JAST (Journal of Animal Science and Technology) TITLE PAGE

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ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Genome-wide Association Studies to Identify Quantitative Trait Loci and Positional Candidate Genes Affecting Meat Quality-related Traits in Pigs
Running Title (within 10 words)	GWAS for pork quality traits
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Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MIST)(No. 2019R1F1A1062342).
Acknowledgements	Not applicable.
Availability of data and material	Upon reasonable request, the datasets of this study can be available from the corresponding author.
Authors' contributions Please specify the authors' role using this form.	Conceptualization: Park HB. Data curation: Lee JB, Lim JH. Formal analysis: Lee JB, Lim JH. Methodology: Lee JB, Park HB. Software: Lee JB, Park HB. Validation: Lee JB, Lim JH. Investigation: Lee JB, Lim JH, Park HB. Writing - original draft: Lee JB, Lim JH. Writing - review & editing: Lee JB, Lim JH, Park HB.
Ethics approval and consent to participate	This investigation followed the guidelines of the Institutional Animal Care and Use Committee of the National Institute of Animal Science, Korea(2015-137)

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Abstract

Meat quality comprises a set of key traits such as pH, meat color, water-holding capacity, tenderness and marbling. These traits are complex because they are affected by multiple genetic and environmental factors. The aim of this study was to investigate the molecular genetic basis underlying nine meat quality-related traits in a Yorkshire pig population using a genome-wide association study (GWAS) and subsequent biological pathway analysis. In total, 45,926 single nucleotide polymorphism (SNP) markers from 543 pigs were selected for the GWAS after quality control. Data were analyzed using a genome-wide efficient mixed model association (GEMMA) method. This linear mixed model-based approach identified two quantitative trait loci (QTLs) for meat color (b^*) on chromosome 2 (SSC2) and one QTL for shear force on chromosome 8 (SSC8). These QTLs acted additively on the two phenotypes and explained 3.92-4.57% of the phenotypic variance of the traits of interest. The genes encoding *HAUS8* on SSC2 and an *lncRNA* on SSC8 were identified as positional candidate genes for these QTLs. The results of the biological pathway analysis revealed that positional candidate genes for meat color (b^*) were enriched in pathways related to muscle development, muscle growth, intramuscular adipocyte differentiation, and lipid accumulation in muscle, whereas positional candidate genes for shear force were overrepresented in pathways related to cell growth, cell differentiation, and fatty acids synthesis. Further verification of these identified SNPs and genes in other independent populations could provide valuable information for understanding the variations in pork quality-related traits.

Keywords : GWAS, meat quality-related traits, *HAUS8*, *lncRNA*, pig

Introduction

As consumer's incomes rise in many countries, there is a general tendency to shift from quantity towards higher pork quality, such as specialty cuts with reddish/pink color, flavor, and tenderness. The color of pork is considered as a key indicator of freshness and quality [1]; Intramuscular fat content (or marbling) is an important trait that is related to meat flavor [2]. Pork tenderness also strongly affects consumer satisfaction and thus, repeat buying [3]. The heritability of meat quality traits is low to moderate [2, 4]. However, it is generally difficult to improve the meat quality-related traits using conventional breeding methods because the measurement of meat quality-related traits, such as pH, meat color, water-holding capacity, tenderness and marbling, can only be recorded and evaluated after slaughter. Therefore, molecular breeding techniques using genetic markers have emerged as potential alternatives for improving meat quality-related traits [5]. For example, *RYR1*, *PRKAG3*, *PHKG1*, and *MYH3* were identified to enhance pork quality in the form of genetic markers [6-9].

Nowadays, DNA array chip technology, which is a highly parallel genomic assay integrated with high-density single nucleotide polymorphism (SNP) markers, has been developed and is available for identifying genes that affect complex and quantitative traits, such as meat quality-related traits [10]. The DNA array chips can provide genotype data for conducting genome-wide association studies (GWAS) to identify quantitative trait loci (QTLs) and their positional candidate genes for marker-assisted selection (MAS), which can also be used for conducting genomic selection (GS) to improve pork quality-related traits. Results from MAS and GS can efficiently and quickly contribute to the genetic improvement of pork quality by selecting piglets with genetic potentials for excellent meat quality-related traits [11].

This study was performed to detect QTL and their positional candidate genes that could be used to develop potential genetic markers to improve pork quality in a purebred Yorkshire population. In addition, we investigated the biological functions of the identified positional candidate genes affecting the variation in the meat quality through the analysis of biological pathways.

Materials and Methods

This study was conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of the National Institute of Animal Science, the Republic of Korea (2015-137).

Animals and DNA extract

Using the OMNI Bead Ruptor (OMNI International, USA) and DNeasy® Blood & Tissue Kit (QIAGEN, Germany), genomic DNA was extracted from 50 mg of frozen muscle tissue of 543 pigs from a purebred Yorkshire population (406 castrated male pigs and 137 female pigs) originated from a GP (grandparents, single multiplier) breeding farm in the Republic of Korea. The experimental animals were born in the GP farm from July 2015 to November 2016 that was a closed population and later transported to nine breeding stock farms and raised until the slaughter. The pigs were slaughtered at an average (±SD) age of 200 (±11.7) days and the mean carcass weight (±SD) was recorded as 88.0 (±9.3) kg.

Meat quality-related phenotypes

Longissimus dorsi muscle samples were isolated from the carcass and meat pH 24 hr postmortem (pH24), redness (a^*), yellowness (b^*), cooking loss (cooking), shear force (shear), National Pork Producers Council (NPPC) meat color (Ncol), and NPPC marbling (Nmar) traits related to the meat quality were measured according to the method reported by Choe et al. [12].

Statistical analyses

Before the GWAS, we obtained the descriptive statistic values and validated the normal distribution of the phenotypic data. Putative outliers were detected based on the ascertainment of normality using the Ryan-Joiner method implemented in the Minitab program (Minitab, USA). The phenotypic values were transformed by natural logarithm [i.e., NPPC meat color, NPPC marbling] or square root [i.e., shear force] as necessary (Table 1). General linear model (GLM) analysis was conducted using the Minitab program (Minitab, USA) to identify sources influencing phenotypic variation. The effects of sex (castrated male, female), farm, season (summer season, and other seasons), and carcass weight were evaluated. A highly significant effect ($p< 0.01$) of carcass weight (in the case of a*, b*, cooking loss, shear force, Ncol, and Nmar) was observed, and, therefore, included in the linear model for GWAS. Significant effects ($p<0.05$) of sex (in the case of b*, Ncol, Nmar), season (ph24, b*, cooking) and farm (ph24, a*, b*, cooking, shear, Ncol) were also observed. Therefore, they were included as cofactors (i.e., sex, farm, and season) and a covariate (i.e., carcass weight) in the GWAS model.

Estimation of heritability and GWAS

SNP marker genotypes were determined from genomic DNA samples of 543 purebred Yorkshire pigs using the Axiom Porcine Breeders array (Thermo Fisher, USA). SNP markers with minor allele frequency (MAF) less than 1%, genotyping error rate greater than 10%, and Hardy-Weinberg equilibrium less than 10^{-6} were removed from the analyzed SNP makers using PLINK program version 1.9 [13]. A total of 45,926 SNP markers on 18 autosomal chromosomes were left after the quality control procedure.

Estimation of the heritability of each meat quality-related trait in this study was conducted using the following linear mixed model (LMM):

$$\mathbf{Y} = \mathbf{Xb} + \mathbf{Zu} + \mathbf{e} \quad (1)$$

where y is the vector of the phenotype of interests and **b** is the vector for fixed cofactors (sex, farm, and season) and a linear covariate (carcass weight); **u** is the vector of random additive genetic effects following a normal distribution $\mathbf{u}\sim N(0, \mathbf{G}\boldsymbol{\sigma}_{\boldsymbol{u}}^{\boldsymbol{2}})$, in which G is the genomic relationship matrix (GRM) whose matrix elements are consisted of pairwise genomic relationship coefficients calculated using the genotypes of 42,399 SNP markers, and $\boldsymbol{\sigma}_{\boldsymbol{u}}^{\boldsymbol{2}}$ is the additive genetic variance component; **e** is the vector of random residuals following a normal distribution $\mathbf{e}\sim N(0, \mathbf{I}\boldsymbol{\sigma}_{\boldsymbol{e}}^{\boldsymbol{2}})$, in which I is the identity matrix, and $\boldsymbol{\sigma}_{\boldsymbol{e}}^{\boldsymbol{2}}$ is the residual variance component. The GEMMA program was used for building up the GRM and the estimation of the $\boldsymbol{\sigma}_{\boldsymbol{u}}^{\boldsymbol{2}}$ and $\boldsymbol{\sigma}_{\boldsymbol{e}}^{\boldsymbol{2}}$ for each meat quality-related trait based on the restricted maximum likelihood method (REML). The **X** and **Z** are the incidence matrices of **b** and **u**, respectively.

The GWAS for the meat quality-related traits was performed for 543 Yorkshire pigs based on a single-marker univariate LMM using GEMMA software [14]. The LMM equation for GWAS (Eq. 2) was as follows:

$$\mathbf{Y} = \mathbf{Xb} + \mathbf{Z_1a} + \mathbf{Z_2u} + \mathbf{e} \quad (2)$$

where **Y** is the univariate phenotype; **a** is the vector of fixed effect of the SNP marker; **b**, **u,** and **e** are the same vectors used in Eq. 1. The **X**, $\mathbf{Z_1}$and $\mathbf{Z_2}$are the incidence matrices of **b**, **a**, and **u**, respectively.

The percentage of phenotypic variance explained by an SNP ($\%Var_{SNP}$) was computed as follows (Eq. 3) [15]:

$$\%Var_{SNP} = 100 \times \frac{2p(1-p)\alpha^2}{\sigma_p^2} \quad (3)$$

where p is the minor-allele frequency of the SNP marker; α is the additive genetic effect of the SNP marker; σ_p^2 is the phenotypic variance for each meat quality-related trait. The p, α and σ_p^2 were estimated using the GEMMA program. To address multiple comparison issues in GWAS, the significance threshold level was determined using the q-value method; a q-value less than 0.1 ($q < 0.1$), which corresponds to $p <$ 2.48E-06, was designated as the threshold of the significance level; a q-value that is 0.1 or more and less than 0.2 ($0.1 \leq q < 0.2$), which corresponds to 2.48E-06 $\leq p <$ 2.55E-05, was designated as the threshold of the suggestive level [16]. The effects of the trait-associated SNP markers on the phenotypes were estimated using the GLM implemented in Minitab (Minitab, USA). Statistical power analysis of the GWAS was conducted using the method developed by Wang and Xu (2015) for LMM-based GWAS under different assumptions regarding the sample size [17]. The CMplot package implemented in R was used to visualize the GWAS results [18]. The Ensembl database (URL: http://asia.ensembl.org/Sus_scrofa/Info/Index) based on *Sus scrofa* 11.1 was used to select the genes that were close to the trait-associated SNPs as positional candidate genes.

Biological pathway analysis

After selecting the SNP marker group with a nominal p-value less than 0.01 based on the results of the GWAS analysis, a list of positional candidate genes of the SNP marker group was prepared using the biomaRt in the R package [19].

The analysis of biological pathways between genes was performed using the KEGG library of the Enrichr database, and a *p*-value of 0.05 was used as a significance level to detect a significant biological pathway [20, 21].

Results and Discussion

Descriptive statistics

Phenotypic data analysis was performed on seven meat quality-related traits collected from 543 purebred Yorkshire pigs. The overall means, standard deviation (SD) and ranges of the traits of interests are presented in Table 1. The observed phenotypes did not display obvious deviations from the normality assumption (Supplementary fig. 1). The phenotypes recorded for this study showed Among them, mean ± SD of yellowness and shear force were 1.81±1.14 and 7.47±0.86, respectively (Table 1). Heritability estimates range from 0.087 (ph24) from 0.28 (a*) (Table 1).

GWAS

As a result of the GWAS for seven traits related to the meat quality using the study population, two trait-associated SNP markers on porcine chromosome 2 (SSC2, AX-116172218, and AX-116679656) and one trait-associated SNP marker on the chromosome 8 (SSC8, AX-116692048) were identified as significant and suggestive QTLs (Fig.1, Table 2). The QTLs identified in SSC2 affected the meat color (b^*). In a study of European commercial pigs by Harmegnies et al. [22], the QTL region was overlapped with the meat color (b^*) QTL identified in this study. The QTL identified on SSC8 was found to affect the shear force trait; however, the QTL identified on SSC8 is yet to be reported based on the Animal QTLdb search [23]. The degree of inflation of the GWAS results (i.e., lambda values) revealed that the effect of population structure due to the genetic relationship between individuals on the association results was insignificant in this Yorkshire study population (Fig. 1). For the rest of meat quality-related phenotypes, our GWAS did not reveal the presence of any statistically significant or suggestive QTLs (Supplementary fig. 2).

The two SNP markers (AX-116172218 and AX-116679656) in the QTL region identified in SSC2 explained 4.25% and 3.92% of the phenotypic variance of the meat color (b^*) trait, respectively, whereas the SNP marker (AX-116692048) in the QTL region on SSC8 accounted for 4.57% of the phenotypic variance in shear force (Table 2). The effects of trait-associated SNP markers on the phenotypes are shown in Fig. 2. The results show that the T alleles is positively associated with b^*, whereas the A allele was positively associated with shear force. The genic action was mostly additive for the two traits examined.

Considering the effects of population structure and polygenic background using the DNA array chip data, we calculated the statistical power of GWAS to detect a QTL under the currently used sample sizes (i.e., 448 for b^* and 537 for shear force) and a significance threshold of a *p*-value = 2.48E-06 corresponding to $q = 0.1$ [17]. When the *p*-value of 2.48E-06 was used, the calculations indicated that a sample size of 537 (the case for shear force) was necessary to achieve 95.0% statistical power to identify a trait-associated marker with a $\%Var_{SNP}$ value of 5.2%. For a sample size of 448 (the case for b^*), the calculations indicated that 74.1% and 81.6% statistical power could be achieved with $\%Var_{SNP}$ values of 3.9% and 4.3%, respectively. This result suggests that the use of the moderate sample sizes in this study provided sufficient statistical power to identify a biologically meaningful QTL [17].

The *HAUS augmin-like complex subunit 8* (*HAUS8*) gene was identified as a positional candidate gene in the QTL region of AX-116679565, and *lncRNA* was detected as a positional candidate gene in the QTL region of AX-116692048. *HAUS8* on SSC2 has been reported to be related to cytoskeletal tissue and the microtubule system, which play essential roles in cell migration [24]. The identified *lncRNA* gene in SSC8 produces a long noncoding RNA consisting of more than 200 nucleotides. *lncRNA* affect post-transcriptional modifications and serves various functions within cells after forming extensive networks of ribonucleoprotein complexes with many chromatin regulators [25, 26]. *lncRNA* is considered a candidate gene for traits related to the muscle development process, such as shear force, because it is expressed during the muscle development process, and is known to regulate the proliferation, differentiation, and fusion of myoblasts [27]. By running the biomaRt for SNP marker groups with a *p*-value less than 0.01, 162 and 142 genes were detected as positional candidate genes that could affect meat color (b*) and shear force traits, respectively.

Biological Pathway analysis

The Enrichr database was used to search for biological pathways related to b^* and shear force. The identified biological pathways and the positional candidate genes belonging to them are shown in Table 3. Among them, four positional candidate genes (integrin-binding sialoprotein; *IBSP*; dentin matrix acidic phosphoprotein 1, *DMP1*; FRAS1 related extracellular matrix 2, *FREM2*; dentin sialophosphoprotein, *DSPP*) were detected for ECM-receptor interactions. ECM-receptor interaction is known to affect the differentiation of intramuscular adipocytes in chicken, and it has been reported that this could be implicated in meat quality [28]. In addition, in the Hippo signaling pathway, four positional candidate genes (discs large MAGUK scaffold protein 2, *DLG2*; frizzled class receptor 4, *FZD4*; discs large MAGUK scaffold protein 4, *DLG4*; bone morphogenetic protein 5, *BMP5*) were also detected. It has been reported that this pathway promotes the proliferation of skeletal muscle stem cells or is closely related to muscle growth and development, and adipocyte proliferation and differentiation [29].

The most significant biological pathway associated with the shear force trait was the glycosaminoglycan degradation (*GAG*) pathway with the hyaluronidase 1 (*HYAL1*) and hyaluronidase 3 (*HYAL3*) genes. This pathway is primarily found in mucopolysaccharides, connective tissues, bone tissues, intercellular mediators, and epithelial tissues and has been reported to be closely related to the regulation of proliferation and differentiation of muscle cells [30]. A significant association with shear force was also detected for the glycerolipid metabolism pathways that include diacylglycerol kinase beta (*DGKB*), glycerol-3-phosphate acyltransferase 3 (*GPAT3*), and diacylglycerol kinase eta (*DGKH*) genes. This pathway is closely related to the synthesis, transportation and esterification of fatty acids involved in the increase of intramuscular fat in chicken breast tissue [31]. The phosphatidylinositol signaling pathway, which includes *DGKB*, phosphatidylinositol-5-phosphate 4-kinase type 2 alpha (*PIP4K2A*), and *DGKH* genes was also found to be associated with shear force. This pathway is involved in cell proliferation, survival, and metabolism; it plays an essential role in cell signaling, such as the insulin signaling pathway, and is closely related to endocytosis and exocytosis [32, 33]. The insulin metabolism plays an important role in the formation of fat, which affects the meat quality of Korean native cattle (Hanwoo) by promoting marbling by accumulating the remaining glucose in the body [34].

In addition, pathways of choline metabolism in cancer, glycerophospholipid metabolism, and ABC transporters were detected, Further studies on the relationship between these pathways and shear force are needed. Based on the

results of the biological pathway analysis, it is thought that candidate genes affecting meat color (b^*) affect differentiation of intramuscular adipocytes, growth, and development of muscles, and fat accumulation in muscles, and that candidate genes for the shear force trait are related to the growth and differentiation of cells and synthesis of fatty acids.

Conclusion

QTLs affecting the meat quality-related traits were to identified using a purebred Yorkshire population, and biological pathways that could affect meat color (b^*) and shear force traits were detected by identifying positional candidate genes in the QTL region. The results of this study can be utilized as basic molecular genetic data to improve the meat quality of pork after passing verification procedures in other independent populations in the future.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MIST) (No. 2019R1F1A1062342).

References

1. Tomasevic I, Djekic I, Font-i-Furnols Maria, Terjung Nino, Lorenzo JM. Recent advances in meat color research. Curr Opin Food Sci. 2021; 41: 81-87. https://doi.org/10.1016/j.cofs.2021.02.012

2. Suzuki K, Irie M, Kadowaki H, Shibata T, Kumagai M, Nishida A. Genetic parameter estimates of meat quality traits in Duroc pigs selected for average daily gain, longissimus muscle area, backfat thickness, and intramuscular fat content. J Anim Sci. 2005;83:2058-2065. https://doi.org/10.2527/2005.8392058x

3. Grunert KG, Bredahl L, Brunso K. Consumer perception of meat quality and implications for product development in the meat sector—a review. Meat Sci. 2004;66:259-272. https://doi.org/10.1016/S0309-1740(03)00130-X

4. Miar Y, Plastow GS, Moore SS, Manafiazar G, Charagu P, Kemp RA, et al. Genetic and phenotypic parameters for carcass and meat quality traits in commercial crossbred pigs. J Anim Sci. 2014;92:2869-2884. https://doi.org/10.2527/jas.2014-7685

5. Gao Y, Zhang R, Hu X, Li N. Application of genomic technologies to the improvement of meat quality of farm animals. Meat Sci. 2007;77:36-45. https://doi.org/10.1016/j.meatsci.2007.03.026

6. Fujii J, Otsu K, Zorzato F, de Leon S, Khanna VK, Weiler JE, O'Brien PJ, MacLennan DH. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science. 1991; 253:448–451. https://doi.org/10.1126/science.1862346

7. Milan D, Jeon JT, Looft C, Amarger V, Robic A, Thelander M, Rogel-Gaillard C, Paul S, Iannuccelli N, Rask L, Ronne H, Lundström K, Reinsch N, Gellin J, Kalm E, Roy PL, Chardon P, Andersson L. A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. Science. 2000; 288:1248–1251. https://doi.org/10.1126/science.288.5469.1248

8. Ma J et al. A splice mutation in the PHKG1 gene causes high glycogen content and low meat quality in pig skeletal muscle. PLoS Genet. 2014; 10: e1004710. https://doi.org/10.1371/journal.pgen.1004710

9. Cho IC, Park HB, Ahn JS, Han SH et al. A functional regulatory variant of MYH3 influences muscle fiber-type composition and intramuscular fat content in pigs. PLoS Genet. 2019; 15: e1008279. https://doi.org/10.1371/journal.pgen.1008279

10. Fan J, Chee MS, Gunderson KL. Highly parallel genomic assays. Nat Rev Genet. 2006;7:632-644. https://doi.org/10.1038/nrg1901

11. Hayes B, Goddard M. Genome-wide association and genomic selection in animal breeding. Genome. 2010; 53: 876-883. https://doi.org/10.1139/G10-076

12. Choe JH, Choi MH, Ryu YC, Go GW, Choi YM, Lee SH, et al. Estimation of pork quality traits using

exsanguination blood and postmortem muscle metabolites. Asian-Australas J Anim Sci. 2015;28:862-869. https://doi.org/10.5713/ajas.14.0768

13. Chang CC, Chow CC, Tellier, Laurent CAM, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience. 2015;4:7. https://doi.org/10.1186/s13742-015-0047-8

14. Zhou X, Stephens M. Genome-wide efficient mixed-model analysis for association studies. Nat Genet. 2012;44:821-824. https://doi.org/10.1038/ng.2310

15. Falcorner DS, Mackay TFC. Introduction to quantitative genetics. 4th ed; Longman Ltd; 1996.

16. Storey JD, Tibshirani R. Statistical significance for genome-wide studies. Proc Natl Acad Sci USA. 2003;100:9440-9445. https://doi.org/10.1073/pnas.1530509100

17. Wang M, Xu S. Statistical power in genome-wide association studies and quantitative trait locus mapping. Heredity. 2019; 123: 287-306. https://doi.org/10.1038/s41437-019-0205-3

18. Lin YL. 2021. The R Package 'CMplot' (URL:https://github.com/YinLiLin/CMplot)

19. Drost H, Paszkowski J. Biomartr: genomic data retrieval with R. Bioinformatics. 2017;33:1216-1217. https://doi.org/10.1093/bioinformatics/btw821

20. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 2016; 44: W90-W97. https://doi.org/10.1093/nar/gkw377

21. Kanehisa M, Sato Y, Kawashima M, Furumichi M, Tanabe M. KEGG as a reference resource for gene and protein annotation. Nucleic Acids Res. 2016;44: D457-D462. https://doi.org/10.1093/nar/gkv1070

22. Harmegnies N, Davin F, De Smet S, Buys N, Georges M, Coppieters W. Results of a whole-genome quantitative trait locus scan for growth, carcass composition and meat quality in a porcine four-way cross. Anim Genet. 2006;37:543-553. https://doi.org/10.1111/j.1365-2052.2006.01523.x

23. Hu Z, Park CA, Reecy JM. Building a livestock genetic and genomic information knowledgebase through integrative developments of Animal QTLdb and CorrDB. Nucleic Acids Res. 2019;47:D701-D710. https://doi.org/10.1093/nar/gky1084

24. Lu Y, Ye Y, Bao W, Yang Q, Wang J, Liu Z, Shi S. Genome-wide identification of genes essential for podocyte cytoskeletons based on single-cell RNA sequencing. Kidney Int. 2017;92(5):1119-1129. https://doi.org/10.1016/j.kint.2017.04.022

25. Rinn JL, Chang HY. Genome regulation by long noncoding RNAs. Annu Rev of Biochem. 2012;81:145-166. https://doi.org/10.1146/annurev-biochem-051410-092902.

281 26. Mercer TR, Mattick JS. Structure and function of long noncoding RNAs in epigenetic regulation. Nat Struct Mol
282 Biol. 2013;20:300-307. https://doi.org/10.1038/nsmb.2480

283 27. Wang S, Jin J, Xu Z, Zuo B. 2019. Functions and Regulatory Mechanisms of lncRNAs in Skeletal Myogenesis,
284 Muscle Disease and Meat Production. Cells. 2019;8(9): 1107. https://doi.org/10.3390/cells8091107

285 28. San J, Du Y, Wu G, Xu R, Yang J, Hu J. Transcriptome analysis identifies signaling pathways related to meat
286 quality in broiler chickens – the extracellular matrix (ECM) receptor interaction signaling pathway. Poult Sci.
287 2021;100(6):101135. https://doi.org/10.1016/j.psj.2021.101135

288 29. Ardestani A, Lupse B, Maedler K. Hippo Signaling: Key Emerging Pathway in Cellular and Whole-Body
289 Metabolism. Trends Endocrinol Metab. 2018;29:492-509. https://doi.org/10.1016/j.tem.2018.04.006

290 30. Velleman SG. MEAT SCIENCE AND MUSCLE BIOLOGY SYMPOSIUM: Extracellular matrix regulation of
291 skeletal muscle formation1,2. J Anim Sci. 2012;90(3):936-941. https://doi.org/10.2527/jas.2011-4497

292 31. Liu L, Cui H, Xing S, Zhao G, Wen J. Effect of Divergent Selection for Intramuscular Fat Content on Muscle
293 Lipid Metabolism in Chickens. Animals. 2019;10:4. https://doi.org/10.3390/ani10010004

294 32. Heath CM, Stahl PD, Barbieri MA. Lipid kinases play crucial and multiple roles in membrane trafficking and
295 signaling. Histol Histopathol. 2003;18:989-998. https://doi.org/10.14670/HH-18.989

296 33. Sun Y, Thapa N, Hedman AC, Anderson RA. Phosphatidylinositol 4,5-bisphosphate: targeted production and
297 signaling. Bioessays. 2013;35:513-522. https://doi.org/10.1002/bies.201200171

298 34. Kim BK, Ha JJ, Yi JK, Oh DY, Jung DJ, Hwang EG, et al. Effect of herbal probiotic supplementation on weight,
299 blood composition, meat quality and immunity in beef. Korean J Food Nutr. 2016;29:860-869.
300 https://doi.org/10.9799/ksfan.2016.29.6.860

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Tables and Figures

Table 1. Summary of meat quality-related traits data and heritability estimates from the study pig population

Phenotype	Label	[1] N	Mean	SD	Range	[2] h^2±SE
ph24	Muscle pH of the meat 24h postmortem	538	5.65	0.17	5.08-6.40	0.087±0.048
a*	Redness	537	6.37	1.21	3.38-9.68	0.28±0.07
b*	Yellowness	488	1.81	1.14	-2.13-5.11	0.12±0.05
cooking	Cooking loss	536	30.13	3.78	14.12-42.30	0.27±0.08
shear^S	Shear force	537	7.47	0.86	5.08-10.33	0.13±0.06
Ncol^N	NPPC meat color	538	1.10	0.19	0.40-2.05	0.17±0.06
Nmar^N	NPPC marbling	538	0.58	0.36	0.00-1.75	0.17±0.08

[1]Number of phenotyped animals. [2]Heritability estimate±standard error; N= natural log transformation; S= Square root transformation.

Table 2. Summary of QTLs affecting meat quality-related traits in Yorkshire pigs

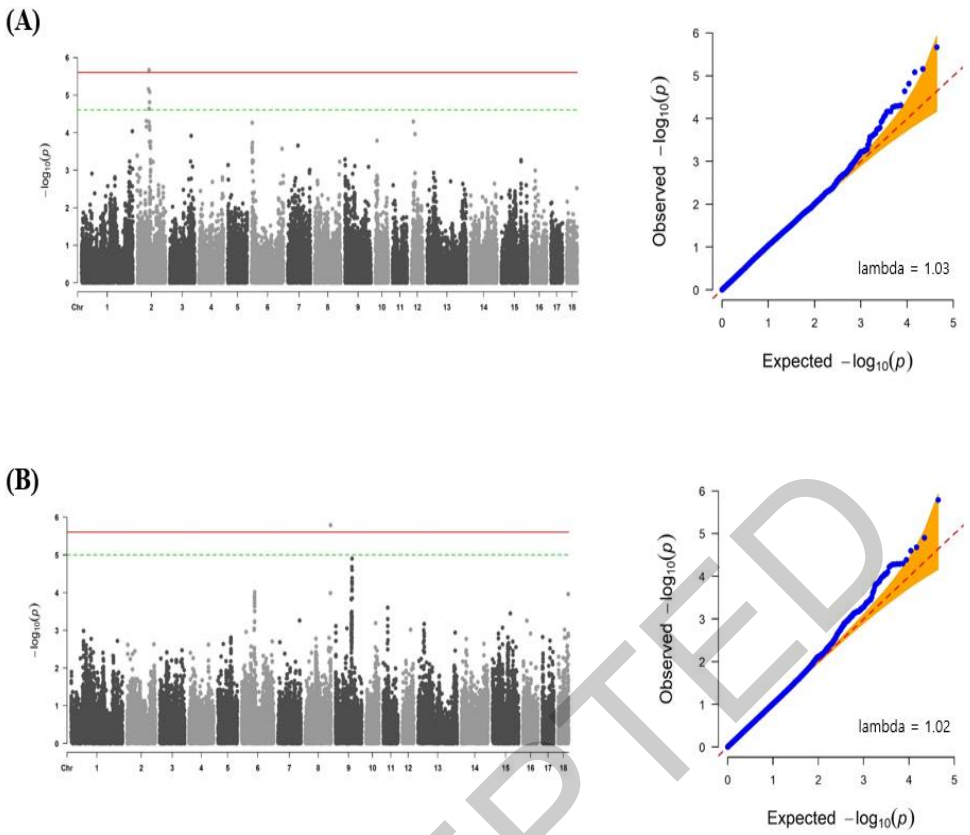
[1]SSC	Traits	[2]N_{snp}	Interval (Mb)	[3]TopSNP	[4]Position (bp)	[5]ß	[6]se	[7]*p*-value	[8]%Var	Known Candidate Genes
2	b*	1	63.4	AX-116172218	63418737	-5.71E-01	1.19E-01	2.16E-06[††]	4.28	_
		4	60.5-66.7	AX-116679656	60582816	-5.41E-01	1.19E-01	6.93E-06[†]	3.92	*HAUS8*
8	shear	1	135.1	AX-116692048	135127121	5.37E-01	1.11E-01	1.63E-06[††]	4.57	*lncRNA*(*ENSSSCG00000047440*)

[1]Pig chromosome, [2]number of significant SNPs at the genome-wide level, [3]ID of top SNPs, [4]genome map position on the *Sus scrofa*11.1 genome assembly, [5]beta, [6]standard error, [7]nominal *p*-values computed using the GEMMA package ([††]q<0.1, [†]0.1≤q<0.2), [8]percentage of phenotypic variance explained by the top SNPs.

Table 3. Top significant pathways for the positional candidate genes located within the QTL for meat color (b) and shear force traits in Yorkshire pigs

Traits	Pathway	*p*-value+	Genes
b*	ECM-receptor interaction	<0.01	*IBSP, DMP1, FREM2, DSPP*
	Folate biosynthesis	<0.05	*QDPR, PAH*
	Hippo signaling pathway	<0.05	*DLG2, FZD4, DLG4, BMP5*
	Vitamin B6 metabolism	<0.05	*AOX1*
	Phenylalanine, tyrosine and tryptophan biosynthesis	<0.05	*PAH*
shear	Glycosaminoglycan degradation	<0.01	*HYAL1, HYAL3*
	Glycerolipid metabolism	<0.01	*DGKB, GPAT3, DGKH*
	Phosphatidylinositol signaling system	<0.05	*DGKB, PIP4K2A, DGKH*
	Choline metabolism in cancer	<0.05	*SLC44A5, DGKB, DGKH*
	Glycerophospholipid metabolism	<0.05	*DGKB, GPAT3, DGKH*
	ABC transporters	<0.05	*ABCA1, ABCC8*

+Nominal *p*-value



317 Fig. 1. The Manhattanplots and quantil-quantil(QQ) plots of the SNP effects for the meat color (b^*) and shear force
318 traits. The red and green horizontal lines represent significant and suggestive thresholds of GWAS, respectively. (A)
319 Manhattan plot and QQ plot for b^*; (B) Manhattan plot and QQ plot for shear force.
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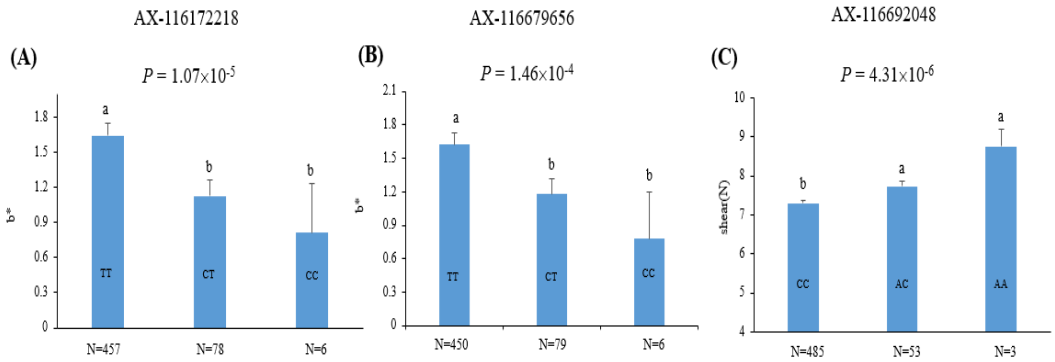
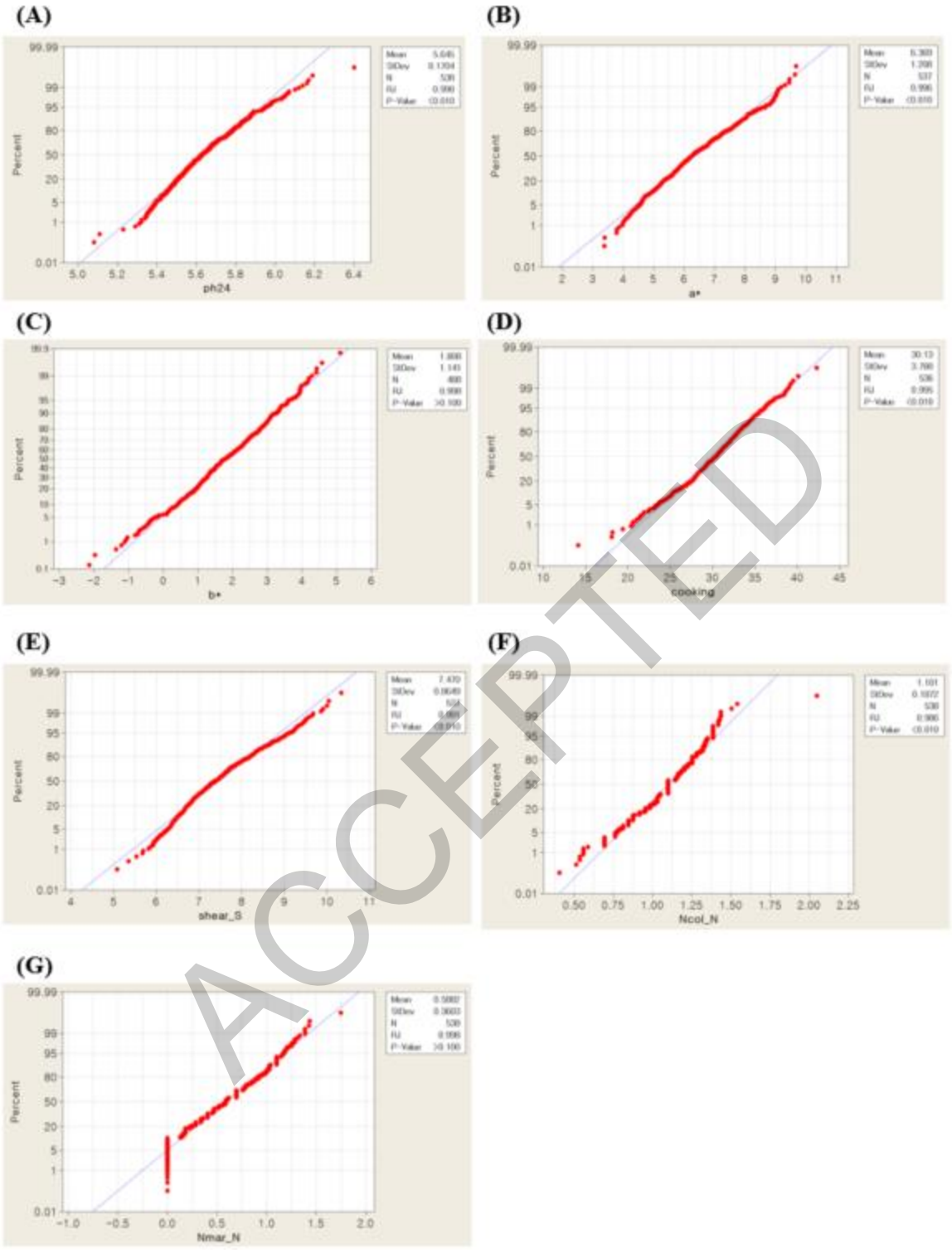
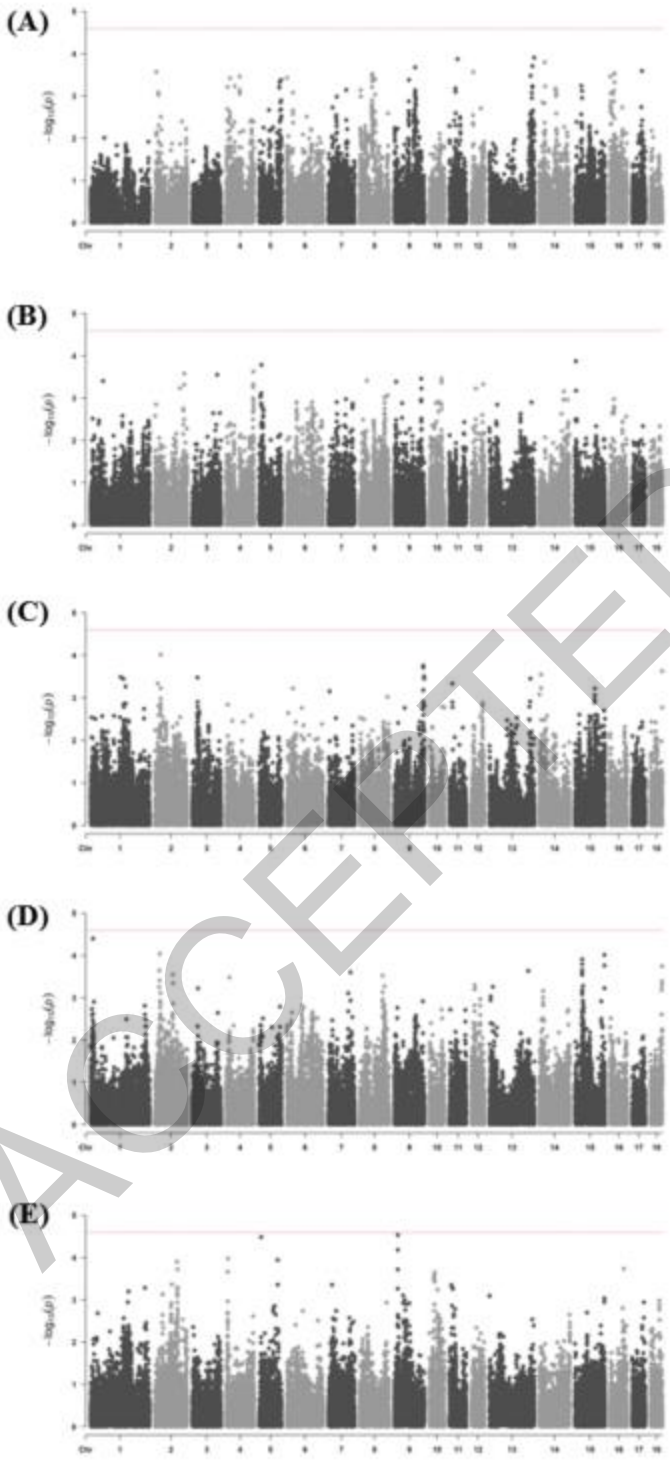


Fig. 2. Least squares means and standard errors by genotype for each top SNP marker affecting meat color traits in the Yorkshire pigs. (A) Bar graph of b trait for AX-116172218; (B) bar graph of b trait for AX-116679656; (C) bar graph of shear trait for AX-116692048. Different alphabets represent statistical significance at $p<0.05$.



Supplementary fig. 1. Normality plots for the pork quality-related traits in this study. The vertical axis represents the scale of probabilities, and the horizontal axis represents the scale of phenotype of interests. (A) ph24; (B) a*; (C) b*; (D) cooking; (E) shear; (F) Ncol; (G) Nmar.



Supplementary fig. 2. The Manhattan plots for the pork quality-related traits in this study. The red horizontal lines represent the suggestive threshold of the GWAS. (A) ph24; (B) a*; (C) cooking; (D) Ncol; (E) Nmar.