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Identification of four SNPs and association analysis with meat quality traits in the porcine *Pitx2c* gene

WU WangJun[1], ZUO Bo[1], REN ZhuQing[1], HAPSARI A.A.R[2], LEI MingGang[1], XU DeQuan[1], LI FengE[1] & XIONG YuanZhu[1*]

[1]*Key Laboratory of Swine Genetics and Breeding, Ministry of Agriculture and Key Laboratory of Agriculture Animal Genetics, Breeding and Reproduction, Ministry of Education, College of Animal Science, Huazhong Agricultural University, Wuhan 430070, China;*
[2]*Faculty of Animal Agriculture, Diponegoro University, Tembalang Campus-Semarang 50275, Indonesia*

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The association of the porcine *Pitx2c* gene with meat quality traits was investigated in the present study. A total of eight single nucleotide polymorphisms (SNPs) were found. Allele frequencies of four SNPs were further detected in four commercial breeds and eight Chinese indigenous breeds. Single SNP and meat quality associations were analyzed in a Yorkshire×Meishan F_2 population. The SNPs c.474C>T (P<0.01) and c.636C>T (P<0.05) showed a significant association with meat color (MCV1). The SNPs c.*37G>A and c.*47G>A were significantly associated with drip loss rate (DLR), water holding capacity (WHC) and meat color value (MCV1) consistently (P<0.05). Linkage disequilibrium (LD) analysis revealed that the adjacent SNPs were in LD. Two major haplotypes were identified, and association analysis between haplotype combinations and meat quality indicated that the presence of two copies of haplotype 1 -CCGG- may improve meat quality.

association, haplotype, *Pitx2c*, meat quality, pig

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Meat is an important requirement for human nutrition and contains essential protein components. Improvement in the quality of meat would likely increase consumer acceptance and perception of meat, and therefore directly impact meat animal breeding programs. In the past, pig breeding research has focused on growth rate, food conversion ratio and lean meat content [1]. However, selection for these traits may lead to dramatic consequences for behavioral, physiological and immunological traits [2], will increase the incidence of pale, soft and exudative meat (PSE) and reduce the intramuscular fat content [3,4]. Thus, in recent years, meat quality traits have received closer attention in pig breeding programs.

Conventional selection methods have been very effective in improving growth rate, food conversion ratio and lean meat content, but very little improvement has been made to meat quality traits. With the development of modern molecular biology and genomic technology, marker-assisted selection (MAS) is one technique that has been considered by numerous breeders. MAS can be used not only to select some quantitative traits by traditional means such as using melanocortin receptor 4 (MC4R) to increase marbling, but also to select sex-limited, age-limited and meat quality traits which cannot be selected by traditional means. Moreover, MAS can help pig breeders to remove the harmful major mutations [5–9], and increase the accuracy of selection and selection response in a population. The identification of major genes, or tightly linked markers, would provide an important base for MAS application. However, until now just a few major genes have been successfully identified

*Corresponding author (email: xiongyz@public.wh.hb.cn)

such as *RYR1*, *RN* and *IGF2* genes [6,10,11]. Single nucleotide polymorphisms (SNPs), the most frequent form of DNA variations in mammals, are well suited for MAS of economic traits in meat animal breeding. So far, a large number of SNPs have been identified to be associated with economic traits in various pig populations [12–16], but the valuable SNP molecular markers are limited. Therefore, more major genes and SNP molecular markers still need to be identified. There are three approaches that can be used to identify major genes [17]: The first method is to observe whether the 'major genes' are segregating in the population; the second is the 'genomic scan' method; and the third is the candidate gene approach, which is more straightforward and cheaper when compared with the other two approaches [18–20].

Pitx2 is a paired-related homeobox gene that was originally identified as one of the genes responsible for human Rieger syndrome, an autosomal dominant condition [21]. Studies on *Pitx2*-deficient mice show disturbances in the development of multiple organs including body-wall, skin, ocular, right pulmonary isomerism, heart, pituitary gland and tooth organogenesis problems [22,23]. It also plays an important role in cell type-specific control of proliferation by combining with general growth factor signal pathways [24], promoting muscle cell proliferation and arresting muscle cell differentiation [25]. In addition, other reports have showed that *Pitx2* has a close relationship with muscle development [26–31]. Recently, the full length cDNA and genomic sequence of porcine *Pitx2c* was isolated and characterized in our laboratory (GenBank accession No. HM030975), and the *Pitx2c* gene was assigned to porcine chromosome 8 in the area of quantitative trait loci for meat quality traits by comparative mapping between pigs and humans. Moreover, tissue distributions showed that the porcine *Pitx2c* gene was highly expressed in skeletal muscle (data not shown).

This information has led us to choose porcine *Pitx2c* as a candidate gene affecting skeletal muscle growth and meat quality traits. Our main objective is to search the SNPs in the porcine *Pitx2c* gene and to examine the associations of its polymorphisms with meat quality traits.

1 Materials and methods

1.1 Animals

Two F_2 populations derived from the intercross of Large White and Meishan pigs that included 334 individual pigs were used for the association study. The pigs were from the Jingpin Pig Station of Huazhong Agricultural University (Wuhan, China). All animals were fed twice daily with diets formulated according to their age under a standardized feeding regimen and had free access to water. The F_2 pigs were slaughtered following a common protocol [32]. The detailed traits of animals and their measurements have been described by Yang *et al.* [33]. Meanwhile, 12 pig breeds containing four Western breeds (100 Yorkshire, 37 Landrace, 28 Pietrain and 27 Duroc pigs) and eight Chinese indigenous breeds (50 Meishan, 25 Qingping, 18 Tongcheng, 19 Erhualian, 19 Jianli, 7 Hezuo, 19 Wannan and 12 Yangxin pigs) were used to assay the allele frequency.

1.2 Identification of SNPs in the porcine *Pitx2c* gene

In an effort to discover the SNPs in the porcine *Pitx2c* gene, two-pair primers (Pitx2c-4F and Pitx2c-4R; Pitx2c-7F and Pitx2c-7R; Table 1) were designed to scan the SNPs existing in the whole coding region using the cDNA from Yorkshire and Meishan pigs. Another pair of primers (Pitx2c-6F and Pitx2c-6R; Table 1) was used to amplify intron 1 of the porcine *Pitx2c* gene using the DNA from Yorkshire and Meishan pigs. PCR was performed in a GeneAmp PCR system 9600 (Perkin Elmer, Foster City, CA, USA) using 1.25 U *LA Taq* polymerase (Takara, Japan), 1×LA PCR buffer II (Mg^{2+} Plus), 0.2 μmol L^{-1} of each dNTP, 0.2 μmol L^{-1}

Table 1 Information of primers used in the study

Gene name	Primer name	Primer sequence (5′–3′)	Binding region	Product size	Annealing temperature
SNP detection primers					
Pitx2c	Pitx2c-7F	CTGGGTGCTGGGAACTGA	5′-UTR	854 bp	58.5°C
	Pitx2c-7R	GCTGGCTGGTGAAGTGAGT	Exon 2		
	Pitx2c-4F	AGCGGACTCACTTCACCAG	Exon 2	787 bp	60.5°C
	Pitx2c-4R	GAGTGCCCACGACCTTCTA	3′-UTR		
	Pitx2c-6F	CTTTGCCCACTCTTGTTTC	Exon 1	1428 bp	57.3°C
	Pitx2c-6R	TCTTCTTGGACGGGTCTTCA	Exon 2		
Genotyping primers					
Pitx2c	PE1-1F	CAAGAATCGCCGGGCCAAAT	Exon 2	296 bp	57.1°C
	PE1-1R	TCACCGCTGAGGGCACCAT	Exon 2		
	PE1-2F	CGCAGTTCAACGGGCTTAT	Exon 2	407 bp	57.4°C
	PE1-2R	GCTGGAGTGCTGCTTTGCT	Exon 2		
	F1	GTGTGAGCTGCGCCCACT	3′-UTR	105 bp	55°C
	R1	TCTTGAGTGCCCACGACCT	3′-UTR		
	S1	GGGATCCTAGGACCGTGC	3′-UTR	–	28°C

of each primer, and 0.5 μL cDNA or 1 μL genomic DNA in 25 μL reactions. The temperature profile was as follows: 4 min for an initial denaturation at 94°C, and then 30 cycles of denaturation at 94°C for 30 s, annealing at optimal temperature (Table 1) for 30 s and extension at 72°C for 1 min. The obtained PCR products were separated by 2.0% agarose gel electrophoresis, and purified using a Gel Extraction Kit (Sangon, Shanghai, China). The purified products were ligated into the pMD18-T vector (Takara, Japan), and transformed into *Escherichia coli* DH5α. At least three independent clones were sequenced from both forward and reverse directions by the Beijing Genomics Institute. Potential SNPs were identified by comparison of the obtained sequences from two pig breeds including Yorkshire and Meishan using DNASTAR software.

1.3 Genotyping protocols

The polymorphism sites c.474C>T and c.636C>T which related to the position of the translation start codon, c.37*G>A and c.47*G>A, which related to the position of the stop codon (positions based on GenBank accession No. HM030975) were further typed in different pig breeds and two Yorkshire×Meishan F_2 reference family using PCR-RFLP and pyrosequencing protocols. A *Bpu*ll02 I and *Hin*d II PCR-RFLP assay was conducted to genotype the SNPs c.474C>T and c.636C>T polymorphisms, respectively. Primer pairs PE1-1F/R and PE1-2F/R (Table 1) were redesigned to detect c.474C>T and c.636C>T polymorphisms, respectively. The PCR reactions were carried out in a 25 μL volume using 1 U polymerase (Fermentas), 1×PCR buffer, 0.2 μmol L^{-1} of each dNTP, 0.2 μmol L^{-1} of each primer, 2 mmol L^{-1} $MgCl_2$ and 1 μL genomic DNA. The thermal profile comprised a denaturation step at 94°C for 4 min, 34 cycles of 94°C for 40 s, optimal annealing temperatures (Table 1) for 40 s and 72°C for 45 s, and a final extension step of 72°C for 10 min. The products were digested with a corresponding enzyme at 37°C for 6 h and the digested products were separated by electrophoresis on 2.5% agarose gels.

A pyrosequencing protocol was employed to genotype the c.37*G>A and c.47*G>A polymorphic sites, both located at 3′ UTR. Pyrosequencing amplification and sequencing primers were designed by Assay Design Software in Pyrosequencing™ Systems (Biotage). The R1 primer was biotin labeled and purified by HPLC, whereas other primers were unlabeled and purified using routine PAGE. A 105 bp fragment was amplified using primers F1 and R1 (Table 1). Polymerase chain reactions were performed in a volume of 35 μL 1×PCR buffer, containing 0.2 μmol L^{-1} of each dNTP, 2 mmol L^{-1} $MgCl_2$, 5 pmol of each primer, and 1 U *Taq* polymerase (Fermentas). The PCR reaction conditions were as follows: 94°C for 4 min, then 40 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 10 s, and a final extension of 72°C for 10 min. Subsequently, the genotyping primer S1 was used in the sequencing of the PCR products. The sequencing reaction was conducted in the PSQ 96MA Pyrosequencing instrument (Biotage) with the Pyro Gold Reagent Kit (Biotage) according to the manufacturer's protocols.

1.4 Statistical analysis

The genotype distributions of four polymorphic sites in the F_2 population were tested for Hardy-Weinberg equilibrium using the online tool chi-square test (http:www.oege.org/software/hwe-mr-calc.shtml). The linkage disequilibrium (LD; r^2) between four SNPs was performed with HAPLOVIEW 4.2 software [34]. Haplotypes of F_2 individuals were obtained by using an efficient computer program PHASE version 2.1 [35].

The general linear model (GLM) procedure in the SAS software package (SAS Institute, Cary, NC, USA) was used to analyze the associations between single SNPs and traits. The differences between the least square means for the porcine *Pitx2c* genotypes were estimated using Duncan's multiple range tests with significance at $P<0.05$. The following GLM statistical model was used:

$$T_{ijk}=\mu+S_i+Y_j+G_k+b_{ijk}X_{ijk}+e_{ijk},$$

where T_{ijk} is the observed values of traits, μ is the least square mean, S_i is the effect of the ith sex (i=1 for male or 0 for female), Y_j is the effect of the jth year (j=1 for year 2003 or 2 for year 2004), G_k is the effect of the kth genotype (k=1, 2 and 3), b_{ijk} is the regression coefficient of carcass weight for the traits, and e_{ijk} is the random residual. The REG procedure was used to estimate the additive and dominance effects. The additive effect was estimated as −1, 0, 1 for one homozygous genotype, heterozygote genotype and the other homozygous genotype, respectively. The dominance effect was represented as −1 for heterozygote genotype, and 1 for homozygous genotype. The association analysis between certain haplotypes and traits was conducted using the statistical model mentioned above.

2 Results

2.1 Genome SNP scanning of the porcine *Pitx2c* gene

Three pairs of primers were used to scan 2489 bp genomic sequences covering the entire coding region, partial UTR regions and intron 1 region. A total of eight SNPs were identified, including two synonymous mutations in the coding region (c.474C>T and c.636C>T; positions based on GenBank accession No. HM030975), two mutations in the 3′ UTR (c.*37G>A and c.*47G>A; positions based on GenBank accession No. HM030975) and four SNPs in the first intron (g.128G>C, g.237G>C, g.451C>G, g.512C>G;

positions based on GenBank accession No. HM030974).

2.2 Allele frequency detection of the porcine *Pitx2c* gene in different breeds

For the c.474C>T polymorphic site, primers PE1-1F and PE1-1R were used to amplify a 296 bp fragment and the amplicon was digested with endonuclease *Bpu*1102 I, resulting in allele C (296 bp) and allele T (247 and 49 bp) (Figure 1A). For the c.636C>T polymorphic site, primers PE1-2F and PE1-2R were used to amplify a 407 bp fragment and the amplicon was digested with endonuclease *Hin*d II, resulting in allele T (407 bp) and allele C (135 and 272 bp) (Figure 1B). Pyrosequencing protocols were implemented to genotype the SNPs c.*37G>A and c.*47G>A present in 3′ UTR. Genotypes at four polymorphic sites and allele frequencies in different breeds are shown in Table 2. At c.474C>T and c.636C>T loci, allele C was over-dominant in Chinese indigenous pigs. While at c.*37G>A and c.*47G>A loci, the G allele was predominant in Chinese indigenous pigs.

2.3 Association analysis of porcine *Pitx2c* single SNP with meat quality traits

Four SNPs were in Hardy-Weinberg equilibrium in the Yorkshire×Meishan F_2 population (Table 3). The results of association analysis between single SNP and meat quality traits are shown in Table 4. The SNP c.474C>T was significantly associated (P<0.01) with meat color value of the *longissimus dorsi* (MCV1) and the SNP c.636C>T had significant associations with MCV1 (P<0.05). Moreover, sig-

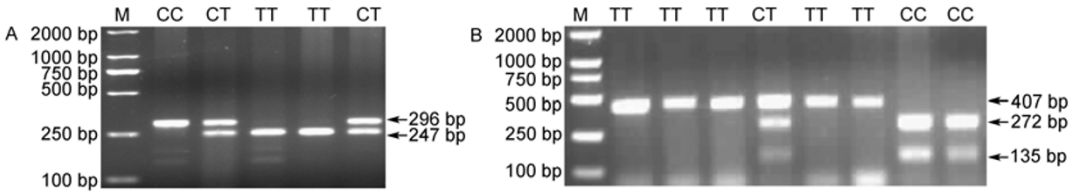

Figure 1 PCR-RFLP analysis of the porcine *Pitx2c* gene c.474C>T (A), c.636C>T (B) polymorphisms. The genotypes are indicated on the top of the lanes. M, DL2000.

Table 2 Genotypic frequencies and allele frequencies of four polymorphisms located in the porcine *Pitx2c* gene[a)]

Breed	Polymorphisms in exon 2											
	c.474C>T						c.636C>T					
		Genotype and frequency			Allele frequency			Genotype and frequency			Allele frequency	
	No.	TT	CT	CC	T	C	No.	TT	CT	CC	T	C
Yorkshire	100	88	9	3	0.945	0.055	34	31	3	0	0.977	0.023
Landrace	37	36	1	0	0.993	0.007	32	32	0	0	1	0
Pietrain	28	16	10	2	0.804	0.196	24	12	10	2	0.763	0.237
Duroc	27	7	17	3	0.608	0.392	27	4	15	8	0.397	0.603
Qingping	25	0	0	25	0	1	22	0	0	22	0	1
Meishan	50	0	0	50	0	1	46	1	0	35	0.030	0.970
Tongcheng	18	0	0	18	0	1	17	0	0	17	0	1
Erhualian	19	0	1	18	0.014	0.986	–	–	–	–	–	–
Jianli	19	0	0	19	0	1	–	–	–	–	–	–
Hezuo	7	0	1	6	0.038	0.962	–	–	–	–	–	–
Wannan	19	0	1	18	0.014	0.986	–	–	–	–	–	–
Yangxin	12	0	0	12	0	1	–	–	–	–	–	–
	Polymorphisms in 3′ UTR											
	c.*37G>A						c.*47G>A					
		Genotype and frequency			Allele frequency			Genotype and frequency			Allele frequency	
Breed	No.	AA	AG	GG	A	G	No.	AA	AG	GG	A	G
Yorkshire	31	31	0	0	1	0	31	27	4	0	0.935	0.065
Landrace	27	27	0	0	1	0	27	26	1	0	0.982	0.018
Pietrain	28	14	14	0	0.750	0.250	28	14	12	2	0.714	0.286
Duroc	27	7	18	2	0.593	0.407	27	7	15	5	0.537	0.463
Qingping	25	0	4	21	0.080	0.920	25	0	4	21	0.080	0.920
Meishan	28	1	4	23	0.107	0.893	28	1	4	23	0.107	0.893

a) – represents the breeds that were not genotyped.

Table 3 Genotype distribution of examined SNP in F_2 populations

Polymorphisms in exon 2					
SNP	Line[a)]	Genotype			HWE[b)]
		CC	CT	TT	
c.474C>T	LMF2	68	146	69	0.590
c.636C>T	LMF2	69	147	73	0.760
Polymorphisms in 3′ UTR					
SNP	Line	Genotype			HWE
		AA	AG	GG	
c.*37G>A	LMF2	74	137	71	0.630
c.*47G>A	LMF2	71	140	71	0.920

a) F_2 populations derived from the intercross of Large White and Meishan pigs. b) *P*-value of a test for deviation from Hardy-Weinberg equilibrium.

Table 4 Association analysis of porcine *Pitx2c* polymorphisms with meat traits[a)]

SNP	Traits	Genotype (mean±SE)			Genetic effect (mean±SE)	
Polymorphisms in exon						
		CC (*n*=68)	CT (*n*=146)	TT (*n*=69)	Additive	Dominance
c.474 C>T	MCV1	18.733±0.140^{AB}	18.966±0.095^{A}	18.516±0.140^{B}	–0.105±0.098	–0.173±0.068*
		CC (*n*=69)	CT (*n*=147)	TT (*n*=73)	Additive	Dominance
c.636 C>T	MCV1	18.911±0.310^{ab}	19.258±0.197^{a}	18.575±0.273^{b}	–0.165±0.098	–0.257±0.069*
Polymorphisms in 3′ UTR						
		AA (*n*=74)	AG (*n*=137)	GG (*n*=71)	Additive	Dominance
c.*37 G>A	DLR%(LD)	6.401±0.178^{ab}	6.806±0.131^{a}	6.338±0.181^{b}	–0.030±0.126	–0.220±0.091*
	WHC%(LD)	91.298±0.235^{ab}	90.753±0.173^{b}	91.379±0.241^{a}	0.040±0.168	0.295±0.121*
	MCV1(LD)	18.645±0.133^{b}	19.014±0.098^{a}	18.682±0.136^{b}	0.015±0.095	–0.173±0.068*
		AA (*n*=71)	AG (*n*=140)	GG (*n*=71)	Additive	Dominance
c.*47 G>A	DLR%(LD)	6.378±0.181^{ab}	6.807±0.129^{a}	6.342±0.181^{b}	–0.020±0.128	–0.225±0.091*
	WHC%(LD)	91.326±0.240^{ab}	90.754±0.171^{b}	91.371±0.241^{a}	0.020±0.170	0.300±0.121*
	MCV1(LD)	18.614±0.135^{b}	19.022±0.097^{a}	18.681±0.136^{b}	0.035±0.096	–0.188±0.068**

a) MCV1, Meat Color Value (m. *longissimus dorsi*, LD); DLR, drip loss rate of *longissimus dorsi*; WHC, water holding capacity of *longissimus dorsi*. Different superscript small letters in one row indicate significant differences at $P < 0.05$; different superscript capital letters in one row indicate extremely significant differences at $P < 0.01$. *, $P < 0.05$; **, $P < 0.01$.

nificant dominance effects were observed for MCV1 at both loci (c.474C>T and c.636C>T). While c.*37G>A and c.*47G>A polymorphisms showed significant associations with drip loss rate (DLR), water holding capacity (WHC) and meat color value (MCV1) of *longissimus dorsi* (P<0.05) and significant dominance effects were detected for all traits at both loci, especially a high significance level was observed for MCV1 at c.*47G>A (P<0.01). Interestingly, the results of c.474C>T and c.636C>T were very similar to MCV1, whereas the results of c.*37G>A and c.*47G>A were very similar to all traits.

2.4 Haplotype analysis

Haplotype blocks and graphical representation of LD structure was generated by HAPLOVIEW 4.2 software. One haplotype block was found in the SNPs genotyped in the porcine *Pitx2c* gene (Figure 2). As expected from the single SNP association results, SNPs c.474C>T and c.636C>T in the coding region were in LD with each other (r^2=0.85), while SNPs c.*37G>A and c.*47G>A within 3′ UTR were detected in high LD (r^2=0.95) and identified in the same amplicon. The SNP c.*37G>A also displayed LD with

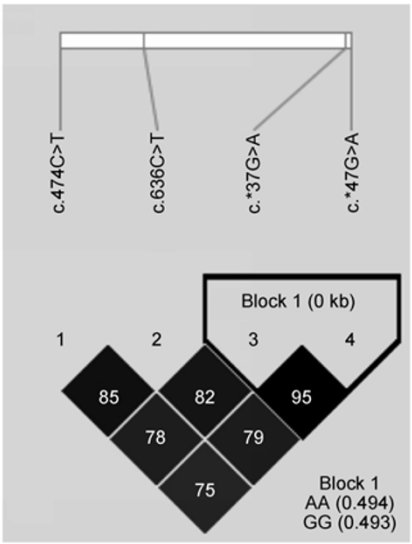

Figure 2 Linkage disequilibrium (LD) for SNPs in the porcine *Pitx2c* gene. Pairwise LD (r^2) values are shown between markers. The shading represents the LD relationships with darker shading indicating higher LD. Block was generated by the default algorithm (confidence intervals) in HAPLOVIEW 4.2. One block was predicted, the block contains markers c.*37G>A and c.*47G>A. The population frequencies are listed in parentheses next to each haplotype.

c.636C>T (r^2=0.82). Two tag SNPs c.474C>T and c.*37G>A were predicted by HAPLOVIEW. In total, twelve haplotypes were constructed by PHASE at four polymorphic sites (Table 5), two major haplotypes accounted for 91.78% of the alleles as follows: haplotype 1, -CCGG- (45.90%); haplotype 2, -TTAA- (45.88%). Subsequently, the association analysis was performed between haplotype combinations and traits (Table 6). Because other haplotypes were very rare among the 12 haplotypes except for haplotypes 1 and 2 in the F_2 population (Table 5), we included only the individuals with haplotypes 1 and 2 in the association analysis. Significant differences (P<0.05) were observed between haplotype combinations on MCV1 and suggestively significant (P<0.1) differences were detected between haplotype combinations and DLR and WHC (Table 6).

Table 5 Haplotype and haplotype frequency of the porcine *Pitx2c* gene

Index	Haplotype	E (freq)[a)]	SE[b)]
1	CCAA	0.016894	0.002761
2	CCAG	0.000146	0.000497
3	CCGA	0.001812	0.000601
4	CCGG	0.459048	0.002680
5	CTAA	0.015679	0.003205
6	CTAG	0.001953	0.001064
7	CTGG	0.004605	0.002106
8	TCAA	0.009468	0.001537
9	TCGG	0.007721	0.001496
10	TTAA	0.458881	0.003064
11	TTAG	0.007223	0.001165
12	TTGG	0.016549	0.002288

a) E (freq) represents the haplotype frequency. b) SE represents the standard errors.

Table 6 Associations of porcine *Pitx2c* haplotype combinations

Trait	LSM (SE)[a)] of haplotype combination			*P*-value		
	11[b)]	12[c)]	22[d)]	11-12	11-22	12-22
MCV1(LD)	18.764 (0.173)	19.026 (0.123)	18.530 (0.180)	0.220	0.352	0.024
DLR%(LD)	6.446 (0.235)	6.853 (0.167)	6.339 (0.244)	0.161	0.753	0.084
WHC%(LD)	91.240 (0.312)	90.697 (0.222)	91.392 (0.324)	0.159	0.737	0.079

a) LSM (SE) represents least squares means and their standard errors. b) 11 indicates haplotype 1, -CCGG- combined with haplotype 1, -CCGG-. (c) 12 indicates haplotype 1, -CCGG- combined with haplotype 2, -TTAA-. (d) 22 indicates haplotype 2, -TTAA- combined with haplotype 2, -TTAA-.

3 Discussion

In meat production systems, skeletal muscle tissue from slaughtered animals becomes meat for human consumption. Skeletal muscle tissue consists of many different cell types, with muscle fibers making up the majority share. Many regulatory factor genes are involved in the formation of skeletal muscle [36]. Therefore, increased knowledge about the relationship between these genes and the development and growth of skeletal muscle fibers is of utmost importance for the improvement of meat quality in pig breeding.

A multitude of studies have shown that the *Pitx2c* gene plays an important role in muscle development [26,27,29, 37,38]. Recently, we also found that porcine *Pitx2c* was expressed at its highest level in skeletal muscle (data not shown). Thus, it is reasonable for us to choose the porcine *Pitx2c* gene as a candidate and investigate the associations of *Pitx2c* polymorphisms with economic traits in Large White×Meishan F_2 populations.

LD analysis indicated that the SNPs c.474C>T and c.*37G>A were in LD with c.636C>T (r^2>0.8), while c.*37G>A and c.*47G>A were detected in high LD (r^2=0.95). The results suggested that these SNPs may have consistent effects on certain traits. As expected, further association analysis results showed that the four polymorphisms had significant associations with MCV1 and significant dominance effects had been observed for MCV1 at four loci (Table 4). Although the SNPs c.474C>T and c.636C>T polymorphisms showed consistent associations with MCV1 (whether considered individually or jointly) and the individuals with the C allele at both loci had higher MCV1 value, there were some different effects on MCV1 between both loci. This result suggested that variations in porcine *Pitx2c* might be having variable effects on MCV1. The allele frequencies of the SNPs c.474C>T and c.636C>T in the *Pitx2c* gene showed significant difference between commercial pigs and Chinese indigenous pigs. At both loci, allele C was over-dominant in Chinese indigenous pigs and the allele frequencies reached 1 in several breeds (Table 2). This is consistent with the fact that the Chinese indigenous pigs have higher meat color value. In addition, we found that both of the SNPs c.*37G>A and c.*47G>A were significantly correlated with DLR, WHC and MCV1 consistently (Table 4) and both loci c.*37G>A and c.*47G>A displayed dominance effects for DLR and WHC, which indirectly support the result that the two loci were highly LD. The allele frequencies of c.*37G>A and c.*47G>A also exhibited remarkable differences between commercial breeds and Chinese indigenous breeds. Chinese indigenous breeds had higher frequencies of the G allele at both loci. The pigs with homozygous GG had higher MCV1 and WHC, and lower DLR when compared with the AA genotype individuals, although no significance had been detected. These coincide with the fact that Chinese indigenous breeds have better meat quality than the commercial breeds. Furthermore, we found that the two loci (c.*37G>A and

c.*47G>A) were significantly over-dominant in actions for MCV1, DLR and WHC. The MCV1 and DLR of AG heterozygote pigs were significantly higher than GG homozygotes while the WHC of AG heterozygote pigs was lower (P<0.05). These results imply that allele G at both loci c.*37G>A and c.*47G>A was associated with good and desirable meat quality, but the heterozygotic genotype was not conducive to the improvement of meat quality. It is well known that meat quality traits such as color, drip loss and water holding capacity are correlated to meat pH. Whereas, in our study the polymorphisms of c.*37G>A and c.*47G>A were not found to be significantly associated with pH, but the pigs carrying the GG had higher pH relative to the AA individuals (data not shown). Actually, our results were consistent with the fact that lower pH will be accompanied by higher DLR and lower WHC [3,39–41]. In addition, we found that the heterozygotic pigs had the lowest pH value, suggesting both of the loci may also display over-dominant effects on pH. Although our results open the possibility that the polymorphisms of c.*37G>A and c.*47G>A are associated with pH, further work is still needed to identify the associations between the c.*37G>A and c.*47G>A polymorphisms and pH in other pig populations.

From the single marker association results, it could be speculated that improving the frequencies of allele C at c.474C>T and c.636C>T loci and allele G at c.*37G>A and c.*47G>A loci would help to improve meat quality. To explore further, we performed the association analysis of haplotype combinations among the four SNPs, where significant associations were also observed between haplotype combinations and MCV1 (P<0.05), and suggestively significant differences with DLR and WHC (P<0.1; Table 6). Therefore, we deduced that variations in the porcine *Pitx2c* gene have an effect on MCV1, DLR and WHC and the differences of the effects among the variations could be caused by the positional differences and possible interactions among these SNPs.

Here, we have identified that the polymorphisms of the porcine *Pitx2c* gene were significantly associated with several meat quality traits and LD was observed between the adjacent SNPs. Interestingly, the polymorphisms of the four SNPs had significant effects on MCV1. Although our results have shown the significant association between the variations of porcine *Pitx2c* and pork quality, further analysis to clarify the significant associations in other commercial populations is needed.

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