

Genome-wide association analysis reveals genetic loci and candidate genes for meat quality traits in Chinese Laiwu pigs

Xinwei Xiong · Xianxian Liu · Lisheng Zhou · Jie Yang · Bin Yang · Huanban Ma · Xianhua Xie · Yixuan Huang · Shaoming Fang · Shijun Xiao · Jun Ren · Congying Chen · Junwu Ma · Lusheng Huang

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Abstract Meat quality traits have economically significant impacts on the pig industry, and can be improved using molecular approaches in pig breeding. Since 1994 when the first genome-wide scan for quantitative trait loci (QTLs) in pig was reported, over the past two decades, numerous QTLs have been identified for meat quality traits by family based linkage analyses. However, little is known about the genetic variants for meat quality traits in Chinese purebred or outbred populations. To unveil it, we performed a genome-wide association study for 10 meat quality traits in Chinese purebred Laiwu pigs. In total, 75 significant SNPs ($P < 1.01 \times 10^{-6}$) and 33 suggestive SNPs ($P < 2.03 \times 10^{-5}$) were identified. On SSC12, a region between 56.22 and 61.49 Mb harbored a cluster of SNPs that were associated with meat color parameters (L^* , lightness; a^* , redness; b^* , yellowness) and moisture content of *longissimus* muscle (LM) and *semimembranosus* muscle at the genome-wide significance level. A region on SSC4 also has pleiotropic effects on moisture content and drip loss of LM. In addition, this study revealed at least five

novel QTLs and several candidate genes including 4-linked *MYH* genes (*MYH1*, *MYH2*, *MYH3*, and *MYH13*), *MAL2*, *LPAR1*, and *PRKAG3* at four significant loci. Except for the SSC12 QTL, other QTLs are likely tissue-specific. These results provide new insights into the genetic basis of meat quality traits in Chinese Laiwu pigs and some significant SNPs reported here could be incorporated into the selection programs involving this breed.

Introduction

Pork is a major meat resource for human. Pork quality has a great influence on consumer preference (Nonneman et al. 2013). So improving pork quality is a high priority in the pork industry (Becker et al. 2013). The major meat quality traits of interest, including pH, drip loss, meat color, moisture, and intramuscular fat contents, are common selection objectives in pig breeding programs (Rosenvold and Andersen 2003; Uimari and Sironen 2014). However, most of these traits show a low to moderate heritability and are difficult and expensive to measure (Suzuki et al. 2005). Their improvement by target breeding programs is challenging and requires knowledge of the genetic and molecular background (Becker et al. 2013).

Since the first genome-wide scan for quantitative trait loci (QTLs) in pigs was reported (Andersson et al. 1994), extensive studies have been conducted to identify QTLs in many experimental crosses. To date, 10,497 QTLs for 649 different traits have been deposited in the Pig QTL database (<http://www.animalgenome.org/cgi-bin/QTLdb/SS/index>). However, most of these QTLs were previously detected by linkage analyses of pig families with low-density microsatellite markers, which lead to lack of

Xinwei Xiong and Xianxian Liu have contributed equally to this work.

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X. Xiong · X. Liu · L. Zhou · J. Yang · B. Yang · H. Ma · X. Xie · Y. Huang · S. Fang · S. Xiao · J. Ren · C. Chen (✉) · J. Ma (✉) · L. Huang
Key Laboratory for Animal Biotechnology of Jiangxi Province and the Ministry of Agriculture of China, Jiangxi Agricultural University, Nanchang 330045, China
e-mail: chcy75@hotmail.com

J. Ma
e-mail: ma_junwu@hotmail.com

precision of their locations (Sanchez et al. 2006; Riquet et al. 2011). As a result, only a handful of genes, including *RYRI*, *PRKAG3*, *IGF2*, *MCR4*, and *PHKG1*, have so far been shown to affect meat quality and production traits in pig (Fujii et al. 1991; Kim et al. 2000; Milan et al. 2000; Van Laere et al. 2003; Ma et al. 2014). Moreover, QTL linkage designs have in most cases based on crosses between divergent populations (Rothschild and Bidanel 2002), resulting in QTL data that are not directly transferable to commercial populations (Sanchez et al. 2014). Compared to linkage mapping method, genome-wide association studies (GWAS) using high-density molecular markers can map QTLs at high resolution and pinpoint the most significantly associated markers for various traits in human and animals. Since the illumina porcine 60 K SNP Beadchip array is available (Ramos et al. 2009), some researchers have recently performed GWAS to detect QTLs in different crossbred and outbred pig populations, particularly in several western commercial pig lines (Ren et al. 2011; Luo et al. 2012; Ma et al. 2013; Ponsuksili et al. 2014; Sanchez et al. 2014; Stratz et al. 2014; Zhang et al. 2014). However, few studies have dissected QTLs for meat quality traits in Chinese indigenous breeds.

The Laiwu breed is the North China type pig representative, with tender meat, bright color, excellent flavor, high water holding capacity, and intramuscular fat content; and it has enjoyed a good reputation for long time. In this work, we carried out a GWAS of meat quality traits which were recorded on two different muscles, *i.e.*, *M. longissimus* (LM) and *M. semimembranosus* (SM) muscles in 316 Laiwu pigs. This study aims to reveal genetic loci associated with meat quality traits and discover appealing candidate genes for follow-up investigations of the underlying genes and variants.

Materials and methods

Ethics statement

All procedures involving animals followed the guidelines for the care and use of experimental animals approved by the State Council of the People's Republic of China. The ethics committee of Jiangxi Agriculture University specially approved this study.

Experimental population

The Laiwu population used in this study comprised 316 offspring produced by 12 boars and 45 sows. They were born and raised for 2–3 months on Laiwu Pig Breeding Center in Laiwu city, Shandong Province. They were then transferred to a farm in Nanchang city in four batches (Sep.

2, Nov. 2, 2012 and Jan. 24, May 10, 2013). All the males were castrated and all piglets were weaned at age of 21 or 22 days. They were fed on a similar diet under a standardized feeding and management regimen, and given free access to water. At the age of 300 days, a total of 316 individuals including 218 barrows and 98 gilts were slaughtered at a commercial abattoir in Nanchang.

Phenotype measurement

The *longissimus* muscle (LM) between the 10th-rib and the first lumbar vertebra and the *semimembranosus* muscle (SM) were evaluated for meat quality traits (Duan et al. 2009; Ma et al. 2009). Here, we analyzed the data of the meat quality traits including pH, drip loss, L^* for lightness, a^* for redness, b^* for yellowness, and moisture content. Muscle pH was measured at 45 min and 24 h postmortem in LM and SM using a Delta 320 pH Meter. Each sample was measured twice and the average of parallel measurements was then used for subsequent analysis. Muscle color measurements were taken on the exposed cut surface of the two muscles with a CM-2600d/2500d Minolta Chroma Meter at 24 h postmortem. Color scores were subjectively assessed according to the color standard (1 = pale; 6 = dark) provided by US National Pork Procedures Council (NPPC) (Berg 2006). Drip loss after 24 h storage of LM and SM was measured using an EZ-DripLoss method (Rasmussen and Andersson 1996). Moisture content was determined by the routine oven-drying method. In this study, 316 Laiwu pigs were phenotyped. We also determined heritability estimates of 15 meat quality traits. Most of the meat quality traits show low to moderate heritabilities. Summary statistics for the 15 traits are in Table 1.

Genotyping and quality control

Genomic DNA was isolated from ear tissue of each sample using a standard phenol/chloroform method. The quality and concentration of DNA were determined by Nanodrop-1000 spectrophotometer (Thermo Fisher, USA). All DNA samples were qualified and standardized into a final concentration of 50 ng/ul.

A total of 316 animals were genotyped for 61,565 SNPs using the illumina porcine 60 K SNP beadchip according to the manufacture's protocol. Quality control was carried out using Plink v1.07 (Purcell et al. 2007). We discarded SNPs with a call rate greater than 90 %, a minor allele frequency (MAF) smaller than 0.01 and a significant deviation from Hardy–Weinberg equilibrium ($P \leq 10^{-5}$). In addition, we removed animals with call rate less than 90 %. After filtering, a final set of 49452 SNPs for 316 Laiwu pigs were included for further statistical analysis. We used

Table 1 Summary statistics of meat quality traits of *longissimus* muscle (LM) and *semimembranosus* muscle (SM) from Chinese Laiwu population

Traits	N	Mean	SD ^a	Min.	Max.	h^2 ^b
pH ^c						
LM_pH45 min	316	6.70	0.20	6.11	7.27	0.08
LM_pH24 h	316	5.91	0.43	4.98	7.02	0.22
SM_pH45 min	316	6.67	0.20	5.99	7.09	0.06
SM_pH24 h	316	6.12	0.36	5.28	6.89	0.30
Drip loss						
LM_DripEZ_24 h (%)	316	0.81	0.69	0.10	3.94	0.06
SM_DripEZ_24 h (%)	316	0.72	0.46	0.13	3.90	0.07
Meat color measures						
LM_ColorM_L24 h	316	47.96	4.62	37.2	64.75	0.2
LM_ColorM_a24 h	316	5.37	1.82	0.07	11.77	0.31
LM_ColorM_b24 h	316	7.92	2.11	2.39	13.28	0.25
LM_ColorScore_24 h (1–6)	316	3.42	0.49	2.00	5.00	0.20
SM_ColorM_L24 h	316	36.87	2.66	30.31	49.25	0.00
SM_ColorM_a24 h	316	6.96	1.44	3.18	11.08	0.20
SM_ColorM_b24 h	316	5.08	2.45	0.16	13.07	0.21
SM_ColorScore_24 h (1-6)	316	4.94	0.59	3.00	6.00	0.02
Moisture content						
LM_MoistureContent (%)	316	66.95	5.90	43.40	87.60	0.52

^a Standard deviation

^b Heritability estimates

^c pH values were measured on the LM and SM at 45 min and 24 h postmortem

BLASTN to determine the positions of the SNPs in the Sscrofa 10.2 genome assembly.

Statistical analyses

The differences in meat quality traits between LM and SM in Laiwu pig were analyzed using the *anov* function of R software. Heritability of meat quality trait was estimated using the *polygenic* function of GenABEL v1.7 (Aulchenko et al. 2007). The GWAS was performed by *polygenic* followed by *mmscore* function of GenABEL v1.7 (Breslow and Clayton 1993; Yu et al. 2006; Bradbury et al. 2007). A generalized linear mixed model was used to estimate associations between SNPs and phenotypic values. Sex and Batch were fitted as fixed effects in the model. The significance thresholds were determined by Bonferroni method, in which the conventional P value was divided by the number of tests performed (Yang et al. 2005). A SNP was considered to have the stringent genome-wide significance at $P < 0.05/N$, and have the suggestive significance at $P < 1/N$, where N stands for the number of SNPs in the data analyses. In this study, the genome-wide and suggestive significance thresholds were $1.01e-06$ ($0.05/49452$) and $2.02e-05$ ($1/49452$), respectively. The phenotypic variance explained by the peak SNP was estimated by $(V_{\text{reduce}} - V_{\text{full}}) / V_{\text{reduce}}$, where V_{full} and V_{reduce} are residual variances of models for association analysis with and without SNP term, respectively.

The problem of population stratification was assessed by examining the distribution of test statistics generated from the thousands of association tests and assessing their deviation from the null distribution (i.e., the distribution expected under the null hypothesis of no SNP associated with the trait) in a quantile–quantile (Q–Q) plot (Pearson and Manolio 2008). In the Q–Q plot, the horizontal axis represents the expected $-\log_{10}P$ and the vertical axis represents the observed $-\log_{10}P$. The diagonal line represents $y = x$, the shaded region shows 95 % confidence interval based on Beta distribution. Overall deviation above the diagonal identity line in the initial stage may suggest a severe population stratification. Deviations from the diagonal line suggest that either the assumed distribution is incorrect or that the sample contains values arising in some other manner, as by a true association (Wellcome Trust Case Control 2007). The Q–Q plot was generated using R software.

Haplotype or linkage disequilibrium (LD) block analyses were performed for the chromosomal regions with multiple significant SNPs clustered around the peak SNP. The LD blocks were determined using Haploview version 4.2 software with default settings (Barrett et al. 2005). A haplotype association study (Druet and Farnir 2011) was also performed to identify genomic regions associated with the LM_MoistureContent, LM_ColorM_L24 h, and LM_ColorM_a24 h. Haplotypes corresponding to a pre-determined number ($K = 20$) of hidden haplotype states

Table 2 Effects of muscle tissues on meat quality traits measured in 316 Laiwu pigs

Traits	LM	SM	Significance
pH45 min	6.70 ± 0.20	6.67 ± 0.20	***
pH24 h	5.91 ± 0.43	6.12 ± 0.36	***
DripEZ_24 h	0.81 ± 0.69	0.72 ± 0.46	***
ColorM_L24 h	47.96 ± 4.62	36.87 ± 2.66	***
ColorM_a24 h	5.37 ± 1.82	6.96 ± 1.44	***
ColorM_b24 h	7.92 ± 2.11	5.08 ± 2.45	***
ColorScore_24 h	3.42 ± 0.49	4.94 ± 0.59	***

LM *Longissimus* muscle

SM *Semimembranosus* muscle

ns not significant

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

(Sartelet et al. 2012) were conducted with a hidden Markov model via PHASEBOOK (Druet and Georges 2010).

Results

Effects of muscle tissues on meat quality traits

Meat quality depends on skeletal muscle structure and metabolic properties (Herault et al. 2014). Although LM and SM are both classified as glycolytic muscles, they exhibit dissimilar myofiber composition and metabolic characteristics. Here, we compared muscle characteristics between LM and SM in the Laiwu pigs. Very significant ($P < 0.001$) differences were found between them in all seven characteristics (Table 2).

Assessment of population stratification

Population stratification is recognized as a major threat to the validity of GWAS results (Pearson and Manolio 2008). The Q–Q plots of test statistics of GWAS are shown in Supplementary Fig. S1. In this study, the average genomic inflation factors (λ) of the GWAS for all meat quality traits were 1.08, which suggests that there were no obvious population stratifications.

GWAS analyses

The GWAS results are illustrated in Fig. 1, Supplementary Fig. S2 and Table 3. A total of 108 SNPs passing the suggestive significance level were identified on six pig chromosomes (SSC) for 10 meat quality traits. Of the 108 SNPs, 75 SNPs and 33 SNPs reached the genome-wide and suggestive significance levels, respectively.

pH and drip loss

For pH and drip loss, a total of six suggestive SNPs were identified, including one on SSC1 for LM_pH45 min, three on SSC15 for LM_pH24 h, one on SSC17 for SM_pH45 min and one on SSC4 for LM_DripEZ_24 h. No significant SNPs were found for pH45 min and DripEZ_24 h of SM.

Meat color and moisture content

We identified 60 and 42 SNPs associated with meat color and moisture content, respectively. Among them, 75 SNPs reached the genome-wide significance level, including 14 for LM_ColorM_a24 h, 14 for LM_ColorM_b24 h, 12 for SM_ColorM_a24 h, and 35 for LM_MoistureContent. On SSC12, the most significant SNP simultaneously associated with LM_ColorM_L24 h ($P = 6.62e-06$), LM_ColorM_a24 h ($P = 1.97e-11$) and LM_MoistureContent ($P = 6.20e-12$) was the SNP *ss131468701* located at 57.95 Mb, which resides only about 100 Kb away from the other two genome-wide significant SNPs *ss131468595* and *ss131468514* for the LM_ColorM_b24 h and SM_ColorM_a24 h, respectively (Fig. 2). These significant SNPs accounted for substantial proportions of phenotypic variances, ranging from 10.8 to 24.8 % (Table 3). Besides SSC12, we also identified associated SNPs on other chromosomes for meat color and moisture content, such as one SNP on SSC18 for LM_ColorM_a24 h, one SNP on SSC4 and one on SSC1 for SM_ColorM_a24 h, one on SSC1 for SM_ColorScore_24 h and eight on SSC4 for LM_MoistureContent. The SNPs on SSC4 for LM_MoistureContent also reached the genome-wide significance level ($P = 3.94e-07$).

Haplotype analyses

We focused our haplotype analysis on the most significant QTL region on SSC12 that spans 500 Kb upstream and downstream of the peak SNP *ss131468701*. The results were illustrated in Fig. 3 and Table 4. We identified two haplotype blocks in which SNPs were in high LD ($r^2 > 0.8$). Block 1 spanned 143 kb and contained five SNPs including the peak SNP *ss131468701*. Block 2 of 13 kb consisted of two SNPs. In the block 1, four distinct haplotypes (ACGAG, AAAGA, GAGGA, and GAAGA, referred to as Hap1, Hap2, Hap3, and Hap4 hereinafter) were identified with frequencies of 22, 18, 15, and 46 %, respectively. Hap2, Hap3, and Hap4 were significantly associated with low moisture content, high L^* and a^* values in LM ($P < 0.05$), while Hap1 displayed opposite effects on these traits (Table 4).

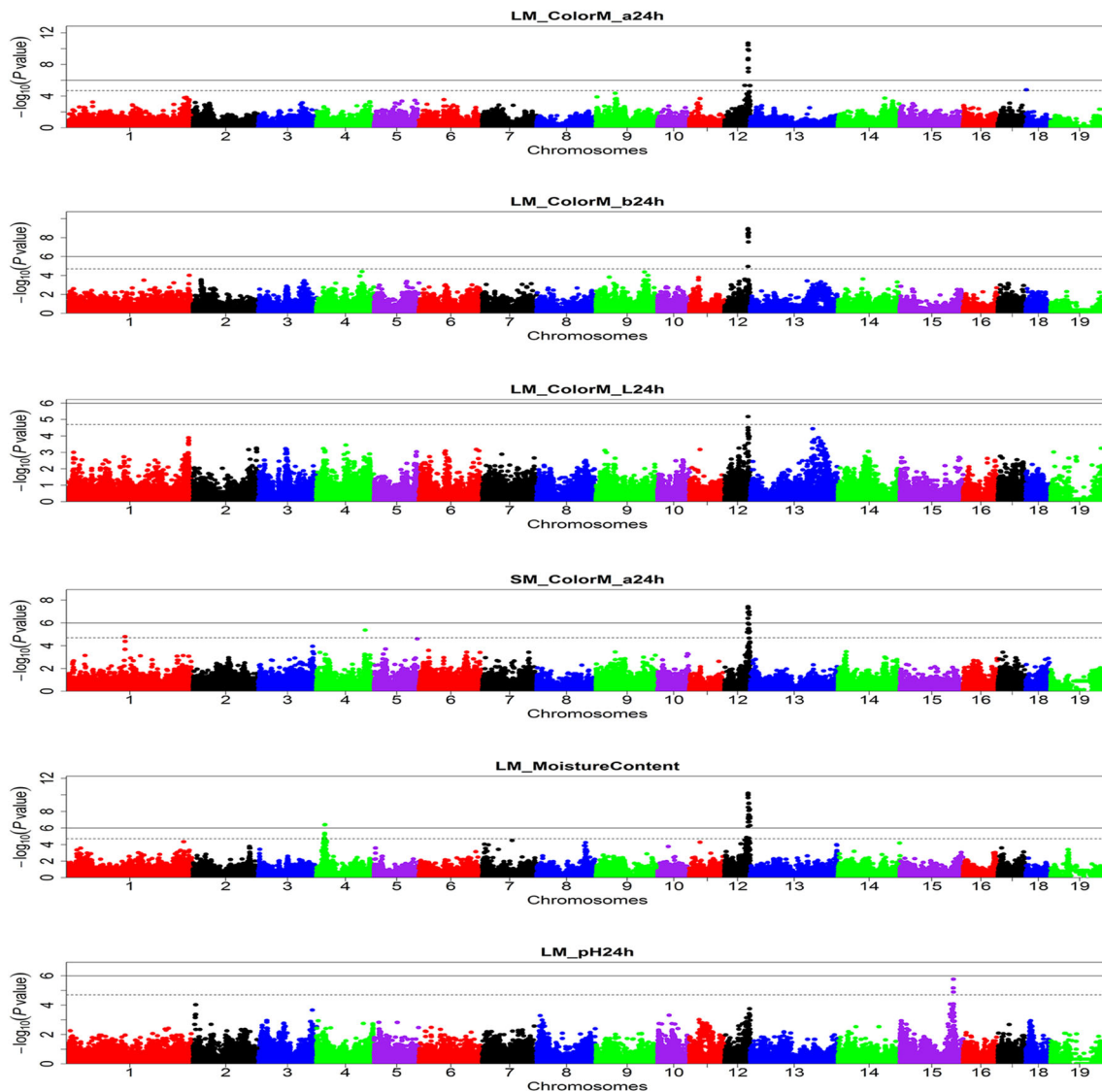


Fig. 1 Manhattans plots of GWAS for meat quality traits. The SNPs on different chromosomes are denoted by different colors. The solid and dashed lines indicate the Bonferroni-corrected thresholds of the genome-wide and suggestive significances, respectively. The most

significant SNP *ss131569519* at 132.80 Mb on SSC15 for LM_pH24 h was close to the *PRKAG3* gene with known effect on this trait

Discussion

Comparison of QTLs identified in this study with those in previous studies

The current GWAS results demonstrated 108 SNPs (24 unmapped) associated with the 10 meat quality traits. Recently, some authors including us have reported GWAS results for meat quality traits in different pig populations. We previously identified 11 significant SNPs and 116 suggestive SNPs associated with 25-related meat quality traits in a White Duroc \times Erhualian F_2 intercross and Chinese Suta pigs (Ma et al. 2013). Most QTLs were located on SSC 2, 3, 4, and X for pH, on SSC 1, 3, 4, 5, and 15 for drip loss, on SSC 2, 4, 5, 6, 7,

10, 12, 13, and 14 for meat color, on SSC 7 and 10 for moisture content. Luo et al. (Luo et al. 2012) also detected a total of 45 significant SNPs associated with one or several meat quality traits in a Large White \times Minzhu F_2 intercross; of these SNPs, 36 were located in a region of approximately 12 Mb (between 43 and 55 Mb) on SSC12. In this study, we confirmed several QTLs (e.g., the SSC12 QTL) that were previously described in those crosses between Chinese and western domestic breeds, which suggests that the alleles at these loci have not fixed in breeds of Chinese or even Western origins. In addition, this study reveals eight novel loci: including *ss131149663* on SSC1 for LM_pH45 min, *ss107838737* on SSC17 for pH24 h, *ss131468701* on SSC12

Table 3 Description of SNPs significantly associated with pH values, drip loss, meat color and moisture content

Traits ^a	Peak SNP ^b	No. ^c	Chr	Pos (bp) ^d	Nearest genes ^e	Freq ^f	Effects ^f	Var (%) ^g	<i>P</i> value ^h
LM_pH45 min	<i>ss131149663</i>	1	1	282,823,080	<i>LPAR1</i>	0.23	0.106	7.0	1.92E-05
LM_pH24 h	<i>ss131569519</i>	3	15	132,803,704	<i>TNSI</i>	0.61	0.110	3.8	1.72E-06
SM_pH24 h	<i>ss107838737</i>	1	17	26,917,006	<i>ENSSSCG00000028750</i>	0.45	0.097	4.8	1.77E-05
LM_DripEZ_24 h	<i>ss131256749</i>	1	4	26,324,825	<i>ENSSSCG00000019239</i>	0.30	0.452	10.7	1.36E-05
LM_ColorM_L24 h	<i>ss131468701</i>	1	12	57,950,908	<i>MYH1</i>	0.75	2.325	12.3	6.62E-06
LM_ColorM_L24 h	<i>ss107905190</i>	1	0	0		0.82	2.891	9.3	1.07E-05
LM_ColorM_a24 h	<i>ss131468706</i>	6	0	0		0.68	0.935	20.6	<u>9.81E-12</u>
LM_ColorM_a24 h	<i>ss131468701</i>	10	12	57,950,908	<i>MYH1</i>	0.75	1.273	24.7	<u>1.97E-11</u>
LM_ColorM_a24 h	<i>ss131465348</i>	1	12	49,872,027	<i>RPA1</i>	0.91	1.462	10.8	4.44E-06
LM_ColorM_a24 h	<i>ss131065155</i>	1	18	528,383	<i>ENSSSCG00000028314</i>	0.98	2.359	8.1	1.58E-05
LM_ColorM_b24 h	<i>ss107812912</i>	6	0	0		0.77	1.328	21.3	<u>8.35E-10</u>
LM_ColorM_b24 h	<i>ss131468595</i>	9	12	57,854,920	<i>MYH13</i>	0.79	1.137	20.1	<u>1.13E-09</u>
SM_ColorM_a24 h	<i>ss131468514</i>	18	12	57,831,831	<i>MYH13</i>	0.77	1.035	14.9	<u>3.68E-08</u>
SM_ColorM_a24 h	<i>ss107812912</i>	4	0	0		0.77	2.069	15.1	<u>4.26E-08</u>
SM_ColorM_a24 h	<i>ss131241209</i>	1	4	120,910,957	<i>LDHB</i>	0.63	0.333	3.7	4.28E-06
SM_ColorM_a24 h	<i>ss131129540</i>	1	1	145,769,945	<i>PIGRAD51</i>	0.01	0.488	1.7	1.60E-05
SM_ColorScore_24 h	<i>ss131179086</i>	1	1	109,082,834	<i>MYO5B</i>	0.92	0.320	6.5	9.64E-06
LM_MoistureContent	<i>ss107905190</i>	7	0	0		0.18	5.712	21.6	<u>1.45E-11</u>
LM_MoistureContent	<i>ss131468701</i>	27	12	57,950,908	<i>MYH1</i>	0.25	3.747	24.8	<u>6.20E-11</u>
LM_MoistureContent	<i>ss131255131</i>	8	4	20,597,887	<i>MAL2</i>	0.93	6.995	2.1	<u>3.94E-07</u>

^a Description of the traits is available in Table 1

^b Chromosomal locations of the most significant SNPs

^c The number of SNP that surpassed the suggestive significance level with the QTL regions

^d Positions of the most significant SNPs according to the Sus Scrofa Build 10.2 assembly

^e Annotated genes nearest to the most significant SNP

^f The frequencies and effects of the advantageous allele that increases phenotype value in the population

^g Phenotypic variance explained by the peak SNP

^h Genome-wide significant associations are underlined

for LM_ColorM_L24 h, *ss131065155* on SSC18 for LM_ColorM_a24 h, *ss131468595* on SSC12 for LM_ColorM_b24 h, *ss131241209* on SSC4, and *ss131129540* on SSC1 for SM_ColorM_a24 h, *ss131179086* on SSC1 for SM_ColorScore_24 h.

Comparison of trait-associated SNPs between LM and SM

Most scientific studies of meat focus on the LM for fresh meat consumption, while SM is also of interest because of its significance for cooked ham production. Table 2 illustrates the marked differences in the tested phenotype between LM and SM in the Laiwu pigs. As for genetic loci, except for the SSC12 QTL affecting meat color, no common QTL for a trait were shared by LM and SM, which was consistent with other studies. Biological mechanisms underlying the discrepancy have not

been elucidated yet. Interestingly, remarkable differences have been observed when comparing the mRNA expression profiles of different pig muscles (Gallardo et al. 2012; Herault et al. 2014), and the differentially expressed genes mainly related to myogenesis process suggests dissimilar post-natal myogenic activity between the two muscles. Furthermore, from the applied perspective, differences in loci controlling meat quality between the two muscles are a relevant aspect to be taken into account when implementing genomic selection schemes devoted to improving these traits (Herault et al. 2014).

Possible pleiotropic QTLs

The present GWAS results showed two regions on SSC4 and SSC12 that were associated with more than one trait. The region between 56.22 and 61.49 Mb on SSC12 harbored a number of

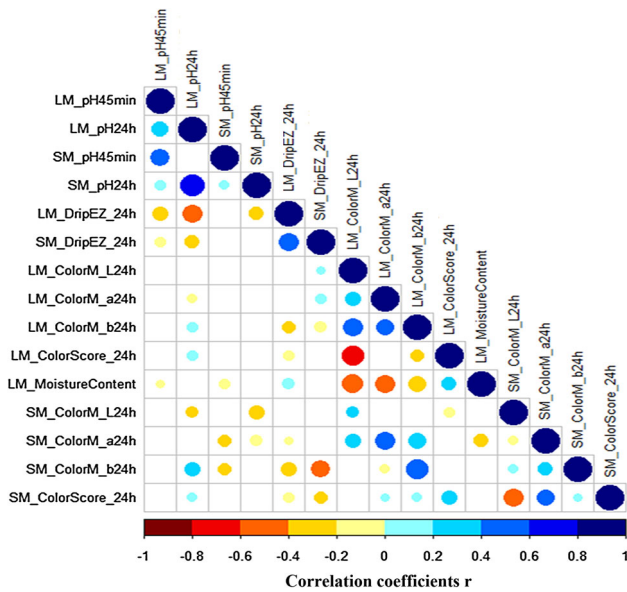


Fig. 2 Magnitudes of correlations between the fifteen meat quality traits. The dots indicate the significant ($P < 0.05$) correlation coefficients between each pair of traits. Their sizes and colors, respectively, represent the degree and direction (positive and negative) of the correlation coefficient (Color figure online)

SNPs affecting five traits, including LM_ColorM_a24 h, LM_ColorM_b24 h, LM_ColorM_L24 h, LM_MoistureContent, and SM_ColorM_a24 h, which supports the findings of Luo et al. (2012) and Lee et al. (2012). The haplotypes in the QTL region also showed significant effects on the multiple traits. Since the correlation coefficients among these traits were significant ($P < 0.05$; Fig. 2), it is very likely that they were influenced by a common causative variant in the SSC12 region.

In addition, a region of 6.86 Mb (between 19.36 and 26.32 Mb) on SSC4 was found to be associated with both LM_DripEZ_24 h and LM_MoistureContent. Thus they may be controlled by the same causative gene on the SSC4 region.

Plausible candidate genes at the identified loci

Based on the GWAS results and information about gene function and expression pattern in muscle tissue, we recognized some interesting candidate genes nearby the association signals. It is known that skeletal muscle is a heterogeneous tissue composed of myofibers, adipose, connective, and vascular and nervous tissues (Herault et al. 2014). The structure and function diversity of contraction protein and metabolic protein cause the different skeletal muscle fiber type. Therefore, within the SSC12 QTL region around 57.95 Mb, the *MYH1*, *MYH2*, *MYH3*, and *MYH13* genes, which belong to the myosin heavy chain gene family (*MYH*) and express highly in skeletal muscle, can be regarded as prime candidates. A targeted null mutation in *MYH1* gene exhibit reduced growth, muscular weakness, kyphosis, and abnormal kinetics of muscle contraction and relaxation in mice (Leinwand et al. 1983; Skarnes et al. 2011). The Glu706Lys mutation in the *MYH2* gene has been reported to be associated with a family congenital myopathy in human (Oldfors et al. 2004). For *MYH3* gene, a structure–function analysis shows that nearly all of the *MYH3* mutations are predicted to interfere with myosin’s catalytic activity and the mutations have been associated with two congenital contracture (arthrogryposis) syndromes, Freeman-Sheldon syndrome and Sheldon-Hall syndrome (Toydemir et al. 2006). So the four members of *MYH* gene family are all related to muscle development (Fan et al. 2011). The *MAL2* gene is located close to the most significant SNPs *ss131255131* for LM_MoistureContent and *ss131256749* for LM_DripEZ_24 h on SSC4. This gene encodes a multispan transmembrane protein, which belongs to the *MAL* proteolipid family. This protein is a component of lipid rafts in polarized cells, and it primarily localizes to endosomal structures beneath the apical membrane (de Marco et al. 2002).

Three QTLs have been found for pH traits. The *LPARI* gene on SSC1 is likely to be responsible for LM_pH45 min. The integral membrane protein encoded by this gene is a lysophosphatidic acid (LPA) receptor from a

Table 4 Haplotype effects of the SSC12 QTL on LM_MoistureContent, LM_ColorM_L24 h and LM_ColorM_a24 h

Haplotype ^a	Frequencies	LM_Moisture Content		LM_ColorM_L24 h		LM_ColorM_a24 h	
		Mean ± SD	<i>P</i> value	Mean ± SD	<i>P</i> value	Mean ± SD	<i>P</i> value
Hap1: ACGAG	0.22	0.71 ± 0.03	ns	46.10 ± 4.36	ns	2.19 ± 0.48	ns
Hap2: AAAGA	0.18	0.64 ± 0.06	***	49.14 ± 4.58	***	5.98 ± 1.19	***
Hap3: GAGGA	0.15	0.66 ± 0.06	***	47.97 ± 4.30	*	5.78 ± 1.48	**
Hap4: GAAGA	0.46	0.66 ± 0.06	***	49.13 ± 4.48	***	5.82 ± 1.61	**

ns not significant

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

^a The haplotypes correspond to haplotype block 1 shown in Fig. 3

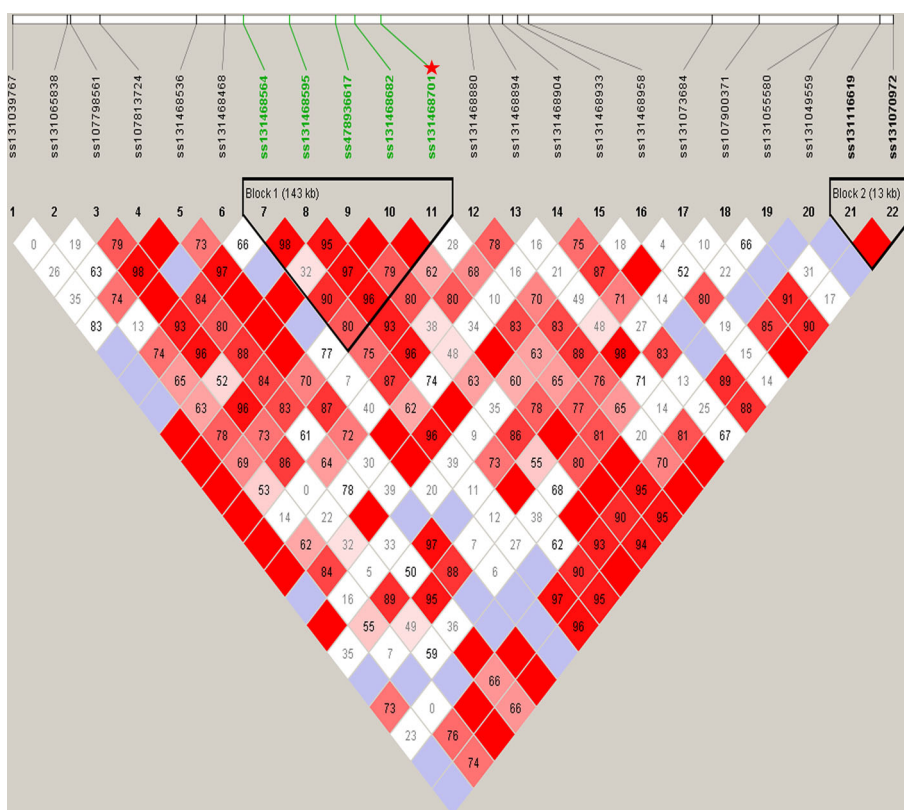


Fig. 3 Haplotype structure of a 1-Mb region on SSC12 containing genome-wide significant SNPs associated with the LM_MoistureContent, LM_ColorM_L24 h and LM_ColorM_a24 h. *Solid lines* mark the identified block

group known as EDG receptors. These receptors are members of the G protein-coupled receptor superfamily. Utilized by LPA for cell signaling, EDG receptors mediate diverse biologic functions, including proliferation, platelet aggregation, and smooth muscle contraction (Moolenaar et al. 1997; Aziziyeh et al. 2009). The most significant SNP *ss131569519* for LM_pH24 h ($P = 1.72e-06$) was detected at 132.80 Mb on SSC15. This QTL overlapped with a known QTL for meat pH values, glycogen content, glycolytic potential, reflectance, tenderness, and flavor score in Western commercial pig breeds, which has been ascribed to different structural or regulatory mutations in *PRKAG3* gene (at 133.80 Mb) (Ciobanu et al. 2001; Otto et al. 2007; Ryan et al. 2012). No obvious candidate genes were found for LM_pH24 h on SSC17.

Conclusions

This GWAS revealed 75 genome-wide significant SNPs and 33 suggestively significant SNPs for 10 meat quality traits in Chinese purebred Laiwu pigs. We not only confirmed several previously reported QTLs but also presented some novel QTLs. Two QTL regions on SSC12 and SSC4 were found to affect more than one trait. A haplotype block

of 143 Kb on SSC12 with five SNPs was identified and displayed four distinct haplotypes with significant association with moisture content and meat color. Several promising candidate genes close to significant SNPs were suggested to underlie the QTL effects. Our findings provide the first insights into the genetic basis of meat quality traits in Chinese indigenous pigs and pave the sound road to identification of causative variants, especially for the major loci on SSC12 and SSC4.

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Conflict of interest The authors declare that they have no conflict of interest.

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