmed that these genes were related to collagen, we predicted that variants in these genes might affect meat tenderness of Hanwoo. We then predicted biological functions and pathways associated with candidate genes to find out how these genes were related to the collagen contents trait. In biological process of GO, we found that CRTC3 (Cyclic adenosine monophosphate [cAMP] -regulated transcriptional coactivator 3) and RELN genes were involved in positive regulation of CREB transcription factor activity (GO:0032793). CRTC3 is a coactivator of cAMP response element binding protein (CREB) that mediates the function of protein kinase A (PKA) signaling pathway. It is involved in various biological processes including lipid and energy metabolism. In porcine, CRTC3 expression is related to fat deposition in vivo. Furthermore, CRTC3 overexpression can increase lipid accumulation and the expression of mature adipocyte-related genes in cultured porcine subcutaneous adipocytes [33]. Lipid accumulation affects meat production and meat quality such as tenderness, juiciness, and flavor [34]. The deposition of subcutaneous and visceral fat directly influences backfat thickness and growth efficiency, while intramuscular fat (IMF) content directly affects meat quality including the flavor, juiciness, tenderness, and fatty acid (FA) composition [35]. In cellular component of GO and KEGG pathway, we found that candidate genes were involved in actin cytoskeleton (GO:0015629) and regulation of actin cytoskeleton (bta04810). A previous study has revealed that actin cytoskeleton-related cell junction is associated with lipid metabolism to influence the deposition of IMF. IMF is one important factor that can influence meat quality. A certain amount of IMF can enhance meat quality traits such as the flavor, juiciness, water holding capacity, and tenderness [36]. MYO1C (Myosin IC) and MYLK4 (Myosin Light Chain Kinase Family Member 4) genes belonging to these biological functions and pathways wererelated to meat tenderness. MYO1C has specialized functions in certain cell types such as muscles. Some researchers have linked myosins to meat tenderness [37]. MYLK4 regulates yak muscle contraction via phosphorylating myosin light chain molecules. Such phosphorylation can positively impact meat tenderness [38].

Variants and genes identified in this study are expected to provide important information for genomic selection of phenotypes to improve meat quality of Hanwoo. Furthermore, they provide a basis for further studies on consumption traits.

REFERENCES

1. Bulik-Sullivan BK, Loh PR, Finucane HK, Ripke S, Yang J, Schizophrenia Working Group of the Psychiatric Genomics Consortium, et al. LD score regression distinguishes confounding from polygenicity in genome-wide association studies. Nat Genet. 2015;47:291-5. https://doi.org/10.1038/ng.3211
2. Strucken EM, Al-Mamun HA, Esquivelzeta-Rabell C, Gondro C, Mwai OA, Gibson JP. Genetic tests for estimating dairy breed proportion and parentage assignment in East African crossbred cattle. Genet Sel Evol. 2017;49:67. https://doi.org/10.1186/s12711-017-0342-1
3. O'Quinn TG, Legako JF, Brooks JC, Miller MF. Evaluation of the contribution of tenderness, juiciness, and flavor to the overall consumer beef eating experience. Transl Anim Sci. 2018;2:26-36. https://doi.org/10.1093/tas/txx008
4. Liu J, Ellies-Oury MP, Stoyanchev T, Hocquette JF. Consumer perception of beef quality and how to control, improve and predict it? Focus on eating quality. Foods. 2022;11:1732. https://

doi.org/10.3390/foods11121732
5. Bedhane M, van der Werf J, Gondro C, Duijvesteijn N, Lim D, Park B, et al. Genome-wide association study of meat quality traits in Hanwoo beef cattle using imputed whole-genome sequence data. Front Genet. 2019;10:1235. https://doi.org/10.3389/fgene.2019.01235
6. McCormick RJ. Extracellular modifications to muscle collagen: implications for meat quality. Poult Sci. 1999;78:785-91. https://doi.org/10.1093/ps/78.5.785
7. Zhu Y, Gagaoua M, Mullen AM, Kelly AL, Sweeney T, Cafferky J, et al. A proteomic study for the discovery of beef tenderness biomarkers and prediction of Warner–Bratzler shear force measured on longissimus thoracis muscles of young Limousin-Sired bulls. Foods. 2021;10:952. https://doi.org/10.3390/foods10050952
8. Lepetit J. Collagen contribution to meat toughness: theoretical aspects. Meat Sci. 2008;80:960-7. https://doi.org/10.1016/j.meatsci.2008.06.016
9. Bernard C, Cassar-Malek I, Le Cunff M, Dubroeucq H, Renand G, Hocquette JF. New indicators of beef sensory quality revealed by expression of specific genes. J Agric Food Chem. 2007;55:5229-37. https://doi.org/10.1021/jf063721
10. Maltin C, Balcerzak D, Tilley R, Delday M. Determinants of meat quality: tenderness. Proc Nutr Soc. 2003;62:337-47. https://doi.org/10.1079/PNS2003248
11. Mullen AM, Stapleton PC, Corcoran D, Hamill RM, White A. Understanding meat quality through the application of genomic and proteomic approaches. Meat Sci. 2006;74:3-16. https://doi.org/10.1016/j.meatsci.2006.04.015
12. Taye M, Kim J, Yoon SH, Lee W, Hanotte O, Dessie T, et al. Whole genome scan reveals the genetic signature of African Ankole cattle breed and potential for higher quality beef. BMC Genet. 2017;18:11. https://doi.org/10.1186/s12863-016-0467-1
13. Meuwissen THE, Hayes BJ, Goddard ME. Prediction of total genetic value using genome-wide dense marker maps. Genetics. 2001;157:1819-29. https://doi.org/10.1093/genetics/157.4.1819
14. Goddard ME, Hayes BJ. Mapping genes for complex traits in domestic animals and their use in breeding programmes. Nat Rev Genet. 2009;10:381-91. https://doi.org/10.1038/nrg2575
15. Lee SH, Kim CN, Ko KB, Park SP, Kim HK, Kim JM, et al. Comparisons of beef fatty acid and amino acid characteristics between Jeju black cattle, Hanwoo, and Wagyu breeds. Food Sci Anim Resour. 2019;39:402-9. https://doi.org/10.5851/kosfa.2019.e33
16. Kolar K. Colorimetric determination of hydroxyproline as measure of collagen content in meat and meat products: NMKL collaborative study. J Assoc Off Anal Chem. 1990;73:54-7. https://doi.org/10.1093/jaoac/73.1.54
17. Hill F. The solubility of intramuscular collagen in meat animals of various ages. J Food Sci. 1966;31:161-6. https://doi.org/10.1111/j.1365-2621.1966.tb00472.x
18. Kim YA, Van Ba H, Dashdorj D, Hwang I. Effect of high-pressure processing on the quality characteristics and shelf-life stability of Hanwoo beef marinated with various sauces. Korean J Food Sci Anim Resour. 2018;38:679-92. https://doi.org/10.5851%2Fkosfa.2018.e4
19. 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. https://doi.org/10.1086/519795
20. Hammami H, Rekik B, Soyeurt H, Bastin C, Bay E, Stoll J, et al. Accessing genotype by environment interaction using within- and across-country test-day random regression sire models. J Anim Breed Genet. 2009;126:366-77.
21. VanRaden PM. Efficient methods to compute genomic predictions. J Dairy Sci. 2008;91:4414-23. https://doi.org/10.3168/jds.2007-0980
22. Srivastava S, Lopez BI, de las Heras-Saldana S, Park JE, Shin DH, Chai HH, et al. Estimation

https://doi.org/10.5187/jast.2022.e110

of genetic parameters by single-trait and multi-trait models for carcass traits in Hanwoo cattle. Animals. 2019;9:1061. https://doi.org/10.3390/ani9121061
23. Aulchenko YS, de Koning DJ, Haley C. Genomewide rapid association using mixed model and regression: a fast and simple method for genomewide pedigree-based quantitative trait loci association analysis. Genetics. 2007;177:577-85. https://doi.org/10.1534/genetics.107.075614
24. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. Nat Genet. 2000;25:25-9. https://doi.org/10.1038/75556
25. Kanehisa M, Goto S. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res. 2000;28:27-30. https://doi.org/10.1093/nar/28.1.27
26. Sherman BT, Hao M, Qiu J, Jiao X, Baseler MW, Lane HC, et al. DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). Nucleic Acids Res. 2022;50:W216-21. https://doi.org/10.1093/nar/gkac194
27. Weston AR, Rogers RW, Althen TG. Review: the role of collagen in meat tenderness. Prof Anim Sci. 2002;18:107-11. https://doi.org/10.15232/S1080-7446(15)31497-2
28. Belbin GM, Odgis J, Sorokin EP, Yee MC, Kohli S, Glicksberg BS, et al. Genetic identification of a common collagen disease in puerto ricans via identity-by-descent mapping in a health system. eLife. 2017;6:e25060. https://doi.org/10.7554/eLife.25060
29. Zhang Z, Bai M, Barbosa GO, Chen A, Wei Y, Luo S, et al. Broadly conserved roles of TMEM131 family proteins in intracellular collagen assembly and secretory cargo trafficking. Sci Adv. 2020;6:eaay7667. https://doi.org/10.1126/sciadv.aay7667
30. Beasley HK, Rodman TA, Collins GV, Hinton A Jr, Exil V. TMEM135 is a novel regulator of mitochondrial dynamics and physiology with implications for human health conditions. Cells. 2021;10:1750. https://doi.org/10.3390/cells10071750
31. McDonough JE, Kaminski N, Thienpont B, Hogg JC, Vanaudenaerde BM, Wuyts WA. Gene correlation network analysis to identify regulatory factors in idiopathic pulmonary fibrosis. Thorax. 2019;74:132-40. https://doi.org/10.1136/thoraxjnl-2018-211929
32. Phelps KJ, Johnson DD, Elzo MA, Paulk CB, Gonzalez JM. Effect of Brahman genetics on myofibrillar protein degradation, collagen crosslinking, and tenderness of the longissimus lumborum. J Anim Sci. 2017;95:5397-406. https://doi.org/10.2527/jas2017.2022
33. Liu J, Wang L, Chen W, Li J, Shan T. CRTC3 regulates the lipid metabolism and adipogenic differentiation of porcine intramuscular and subcutaneous adipocytes by activating the calcium pathway. J Agric Food Chem. 2021;69:7243-55. https://doi.org/10.1021/acs.jafc.1c02021
34. Thompson JM. The effects of marbling on flavour and juiciness scores of cooked beef, after adjusting to a constant tenderness. Aust J Exp Agric. 2004;44:645-52. https://doi.org/10.1071/EA02171
35. Nian Y, Moloney AP, Li C, Allen P, Harrison SM, Prendiville R, et al. A comparison of meat composition, tenderness and the fatty acid profile of three muscles from Holstein-Friesian bulls from production system resulting in final ages of either < 16 or 19 months. Anim Prod Sci. 2022;62:368-80. https://doi.org/10.1071/AN20697
36. Cui HX, Liu RR, Zhao GP, Zheng MQ, Chen JL, Wen J. Identification of differentially expressed genes and pathways for intramuscular fat deposition in pectoralis major tissues of fast-and slow-growing chickens. BMC Genomics. 2012;13:213. https://doi.org/10.1186/1471-2164-13-213
37. Zhao C, Ji G, Carrillo JA, Li Y, Tian F, Baldwin RL, et al. The profiling of DNA methylation and its regulation on divergent tenderness in Angus beef cattle. Front Genet. 2020;11:939. https://doi.org/10.3389/fgene.2020.00939
38. Yang Y, Yang J, Ma J, Yu Q, Han L. iTRAQ-mediated analysis of the relationship between proteomic changes and yak longissimus lumborum tenderness over the course of postmortem storage. Sci Rep. 2021;11:10450. https://doi.org/10.1038/s41598-021-90012-0