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Implementing meta-analysis from genome-wide association studies for pork quality traits[1]

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ABSTRACT: Pork quality plays an important role in the meat processing industry. Thus, different methodologies have been implemented to elucidate the genetic architecture of traits affecting meat quality. One of the most common and widely used approaches is to perform genome-wide association (GWA) studies. However, a limitation of many GWA in animal breeding is the limited power due to small sample sizes in animal populations. One alternative is to implement a meta-analysis of GWA (MA-GWA) combining results from independent association studies. The objective of this study was to identify significant genomic regions associated with meat quality traits by performing MA-GWA for 8 different traits in 3 independent pig populations. Results from MA-GWA were used to search for genes possibly associated with the set of evaluated traits. Data from 3 pig data sets (U.S. Meat Animal Research Center, commercial, and Michigan State University Pig Resource Population) were used. A MA was implemented by combining *z*-scores derived for each SNP in every population and then weighting them using the inverse of estimated variance of SNP effects. A search for annotated genes retrieved genes previously reported as candidates for shear force (calpain-1 catalytic subunit [*CAPN1*] and calpastatin [*CAST*]), as well as for ultimate pH, purge loss, and cook loss (protein kinase, AMP-activated, γ 3 noncatalytic subunit [*PRKAG3*]). In addition, novel candidate genes were identified for intramuscular fat and cook loss (acyl-CoA synthetase family member 3 mitochondrial [*ACSF3*]) and for the objective measure of muscle redness, CIE a* (glycogen synthase 1, muscle [*GYS1*] and ferritin, light polypeptide [*FTL*]). Thus, implementation of MA-GWA allowed integration of results for economically relevant traits and identified novel genes to be tested as candidates for meat quality traits in pig populations.

Key words: candidate genes, genome-wide association, meat quality, meta-analysis, pigs

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INTRODUCTION

Pork quality is one of the most important aspects for all segments of the swine industry (Rosenvold and Andersen, 2003). Genomic selection (Meuwissen et al., 2001) and whole-genome regression methods such as Genomic Best Linear Unbiased Prediction (**GBLUP**) have allowed estimation of genomic breeding values (GEBV) for pork (H. Wang et al., 2012) and beef quality traits (Akanno et al., 2014). Genomic Best Linear Unbiased Prediction assumes that SNP effects are independent and normally distributed, assigning the same variance to all SNP (VanRaden, 2008; Habier et al.,

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2010); GBLUP based on REML and BLUP programs is straightforward to implement and less computationally demanding than other genomic prediction methodologies (Guo et al., 2014). A common practice after implementation of genomic evaluations is to perform a genome-wide association study (GWA; Ma et al., 2013; Jung et al., 2014; Stratz et al., 2014). However, the power of GWA has been limited because of small sample sizes (less than 2000 animals) and the complex population structures characterizing animal data sets (Muñoz et al., 2009; Minozzi et al., 2012). One alternative to reduce false-positive associations and to increase the power of association studies is to perform a meta-analysis of GWA (**MA-GWA**), defined as the statistical methodology that integrates results from individual association studies in a unique statistic across data sets (Evangelou and Ioannidis, 2013). In animal breeding, different implementations of MA have been done (Wood et al., 2006; Salmi et al., 2010; Silva et al., 2011; Akanno et al., 2013; Bolormaa et al., 2014). However, implementation of MA considering results from independent GWA is still emerging. As GBLUP is the most commonly used methodology to perform genomic evaluations, a MA-GWA of several populations using GBLUP is an attractive approach to increase power of detection of variants with small effects across populations. The goal of this research was to implement a MA from multiple independent GBLUP evaluations to identify and suggest potential candidate genes for pork quality traits.

MATERIALS AND METHODS

Data Sets

Animal protocols were approved by the Michigan State University All University Committee on Animal Use and Care (AUF number 09/03-114-00). Phenotypes related to pork quality traits from 3 different pig populations were analyzed, and a brief description follows.

Michigan State University Pig Resource Population (MSUPRP). An experimental population was developed at the Michigan State University Swine Teaching and Research Center (Edwards et al., 2008). Four unrelated Duroc sires and 15 Pietrain sows were mated to produce F_1 animals. From all resulting F_1 animals, 50 females and 6 males (sons of 3 F_0 sires) were kept as parents to produce F_2 pigs. In this case, 1,259 F_2 pigs were obtained and were measured for growth, carcass composition, and pork quality traits (Edwards et al., 2008). Animals were genotyped using high- and low-density panels; that is, 411 F_0, F_1, and F_2 animals were genotyped with the PorcineSNP60 BeadChip (Illumina Inc., San Diego, CA.; Ramos et al., 2009) while 612 additional F_2 animals were genotyped with the 9K tagSNP set (GGP-Porcine LD version 1, GeneSeek, Lincoln, NE; Badke et al. 2013) and imputed with high accuracy (Gualdrón Duarte et al., 2013).

Meat Animal Research Center Population (MARC). This population was developed at the U.S. Meat Animal Research Center (USMARC, Clay Center, NE) and was created from the mating of Yorkshire-Landrace females (n = 220) with Duroc or Landrace sires (12 sires of each breed). Twelve sires of each breed were assigned randomly to Yorkshire-Landrace females (n = 220). In the next generations of matings, Duroc-sired pigs were mated with Landrace-sired pigs, and further matings were done at random, avoiding those within sire line. This multigenerational population consists of 1,237 phenotyped animals, sampled in different generations and genotyped using the PorcineSNP60 BeadChip (Illumina Inc.; Ramos et al., 2009). Sporadically missing genotypes (<2%) were imputed using University of Washington, Seattle, WA. BEAGLE version 3.3.1 (Browning and Browning, 2009; estimated imputation accuracy >98%). Carcass composition and pork quality trait records were collected as previously described (Nonneman et al., 2013).

Commercial Population. Pork quality traits were measured from boneless loins, obtained from 4 large-scale processing facilities and sampled at approximately 24 h postmortem (King et al., 2011; Shackelford et al., 2012). Loins were part of previous research projects and, specifically, provided by King et al. (2011; 1,208 loins from 4 plants), Shackelford et al. (2011; 112 loins from a single packing plant), and Shackelford et al. (2012; 600 loins from 3 plants). Some plants were common among studies, and thus, 5 locations were included. Loins were combined from the 3 studies, involving pigs of similar genetic origin (Shackelford et al., 2011). Loins were vacuum packaged, boxed, and transported to USMARC, and at 14 d postmortem, color was determined objectively (Minolta ColorTec PCM; color-tec.com, Clinton, NJ). In this data set, 480 loins (25% of the samples) were selected at random to be genotyped using the PorcineSNP60 BeadChip (Illumina Inc.; Ramos et al., 2009), whereas 1,440 loins were genotyped using the 9K tagSNP set (GGP-Porcine LD version 1, GeneSeek, Lincoln, NE; Badke et al., 2013) and imputed using BEAGLE version 3.3.1 (Browning and Browning, 2009) as described in Badke et al. (2013). Imputation accuracy (97%) was determined as the squared correlation between the observed and imputed allelic dosage in randomly masked and reimputed genotypes.

Phenotype Data

Pork quality traits were measured in the LM, including ultimate pH 24 h postslaughter (**pHu**), slice shear force (**SF**) in kilograms or Warner-Bratzler shear force

Table 1. Number of records, mean, and SD for each trait within data sets

	Commercial		MARC[2]		MSUPRP[3]		
Trait[1]	*N*[5]	Mean (SD)[6]	*N*	Mean (SD)	*N*	Mean (SD)	Total *N*[4]
pHu	1,857	5.63 (0.166)	530	5.812 (0.168)	904	5.512 (0.139)	3,291
SF, kg	1,892	16.81 (5.628)	1,234	13.79 (3.435)	911	3.205 (0.683)	4,037
PRL,%	1,780	0.885 (0.763)	673	2.955 (1.259)	920	1.838 (1.175)	3,373
IMF,%	700	2.154 (0.770)	1,234	2.276 (1.047)	910	3.182 (1.406)	2,844
CKL,%	1,780	17.24 (2.257)	1,234	20.18 (3.062)	912	22.73 (2.836)	3,926
CIE L*	1,780	57.67 (3.343)	704	56.13 (3.756)	874	53.79 (2.238)	3,358
CIE a*	1,780	14.49 (1.495)	704	6.746 (1.428)	874	17.26 (1.827)	3,358
CIE b*	1,780	21.03 (2.07)	704	12.92 (1.686)	874	9.107 (1.603)	3,358

[1]Trait: pHu = ultimate pH; SF = shear force; PRL = purge loss; IMF = intramuscular fat; CKL = cook loss; CIE L*, a* and b* = color traits related to measures of lightness, redness, and yellowness, respectively.

[2]U.S. Meat Animal Research Center population.

[3]Michigan State University Pig Resource population.

[4]Total number of records for each trait across data sets.

[5]Number of records for each trait and for each population.

[6]Mean and SD for each trait and population.

(**WBS**) in kilograms, purge loss percentage (**PRL**), intramuscular fat percentage (**IMF**), cook loss percentage (**CKL**), and objective color measures of lightness (CIE L*), redness (CIE a*), and yellowness (CIE b*). Table 1 presents descriptive statistics for the traits within each data set. The Meat Animal Research Center population and MSUPRP reported the smallest number of records for all traits except for IMF, whereas the commercial population had the lowest sample size. Across populations, the largest number of records was observed for SF (n = 4,037), in contrast to those available for IMF (n = 2,844). Homogeneous mean values for pHu were observed across data sets in comparison with values reported for the remaining traits. Smaller values for WBS in MSUPRP are related to differences in measurement instruments, that is, SF in MARC and commercial populations vs. WBS in MSUPRP.

Data Edit. In all data sets, individuals with low genotyping rate (<90%) and more than 10% of missing genotypes before imputation were discarded. After imputation, SNP with low minor allele frequency, determined within each data set (Minor Allele frequency < 5%), were discarded. Table 2 summarizes the total number of animals and SNP after applying edit criteria independently in each data set, as well as the imputation accuracy observed in the pig data sets.

Statistical Analysis

Genome-Wide Association for Pork Quality Traits. To perform a GWA for pork quality traits within data sets, variance components and breeding values were estimated following the animal centric model for genomic evaluation given by

$$\boldsymbol{y} = \boldsymbol{X}\boldsymbol{\beta} + \boldsymbol{a} + \boldsymbol{e}, \quad [1]$$

where $\boldsymbol{y}$ is the vector of phenotypes; $\boldsymbol{X}$ is the incidence matrix relating records to the vector of fixed effects $\boldsymbol{\beta}$; $\boldsymbol{e}$ is the vector of residual effects, with $\boldsymbol{e} \sim N(0, \boldsymbol{I}\sigma_e^2)$ and σ_e^2 being the residual variance; $\boldsymbol{a}$ is the vector of random breeding values, with $\boldsymbol{a} \sim N(0, \boldsymbol{G}\sigma_a^2)$ and σ_a^2 being the additive genetic variance; and $\boldsymbol{G}$ is the genomic relationship matrix ($n \times n$), with n being the number of animals and obtained as $\boldsymbol{G} = \boldsymbol{ZZ}'$. In this case $\boldsymbol{Z}$ ($n \times m$), with m being equal to the number of SNP, is the matrix containing normalized allelic dosages (counts of allele "B" minus its expected value divided by expected standard deviation). A preliminary

Table 2. Summary of genotypic information for commercial, U.S. Meat Animal Research Center (MARC), and Michigan State University Pig Resource (MSUPRP) populations

	Population		
Item	Commercial	MARC	MSUPRP
Number of SNP after filtering[1]	45,688	44,020	40,569
Number of individuals genotyped at HD[2]	474	1234	324
Number of individuals genotyped at LD[3]	1418	0	604
Total number of individuals[4]	1892	1234	928
Imputation accuracy[5]	0.97	—	0.99

[1]Number of SNP after quality control filters.

[2]High-density genotyped animals, kept after quality control filters. High-density genotyping performed using the PorcineSNP60 BeadChip (Illumina Inc. San Diego, CA; Ramos et al., 2009).

[3]Low-density genotyped animals, kept after quality control filters. Low-density genotyping performed using the GGP-Porcine LD (GeneSeek, Lincoln, NE; Badke et al., 2013)

[4]Final number of animals for each population

[5]Imputation accuracy obtained for commercial (following Badke et al., 2013) and MSUPRP (Gualdrón Duarte et al., 2013).

nary study was conducted to determine fixed effects that accounted for systematic variation of each trait in each population. As a result, different fixed effects were included depending on the population (see Supplementary Table 1).

Population Structure Analysis. According to Janss et al. (2012), inclusion of principal components in model [1] to account for population structure is not required, mainly because of the genetic variation accounted for after the genomic relationship matrix $\boldsymbol{G}$ has been considered in the model. Thus, principal components were not incorporated in individual population analyses.

Population Test Statistics. For each SNP and trait within population, estimates of SNP effects $\hat{\boldsymbol{g}}$ were computed from a linear transformation of estimated breeding values $\hat{\boldsymbol{a}}$: $\hat{\boldsymbol{g}} = \boldsymbol{Z}'\mathbf{G}^{-1}\hat{\boldsymbol{a}}$, with their variances obtained as $\mathrm{Var}(\hat{\boldsymbol{g}}) = \boldsymbol{Z}'\boldsymbol{G}^{-1}\boldsymbol{Z}\sigma_a^2 - \boldsymbol{Z}'\boldsymbol{G}^{-1}\boldsymbol{C}^{\boldsymbol{aa}}\boldsymbol{G}^{-1}\boldsymbol{Z}$ (Gualdrón Duarte et al., 2014). In this case, $\boldsymbol{\sigma}_a^2$ is the genetic variance, and ***Caa*** is the portion of the inverse of the mixed model equations associated with model [1]. Then, test statistics were obtained standardizing estimated SNP effects divided by the square root of their variance (Bernal Rubio et al., 2014):

$$z_{ij} = \frac{\hat{\boldsymbol{g}}_{ij}}{\sqrt{\mathrm{Var}\left(\hat{\boldsymbol{g}}_{ij}\right)}}, \qquad [2]$$

where z_{ij} corresponds to the z-score for jth SNP in population i. Furthermore, on the basis of these z-scores, P-values for significance of SNP effects were obtained as follows:

$$P\text{-value}_{ij} = 2\left[1 - \Phi\left(\left|\frac{\hat{\boldsymbol{g}}_{ij}}{\sqrt{\mathrm{Var}\left(\hat{\boldsymbol{g}}_{ij}\right)}}\right|\right)\right], \qquad [3]$$

where $P\text{-value}_{ij}$ is the P-value associated with the jth SNP in population i and $\phi(\cdot)$ is the standard normal cumulative distribution (Gualdrón Duarte et al. 2014).

Meta-analysis of Genome-Wide Association Studies. Following the approach presented by Bernal Rubio et al. (2014), we combined population z-scores obtained from multiple independent GWA studies (Eq. [2]) into a single z-score, considering the Hedges and Vevea estimator (Hedges and Vevea, 1998). In this approach, weights are based on the estimation of the inverse variance of each effect size. It has been shown that the test statistic presented in Eq. [2] is equivalent to a test statistic based on a SNP fixed effects model (Bernal Rubio et al., 2015). Thus, to optimally weight test statistics given in Eq. [2], variance of the fixed SNP effect estimate $\mathrm{Var}\left(\hat{\boldsymbol{b}}_{ij}\right)$ has to be computed from the variance of the random effect $\mathrm{Var}\left(\hat{\boldsymbol{g}}_{ij}\right)$ (Bernal Rubio et al., 2015). That is,

$$\mathrm{Var}\left(\hat{\boldsymbol{b}}_{ij}\right) = \frac{\left(\sigma_a^2\right)^2}{\mathrm{Var}\left(\hat{\boldsymbol{g}}_{ij}\right)}. \qquad [4]$$

Thus, considering the result presented in Eq. [4], the weight for inverse-variance criterion is

$$w_{ij} = \frac{1}{\mathrm{Var}\left(\hat{\boldsymbol{b}}_{ij}\right)}. \qquad [5]$$

This weighting approach requires a consistent scale of measurement across data sets. However, in the case of SF, different units were used for the commercial and MARC populations in comparison with MSUPRP (WBS). Thus, to combine test statistics obtained for SF across populations, we transformed genetic variance and variance of each SNP effect obtained within population, using the following equation given by Shackelford and Wheeler (2009):

$$\mathrm{WBS} = (0.1063 \times \mathrm{SF}) + 2.2718. \qquad [6]$$

Combined z-Score. Weights obtained from Eq. [5] were used to generate a unique z-score z_j^*, computed as a weighted combination of the population specific z-scores z_{ij} obtained with Eq. [2]:

$$z_j^* = \sum_{i=1}^{k}\left[z_{ij}\left(\frac{\sqrt{w_{ij}}}{\sqrt{\sum_{i=1}^{k} w_{ij}}}\right)\right]. \qquad [7]$$

Finally, meta-analysis P-values of association were computed as presented in Eq. [3].

Candidate Gene Search Based on Linkage Disequilibrium Blocks. Following a similar approach to the one presented by Do et al. (2014), intervals for candidate gene search were defined according to linkage disequilibrium (**LD**) blocks, constructed around QTL peaks. Considering that the goal was to find LD patterns consistent across populations, LD blocks were constructed on the basis of the creation of a unique genotype file, which included common SNP after quality checks within data sets (m = 36,876). After that, annotated genes contained in the window were identified using Ensembl with the *Sus scrofa* Build 10.2 assembly (http://ensembl.org/Sus_scrofa/Info/Index).

RESULTS AND DISCUSSION

Genetic Variation between Populations. Genetic and residual variances, as well as heritabilities and SE estimates of variance components and of heritabilities

Table 3. Variance components, heritabilities, and SE estimates for each trait and data set

	Genetic variance (SE)			Residual variance (SE)			Heritability (SE)[2]		
Trait[1]	Com[3]	MARC[4]	MSU[5]	Com	MARC	MSU	Com	MARC	MSU
pHu	0.009 (0.001)	0.007 (0.002)	0.003 (0.001)	0.017 (0.001)	0.019 (0.002)	0.013 (0.001)	0.340 (0.044)	0.267 (0.076)	0.169 (0.044)
SF, kg	7.584 (1.287)	3.329 (0.610)	0.112 (0.025)	18.122 (0.984)	7.499 (0.461)	0.286 (0.018)	0.295 (0.043)	0.307 (0.046)	0.282 (0.050)
PRL, %	0.184 (0.027)	0.528 (0.116)	0.289 (0.065)	0.313 (0.019)	0.801 (0.083)	0.797 (0.050)	0.370 (0.045)	0.397 (0.069)	0.266 (0.049)
IMF, %	0.275 (0.057)	0.445 (0.055)	0.917 (0.134)	0.324 (0.037)	0.361 (0.028)	0.794 (0.061)	0.459 (0.073)	0.552 (0.043)	0.536 (0.048)
CKL, %	1.483 (0.236)	1.270 (0.206)	2.230 (0.476)	2.929 (0.172)	2.148 (0.140)	5.363 (0.343)	0.336 (0.045)	0.372 (0.047)	0.294 (0.050)
CIE_L*	3.712 (0.557)	2.882 (0.709)	1.625 (0.316)	6.545 (0.393)	6.325 (0.536)	2.957 (0.203)	0.362 (0.045)	0.313 (0.064)	0.355 (0.052)
CIE_a*	0.900 (0.114)	0.131 (0.049)	0.552 (0.075)	1.103 (0.072)	0.688 (0.050)	0.363 (0.030)	0.449 (0.044)	0.160 (0.055)	0.603 (0.045)
CIE_b*	1.072 (0.166)	0.190 (0.065)	0.110 (0.029)	2.009 (0.119)	0.846 (0.063)	0.408 (0.026)	0.348 (0.045)	0.184 (0.057)	0.213 (0.048)

[1]Trait: pHu = ultimate pH; SF = shear force; PRL = purge loss; CKL = cook loss; IMF = intramuscular fat; CIE L*, a* and b* = color traits related to measures of lightness, redness and yellowness, respectively.

[2]Heritability estimates and standard error (Visscher and Goddard, 2015).

[3]Commercial population.

[4]U.S. Meat Animal Research Center (MARC) population.

[5]Michigan State University Pig Resource population.

for evaluated phenotypes, are presented in Table 3. In general, heritability estimates for evaluated traits had a wide range, with minimum and maximum values of 0.16 and 0.60 for CIE a* in MARC and MSUPRP, respectively. In our data sets, the widest range of heritabilities was observed for CIE a*. This can be explained by the difference in variance component estimates across populations. Specifically, genetic variance estimates were larger in commercial and MSUPRP data sets, leading to larger heritabilities for CIE a* in those populations, regardless of the large value of residual variance observed in the commercial population (Table 2). However, globally, our estimates were moderate to high and similar to results reported for these traits in the literature. For instance, Gjerlaug-Enger et al. (2010) reported heritabilities ranging from 0.23 to 0.33 for PRL, 0.19 to 0.27 for pHu, 0.50 to 0.62 for IMF, and between 0.28 and 0.41, 0.43 and 0.46, and 0.31 and 0.33 for CIE L*, CIE a*, and CIE b*, respectively. These values are similar to estimates presented in this paper. The importance of accounting for variance component heterogeneity in GWA has been shown (Bernal Rubio et al., 2015). Specifically, meta-analysis of independent genomic evaluation models for each population under study will account for heteroscedasticity across populations. Our methods for combining SNP effects into a MA-GWA considers the differences in variance components, and it increases power compared to a joint analysis in which records are pooled and analyzed together (Walling et al., 2000; Zhou et al., 2011; Bernal Rubio et al., 2015).

Meta-analysis of GWA. Figure 1 presents Manhattan plots for MA of associations of pork quality traits. Significant association peaks detected by MA-GWA are summarized in Table 4. A significant QTL at 308.9 Mb on chromosome 1 (SSC1; ALGA0103022) was associated with CIE a*. On SSC2, significant associations with SF were observed at 5.4 Mb (H3GA0055977) and at 106.4 Mb (ASGA0011029). In the case of SSC6, a QTL associated with IMF and CKL was identified at 0.013 Mb (ALGA0109178), along with a significant second association with CIE a* at 49.8 Mb (DIAS0000492). Finally, a region identified on SSC15 was associated with several traits, pHu at 135.2 Mb (H3GA0052416) and PRL and CKL at 133.9 Mb (M1GA0020450). No significant associations with CIE b* or CIE L* were identified by MA.

Some of the significant SNP are in close proximity to previously reported QTL regions. On SSC2, a significant association detected at 5.4 Mb for SF (included in the region between 5.1 and 6.2 Mb) was 0.5 Mb away from a significant SNP reported by Nonneman et al. (2013) for the same trait. Likewise, on SSC15, significant associations for pHu observed in this paper (133.2 to 135.7 Mb) overlapped the associations reported by Uimari et al. (2013; between 133 and 134 Mb). On the same chromosome, a significant interval observed for CKL (133.2 to 135.2 Mb) included the region reported by Nonneman et al. (2013), located between 133.4 and 133.8 Mb.

We also discovered numerous novel associations. Specifically, a significant SNP associated with CIE a* on SSC1 (308.9Mb) has not been reported. A significant SNP located at 106.4 Mb on SSC2 that is associated with SF is near the QTL previously reported by Rohrer et al. (2006) and by Stearns et al. (2005) on SSC2 for shear force. In the case of SSC6, a significant SNP located at 0.013Mb associated with CKL and IMF is distant from the region previously reported by Ponsuksili et al. (2010) for CKL (around 71 Mb apart from our reported SNP) and by Markljung et al. (2008) for IMF (between 22 and 26 Mb apart from our SNP). Also on SSC6, the significant interval identified between 48.5 and 63.1 Mb for CIE a* was 12 Mb upstream from the region previously reported

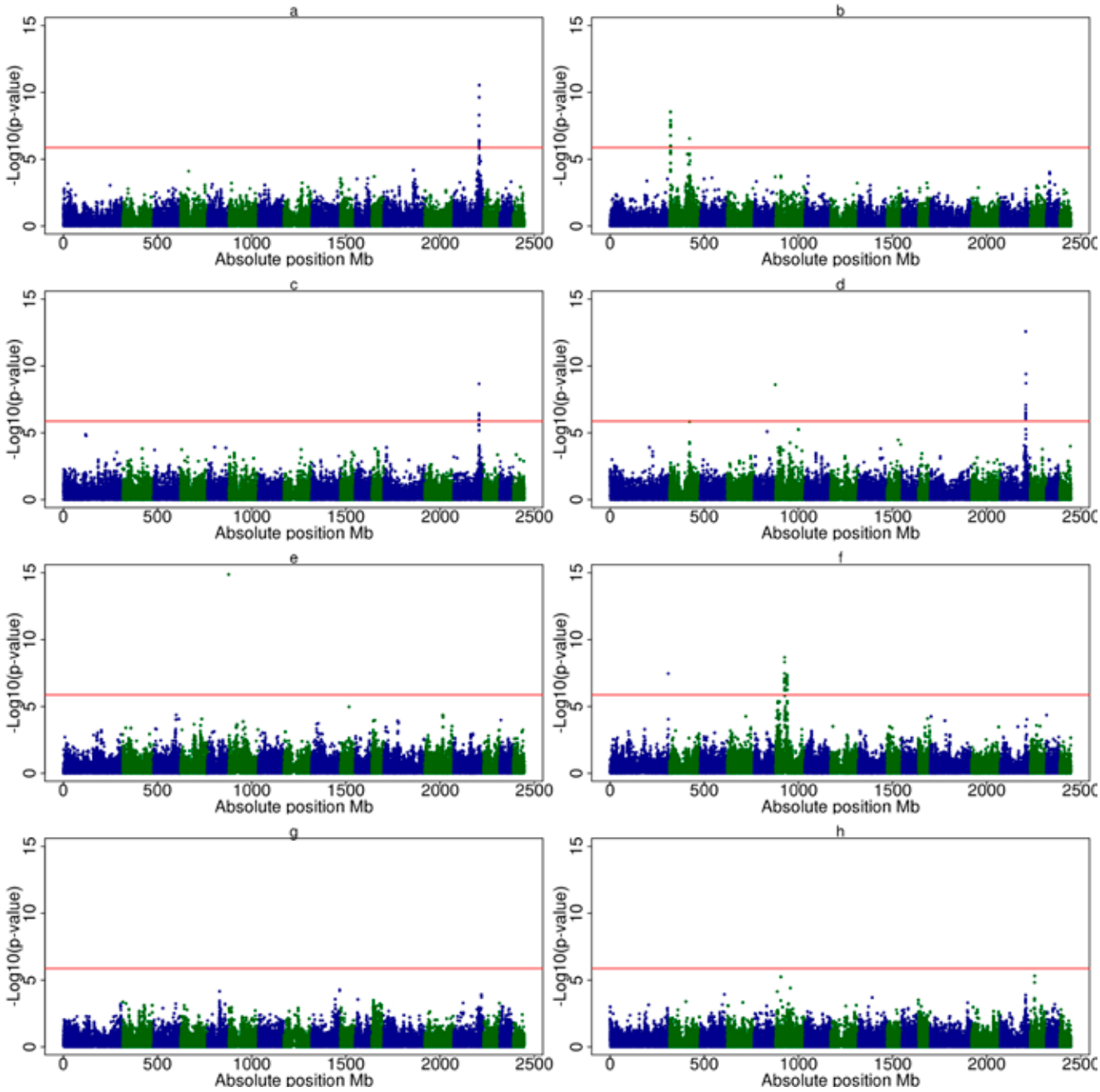

Figure 1. Significant associations for pork quality traits using inverse variance meta-analysis (MA). Manhattan plots for SNP associations using inverse variance MA for (a) ultimate pH 24 h postslaughter (pHu), (b) slice shear force (SF), (c) purge loss (PRL), (d) cook loss (CKL), (e) intramuscular fat (IMF), (f) CIE a*, (g) CIE b*, and (h) CIE L*. $-\text{Log}_{10}$(*P*-value; *y* axis) vs. absolute SNP position in megabases (*x* axis). Alternating colors indicate autosomes (1–18). Genome-wide significance threshold is in red ($P < 0.05$).

by Cherel et al. (2011) for the same trait. Associations for PRL on SSC15, located between 133.7 and 134 Mb, were distant from the significant QTL reported by Ma et al. (2013) at 96.4 Mb. Therefore, significant QTL and genomic regions identified by MA-GWA in this paper provide additional evidence of associations previously reported and also contribute novel evidence in regard to candidate regions associated with pork quality traits.

Candidate Genes Queried for Pork Quality Traits. For each QTL interval observed with MA and defined according to LD blocks around QTL peaks, several annotated genes were identified as possible candidates for pork quality traits (Table 5). One exception was SSC1 associated with CIE a* (peak at 308.9 Mb). In this case, the initial interval was defined between 307.9 and 309.9 Mb (1 Mb upstream and downstream from association peak), which was characterized by high LD between SNP around the peak and in neighboring LD blocks but with low LD in flanking regions. Thus, we narrowed down the genomic interval considering LD around a significant peak (308.9 to 309.1 Mb) and found 1 gene (vomeronasal 1 receptor 2 [***VN1R2***]; SSC1: 308.9 Mb)

Table 4. Summary of significant regions associated with pork quality traits using inverse variance meta-analysis

Chr[1]	Peak SNP[2]	Peak position,[3] Mb	Searching interval,[4] Mb	Trait[5]
1	ALGA0103022	308.9	308.9–309.1	CIE a*
2	H3GA0055977	5.4	5.3–6.5	SF
2	ASGA0011029	106.4	105.02–109.6	SF
6	ALGA0109178	0.013	0–1	IMF
				CKL
6	DIAS0000492	49.8	47.3–52.7	CIE a*
15	H3GA0052416	135.2	131.9–135.7	pHu
	M1GA0020450	133.9		PRL
	M1GA0020450	133.9		CKL

[1]Chromosome relative to *Sus scrofa* genome build 10.2.

[2]SNP name in the peak.

[3]Position of the peak expressed in megabases.

[4]Interval for candidate genes search, established after linkage disequilibrium block construction (expressed in megabases).

[5]Evaluated pork quality traits. Only those traits with significant QTL under inverse variance meta-analysis are included (pHu = ultimate pH; SF = shear force; PRL = purge loss; CKL = cook loss; IMF = intramuscular fat; CIE a* = color trait related to redness).

and 3 uncharacterized proteins. However, there is no evidence of the biological association between the *VN1R2* gene and CIE a*, and thus, the genetic cause of this association peak remains unknown. With the small LD block in the region (0.2 Mb), no strong candidate gene exists.

Candidate Genes on Chromosome 2 (SF). In the case of SF and specifically for the first region on SSC2 (5.3 to 6.5Mb), the gene calpain-1 catalytic subunit (***CAPN1***; SSC2: 6.12 to 6.15 Mb) can be suggested as a potential candidate gene in pigs. The region near *CAPN1* has previously been shown to be associated with SF in pigs (Nonneman et al., 2013), and SNP in bovine *CAPN1* have been associated with tenderness traits in several cattle populations (Page et al., 2004; White et al., 2005; McClure et al., 2012). According to Huff-Lonergan et al. (1996), the calpain protease system has an important role in postmortem proteolysis through degradation of myofibrillar and myofibril-associated proteins. In general, mechanisms controlling meat tenderness depend on a complex interaction of cellular processes, myofibrillar degradation, and activity of enzymes, including cathepsins, the calpain/calpastatin system, and the proteasome (Bendixen, 2005). Therefore, we searched for further genes in the same region possibly having an effect on meat tenderness. First, the gene cystatin E/M (***CST6***; SSC2: 5.395 to 5.396 Mb) encodes cystatin M or E/M, which is an endogenous inhibitor of lysosomal cysteine proteases that functions to regulate and protect cells against uncontrolled proteolysis by the cysteine proteases cathepsins L and V (cathepsin L [**CTSL**], cathepsin V [**CTSV**]; Turk and Bode, 1991; Cheng et al., 2006; Zeeuwen et al., 2009). One of those protease cathepsins is encoded by the gene cathepsin W (***CTSW***; SSC2: 5.550 to 5.554 Mb), also found in the genomic region significantly associated with SF. Cathepsins have been suggested to contribute to the overall net myofibrillar proteolysis in porcine LM at the normal pHu, having an effect on protein degradation in muscle (Ertbjerg et al., 1999) and thus emerging as potential candidate genes for SF. In addition, the gene synovial apoptosis inhibitor 1, synoviolin (***SYVN1***; SSC2: 6.19 to 6.2 Mb) is an E3 ubiquitin ligase that encodes a protein involved in endoplasmic reticulum (**ER**) degradation and uses the ubiquitin-proteasome system for mediated proteolysis of unfolded proteins. Those unfolded proteins have been highlighted in mice to decrease ER stress during chondrocyte hypertrophy (cells found in cartilage) by improving the protein-folding capacity of the ER (Kaufman, 1999; Malhotra and Kaufman, 2007; Ron and Walter, 2007; Cameron et al., 2011). All in all, *CAPN1* remains the most likely candidate gene for SF in this region of SSC2, but we also wanted to call attention to colocalized genes that code for biochemically related proteins and could also be potential candidate genes for SF not reported before.

In the second region associated with SF located on SSC2 between 105.02 and 109.6 Mb, the gene calpastatin (***CAST***; SSC2:106.9 to 107.1 Mb) is a candidate gene, considering that markers in *CAST* have been associated with tenderness in pork (Ciobanu et al., 2004; Meyers and Beever, 2008; Lindholm-Perry et al., 2009) and beef (Schenkel et al., 2006; McClure et al., 2012; Tait et al., 2014). Some SNP in transcription factor binding sites in the promoter region of porcine calpastatin that were consistently associated with tenderness across commercial populations showed allele-specific binding to nuclear proteins that may be functional (Nonneman et al., 2011). Calpastatin is the endogenous inhibitor of μ- and m-calpain, which regulates calpain activity in postmortem muscle (Koohmaraie, 1992). In pork, a high activity of calpastatin has been associated with reduced degradation of muscle proteins (Lonergan et al., 2001). In addition, Ciobanu et al. (2004) identified several variants of the porcine calpastatin gene that had significant effects on tenderness and other commercially important pork quality traits. Thus, the present study allowed us to replicate previous findings, providing more evidence of the association between markers close to the calpastatin gene and tenderness in pork.

Candidate Genes Chromosome 6 (CKL and IMF). In the region significantly associated with CKL and IMF (SSC6 0 to 1 Mb), the gene acyl-CoA synthetase family member 3 mitochondrial (***ACSF3***; SSC6: 0.82 to 0.84 Mb) is a candidate gene positioned close to the association peak identified for those traits (0.013 Mb). The gene *ACSF3* belongs to a family of enzymes that activate fatty acids (Bovo et al., 2015) and has been observed to be

Table 5. Candidate genes queried for pork quality traits

Chr[1]	Significant region[2]	Trait[3]	Symbol[4]	Candidate gene name[5]
2	5.3–6.5	SF	*CAPN1*	calpain-1 catalytic subunit
2	105.02–109.6	SF	*CAST*	calpastatin
6	0–1	IMF, CKL	*ACSF3*	acyl-CoA synthetase family member 3
6	47.3–52.7	CIE a*	*GYS1*	glycogen synthase 1 (muscle)
			FTL	ferritin light polypeptide.
15	131.9–135.7	pHu, PRL, CKL	*PRKAG3*	protein kinase, AMP-activated, γ3 noncatalytic subunit

[1]Chromosome.

[2]Significant region in megabases.

[3]Trait: PRL = purge loss; pHu = ultimate pH; SF = shear force; CKL = cook loss; IMF = intramuscular fat; CIE a* = color trait (measure of redness).

[4]HGNC (Human genome organization Gene Nomenclature Committee) symbol of candidate gene (http://www.genenames.org/).

[5]Name of candidate gene (http://www.genenames.org/).

overexpressed in pig semimembranosus muscle and related to the mitochondrial fatty acid β-oxidation pathway (Herault et al., 2014). With regard to CKL, we did not find previous results supporting a potential association with *ACSF3*. However, a relationship between IMF and CKL has been described before, showing that meat with a high content of IMF had a low CKL at 60°C and 70°C (temperature at center of loin chop), whereas at 80°C the meat with the lowest IMF content had a significantly higher CKL (Aaslyng et al., 2003). Hence, results obtained in the present study for this region on SSC6 are relevant in different aspects: First, identification of the *ACSF3* gene within a genomic interval significantly associated with IMF provides additional support for the relation between the *ACSF3* gene and IMF. Moreover, our results provide potential evidence for the association between the *ACSF3* gene and CKL, which was not previously reported. Finally, detection of a common significant QTL for IMF and CKL (the same SNP was significantly associated with both traits in MA-GWA) partially explains the possible biological relationship between IMF and CKL and resulting meat quality in pigs.

Candidate Genes Chromosome 6 (CIE a*). Two genes were identified as candidates for the objective measure of redness, CIE a*. The gene glycogen synthase 1, muscle (***GYS1***; SSC6: 50.07 to 50.09 Mb), which is the skeletal muscle form of glycogen synthase (Wang et al., 2012), accounts for storage of circulating glucose. The anaerobic degradation of glycogen controls the rate and extent of pH decline in muscle affecting pork quality attributes, including color (Scheffler et al., 2011). In addition, the light chain ferritin gene ferritin, light polypeptide (***FTL***; SSC6: 50.09 Mb) has subunits along with H-ferritin (***FTH1***), integrating the iron storage protein complex ferritin. Iron content in pork is highly genetically correlated with a* and other color values (Hermesch and Jones, 2012). However, previous associations between *GYS1* and *FTL* genes and CIE a* have not been reported. Consequently, results presented in this paper along with the biological pathways of these genes serve as precedent for the possible relationship between *GYS1* and *FTL* with measure of redness in pork.

Candidate Genes Chromosome 15 (pHu, CKL, and PRL). On SSC15, the gene protein kinase, AMP-activated, γ 3 noncatalytic subunit (***PRKAG3***; SSC15: 133.8 Mb), widely reported to affect variation in glycogen content, PRL, CKL, pHu, and subsequent pork quality (Milan et al., 2000; Ciobanu et al., 2001; Otto et al., 2007; Rohrer et al., 2012; Nonneman et al., 2013), is the strongest candidate gene for pHu, PRL, and CKL. The *PRKAG3* gene has been widely associated with variation in muscle glycogen affecting production of lactate in postmortem muscle, the resulting pH, and, subsequently, the ability of meat to retain water during cutting, heating, and processing (water-holding capacity [**WHC**]) in fresh pork (Huff-Lonergan and Lonergan, 2005; Ryan et al., 2012). Additionally, relationships between pHu, CKL, and PRL have been reviewed in the literature. Specifically, Hamm (1960) observed that the rate and extent of pH decline affected proteolysis of cytoskeletal proteins and WHC in pork. High PRL and development of low WHC is related to low pHu, a decline that results in denaturation (loss of functionality and water-binding ability) of many proteins, including those involved in binding water (Huff-Lonergan and Lonergan, 2005). In regard to CKL, Aaslyng et al. (2003) have indicated that WHC and pH influenced CKL, where low WHC and low pH resulted in high CKL. Altogether, considering the physiological association between pHu, PRL, and CKL, the detection of a common genomic region for those traits in our populations is an expected result. As a consequence, identification of the *PRKAG3* gene provides further evidence for its association with pork quality traits such as pHu, PRL, and CKL.

Conclusion

In conclusion, implementation of MA-GWA allowed the identification of significant genetic associations with pork quality traits that were consistent across

3 populations of diverse genetic backgrounds by combining results from independent genomic evaluations. Significant regions included associations on SSC1 for CIE a*; on SSC2 for SF; on SSC6 for IMF, CKL, and CIE a*; and on SSC15 for PRL, CKL, and pHu. The MA-GWA confirmed candidate genes previously reported for pHu, PRL, and CKL (*PRKAG3*) and SF (*CAPN1* and *CAST*), and it identified novel candidate genes for CKL and IMF (*ACSF3*) and for CIE a*(*GYS1* and *FTL*). Further research is required to test biological relationships between novel candidate genes' suggested and reported QTL. Results presented in this paper illustrate the merits of MA-GWA for detection of significant regions associated with economically relevant traits.

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