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Research Paper

Genome-Wide Association Analysis of Meat Quality Traits in a Porcine Large White × Minzhu Intercross Population

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Abstract

Pork quality is an economically important trait and one of the main selection criteria for breeding in the swine industry. In this genome-wide association study (GWAS), 455 pigs from a porcine Large White × Minzhu intercross population were genotyped using the Illumina PorcineSNP60K Beadchip, and phenotyped for intramuscular fat content (IMF), marbling, moisture, color L*, color a*, color b* and color score in the longissimus muscle (LM). Association tests between each trait and the SNPs were performed via the Genome Wide Rapid Association using the Mixed Model and Regression-Genomic Control (GRAMMAR-GC) approach. From the Ensembl porcine database, SNP annotation was implemented using *Sus scrofa* Build 9. A total of 45 SNPs showed significant association with one or multiple meat quality traits. Of the 45 SNPs, 36 were located on SSC12. These significantly associated SNPs aligned to or were in close approximation to previously reported quantitative trait loci (QTL) and some were located within introns of previously reported candidate genes. Two haplotype blocks ASGA0100525-ASGA0055225-ALGA0067099-MARC0004712-DIAS0000861, and ASGA0085522-H3GA0056170 were detected in the significant region. The first block contained the genes *MYH1*, *MYH2* and *MYH4*. A SNP (ASGA0094812) within an intron of the *USP43* gene was significantly associated with five meat quality traits. The present results effectively narrowed down the associated regions compared to previous QTL studies and revealed haplotypes and candidate genes on SSC12 for meat quality traits in pigs.

Key words: F2 design; genome-wide association study; meat quality trait; pig; SNP.

Introduction

Consumer attitudes towards pork are often influenced by sensory attributes such as odor, flavor, tenderness and juiciness, in addition to physical and biochemical parameters such as pH, shear force, water holding capacity and intramuscular fat content [1]. Even though some parameters, such as fat distribution, can be predicted using computed tomography (CT) scanning, others such as moisture and meat color

can only be measured after slaughtering the pigs. For breeding stock, some traits can only be predicted using related animal information. The detection of markers associated with these traits is necessary for marker-assisted selection (MAS) which could improve early selection and enormously decrease the cost of breeding for meat quality trait optimization. Since the initial report of quantitative trait loci (QTLs) for meat quality traits by Andersson-Eklund [2], approximately 4,434 off these QTLs were identified via genome scanning based on linkage analyses (http://www.animalgenome.org/cgi-bin/QTLdb/SS/index, Apr 20, 2011). As a result of the low density of currently detected microsatellite markers, QTLs are often mapped to a large interval of 20 centimorgans (cM) or more. Only a few quantitative trait nucleotides (QTN) have been identified on the basis of results for complex traits in domestic animals via QTL fine mapping analysis [3-6]. The current porcine 60K SNP panel provides more density than the available microsatellite markers and contributes to improved accuracy in finding the exact QTL locations.

Genome-wide panels of SNPs have been developed in many species. Genome-wide association studies (GWAS) that survey most of the genome using genetic variants [7] have been conducted and applied widely in the analysis of human diseases and complex traits. Furthermore, this approach has been applied to detect SNPs associated with many complex traits in livestock [8-13]. In the present study, a GWAS was performed using the PorcineSNP60 Genotyping BeadChip (Illumina, San Diego, CA, USA) to detect potential genetic variants associated with meat quality traits in a porcine Large White × Minzhu intercross population.

Materials and methods

Animals

Minzhu is a pig breed indigenous to northeast China. Average environmental temperatures of 4°C/year are experienced in this region and in response, the Minzhu breed has developed excellent characteristics of fat deposition, with 5.1 cm back fat thickness and 5% intramuscular fat content (IMF) in the longissimus muscle (LM) at 240 days of age [14].

In this study, a three-generation resource population was produced by intercrossing Large White boars and Minzhu sows during the period from 2007 to 2011. Four Large White boars were mated with 16 Minzhu sows. The resulting F1 generation, comprising nine sires and 46 dams were mated (avoiding full-sib mating) to produce 455 F2 animals (88 litters) in three parities. Most sows were mated to the same boar for all three parities to provide large, full-sib populations. The average number of offspring per sire was 51. Male pigs of the F2 generation were castrated. All F2 animals were reared on the same feeding conditions at the pig research station of the Institute of Animal Science at the Chinese Academy of Agricultural Sciences.

Phenotypic data

Phenotypic data of seven meat quality traits were recorded by trained personnel for all F2 individuals following the guidelines of the National Pork Producers Council (NPPC 1991) of the USA. All F2 animals were slaughtered at the age of 240 ± 7 d in 48 batches (slaughter groups). After slaughter, carcasses were divided into ham, back, belly and shoulder portions, which were defatted and fully dissected. Chilled meat quality traits were evaluated 24 h post-slaughter. These traits included the subjective quality traits (marbling and color score) and objective quality traits (intramuscular fat content, moisture, color L*, color a* and color b*) in the LM (located between the 6th rib and the last lumbar vertebra). Meat color was assessed subjectively in terms of color score (CS) according to the color standard (1 = pale; 6 = dark) provided by the NPPC [15] and evaluated objectively using a CM-2600d/2500d Minolta Chroma Meter, where color L* represented lightness, color a* represented redness and color b* represented yellowness on the cut surface of the LM. Percentage meat moisture content was determined by the routine oven-drying method. Intramuscular fat (IMF) content was analyzed by a subjective NPPC photographic reference standard (1–10, with 1 = devoid, 10 = overly abundant) to determine marbling scores of LM at 24 h post-mortem and objectively using an ether extraction method (Soxtec Avanti 2055 Manual Extraction Unit, Foss Tecator).

Genotyping and quality control

Whole blood was collected from 20 F0, 55 F1 and 455 F2 animals for DNA isolation. Genotyping was performed using the PorcineSNP60 Genotyping BeadChip technology (Illumina), which contained 62,163 SNPs across the whole genome. BEADSTUDIO software (Illumina) was used to call the genotypes for all samples. Before quality control, the maximum likelihood method was applied using the Cervus program [16] to check pedigree mismatching using SNP information. After parentage identification, quality control procedures were performed for the 455 F2 animals within the R statistical environment using the GenABEL package [17]. Data were quality controlled for sample call rate, SNP call rate, minor allele

frequency (MAF) and deviations from Hardy-Weinberg Equilibrium (HWE). The quality control procedure could be split into two steps: Firstly, gender errors were identified and secondly the residual errors were removed iteratively. At the first step of the iterative procedure, SNPs were excluded according to the following criteria: (1) call rate <90%; (2) MAF <3%; and (3) significant divergence from HWE with *P*-values lower than 10^{-6}. At the second step of the iterative procedure, individuals were excluded with call rates <90%. The recursive procedure was applied till no further markers and individuals were eliminated. Application of the quality control procedures resulted in the following exclusions: one individual with a call rate <90%; 112 X-linked SNPs that were likely to be autosomal (odds >1,000), 3,989 SNPs with call rates <90%, 11,252 SNPs with MAF <3% and 1,466 SNPs with extreme HWE values ($P<10^{-6}$).

Table 1. Distribution of SNPs after quality control and average distances on each chromosome.

Chromosome	No. SNPs	Average distance (kb)[a]
1	5155	57.33
2	2112	72.58
3	1659	88.11
4	2903	49.73
5	1776	58.27
6	1505	114.05
7	2838	46.96
8	1770	84.73
9	2080	74.2
10	1094	70.97
11	1478	56.95
12	893	76.41
13	2860	76.09
14	3150	47.18
15	2025	83.67
16	1264	69.04
17	1314	45.26
18	901	65.31
X	668	197.74
Y	1	
0[b]	10792	
Total	48238	

[a]Derived from *Sus scrofa* Build 9 (http://pre.ensembl.org/Sus_scrofa_map/Info/Index).
[b]These SNPs are not assigned to any chromosomes in the Illumina data.

The final data set that passed the quality control procedures and was used in the analysis contained 48,238 SNPs and 454 F2 individuals. The distribution of SNPs after quality control and the average distance between adjacent SNPs on each chromosome are shown in Table 1.

Statistical analysis

Genome-wide association analysis was performed via Genome-wide Rapid Association using the Mixed Model and Regression-Genomic Control (GRAMMAR-GC) approach [17, 18]. The procedure involved three steps:

Step 1: Data were analyzed using the mixed model:

$$y = 1\mu + Xb + pw + Tc + Za + e$$

where y is the vector of phenotypes of 454 F2 individuals, b is the vector of fixed effects (consisting of the sex, parity and batch which contained the herd-year-season effect), w is the vector of body weights of the individuals (considered as a covariate), c is the vector of litter effect (considered as a random effect, $c \sim N(0,\sigma_c^2)$, a is the vector of random additive genetic effects with $a \sim N(0,A\sigma_a^2)$ (where A is the relationship matrix calculated from the corrected pedigree and σ_a^2 is the additive genetic variance), X, T and Z are incidence matrices relating records in y to fixed and random effects, p is the regression coefficient of body weight and e is the vector of residual errors with $e \sim N(0,I\sigma_e^2)$,where I is the identity matrix and σ_e^2 is the residual variance. The vector of residuals y^* is estimated as

$$y^* = y - (1\mu\hat{} + X\,b\hat{} + p\hat{}w + Tc\hat{} + Za\hat{})$$

where $b\hat{}$, $p\hat{}$, $c\hat{}$ and $a\hat{}$ are estimates and predictors for b, p, c and a, respectively.

Step 2: The residuals are used as the dependent trait and the associations are tested using single locus regression analysis:

$$y^* = 1\mu + kg + e^*$$

where g is the vector of genotypes, k is the regression coefficient and e^* is the vector of random residuals.

Step 3: In the GC procedure, the unadjusted test statistic factor of the ith SNP T_i^2 is calculated as:

$$T_i^2 = \hat{k}_i^2 / \mathrm{var}(\hat{k}_i)$$

where $\hat{k}_i$ and $\mathrm{var}(\hat{k}_i)$ are the estimate and sample variance of k, respectively. The deflation factor λ is estimated as $\lambda = \mathrm{median}(T_1^2, T_2^2, \ldots, T_i^2)$, where 0.456 is the median of $\chi_{(1)}^2$ [19]. Association of the ith SNP with the trait is examined by comparison of $T_1^2/\hat{\lambda}$ with $\chi_{(1)}^2$.

Step 1 was performed using DMU software [20]

and the remainder of the analysis was performed within the R statistical environment using the GenABEL package [17]. The genome-wide significance threshold was determined by the Bonferroni method, in which the conventional *P*-value was divided by the number of tests performed [21]. A SNP was considered to have genome-wide significance at $P<0.05/N$, where N is the number of SNPs tested in the analyses. In this study, N was 48,238 and the significant threshold was 1.037e-6.

Phenotypic correlations among the traits were calculated to investigate whether they reflect the correlation among GWAS results. Pearson correlation among the meat quality traits and significance tests were performed within the R statistical environment.

Haplotype block detection was performed on the chromosomal region which contained all the SNPs that were significantly associated with meat quality traits. The genotypes of those significant SNPs loci for 454 F2 individuals and their parents (55 F1 individuals) were used to detect the haplotype blocks. The HAPLOVIEW V3.31 program [22] was used to detect and visualize the haplotype blocks in this work. The procedure was run with default parameters following the manual for HAPLOVIEW program [22].

Association analysis of detected haplotype blocks and meat quality traits of 454 F2 individuals were performed using the Haplo.Stats package [23] within the R statistical environment. A score for each haplotype (hap-score) was calculated and *P*-value was also calculated for the significance of each hap-score. A positive/negative score for a particular haplotype would have suggested that the haplotype was associated with increased/decreased risk of the trait. The index of global score statistic, which had an asymptotic distribution with degrees of freedom (df) and *P*-value, was calculated to test overall associations among haplotype blocks and traits.

Population stratification

Population stratification is recognized as a major threat to the validity of GWAS results [24]. In this study, the influence of population stratification was assessed in a quantile-quantile (Q-Q) plot by examining the distribution of test statistics generated from association tests and the deviation from the null hypothesis of no SNP association with the trait was assessed. Overall deviation above the diagonal identity line in the initial stage may suggest population stratification. GWAS results with and without performing the genomic control (GC) procedures were compared in the "Q-Q" plot to access the effect of the method for population stratification adjustment. The "Q-Q" plot was constructed within the R statistical environment.

Results

Phenotype description and correlation among the traits

Means, standard deviations, minimum and maximum values of the traits measured in the current experiment are presented in Table 2. Means for IMF, marbling, moisture, color L*, color a*, color b* and color score were 2.85%, 2.88, 73.31%, 50.2, 14.08, 7.76 and 3.31, respectively. Phenotypic correlation coefficients among IMF, marbling, moisture, color L*, color a*, color b* and color score are shown in Table 3. High correlation coefficients were identified between IMF and marbling ($r = 0.60$; $P < 0.01$), IMF and moisture ($r = -0.72$; $P < 0.01$) and color L* and color score ($r = -0.60$, $P < 0.01$). Moderate correlation coefficients were identified between moisture and marbling ($r = -0.43$; $P < 0.01$), color L* and color a* ($r = -0.30$; $P < 0.01$), color L* and color b* ($r = 0.41$; $P < 0.01$), color a* and color b* ($r = -0.46$; $P < 0.01$) and color score and color a* ($r = 0.39$; $P < 0.01$). Low phenotypic correlation coefficients were identified between all other traits.

IMF

Of the 40 genome-wide significant SNPs for IMF, 35 were located within an 11.97 Mb segment (between 43.25 and 55.22 Mb) on SSC12 in *Sus scrofa* Build 9 (Table 4). Nine of these (M1GA0016908, ASGA0102838, ALGA0066986, ASGA0055169, M1GA0017055, ASGA0094812, CASI0008458, ALGA0067099 and DIAS0000861) were located in the introns of nine annotated genes: *solute carrier family 13, member 5* (*SLC13A5*), *dynein, axonemal, heavy chain 2* (*DNAH2*), *nudE nuclear distribution gene E homolog-like 1* (*NDEL1*), *phosphoinositide-3-kinase, regulatory subunit 5* (*PIK3R5*), *netrin 1* (*NTN1*), *ubiquitin specific peptidase 43* (*USP43*), *glucagon-like peptide 2 receptor* (*GLP2R*), *myosin, heavy chain 4* (*MYH4*) and *myosin, heavy chain 3* (*MYH3*), respectively. The remainder were located 5.6 Kb to 110.4 Kb from the nearest identified genes (Table 4 and Fig. 1A). The most significant SNP (MARC0017000) was located 28.6 Kb from the *PIRT* gene on SSC12.

Marbling

Of the 37 SNPs associated with marbling, 32 were located within an 8.32 Mb segment (between 46.90 Mb and 55.22 Mb) on SSC12, while the remainders were not mapped to a chromosome in the *Sus scrofa* Build 9 (Table 5 and Fig. 1B). The segment significantly associated with marbling was almost consistent to that for IMF, with the exception of the segment of 43.25 Mb to 46.90 Mb. The most significant SNP was also MARC0017000. The subsequent two

significant SNPs, ASGA0094812 and ALGA0066945, were located within an intron of *USP43* gene and 5.6 Kb from the *EIF5A* gene, respectively.

Moisture

Six SNPs were significantly associated with moisture and these SNPs were located from 49.78 Mb to 54.91 Mb on SSC12 (Table 6). The Manhattan plot is shown in Fig.1C. Only one SNP (ASGA0094812) was located in the *USP43* gene (Table 6). The most significant SNP (ALGA0067173) was located in an uncharacterized gene *ENSSSCG00000018022* (Ensembl).

Meat color

The GWAS was conducted for four meat color traits (color L*, color a*, color b* and color score). No SNP was significantly associated with color L* and color b*. The SNPs significantly associated with color a* and color score are displayed in Tables 7 and 8. The Manhattan plots of the four traits are shown in Fig. 1D-1G. For color a*, four out of six genome-wide significant SNPs were located within a 1.38 Mb segment (between 50.56 Mb and 51.94 Mb) on SSC12. The most significant SNP (ASGA0100525) was located 77.2 Kb from the uncharacterized gene *ENSSSCG00000018002* (Ensembl) on SSC12. For color score, four significant SNPs were identified in the segment between 49.78 Mb and 52.64 Mb on SSC12. The most significant SNP (ASGA0094812) was located in the *USP43* gene on SSC12. Another SNP (ASGA0102838) located in the intron of *DNAH2* gene showed a significant association with both color a* and color score.

Table 2. Descriptive statistics of meat quality traits for 455 individuals.

Traits	Mean	Standard deviation	Minimum	Maximum
IMF	2.85	1.79	0.73	12.70
Marbling	2.88	1.01	1.00	8.00
Moisture	73.31	1.91	61.28	85.36
Color L*	50.20	3.97	27.49	62.56
Color a*	14.08	1.77	9.62	19.64
Color b*	7.76	1.71	2.38	14.53
Color score	3.31	0.64	1.50	5.00

IMF, intramuscular fat content.

Table 3. Correlation coefficients of phenotypes for meat quality traits.

Traits	Marbling	Moisture	Color L*	Color a*	Color b*	Color Score
IMF	0.60**	-0.72**	0.04	0.14**	0.20**	0.23**
Marbling		-0.43**	0.03	-0.0017	0.17**	0.29**
Moisture			0.01	-0.18**	-0.03	-0.16**
Color L*				-0.30**	0.41**	-0.60**
Color a*					-0.46**	0.39**
Color b*						-0.19**

**$P < 0.01$

IMF, intramuscular fat content

Color L*, color a* and color b* represented three meat color traits lightness, redness and yellowness on the cut surface of the LM, respectively.

Table 4. Genome-wide significant SNPs associated with IMF.

SNP	Chr.[1]	Adjust Chr.[2]	Position[3]	Nearest gene[4]	Distance (bp)[5]	GWAS *P*-value
ASGA0054854	12	12	43252014	*ENSSSCG00000017784*	28775	2.73E-08
M1GA0016908	12	12	47940166	*SLC13A5*	intron	1.99E-11
ALGA0117904	0	12	48302347	*WSCD1*	125682	8.67E-07
ALGA0066945	12	12	49784913	*EIF5A*	5646	1.27E-14
ASGA0102838	0	12	50233550	*DNAH2*	intron	1.92E-13
ALGA0066986	12	12	50677511	*NDEL1*	intron	1.80E-07
ALGA0067016	12	12	50961587	*ENSSSCG00000017990*	intron	7.31E-07
ASGA0055169	12	12	51146021	*PIK3R5*	intron	5.31E-07
M1GA0017055	12	12	51354730	*NTN1*	intron	4.38E-07
ASGA0094812	12	12	51682689	*USP43*	intron	3.69E-15
CASI0008458	12	12	51754735	*GLP2R*	intron	9.09E-07
ALGA0067072	12	12	51869438	*ENSSSCG00000018002*	10583	5.72E-07

ASGA0100525	12	12	51936026	*ENSSSCG00000018002*	77171	1.03E-06
ASGA0055225	12	12	52168096	*MYH4*	6253	6.84E-07
ALGA0067099	12	12	52194871	*MYH4*	intron	4.00E-07
MARC0004712	12	12	52254677	*ENSSSCG00000018004*	23808	1.87E-07
DIAS0000861	12	12	52424001	*MYH3*	intron	1.21E-07
ASGA0055256	12	12	52542002	*TMEM220*	20236	7.91E-09
ALGA0107518	12	12	52555184	*TMEM220*	33418	7.62E-08
MARC0017000	12	12	52643400	*PIRT*	28620	2.82E-15
ASGA0085522	12	12	52693097	*PIRT*	78316	4.63E-08
H3GA0056170	12	12	52694087	*PIRT*	79306	7.62E-08
ASGA0096690	12	12	52921855	*SHISA6*	110471	1.48E-07
MARC0030345	12	12	53079696	*ENSSSCG00000018013*	intron	5.02E-07
MARC0009546	12	12	53088251	*ENSSSCG00000018013*	intron	4.27E-07
H3GA0022758	12	12	53577424	*ENSSSCG00000018016*	42532	3.19E-07
ALGA0119023	12	12	53581965	*ENSSSCG00000018016*	47073	2.11E-07
ALGA0067173	12	12	54700447	*ENSSSCG00000018022*	39023	2.99E-08
M1GA0017151	12	12	54761424	*ENSSSCG00000018022*	intron	3.34E-07
ALGA0067189	12	12	54794612	*ENSSSCG00000018022*	intron	7.90E-10
ALGA0067220	12	12	54915217	*ENSSSCG00000018022*	77270	1.09E-08
ASGA0099873	12	12	55014273	*ENSSSCG00000018023*	intron	9.53E-08
ALGA0109745	12	12	55167626	*ENSSSCG00000018025*	intron	8.54E-10
ASGA0084548	0	0				8.17E-08
ASGA0089507	0	0				1.24E-08
ASGA0093543	0	0				8.63E-08
M1GA0026329	0	0				8.63E-08
M1GA0026465	0	0				8.63E-08
ALGA0107077	0	0				4.76E-09
ALGA0108818	0	0				4.11E-08

[1]SNP location on chromosome in the PorcineSNP60 array.
[2]SNP location adjusted on chromosomes in the *Sus scrofa* Build 9 assembly.
[3]SNP position derived from the *Sus scrofa* Build 9 assembly.
[4]Gene location on the *Sus scrofa* Build 9 assembly; gene names start with ENSSSCG as in Ensembl while the other gene symbols are as in GenBank.
[5]SNP designated as in a gene intron or distance from a gene coding region in the *Sus scrofa* Build 9 assembly.

Table 5. Genome-wide significant SNPs associated with marbling.

SNP	Chr.[1]	Adjust Chr.[2]	Position[3]	Nearest gene[4]	Distance bp[5]	GWAS *P*-value
ASGA0054989	12	12	46896795	*ENSSSCG00000017860*	834	3.04E-07
MARC0051399	0	12	47553159	*UBE2G1*	intron	9.94E-07
ALGA0066905	12	12	48052636	*KIAA0753*	13564	8.70E-07
ALGA0066945	12	12	49784913	*EIF5A*	5646	2.72E-09
ASGA0102838	0	12	50233550	*DNAH2*	intron	7.00E-09
ALGA0066986	12	12	50677511	*NDEL1*	intron	7.54E-07
ASGA0094812	12	12	51682689	USP43	intron	2.70E-12
ALGA0067072	12	12	51869438	*ENSSSCG00000018002*	10583	3.84E-09
MARC0027759	12	12	51932407	*ENSSSCG00000018002*	73551	3.13E-07
ASGA0100525	12	12	51936026	*ENSSSCG00000018002*	77171	6.45E-09

ASGA0055225	12	12	52168096	*MYH4*	6253	4.49E-08
ALGA0067099	12	12	52194871	*MYH4*	intron	5.91E-09
MARC0004712	12	12	52254677	*ENSSSCG00000018004*	23808	4.97E-09
DIAS0000861	12	12	52424001	*MYH3*	intron	5.09E-09
ASGA0055256	12	12	52542002	*TMEM220*	20236	4.41E-08
ALGA0107518	12	12	52555184	*TMEM220*	33418	7.37E-09
MARC0017000	12	12	52643400	*PIRT*	28620	7.00E-11
ASGA0085522	12	12	52693097	*PIRT*	78316	2.49E-08
H3GA0056170	12	12	52694087	*PIRT*	79306	7.37E-09
MARC0030345	12	12	53079696	*ENSSSCG00000018013*	intron	1.45E-08
MARC0009817	0	12	53082355	*ENSSSCG00000018013*	intron	2.60E-08
MARC0009546	12	12	53088251	*ENSSSCG00000018013*	intron	6.82E-09
ASGA0035681	7	12	53515215	*ENSSSCG00000018016*	intron	2.74E-08
H3GA0022758	12	12	53577424	*ENSSSCG00000018016*	42532	1.99E-08
ALGA0119023	12	12	53581965	*ENSSSCG00000018016*	47073	8.40E-07
MARC0048623	0	12	53815574	*ENSSSCG00000018016*	280682	4.26E-07
M1GA0017151	12	12	54761424	*ENSSSCG00000018022*	intron	2.79E-08
ALGA0067189	12	12	54794612	*ENSSSCG00000018022*	intron	1.05E-07
ALGA0067220	12	12	54915217	*ENSSSCG00000018022*	77270	5.03E-07
ASGA0099873	12	12	55014273	*ENSSSCG00000018023*	intron	5.53E-10
ALGA0109745	12	12	55167626	*ENSSSCG00000018025*	intron	5.54E-10
ASGA0100497	12	12	55223789	*ENSSSCG00000018025*	intron	2.67E-07
ASGA0084548	0	0				6.99E-10
ASGA0093543	0	0				3.96E-08
M1GA0026329	0	0				3.96E-08
M1GA0026465	0	0				3.96E-08
MARC0093869	0	0				9.42E-07

[1]SNP location on chromosome in the PorcineSNP60 array.
[2]SNP location adjusted on chromosomes in the *Sus scrofa* Build 9 assembly.
[3]SNP position derived from the *Sus scrofa* Build 9 assembly.
[4]Gene location on the *Sus scrofa* Build 9 assembly; gene names start with ENSSSCG as in Ensembl while the other gene symbols are as in GenBank.
[5]SNP designated as in a gene intron or distance from a gene coding region in the *Sus scrofa* Build 9 assembly.

Table 6. Genome-wide significant SNPs associated with moisture.

SNP	Chr.[1]	Adjust Chr.[2]	Position[3]	Nearest gene[4]	Distance bp[5]	GWAS *P*-value
ALGA0066945	12	12	49784913	*EIF5A*	5646	7.41E-07
ASGA0094812	12	12	51682689	*USP43*	Intron	8.02E-09
MARC0017000	12	12	52643400	*PIRT*	28620	6.76E-08
ALGA0067173	12	12	5470044	*ENSSSCG00000018022*	39023	4.37E-09
ALGA0067189	12	12	54794612	*ENSSSCG00000018022*	Intron	7.69E-08
ALGA0067220	12	12	54915217	*ENSSSCG00000018022*	77270	1.00E-07

[1]SNP location on chromosome in the PorcineSNP60 array.
[2]SNP location adjusted on chromosomes in the *Sus scrofa* Build 9 assembly.
[3]SNP position derived from the *Sus scrofa* Build 9 assembly.
[4]Gene location on the *Sus scrofa* Build 9 assembly; gene names start with ENSSSCG as in Ensembl while the other gene symbols are as in GenBank.
[5]SNP designated as in a gene intron or distance from a gene coding region in the *Sus scrofa* Build 9 assembly.

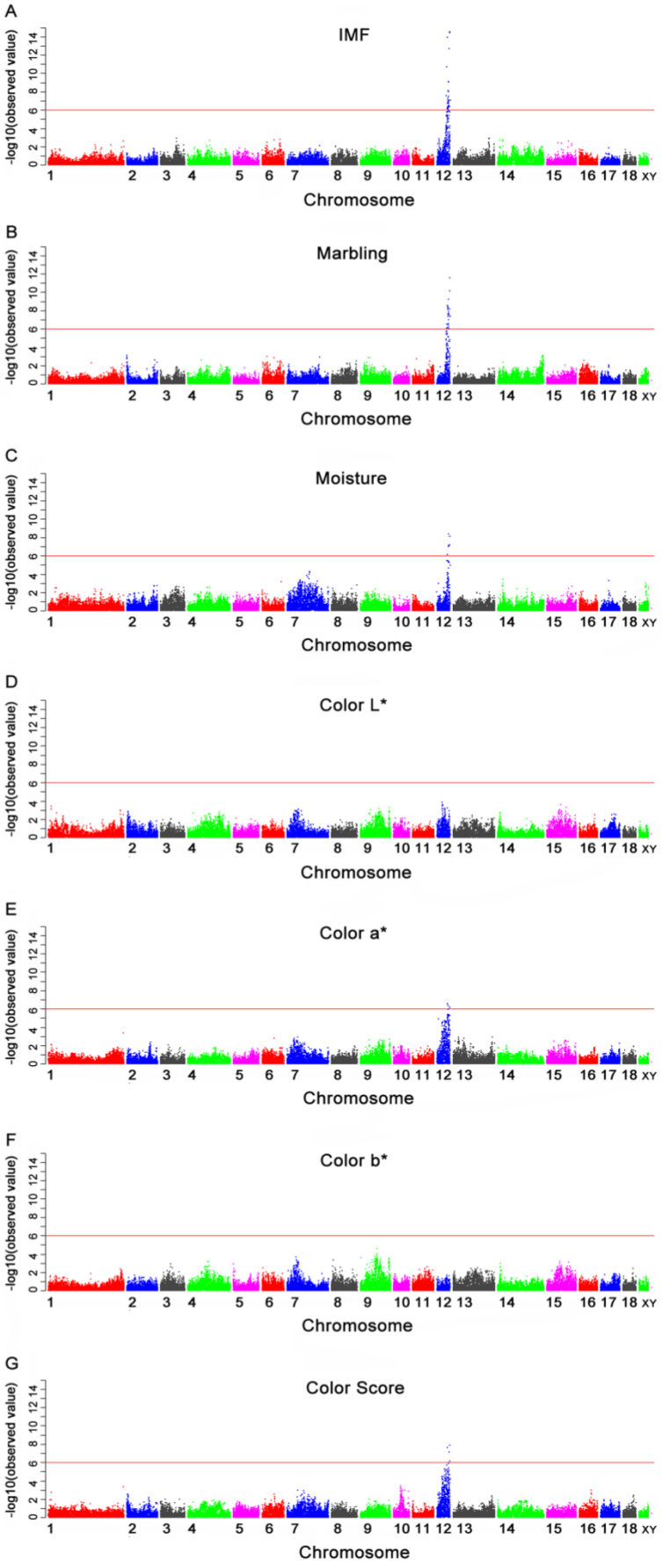

Figure 1. Manhattan plots of genome-wide association study with seven meat quality traits. Chromosomes 1-18, X and Y are shown separated by color. A, B, C, D, E, F and G refer to plots for IMF, marbling, moisture, color L*, color a*, color b* and color score, respectively. Values above -log10(observed value) >5.98 (red horizontal) are genome-wide significant.

Table 7. Genome-wide significant SNPs associated with color a*.

SNP	Chr.[1]	Adjust Chr.[2]	Position[3]	Nearest gene[4]	Distance bp[5]	GWAS *P*-value
MARC0093869	0	12	50558797	*ALOXE3*	772	1.03E-06
ASGA0094812	12	12	51682689	*USP43*	intron	5.94E-07
ALGA0067072	12	12	51869438	*ENSSSCG00000018002*	10583	4.34E-07
ASGA0100525	0	12	51936026	*ENSSSCG00000018002*	77171	4.08E-07
ASGA0089507	0	0				1.68E-07
M1GA0016964	0	0				2.83E-07

[1]SNP location on chromosome in the PorcineSNP60 array.
[2]SNP location adjusted on chromosomes in the *Sus scrofa* Build 9 assembly.
[3]SNP position derived from the *Sus scrofa* Build 9 assembly.
[4]Gene location on the *Sus scrofa* Build 9 assembly; gene names start with ENSSSCG as in Ensembl while the other gene symbols are as in GenBank.
[5]SNP designated as in a gene intron or distance from a gene coding region in the *Sus scrofa* Build 9 assembly.

Table 8. Genome-wide significant SNPs associated with color score.

SNP	Chr.[1]	Adjust Chr.[2]	Position[3]	Nearest gene[4]	Distance bp[5]	GWAS *P*-value
ALGA0066945	12	12	49784913	*EIF5A*	5646	2.25E-08
ASGA0102838	0	12	50233550	*DNAH2*	Intron	6.99E-08
ASGA0094812	12	12	51682689	*USP43*	Intron	1.33E-08
MARC0017000	12	12	52643400	*PIRT*	28620	7.32E-07

[1]SNP location on chromosome in the PorcineSNP60 array.
[2]SNP location adjusted on chromosomes in the *Sus scrofa* Build 9 assembly.
[3]SNP position derived from the *Sus scrofa* Build 9 assembly.
[4]Gene location on the *Sus scrofa* Build 9 assembly; gene names start with ENSSSCG as in Ensembl while the other gene symbols are as in GenBank.
[5]SNP designated as in a gene intron or distance from a gene coding region in the *Sus scrofa* Build 9 assembly.

Haplotype block

Within the 8.3 Mb region containing all the significant SNPs associated with the five meat quality traits, two haplotype blocks were identified (Fig. 3). Block1 was ASGA0100525-ASGA0055225-ALGA0067099-MARC0004712-DIAS0000861 for 325 Kb and block2 was ASGA0085522-H3GA0056170 for 0.99 Kb.

Haplotype frequencies were calculated and association analysis was performed for the two haplotype blocks. For block1, the AGAAG (47.7% and positive effect) and CAGGA (37.0% and negative effect) haplotypes were significantly associated ($P < 0.001$) with IMF, marbling, color a* and color score (Table 9). Although there were significant associations of AGAAG and CAGGA haplotypes ($P < 0.01$), opposite trend of effect was found in moisture comparing to the above four traits. Only the haplotype AGAAG (negative effect) was associated with color L* ($P = 0.02636$). The haplotypes of CGGGA (6.4%), CGAAG (6.1%), CGAAA (1.8%) and AAGGA (0.9%) showed no significant association with any trait. The global score *P*-values for IMF, marbling, moisture, color L*, color a*, color b* and color score were <1e-5, <1e-5, 0.01601, 0.07871, 0.00012, 0.86210 and <1e-5, respectively.

For block2, the haplotype AA (58.92%) was associated with IMF ($P < 1e-5$), marbling ($P < 1e-5$), color a* ($P = 0.00001$) and color score ($P < 1e-5$) for positive hap-score, while associated with moisture ($P = 0.00002$) and color L* ($P = 0.01263$) for negative hap-score. The haplotype GG (32.82%, negative effect) showed significant association with those above traits except for color L* ($P = 0.05162$). The global score *P*-values for IMF, marbling, moisture, color L*, color a*, color b* and color score were <1e-5, <1e-5, 0.00008, 0.04241, 0.00001, 0.70281 and <1e-5, respectively.

Population stratification assessment

The "Q-Q" plots of the ranked Chi-square statistic values of the association tests versus expected values sampled from a Chi-square distribution for all

of the 48,238 SNPs obtained from 454 F2 offspring for IMF, marbling, moisture content, color L*, color a*, color b* and color score are shown in Fig. 2A to 2G. The deflation factors for IMF, marbling, moisture, color L*, color a*, color b* and color score were 1.16, 1.05, 1.08, 1.17, 1.18, 1.07 and 1.33, respectively. The deviation of color score was reduced via GC procedure applied (black line). The deflation factors for other traits were closed to 1 and the lines of the two methods overlapped to a certain extent. These results indicated that by using the GRAMMAR-GC method, the potential population stratification could be reduced to a certain degree.

Table 9. Results of haplotype association analysis of block1.[1]

Trait	Haplotype	Hap-Freq[2]	Hap-score[3]	Haplotype-Specific score *P*-value[4]	Global Score Statistic[5]
IMF	CAGGA	0.37022	-5.18999	<1e-5	χ^2=34.79913
	AAGGA	0.00941	-1.46451	0.14305	df=5
	CGGGA	0.06420	-0.70883	0.47843	*P*-value<1e-5
	CGAAA	0.01788	-0.22010	0.82579	
	CGAAG	0.06092	0.24839	0.80383	
	AGAAG	0.47737	5.50622	<1e-5	
Marbling	CAGGA	0.37022	-5.61951	<1e-5	χ^2=39.70068
	CGGGA	0.06420	-0.99881	0.31789	df=5
	AAGGA	0.00941	-0.66130	0.50842	*P*-value<1e-5
	CGAAG	0.06092	-0.28076	0.77889	
	CGAAA	0.01788	0.16462	0.86925	
	AGAAG	0.47737	6.03922	<1e-5	
Moisture	AGAAG	0.47737	-3.50341	0.00046	χ^2=13.93724
	CGAAA	0.01788	-0.18704	0.85163	df=5
	CGAAG	0.06092	-0.03311	0.97358	*P*-value=0.01601
	AAGGA	0.00941	0.66883	0.50360	
	CGGGA	0.06420	1.54321	0.12278	
	CAGGA	0.37022	2.83508	0.00458	
Color L*	AGAAG	0.47737	-2.22089	0.02636	χ^2=9.87991
	AAGGA	0.00941	-1.48342	0.13796	df=5
	CGGGA	0.06420	-0.20138	0.84041	*P*-value=0.07871
	CGAAA	0.01788	-0.11724	0.90667	
	CGAAG	0.06092	1.72188	0.08509	
	CAGGA	0.37022	1.91811	0.05510	
Color a*	CAGGA	0.37022	-3.36812	0.00076	χ^2=25.29306
	CGGGA	0.06420	-1.75901	0.07858	df=5
	AAGGA	0.00941	-1.47089	0.14132	*P*-value=0.00012
	CGAAG	0.06092	-1.22661	0.21997	
	CGAAA	0.01788	-0.00380	0.99697	
	AGAAG	0.47737	4.90989	<1e-5	
Color b*	CAGGA	0.37022	-0.71172	0.47664	χ^2=1.90519
	AAGGA	0.00941	-0.60538	0.54492	df=5
	CGGGA	0.06420	-0.39088	0.69589	*P*-value=0.86210
	CGAAA	0.01788	-0.21136	0.83261	
	AGAAG	0.47737	0.59505	0.55181	
	CGAAG	0.06092	0.94374	0.34530	

Trait	Haplotype	Hap-Freq[2]	Hap-score[3]	Haplotype-Specific score *P*-value[4]	Global Score Statistic[5]
Color score	CAGGA	0.37022	-4.88339	<1e-5	χ^2=35.55778
	CGAAG	0.06092	-1.53435	0.12494	df=5
	CGGGA	0.06420	-1.28754	0.19791	*P*-value<1e-5
	AAGGA	0.00941	-0.11132	0.91136	
	CGAAA	0.01788	0.65579	0.51196	
	AGAAG	0.47737	5.81331	<1e-5	

[1]Block1: ASGA0100525-ASGA0055225-ALGA0067099-MARC0004712-DIAS0000861

[2]Estimated frequency of each haplotype in the population.

[3]The score for the haplotype, which is the statistical measurement of association of each specific haplotype with the trait. The results are sorted by this value.

[4]The asymptotic chi-square (1 df) *P*-value, calculated from the square of the score statistic.

[5]The overall association between haplotypes and the response.

Table 10. Results of haplotype association analysis of block2.[1]

Trait	Haplotype	Hap-Freq[2]	Hap-score[3]	Haplotype-Specific score *P*-value[4]	Global Score Statistic[5]
IMF	GG	0.32819	-5.79716	<1e-5	χ^2=37.12951
	GA	0.08258	-0.30975	0.75675	df=2
	AA	0.58923	5.90899	<1e-5	*P*-value<1e-5
Marbling	GG	0.32819	-5.93800	<1e-5	χ^2=37.96553
	GA	0.08258	-0.02525	0.97985	df=2
	AA	0.58923	5.91486	<1e-5	*P*-value<1e-5
Moisture	AA	0.58923	-4.23732	0.00002	χ^2=18.83514
	GA	0.08258	0.29517	0.76786	df=2
	GG	0.32819	4.09874	0.00004	*P*-value=0.00008
Color L*	AA	0.58923	-2.49408	0.01263	χ^2=6.32093
	GA	0.08258	1.02990	0.30306	df=2
	GG	0.32819	1.94625	0.05162	*P*-value=0.04241
Color a*	GG	0.32819	-4.41599	0.00001	χ^2=22.87828
	GA	0.08258	-0.61689	0.53731	df=2
	AA	0.58923	4.72064	<1e-5	*P*-value=0.00001
Color b*	GG	0.32819	-0.81572	0.41466	χ^2=0.70534
	GA	0.08258	0.40514	0.68538	df=2
	AA	0.58923	0.58519	0.55842	*P*-value=0.70281
Color score	GG	0.32819	-4.70167	<1e-5	χ^2=27.44276
	GA	0.08258	-1.02013	0.30766	df=2
	AA	0.58923	5.20804	<1e-5	*P*-value<1e-5

[1]Block2: ASGA0085522-H3GA0056170

[2]Estimated frequency of each haplotype in the population

[3]The score for the haplotype, which is the statistical measurement of association of each specific haplotype with the trait. The results are sorted by this value.

[4]The asymptotic chi-square (1 df) *P*-value, calculated from the square of the score statistic.

[5]The overall association between haplotypes and the response.

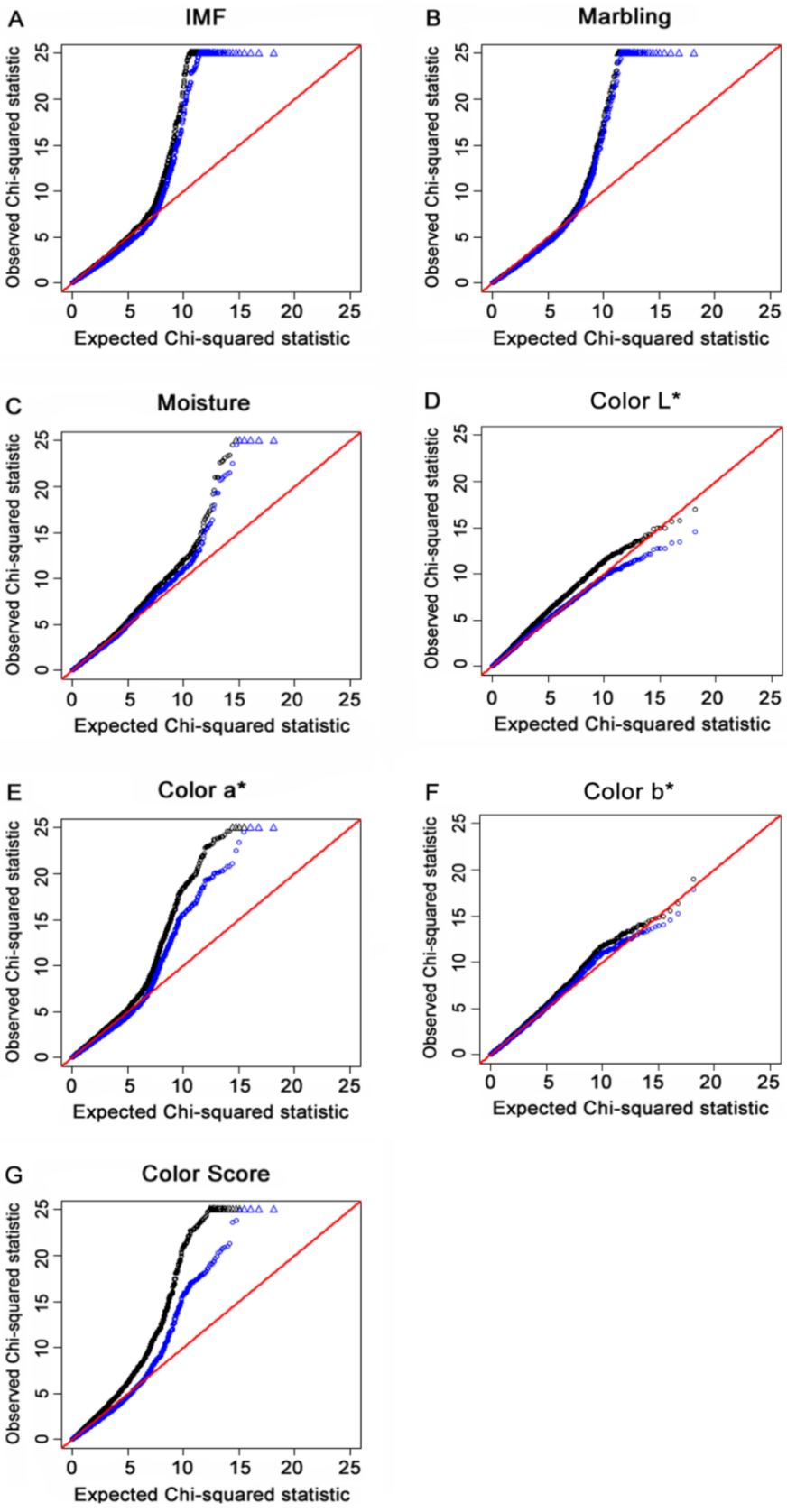

Figure 2. For each of the seven meat quality traits, a quantile-quantile (Q-Q) plot of the results derived without adjustment for the inflation factor (λ) are shown in black. Results derived using the genomic control (GC) procedure are shown in blue. SNPs for which the test statistic exceeds 25 are represented by triangles. A, B, C, D, E, F and G refer to Q-Q plots for IMF, marbling, moisture, color L*, color a*, color b* and color score, respectively. Results indicated that population stratification was reduced to a certain degree by using the GC method.

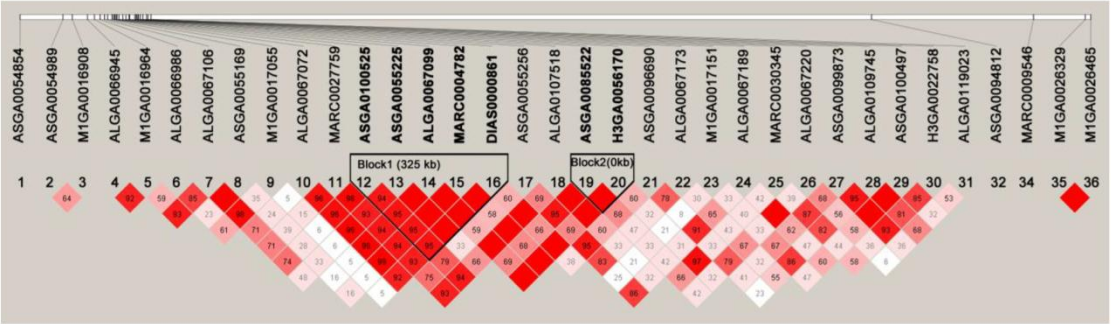

Figure 3. Haplotypes on an 8.3-Mb region on SSC12 containing all the significant SNPs associated with the five meat quality traits obtained with the HAPLOVIEW 3.31 program. Solid lines mark the two blocks identified.

Discussion

Some disease risk genes have been identified using genome-wide association studies (GWAS) in humans [25]. Unlike unrelated populations or small families employed in human studies, a large number of half-sibs and full-sibs can be obtained in livestock. In the current work, GWAS for meat quality traits was performed in a porcine Large White × Minzhu intercross population. As a result of the large number of half and full sibs present in the studied population, the ignorance of pedigree information could lead to an increased false discovery rate [26]. Using the GRAMMAR-GC method in this work, intra- and inter-family variations were considered. Furthermore, phenotypes were adjusted using fixed and random effects, and the population stratification was adjusted. These results indicated that the GRAMMAR-GC method was robust for population stratification and the F2 intercross population was suitable for GWAS in the present experiment.

In this study, most significant SNPs for IMF, marbling, meat color and moisture were located on SSC12 in proximal regions. A QTL for color score has previously been mapped to the region between SWC62 (37.9Mb) and S0106 (43.7Mb) on SSC12 [27]. QTLs for IMF, marbling and moisture have been reported within SSC12 at 95 cM (42-43 Mb), 18.2-93.9 cM (35.4-51.1 Mb), and 64.7-80.2 cM (21.3-35.4 Mb), respectively [28, 29]. The QTLs for color a* have been reported to be on SSC 1, 4, 6, 7, 8, 13, 14, 15, 16 and 17 [28, 30-38]. However, this GWAS revealed novel loci for color a* on SSC12.

In the current study, a haplotype block, ASGA0100525-ASGA0055225-ALGA0067099-MARC0004712-DIAS0000861, was identified within a 325 Kb fragment on SSC12. This region encompasses five annotated genes (*GAS7*, *MYH1*, *MYH2*, *MYH3* and *MYH4*) in the pig genome region. The *MYH1*, *MYH2*, *MYH3* (predicted gene in GenBank) and *MYH4* genes belong to the myosin heavy chain gene family (MYH), which are located on chromosomes 7 and 12 [39-41]. These different isoforms may partially reflect skeletal muscle fiber type diversity. Four adult MYH isoforms are expressed in the skeletal muscle of pigs: types I, IIa, IIx and IIb, which are encoded by the *MYH7*, *MYH2*, *MYH1* and *MYH4* genes, respectively [42]. With the exception of *MYH7* on SSC7, the *MYH1*, *MYH2* and *MYH4* genes were all identified on the region of haplotype block1 on SSC12 in this study. Davoli *et al.* [43] found that a SNP in 3'-UTR of *MYH4* gene was potentially associated with expected breeding value (EBV) for visible intermuscular fat (VIF) in one group of Duroc pigs (P = 0.059). The Glu706Lys mutation in the *MYH2* gene has been reported to be associated with a familial congenital myopathy in humans [44]. The *MYH1*, *MYH2* and *MYH4* are related to muscle development [45]. Fat type (indigenous Chinese pig breeds) and meat type (western commercial pig breeds) pig breeds show obvious differences in muscle development [46]. Furthermore, the fat type pig breeds, such as Meishan and Laiwu pigs, are known to have superior IMF and marbling compared to meat type pig breeds, including Large White and Duroc. Comparing the expression of genes in the two type pigs, *MYH4* was decreased ($P < 0.05$), while *MYH1* ($P < 0.05$) and *MYH2* ($P < 0.05$) were increased in LM of fat type pigs [47, 48]. According to both the expressions and SNPs showing significant association with meat quality traits, they could be used as potentially strong candidate genes.

Besides the genes in this haplotype block, there were eight significant SNPs located within introns of eight annotated genes, *UBE2G1*, *SLC13A5*, *DNAH2*, *NDEL1*, *PIK3R5*, *NTN1*, *USP43* and *GLP2R*. A signifi-

cant SNP (ASGA0094812) within the *USP43* gene was detected for five meat quality traits. The SNP MARC0051399 was located in the *UBE2G1* gene, which is expressed in skeletal muscle [49], and showed a significant association with marbling. The associations of SNPs in *USP43* and *UBE2G1* genes with meat quality traits were not reported in previous studies. For the remaining genes, neither expression in skeletal muscle nor SNPs within genes association with muscle development traits were reported previously.

IMF in pork is considered as a key factor that influences meat quality and associates with marbling, juiciness, tenderness and flavor [50]. Reduction of IMF in pork leads to increased water content. Similar to previous reports [51, 52], a strong negative phenotypic correlation was identified between IMF and moisture. Furthermore, the results from this GWAS revealed the opposite effect of significant SNPs associated with these two traits (data not shown). The strongest correlation coefficient among the measured traits was observed between IMF and marbling. The present GWAS results that 28 SNPs were significantly associated with both traits could support this strong correlation.

In summary, this GWAS demonstrated that 36 of the 45 SNPs that were significantly associated with meat quality traits were located in a region that is approximately 12 Mb in length (43 to 55 Mb) on SSC12. These SNPs were located within previously reported QTLs. These results narrow down the previously detected QTL intervals. Furthermore, the haplotype block containing four MYH gene family members that were significantly associated with meat quality traits proved these QTL effects.

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Competing Interests

The authors have declared that no competing interest exists.

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