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Potential of eight mutations for marker-assisted breeding in Chinese Lulai black pigs

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Abstract

Molecular marker-assisted selection (MAS) provides an efficient tool for pig breeding. In this study, according to the literature, we selected eight effective or causal mutations from eight functional genes, including five causal mutations in *PHKG1* (*rs330928088*), *MUC13* (*rs319699771*), *IGF2* (*g.3072G>A*), *VRTN* (*g.20311_20312ins291*), and *MYH3* (*XM_013981330.2:g.-1805_-1810del*) genes, and three effective mutations in *LIPE* (*rs328830166*), *LEPR* (*rs45435518*), and *MC4R* (*rs81219178*) genes, to investigate their potential breeding effect in 418 Lulai pigs. The linear model was used to analyse the association between mutations and intramuscular fat (IMF) content, average backfat thickness, and muscle moisture %. The results revealed that amongst the three effective mutations, only the mutation in the *LEPR* gene, which affects IMF deposition, was significantly associated with IMF content. However, the other molecular markers were not significantly associated with the affected traits reported in previous studies, and these mutations are ineffective for MAS in the Lulai black pig population. Therefore, causal mutations in *PHKG1*, *IGF2*, and *VRTN* genes, and an effective mutation in *LEPR* gene could be used as effective breeding makers for MAS in Lulai pigs. These results can provide helpful information for further breeding in Lulai black pigs.

Key words: Lulai black pigs, effective mutations, causal mutations, marker-assisted selection

Résumé

La sélection assistée par marqueur moléculaire (MAS — « molecular marker-assisted selection ») offre un outil efficace pour la reproduction des porcs. Dans cette étude, selon la littérature, nous avons choisi huit mutations efficaces et causales provenant de huit gènes fonctionnels, incluant cinq mutations causales dans les gènes *PHKG1* (*rs330928088*), *MUC13* (*rs319699771*), *IGF2* (*g.3072G>A*), *VRTN* (*g.20311_20312ins291*) et *MYH3* (*XM_013981330.2:g.-1805_-1810del*), et trois mutations efficaces dans les gènes *LIPE* (*rs328830166*), *LEPR* (*rs45435518*) et *MC4R* (*rs81219178*), afin d'étudier les effets potentiels de celles-ci sur la reproduction de 418 porcs Lulai. Le modèle linéaire a été utilisé pour analyser l'association entre les mutations et la teneur en gras intramusculaire (IMF — « intramuscular fat »), l'épaisseur moyenne du gras dorsal (ABT — « average backfat thickness »), et le pourcentage d'humidité dans le muscle (MMP — « muscle moisture percent »). Les résultats ont révélé que parmi les trois mutations efficaces, seule la mutation dans le gène *LEPR*, qui a un effet sur le dépôt d'IMF, était associée de façon significative à la teneur en IMF. Par contre, les autres marqueurs moléculaires n'étaient pas associés de façon significative avec les caractéristiques affectées rapportées dans les études préalables, et ces mutations sont non efficaces pour la MAS dans la population de porcs noirs Lulai. Donc, les mutations causales dans les gènes *PHKG1*, *IGF2* et *VRTN*, et une mutation efficace dans le gène *LEPR* pourraient être utilisées comme marqueurs efficaces de reproduction pour la MAS chez les porcs Lulai. Ces résultats pourraient offrir de l'information utile pour la reproduction ultérieure chez les porcs noirs Lulai. [Traduit par la Rédaction]

Mots-clés : porcs noirs Lulai, mutations efficaces, mutations causales, sélection assistée par marqueur moléculaire

Introduction

Breeders in China have devoted themselves to the genetic improvement of pigs for a long time and have achieved remarkable achievement through steadily advancing in the last decade (Palombo et al. 2021). However, the improvement of breeding efficiency and accuracy is still a challenge. With the development of molecular marker techniques, marker-assisted selection (MAS) has become a rapid and simple method for molecular breeding (Chen et al. 2019; Yang et al. 2020). It uses the linkage between phenotypes of a certain trait and molecular markers in the genomic. By selecting target genotype, individuals with target traits were quickly and accurately selected. It is regarded as one of the effective means of improving breeding efficiency.

In pigs, some causal genes or tightly linked genes affecting crucial traits and used in practical production have been

reported in previous studies, such as *PHKG1* (*rs330928088*) (Ma et al. 2014), *MUC13* (*rs319699771*) (Zhang et al. 2008), *IGF2* (*g.3072G>A*) (Burgos et al. 2012), *VRTN* (*g.20311_20312ins291*) (Ren et al. 2012*a*; Fan et al. 2013), *MYH3* (*XM_013981330.2:g.-1805_-1810del*) (Cho et al. 2019), *LIPE* (*rs328830166*) (Zhao et al. 2009), *LEPR* (*rs45435518*) (Ros-Freixedes et al. 2016), and *MC4R* (*rs81219178*) (Schroyen et al. 2015). The mutations in these genes could be used as markers for MAS to accelerate the breeding progress in pigs.

Amongst the genes mentioned above, *PHKG1* is a vital candidate gene affecting pork water-holding capacity, pH value, pork colour, and glycolysis (Zappaterra et al. 2019). C to A substitution at position 8283 in intron 9 of *PHKG1* gene will lead to the decrease of the rate of glycogenolysis in muscle tissue. However, excessive glycogen could induce the accumulation of lactate, which leads to the decrease of final pH value and the increase of drip loss (Ma et al. 2014; Florowski et al. 2017; Liu et al. 2019*b*). *MUC13* is a major gene that affects diarrhoea in neonatal and young pigs. The mutation *rs319699771* in *MUC13* can influence the adhesion of enterotoxigenic *Escherichia coli* f4ac to the small intestinal villous of pigs. Amongst the genotypes, individuals with GG genotype are resistant ones, and individuals with AG and AA genotypes are susceptible ones (Ren et al. 2012*b*; Shi et al. 2017; Liu et al. 2019*a*). *IGF2* is a causal gene affecting the lean meat rate in pigs. Base change of G to A in intron 3 of *IGF2* gene can increase muscle production and reduce backfat (BF) deposition (Van Laere et al. 2003). The indel *g.20311_20312ins291* in *VRTN* gene is the causative mutation affecting the thoracic vertebrae number in pigs, and individuals with this mutation have more than one thoracic vertebra than the wild type (Fan et al. 2013; Yang et al. 2016). *MYH3* gene encodes the embryonic heavy chain myosin, which is mainly used to control the traction and slide of muscles (Knight and Molloy 2000; Maheshwari et al. 2017; Cope et al. 2020). Recently, Cho et al. (2019) reported that the causal mutation XM 013981330.2:g.-1805-1810del, which is a 6 bp deletion variant in the promoter region of *MYH3* gene, could affect intramuscular fat (IMF) content and red flesh colour (a$*$) in pigs. The protein encoded by the hormone-sensitive lipase (*LIPE*) gene is one of the lipolytic enzyme. The gene plays a crucial role in controlling lipid deposition (Lampidonis et al. 2011; Piórkowska et al. 2018; Al-Thuwaini et al. 2020). Several studies have suggested that the mutation (*rs328830166*) of *LIPE* is significantly correlated with IMF content in the crossbred pigs (Duroc $\times$ Shanzhu pigs), and the IMF content of individuals with allele A was higher (Xue et al. 2015). *LEPR* gene encoding proteins binding to leptin protein could regulate several physiological processes including body energy balance and fat metabolism (Zhang et al. 2016). Individuals with the TT genotypes of *rs45435518* in *LEPR* have more saturated fatty acids (SFA) than ones with wild type. In addition, genome-wide association studies have confirmed that *LEPR* gene is one of the major loci that affect IMF content and fatty acid composition in Duroc (Rodriguez et al. 2010; Galve et al. 2012; Ros-Freixedes et al. 2016). *MC4R* gene is one of the candidate genes affecting growth, BF thickness, and meat quality. When a G/A substitution at position 298 of *MC4R* gene occurs, it will significantly increase BF thickness, carcass weight, moisture content, and the content of SFA (Fontanesi et al. 2013; Hirose et al. 2014; Choi et al. 2016).

China is rich in pig species resources and has many local pigs. Most local pigs have high fecundity and the characteristics of strong stress resistance, and especially the meat quality is significantly better than foreign pig breeds (Cheng-yi et al. 2003). Lulai black pig is a new variety with the comprehensive advantages of local pigs and foreign pigs from the cross between Laiwu pig (representative of North China black pig) and Yorkshire pig after six generations of breeding (Chen et al. 2017; Wang et al. 2019). Known for its high IMF content, it is the best animal model to study fat deposition and animal breeding. It was released by the National livestock and Poultry Genetic Resources Management Committee in 2006. And it was announced by the Ministry of Agriculture of the People's Republic of China and issued the certificate as a new variety of Lulai black pig. In this study, Lulai black pigs were used to analyse the frequency distribution of the abovementioned eight effective or causal mutation sites in the abovementioned eight genes by restricted fragment length polymorphism (RFLP) analysis or sequencing. Moreover, we investigated the correlation between the effective mutation sites and the meat quality traits in the Lulai black pig population. The aim of the present study was to provide a theoretical basis for Lulai black pig breeding about the disease resistance, meat quality, and growth performance. In addition, the insights provided are valuable for the application and improvement of MAS in pig breeding.

Materials and methods

Ethics statement

All the methods during slaughter were carried out following the national standards (GB/T 17236-2019) from the State Administration for Market Regulation of the People's Republic of China. The Ethical Committee approved all procedures of animal handling of Qingdao Agricultural University.

Animals

In this study, 418 Lulai black pigs, including 267 males and 151 females, were used. All pigs were fed in the breeding pig farm of Jinan Laiwu Pig Breeding Co., Ltd. Ear tissues of each pig were collected in tubes containing 75% ethanol and stored at -20 °C for DNA extraction.

Phenotypic determination and genomic DNA extraction

Lulai black pigs were slaughtered when their body weight (BW) reached approximately 90 kg. Pigs were fasted for 24 h before slaughter, then BW was measured. After slaughtering, the BF thicknesses of the dorsal line shoulder, the BF thicknesses of the thoracolumbar junction, and the BF thicknesses of the last lumbar vertebrae were measured using Vernier callipers. The average of three values was regarded as the result. The longissimus dorsi (LD) muscle tissues at the last rib were collected to measure IMF content and muscle moisture % (MMP) of LD. Before the measurements, the peripheral fascia was separated. Then, the LD was minced using a

meat grinder and oven-dried at 65 °C to a constant weight in a dryer. Finally, the moisture content was calculated by dividing the weight difference before and after mass by fresh weight. The IMF content was analysed using the Soxhlet extraction method (Wang et al. 2019).

Collected ear tissues were used to isolate genomic DNA using TIANamp Genomic DNA Kit from TIAGEN Biotech (Beijing, China) following the manufacturer's instructions. DNA quality and concentrations were checked using SpectraMax QuickDrop Micro-Volume Spectrophotometer (Molecular Device, USA). The DNA samples with an A260/A280 ratio between 1.8 and 2.0 and an A260/A230 ratio greater than 2.0 were qualified DNA. The DNA concentration in multiple samples was diluted to a range of 20−50 ng/μL by ddH_2O and the samples were used for a subsequent test.

Primer design and PCR (Polymerase Chain Reaction) amplification

The primers containing *IGF2* (*g.3072G>A*), *VRTN* (*g.20311_20312ins291*), and *MYH3* (*XM_013981330.2:g.-1805_-1810del*) mutation sites were designed using Premier 5.0 based on the *IGF2*, *MYH3*, and *VRTN* sequence of GenBank. The primers are described in Table 1. All primers were synthesized by the TSINGKE Company (Qingdao, China).

The multiplex PCR reaction of *MYH3* and *VRTN*, performed in a 25 μL total volume, contained 12.5 μL of Dream Taq™ Green PCR Master Mix (2×), 8.5 μL of ddH_2O, 1 μL of Primer F and Primer R, and 2 μL of genomic DNA. The PCR programme was as follows: 94 °C for 5 min, 35 cycles (94 °C for 30 s, 61.5 °C for 30 s, and 72 °C for 1 min), 72 °C for 10 min, and hold at 4 °C. The multiplex PCR reaction of IGF2, performed in a 25 μL total volume, contained 0.25 μL of Ex Taq DNA Polymerase (5 U/μL), 12.5 μL of 2× GC Reaction Buffer, 5 μL of GC Enhancer Buffer (5 mol/L), 1 μL of dNTP Mix (10 mmol/L each), 1 μL of Primer F and Primer R, 2.25 μL of RNase free water, and 2 μL of genomic DNA. The PCR programme was as follows: 94 °C for 1 min, 35 cycles (98 °C for 10 s, 63.2 °C for 30 s, and 72 °C for 30 min), 72 °C for 5 min, and hold at 4 °C. PCR products obtained were electrophoresed on 1.5% agarose gel, and the gel running conditions were 180 V for 35 min. PCR products with bright and single bands were used for subsequent tests.

Genotyping

The PCR products of *IGF2* were sent to TSINGKE Company (Qingdao, China) for Sanger sequencing. The sequencing results were analysed by using DNAMAN 8.0.8 (Lynnon Corporation, Canada) and Chromas 2.6.5 (Technelysium Pty, Ltd., Ireland) to identify mutations. The *VRTN* (*g.20311_20312ins291*) genotypes results were determined by electropherograms. The mutant (QQ) genotype generates a band of 411 bp, whereas the wild type (qq) genotypes a band of 120 bp and two bands for heterozygous (Qq) genotype (Fig. 1). Genotyping of the *MYH3* gene was performed by PCR–RFLP. The multiplex PCR reaction of HpyCH4IV in a 25 μL total volume contained 10 μL of PCR product, 0.5 μL of HpyCH4IV, 2.5 μL of 10× NEBuffer, and 12 μL of ddH_2O. The reaction was incubated for 15 min at 37 °C. The genotypes were determined by electrophoresis on a 3% agarose gel (one band: 143 bp for wild type (qq) genotype; two bands: 91 and 46 bp for the mutant (QQ) genotype; and three bands: 143, 91, and 46 bp for heterozygous (Qq) genotype) (Fig. 2). The remaining five sites were genotyped by Tianhao Biotechnology Company (Shanghai, China) through SNaPshot, including *PHKG1* (*rs330928088*), *MUC13* (*rs319699771*), *LIPE* (*rs328830166*), *LEPR* (*rs45435518*), and *MC4R* (*rs81219178*).

Statistical analyses

The total number of animals analysed here was 418. Allelic and genotypic frequencies were calculated by genetic equilibrium law. Polymorphic information content (PIC), observed heterozygosity (HO), and expected heterozygosity (HE) were computed by using $H_0 = \sum_{i=1}^{k} P_i^2$, $H_e = 1 - \sum_{i=1}^{k} P_i^2$, and $\text{PIC} = 1 - \sum_{i=1}^{k} P_i^2 - \sum_{i=1}^{k} P_i^2 \sum_{j=i+1}^{k} 2P_i^2 P_j^2$, where P_i and P_j are the frequencies of the *i*-th and *j*-th alleles, respectively, and *k* is the number of alleles. A PIC value from 0 to 0.25 indicates low polymorphism, a PIC value from 0.25 to 0.5 indicates intermediate polymorphism, and a PIC value from 0.5 to 1 indicates high polymorphism.

Normality distribution test was performed on the data using SPSS software (Version 26). Correlation function within the R package "Performance Analytics" was used to calculate the correlation between IMF, MMP, and average backfat thickness (ABT) and to generate the correlation scatter plot. And the sex and weight were adjusted when calculating the phenotypic correlation. Association analyses were done using the linear model in PLINK software (Purcell et al. 2007). Sex and weight were included as fixed effects. The full model is $y = \alpha \text{SNP} + xb + e$. y denotes the phenotypic values. α is the marker effects for each SNP. x is design matrices for the fixed effects, and b is the sex and weight fixed-effect vectors. e is the residual error. Excel was used to calculate the FDR (False Positive Rate) value of the statistical results, so as to perform multiple tests on the p value. In general, FDR = Q value = adjusted p value. The calculation formula is Q value = $p \times (m/k)$, where m is the number of tests and k is the ranking of p value of this test amongst all tests. The individuals with significant differences were analysed by ANOVA (Analysis of Variance) using the SPSS (Version 26).

Results

Phenotypic measurement results of Lulai black pig and descriptive statistics

The results of measurements for traits of 418 Lulai black pigs are shown in Table 2. The means of ABT and MMP of the Lulai black pig population were 38.50 ± 5.66 mm and 71.15 ± 2.83%, respectively, and the means of overall IMF content were 5.02 ± 3.10%. MMP had the smallest coefficient of variation (CV; 3.98%), and IMF was the most remarkable (61.94%) in the population. That suggested that interindividual variation in IMF content was wide.

Table 1. Primer sequences and annealing temperature.

Prime	Sequence of the primer (5′–3′)	Length of the fragment (bp)	Annealing temperature (°C)
VRTN-F	GGCAGGGAAGGTGTTTGTTA	411	61.5
VRTN-R	GACTGGCCTCTGTCCCTTG		
IGF2-F	ACTGTTGAAGTCCCCGAGAG	283	63.2
IGF2-R	GAAGGGAGGAAGCCGAGAG		
MYH3-F	GGGCTACCTCCCTCTCA	143	61
MYH3-R	GTTGTGGCAGGAATGTGT		

Fig. 1. The electrophoretogram of the PCR amplification of *VRTN* gene.

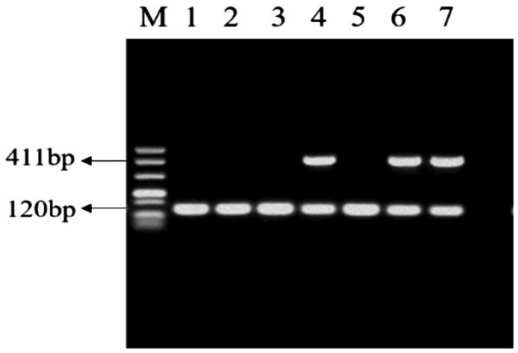


Fig. 2. The electrophoretogram of the PCR amplification of *MYH3* gene.

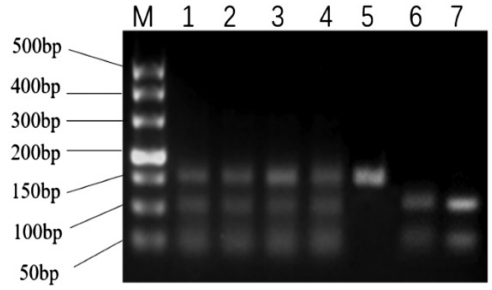


Table 2. Statistical description of carcass and meat quality traits in Lulai black pig population.

Traits	*N*	Min	Max	Mean ± SD	CV
ABT (mm)	418	21.15	61.39	38.50 ± 5.66	17.81%
MMP (%)	418	54.10	75.79	71.15 ± 2.83	3.98%
IMF (%)	418	1.02	18.78	5.02 ± 3.10	61.94%

Note: Mean ± SD represents the means with standard deviations for diverse genotypes; Max refers to the maximum value of the phenotype; Min refers to the minimum value of the phenotype; CV = coefficient of variation.

Phenotype correlation analysis of Lulai black pigs

The degree of phenotypic correlation amongst traits of Lulai black pigs is shown in Figs. 3 and 4. Q–Q plots are approximately linear. And tests for normality indicated that three traits followed an approximately normal distribution. The correlation was strongest for IMF and MMP, showing a negative correlation, with a correlation coefficient of −0.87. However, there is a weak correlation between IMF and ABT, with a correlation coefficient of 0.22. As the ABT and MMP changed, the results revealed that a corresponding increase/decrease was seen in IMF content.

Genetic analysis of effective/causal mutations of Lulai black pigs

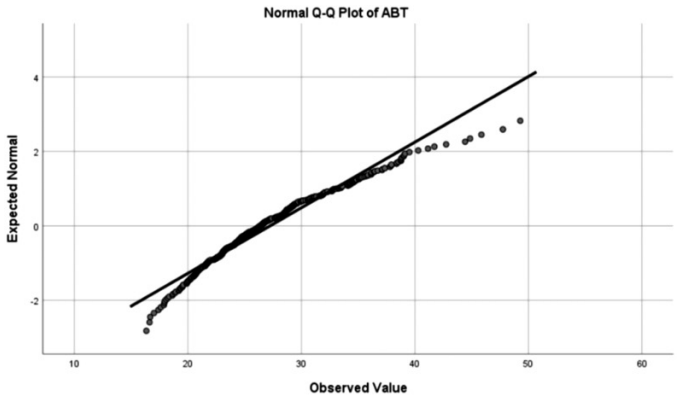
The allele frequency, genotype frequency, and genetic diversity parameters (HO, HE, and PIC) of the effective/causal sites in the *PHKG1*, *VRTN*, *MYH3*, *LIPE*, and other genes are summarized in Table 3. The frequencies of favourable alleles were higher in mutation sites of causal genes *PHKG1*, *VRTN*, and *MYH3*, means 0.927 (C), 0.854 (q), and 0.523 (Q), respectively. And the population lacks individuals with homozygous genotype from *VRTN* unfavourable alleles. The frequencies of favourable alleles were lower in *MUC13* and *IGF2*, means 0.394 (G) and 0.075 (A), respectively. The favourable homozygous genotype of the *IGF2* gene was only 5 cases in 418 Lulai black pigs. For other candidate genes, the frequencies of favourable alleles were higher in *LIPE*, mean 0.737 (A); the frequencies of favourable alleles were lower in *LEPR* and *MC4R*, means 0.275 (T) and 0.122 (A), respectively. The favourable homozygous genotype of the *MC4R* gene was only one case.

In the Lulai black pig population, the homozygosity of all effective/causal mutation sites was higher than the heterozygosity. The effective/causal mutations of *MUC13*, *MYH3*, *LIPE*, and *LEPR* genes were moderately polymorphic, with 0.364, 0.375, 0.313, and 0.319 PIC values. The rest of the sites belonged to a low polymorphic locus ($0 < PIC < 0.25$). The values of HE and PIC of the *PHKG1*, *IGF2*, and *MC4R* were low due to the small number of homozygous mutants.

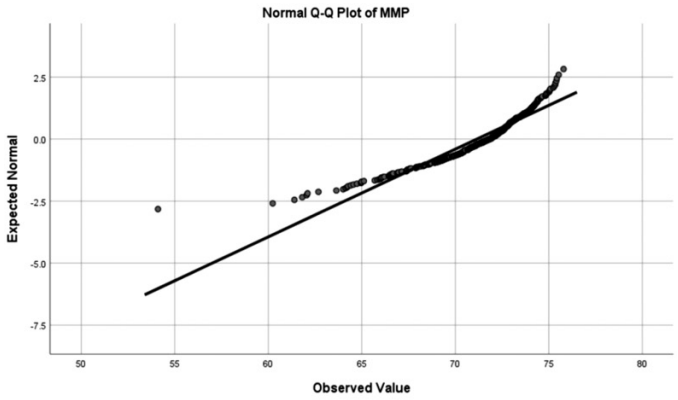
Association analysis between Lulai black pigs' effective mutation sites and ABT, MMP, and IMF

In the population, we performed association analysis between effective mutation sites and ABT, MMP, and IMF. Association analysis results of effective mutation sites *LIPE* (*rs328830166*), *LEPR* (*rs45435518*), and *MC4R* (*rs81219178*) with ABT, MMP, and IMF are shown in Table 4. The results revealed that the effective mutation site of the *LEPR* gene was significantly associated with MMP and IMF ($p < 0.05$) with sex and baseline weight as fixed effects, and IMF of the CC genotype (5.32% ± 0.22%) was significantly higher than that of the TT genotype (4.29% ± 0.42%). However, the effective mutation sites of the *LIPE* and *MC4R* genes had no significant association with ABT, MMP, and IMF.

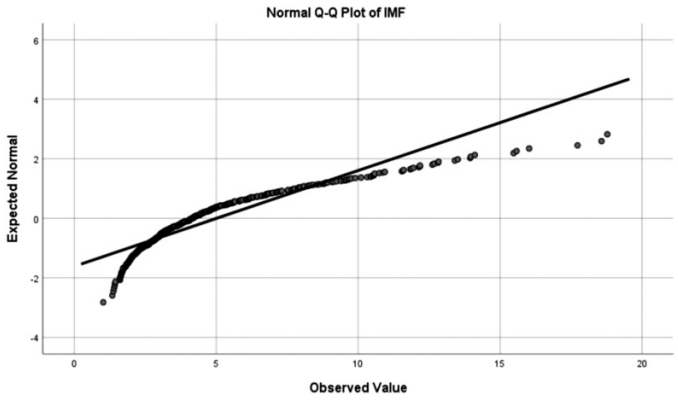
Fig. 3. Examining plots of normal distribution. (A) Normal Q–Q plot of ABT; (B) normal Q–Q plot of MMP; and (C) normal Q–Q plot of IMF.



A



B



C

In addition, amongst the five causal genes, Huang et al. (2021) discovered that the *XM_013981330.2:g.-1805_-1810del* mutation in *MYH3* was not a causal mutation. Therefore, in this study, the association analyses of genotypes with meat quality traits were conducted. It was found that the mutant genotype reported in *MYH3* was not significantly associated with IMF.

Discussion

Improving meat quality has become the aim of pig farming and the primary goal of pig genetic breeding programmes (Huang et al. 2021). In this study, we analysed the phenotypic data of IMF, MMP, and ABT of Lulai black pigs. The results revealed that the value of the CV% of MMP was deficient and stable in the group, and that of IMF was considerably high, which may be due to a large separation of IMF phenotypes in the Laiwu pig population (Chen et al. 2017; Wang et al. 2020). The ancestor of Lulai black pigs may not have been intensively selected for the IMF during the breeding process.

In addition, to screen the molecular markers that were valuable for meat quality trait improvement, this test analysed the genotype distribution and genetic variation of eight effective/causal mutation sites, including *PHKG1* (*rs330928088*), *MUC13* (*rs319699771*), and *MYH3* (*XM_013981330.2:g.-1805_-1810del*), and others, in the Lulai black pig population. The study further explored the association between genotypes at the effective mutation sites and meat quality traits. In the five causal mutations, the *PHKG1* gene mutation is a loss-of-function mutation, resulting in muscle glycogen degradation defects, forming sour meat, reducing pork quality, and bringing significant economic losses for pork production (Ma et al. 2014). The unfavourable mutant allele was fixed in the Duroc and pigs with Duroc kinship (Liu et al. 2019*b*). The mapping population used in this study was Lulai black pig, a new hybrid of Laiwu pigs and Yorkshire pigs. Thus, the unfavourable allelic frequency of the A allele was considerably low, and the number of "Sour Meat" alleles, AA genotypes, was smaller. Still, 12% of Lulai black pigs with unfavourable alleles (AA and AC) were observed, phasing out during the follow-up breeding. *MUC13* disease resistance gene has been extensively used for identifying susceptible and resistant individuals with piglet diarrhoea (Ren et al. 2012*b*). The results revealed that the frequency of the favourable gene G was lower than A in the Lulai black pig population, and the number of GG genotypes was smaller. Therefore, in the breeding process of Lulai black pigs, individuals with the AA genotypes should be appropriately eliminated. Individuals with the G allele could be selected from generation to generation to establish a core group of the pig individuals with homozygous resistant alleles. The process can effectively reduce the diarrhoea rate of piglets. A causal mutation in *IGF2* (*g.3072G>A*) is paternally expressed. The mutant AA genotype could increase lean meat production by 3–4% (Van Laere et al. 2003), improving remarkable economic benefits. Studies indicate that these gene polymorphisms were distributed in Landrace, Large White breed, and Erhualian pig. The A allele was the superior allele in the Landrace and Large White breed (Yang et al. 2006). The study revealed that the frequency of the favourable allele A was only 0.075 in the Lulai black pig population, responsible for the characteristics of low lean meat. A method in which paternal AA type and maternal GG type can be cultivated in breeding exists, increasing the frequency of the A alleles

Fig. 4. Phenotypic correlation coefficients between carcass and meat quality traits in Lulai black pig population. $|r| \leq 0.3$, weak correlation; $0.3 < |r| \leq 0.4$, moderate correlation; and $|r| > 0.4$, strong correlation. $^{**}p < 0.05$; $^{***}p < 0.01$. [Colour online.]

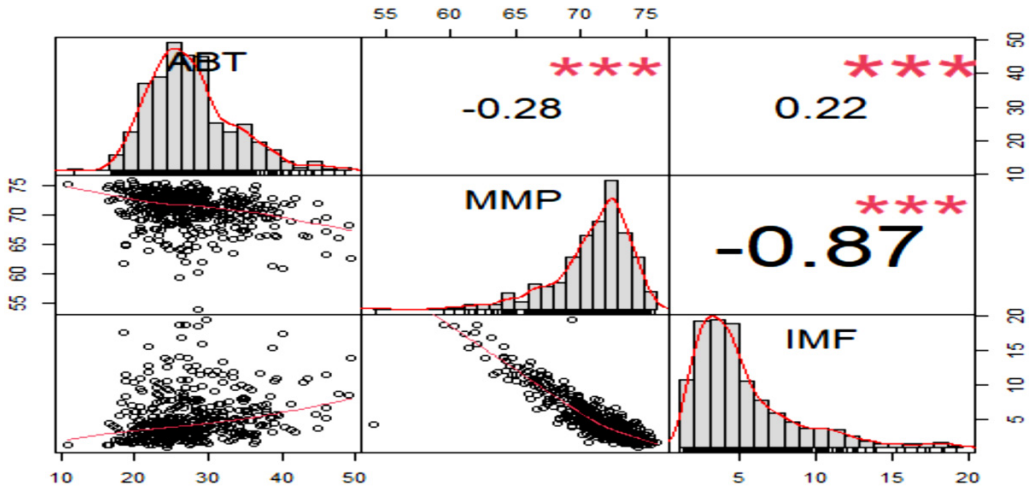


Table 3. Genetic parameters of eight causal or effective mutations in 12 genes of Lulai black pigs.

Gene (SNP)	Chromosome	Genotype (number)	Frequency		HO	HE	PIC
			Genotype	Allele			
PHKG1	3	CC (368)	0.880	0.927 (C)	0.865	0.135	0.126
rs330928088		CA (39)	0.094				
		AA (11)	0.026	0.073 (A)			
MUC13	13	AA (144)	0.344	0.606 (A)	0.522	0.478	0.364
rs319699771		AG (219)	0.524				
		GG (55)	0.132	0.394 (G)			
IGF2	2	GG (358)	0.863	0.925 (G)	0.861	0.139	0.129
g.3072G>A		GA (52)	0.125				
		AA (5)	0.012	0.075 (A)			
VRTN	7	QQ (0)	0	0.146 (Q)	0.750	0.250	0.180
g.20311_20312ins291		Qq (121)	0.292				
		qq (294)	0.708	0.854 (q)			
MYH3	12	qq (41)	0.098	0.477 (q)	0.501	0.499	0.375
XM_013981330.2:g.-1805_-1810del		Qq (318)	0.759				
		QQ (60)	0.143	0.523 (Q)			
LIPE	6	GG (15)	0.036	0.263 (G)	0.612	0.388	0.313
rs328830166		GA (190)	0.454				
		AA (213)	0.510	0.737 (A)			
LEPR	6	CC (221)	0.529	0.725 (C)	0.601	0.399	0.319
rs45435518		CT (164)	0.392				
		TT (33)	0.079	0.275 (T)			
MC4R	1	GG (317)	0.758	0.878 (G)	0.786	0.214	0.191
rs81219178		GA (100)	0.239				
		AA (1)	0.003	0.122 (A)			

and improving the shortboard with a low lean meat rate of cultivated varieties with high consanguinity. In addition, our results revealed extremely high vertebra number alleles *VRTN* (*g.20311_20312ins291*) in this population. No homozygous genotyped individuals with unfavourable alleles of the *VRTN* gene were observed. Vertebra numbers are an essential economic trait in a pig that can influence carcass length meat production, and its heritability was remarkably high (Borchers et al. 2004). Chinese and Western purebred pigs, such as Laiwu pigs and Landrace, harbour beneficial alleles, and the frequency is higher in Western pigs. Several studies indicate that mutation leading to changes in vertebra numbers originated from Chinese native pigs (Fan et al. 2013; Yang et al. 2016). Results of this trial provided a basis for the favourable mutant allele derived from Chinese native pigs.

Table 4. Association analysis of eight effective/causal mutations in eight genes with ABT, MMP, and IMF of Lulai black pigs.

Gene (SNP)	Genotype (number)	ABT (mm)			MMP (%)			IMF (%)		
		Mean ± SE	*p* value	Q value	Mean ± SE	*p* value	Q value	Mean ± SE	*p* value	Q value
PHKG1rs330928088	CC (368)	38.52 ± 0.35	0.3421	1.19E-02	71.12 ± 0.15	0.3349	1.20E-02	5.05 ± 0.16	0.4114	1.12E-02
	CA (39)	39.63 ± 1.21			71.02 ± 0.45			5.08 ± 0.51		
	AA (11)	35.56 ± 1.87			72.62 ± 0.82			3.69 ± 0.81		
MUC13rs319699771	AA (144)	38.31 ± 0.54	0.1421	9.75E-03	71.38 ± 0.21	0.4748	1.11E-02	4.79 ± 0.23	0.8351	1.01E-02
	AG (219)	38.53 ± 0.50			71.01 ± 0.21			5.23 ± 0.23		
	GG (55)	39.26 ± 0.72			71.11 ± 0.30			4.77 ± 0.35		
IGF2g.3072G>A	GG (358)	38.47 ± 0.36	0.7542	1.01E-02	71.02 ± 0.16	0.07196	9.00E-03	5.18 ± 0.17	0.05221	8.50E-03
	GA (52)	39.09 ± 1.01			72.01 ± 0.24			3.89 ± 0.23		
	AA (5)	36.81 ± 1.55			70.72 ± 1.11			5.97 ± 1.78		
VRTNg.20311_20312ins291	QQ (0)	0	0.6412	1.03E-02	0	0.5577	1.05E-02	0	0.4428	1.09E-02
	Qq (121)	38.89 ± 0.62			70.96 ± 0.27			5.27 ± 0.31		
	qq (294)	38.58 ± 0.40			71.21 ± 0.16			4.93 ± 0.17		
MYH3XM_013981330.2: g.-1805_-1810del	qq (60)	27.14 ± 0.68	0.8756	1.01E-02	70.98 ± 0.38	0.4976	1.09E-02	5.32 ± 0.46	0.6493	1.03E-02
	Qq (318)	27.20 ± 0.32			71.15 ± 0.16			4.96 ± 0.17		
	QQ (41)	27.80 ± 0.98			71.45 ± 0.43			5.01 ± 0.48		
LIPErs328830166	GG (15)	37.02 ± 1.59	0.2218	1.12E-02	71.61 ± 0.56	0.2068	1.09E-02	4.91 ± 0.57	0.3040	1.17E-02
	GA (190)	38.30 ± 0.50			71.32 ± 0.21			4.81 ± 0.21		
	AA (213)	38.88 ± 0.47			70.97 ± 0.19			5.21 ± 0.23		
LEPRrs45435518	CC (221)	38.59 ± 0.51	0.8690	1.00E-02	70.85 ± 0.19^{b}	0.02007	7.81E-03	5.32 ± 0.22^{a}	0.03396	8.80E-03
	CT (164)	38.61 ± 0.44			71.42 ± 0.23^{ab}			4.76 ± 0.24^{ab}		
	TT (33)	37.97 ± 1.33			71.84 ± 0.41^{a}			4.29 ± 0.42^{b}		
MC4Rrs81219178	GG (317)	38.60 ± 0.38	0.6626	1.03E-02	71.14 ± 0.16	0.8237	1.02E-02	5.07 ± 0.18	0.5198	1.09E-02
	GA (100)	38.26 ± 0.71			71.18 ± 0.26			4.88 ± 0.29		
	AA (1)	52.32 ± 0.00			72.25 ± 0.00			2.87 ± 0.00		

Note: Mean ± SE represents the means with standard errors for different genotypes; superscripts with different lowercase letters indicate a significant difference between genotypes ($p < 0.05$); $p < 0.05$, significant; $p < 0.01$, extremely significant

Studies have indicated that the causal mutation affects IMF in pigs, a 6 bp deletion variant in the porcine *MYH3* promoter region. Still, the results of this study revealed that the mutation was not significantly associated with IMF content. Thus, the result could not support that the mutation occurred at a causal mutation site, affecting the IMF content of Lulai black pigs. The result is consistent with previous studies (Huang et al. 2021). The finding of the causal mutation for the QTL (Quantitative Trait Locus) on SSC8 affecting IMF content by Cho et al. (2019) must be further studied.

For other essential three candidate genes, the results of our study revealed that individuals with the CC genotype of the *LEPR* (*rs45435518*) gene had higher IMF content, further confirming that the mutation in the *LEPR* gene (*rs328830166*) was an effective mutation affecting the IMF content. However, its specific mechanism of action needs further research in Lulai black pig population. Although the *MC4R* and the *LIPE* genes were candidate genes associated with meat quality traits and lipid deposition control, the association between *MC4R* (*rs81219178*) and *LIPE* (*rs328830166*) mutation genotypes and ABT, MMP, and IMF meat quality traits exhibited no significant level in Lulai black pigs. Therefore, the *MC4R* and *LIPE* effective mutations could not be used as a molecular marker for improving the ABT, MMP, and IMF meat quality traits of Lulai black pigs.

Conclusions

In this study, the eight effective/causal mutation sites affecting fat deposition, meat quality, growth, and disease resistance traits were detected and analysed in 418 Lulai pigs. The results indicated that the eight effective/causal mutation sites were all genetically polymorphic in Lulai black pig population. The causal mutation of *MYH3* (*XM_013981330.2:g.-1805_-1810del*) was not significantly associated with IMF content ($p > 0.05$). In contrast, amongst the three effective mutation sites, the effective mutation of the *LEPR* gene was significantly associated with IMF content in Lulai black pigs ($p < 0.05$). With overall evaluation, the causal mutation sites of *PHKG1* (*rs330928088*), *MUC13* (*rs319699771*), *IGF2* (*g.3072G>A*), and *VRTN* (*g.20311_20312ins291*), and the effective mutation site of *LEPR* (*rs45435518*) have critical breeding values to Lulai black pigs.

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Competing interests

The authors have declared that no competing interests exist.

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