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Research Article

Associations of *MYF*5 gene polymorphisms with meat quality traits in different domestic pig (*Sus scrofa*) populations

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Abstract

The *MYF*5 gene is first inducibly expressed in muscle cell during embryonic muscle development and plays an important role in regulating the differentiation of skeletal muscle precursors. In this study we used PCR-RFLP to investigate two pig (*Sus scrofa*) populations (n = 302) for two *MYF*5 gene polymorphisms, a previously unreported novel Met-Leu shift single nucleotide polymorphism (SNP) *MYF*5/*Hsp92II* located on exon 1 and the previously identified intron 1 *MYF*5/*HinfI* SNP. Haplotype and association analysis showed that haplotypes of the two SNPs were significantly associated with drip loss rate (DLR, $p < 0.05$), water holding capacity (WHC, $p < 0.05$), *biceps femoris* meat color value (MCV2, $p < 0.05$), *biceps femoris* marbling score (MM2, $p < 0.01$), *longissimus dorsi* intramuscular fat percentage (IMF, $p < 0.01$) and *longissimus dorsi* Water moisture content (WM, $p < 0.01$) in the population 2. However, further studies are needed to confirm these preliminary results.

Key words: meat quality, *MYF*5 gene, pigs, polymorphism, *Sus scrofa*).

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Introduction

Continued genetic improvement of domestic pigs (*Sus scrofa*) requires molecular markers to assist selection. In some cases both genes and the underlying causal mutation have been identified by the candidate gene approach (Harlizius and van der Lende, 2001; Li *et al.*, 2002; Xu *et al.*, 2005). Animal breeders have started applying marker-assisted selection to improve the quality and performance of livestock (van der Steen *et al.*, 2005) and genetic polymorphisms (marker loci) significantly associated with important traits have become very useful tools.

After slaughtering an animal muscle tissue becomes meat and meat quality traits, controlled by multiple genes, are economically important traits in all animals reared for meat production, including pigs (Zhao *et al.*, 2004). Myogenic regulatory factors (MRF) are involved in muscle development from commitment and proliferation through muscle fiber formation and postnatal muscle maturation and function (Hughes *et al.*, 1999). The known myogenic regulatory factors are the *MYOD1*, *MYF*5, *MYOG* (*myogenin*) and *MYF6* (*MRF4*) genes which encode highly conserved basic helix-loop-helix (bHLH) proteins (Olson and Klein, 1994). The *MYF*5 gene (Ott *et al.*, 1991) is first inducibly expressed in muscle cells during embryonic muscle development, with many discrete regulatory elements being involved in the activation and maintenance of *MYF*5 gene expression in the various muscle precursor populations (Teboul *et al.*, 2002). The effects of *MYF*5 gene restriction fragment length polymorphism (RFLP) on carcass traits have been described by various authors (Stratil and Cepica, 1999; te Pas *et al.*, 1999; Cieslak *et al.*, 2002; Urbanski and Kuryl, 2004) and this gene has been considered a candidate gene for meat production and meat quality (te Pas, 2004; Carmo *et al.*, 2005). However, few studies on the effect of *MYF*5 gene polymorphisms on meat quality have, in fact, been published and before such polymorphisms can be used efficiently in breeding and management decisions studies with different polymorphisms in different populations are required to properly characterize any associations of this gene with economically important traits across pig populations.

During the study described in this paper we used the polymerase chain reaction (PCR) and RFLP to identify novel *MYF*5 gene polymorphisms in pure and crossbred pig populations with the aim of elucidating the relationship between *MYF*5 genotypes and meat quality traits.

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Materials and Methods

Animals and data collection

We examined 302 pigs with documented records, the pigs being divided into two populations: population 1 (P1, n = 130), composed of 28 Yorkshire (Y), 46 Landrace (L), 21 Yorkshire Landrace (YL) and 35 Landrace Yorkshire (LY) pigs; and population 2 (P2, n = 172), consisting of 50 Meishan (M), 78 Yorkshire Meishan (YM) and 44 Meishan Yorkshire (MY) pigs. All the pigs were born and raised at Jingpin pig station, Huazhong Agriculture University, Peoples Republic of China.

At slaughter the pigs were stunned using a head-only electric stun-tong apparatus (SFK Meat Systems, a.m.b.a, Kolding, Denmark) after at least two hours rest, slaughtered by the sticking method, exsanguinated, scalded, mechanically dehaired, eviscerated and weighed. The left side of each carcass was used to assess meat quality. At 45 min postmortem we used a portable digital pH-Meter (model 646, Knick, Berlin) to measure the pH of the last thoracic vertebral *longissimus dorsi* (LD), the *biceps femoris* (BF) and the *semispinalis capitis* (SC) muscles. At the same time, we measured the drip loss rate (DLR, %) and water holding capacity (WHC, %) by the press technique (Wierbicki and Deatherage, 1958) using a 2.523 cm diameter 1 cm high columnar meat sample pressed for 5 min between 36 medium-speed filter papers (Xinhua Paper Industry Co., LTD, China) using a swelling press (Qinchuan Electric Apparatus Factory, China) and an applied force of 35 kg (Xiong and Deng, 1999).

At one to two hours postmortem we used a reflectometer (Model 43, Diffusion Systems Ltd, UK) to measure the objective meat color value (MCV, normal color value is 15 to 25) of the LD muscle (MCV1) on the freshly cut surface of a one cm thick chop removed from the thorax-waist LD (Hornsey, 1956) and the same chop was also given a subjective color score using a standard 1 to 5 color scale (NPPC, 1991) in which 1 represented a very bright color, 3 normal quail-meat color and 5 a very dark color. The same system was used to score the BF MCV (MCV2) of muscle from the core of a hind leg. A subjective meat marbling (MM) score was also given to the thorax-waist LD (MM1) and BF (MM2) muscles using the international marbling standard (American system) 5-grade marking system scale of 1 to 5 in which 1 indicates that the muscle is devoid of marbling, 2 that it is practically devoid of marbling, 3 that marbling is moderately abundant, 4 that marbling is abundant and 5 that marbling is overly abundant (NPPC, 1991).

One day after slaughter, intramuscular fat percentage (IMF, %) of the last thoracic vertebral LD was determined by chloroform-methanol extraction (Bligh and Dyer, 1959). Water moisture content (WM, %) was measured in a drying oven at 102 °C for 18 h (Bourke *et al.*, 1970). Genomic DNA was isolated from blood samples using a standard phenol: chloroform extraction method (Blin and Stafford, 1976).

Primers, amplification and PCR-RFLP analysis

We designed two PCR primers based on the porcine *MYF*5 gene sequence (GenBank, accession number Y17154), the *MYF*5-p1 primer (5'CGGAGAAGATGGA CCTGAT3' and 5'ATTTCCTCTTGCACGCTTT3') amplifying 243 base pairs (bp) of exon 1 of the *MYF*5 gene and the *MYF*5-p2 primer (5'GAGACGGGTGGCTGTGAA T3' and 5'AGGCTGAG AATCGGTGCTG3') amplifying 1193 bp of intron 1 and 2 and exon 2 of the *MYF*5 gene.

The PCR amplification was carried out in a 20 μL final volume containing 25 ng of genomic DNA as template, 0.25 μM of each dNTP (MBI Fermentas, Lithuania), 0.25 μM of each primer and 1 unit of *Taq* polymerase (Biostar Internation, Canada) in 1PCR reaction buffer (Biostar Internation, Canada). The PCR conditions consisted of an initial denaturation at 95 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 45 s, annealing at 57 °C for *MYF*5-p1 or 63 °C for *MYF*5-p2 for 45 s and extension at 72 °C for 1 min, with a final 72 °C extension for 10 min.

The *MYF*5 alleles were analyzed using a RFLP protocol in which 8.5 μL of the PCR products were digested with 5 units of restriction enzyme (*Hsp92II* for the *MYF*5-p1 primer, *HinfI* for the *MYF*5-p2 primer) at 37 °C for 4 h in 10 μL of 1 buffer and the digestion products separated by electrophoreses on 1.5% (w/v) agarose gels using 1TAE buffer (Sambrook *et al.*, 1989), the gels being stained with ethidium bromide.

Statistical analysis

Associations between the *MYF*5 gene haplotypes and meat quality traits were evaluated using the least square method of the GLM (General Linear Models) procedure and the Statistical Analysis Software version 8.0 (SAS Institute Inc., Cary, USA). The model used to analyze the data was assumed to be:

$$Y_{ijklm} = \grave{\imath} + S_i + B_j + G_k + D_l + P_m + b_{ijklm}X_{ijklm} + e_{ijklm}$$

where Y_{ijklm} is the observation of the trait; i is the population mean, S_i is the effect of ith sex (i = 1 for male or 0 for female), B_j is the effect of j^{th} breed, G_k is the effect of kth haplotype, D_l is the effect of boars, P_m is the batch effect, b_{ijklm} is the regression coefficient of the slaughter age and e_{ijklm} is the random residue.

Results

Genotype frequencies in the different populations

Two fragments, *MYF*5-p1 (243 bp) and *MYF*5-p2 (1193 bp) were PCR amplified and sequenced from genomic DNA. Multiple alignments of the sequences of six indi-

vidual samples (2 Yorkshire, 2 Landrace and 2 Meishan pigs) allowed the identification of a novel single nucleotide polymorphism (SNP) adenine to cytosine (methionine (ATG) to leucine (CTG) shift) substitution (*MYF5*/*Hsp92II*, detected with the *MYF5*-p1 primer) in exon 1 and a previously described cytosine/guanine mutation (*MYF5*/*HinfI*, detected with the *MYF5*-p2 primer) in intron 1 (te Pas *et al.*, 1999). We applied the *Hsp92II* and *HinfI* PCR-RFLP protocols to genotyping the exon 1 and intron 1 mutations in 302 pigs with phenotypic records and designated the *MYF5*-p2 primer *MYF5*/*HinfI* genotypes as AA (1.19 kb), AB (1.19 kb + 729 bp + 464 bp) and BB (729 bp + 464 bp), while the *MYF5*-p1 primer *MYF5*/*Hsp92II* genotypes as CC (243 bp), CD (243 bp + 210 bp + 33 bp) and DD (210 bp + 33 bp).

The combined effects of the exon 1 and intron 1 substitutions were estimated as haplotype substitution effects. The combined genotype and haplotype frequencies of the *Hsp92II* and *HinfI MYF5* gene polymorphisms for pigs with records in the two populations studied are shown in Table 1. The *HinfI* locus had three genotypes in the population 1 and two genotypes (AA and AB) in population 2. The *Hsp92II* locus was monomorphic in population 1, where only the DD genotype was found, but polymorphic in population 2 where all three genotypes (CC, CD and DD) were detected (Table 1). In population 2 the values in Table 1 suggest the presence of six haplotypes (AA/CC, AA/CD, AA/DD, AB/CC, AB/CD and AB/DD) but since the AB/CC haplotype was detected in only one pig we excluded this pig and the AB/CC haplotype from the association analyses. Moreover, only three of the nine possible genotypic combinations of the two polymorphisms were recorded in population 1. Table 1 also shows that the AA/DD haplotype was present at high frequencies in both population 1 (0.785) and 2 (0.529).

Table 1 - Genotypic frequency distributions for the *MYF5* gene *HinfI* and *Hsp92II* polymorphic loci haplotypes for two pig populations. The number of pigs with records for each genotype frequency is shown in parentheses. Population 1 (n = 130), composed of 28 Yorkshire, 46 Landrace, 21 Yorkshire¡ x Landrace and 35 Landrace x Yorkshire pigs; and population 2 (n = 172), consisting of 50 Meishan, 78 Yorkshire x Meishan and 44 Meishan x Yorkshire pigs.

Population and *HinfI* genotype	*Hsp92II* genotype frequency			
	CC	CD	DD	Combined
Population 1				
AA	0	0	0.785 (102)	0.785 (102)
AB	0	0	0.192 (25)	0.192 (25)
BB	0	0	0.023 (3)	0.023 (3)
Combined	0	0	1.00 (130)	1.000 (130)
Population 2				
AA	0.052 (9)	0.285 (49)	0.529 (91)	0.866 (149)
AB	0.006 (1)	0.047 (8)	0.081 (14)	0.134 (23)
BB	0	0	0	0
Combined	0.058 (10)	0.332 (57)	0.610 (105)	1.000 (172)

Relationship between *MYF5* genotypes and meat quality traits

In population 1 there were only three *MYF5* haplotypes (AA/DD, AB/DD and BB/DD) but these did not differ for any meat quality trait analyzed (data not shown). We focused our association analysis on the five population 2 haplotypes (AA/CC, AA/CD, AA/DD, AB/CD and AB/DD) and found statistically highly significant associations with the MM2, IMF and WM traits and significant associations with the DLR, WHC and MCV2 traits (Table 2). For the DLR and WHC traits the effect for the AA/CD haplotype was significantly different ($p < 0.05$) to the AA/DD haplotype, replacing the AA/DD haplotype by the AA/CD haplotype would significantly decrease WHC by 0.78% and increase the DLR by 0.583%. For the MCV2 trait, the effect of the AB/CD haplotype was significantly different ($p < 0.05$) to the AB/DD haplotype in population 2 and replacing the AB/DD with AB/CD would significantly decrease the MCV2 by 0.597. The AA/CC haplotype was associated with the highest significant IMF value ($p < 0.01$) and the lowest significantly WM value ($p < 0.01$). In contrast, pigs carrying the AB/CD haplotype had the lowest IMF and MM2 values but the highest WM values of all the haplotypes.

Discussion

Research on mutations in targeted functional genes (candidate genes) and their association with economic traits has been performed to ascertain the genetic basis of production traits and to develop DNA tests as selection tools in pig breeding schemes (de Vries *et al.*, 1998). This approach is a very promising route for the improvement of meat quality, since direct meat quality records are not available for potential breeding animals (Óvilo *et al.*, 2006). A well known example is the gene tests used to remove the Halothane (*HAL*) and ryanodine receptor (*RN*) mutations which have undesirable effects on meat quality, these tests having resulted in a clear improvement of the technological quality of the pork produced in many countries (Fujii *et al.*, 1991; Le Roy *et al.*, 2000). In order to deal with growing market segmentation new genetic techniques are needed to adapt technological and sensory qualities to the requirements of processors and consumers (Monin, 2003; Óvilo *et al.*, 2006).

The *MYF5* gene plays a key regulatory role in the initiation and development of skeletal muscle and the maintenance of its phenotype, and is thus a candidate gene for involvement in traits related to growth and meat quality (Maak *et al.*, 2006). In our study we constructed and used the *MYF5*-p1 primer to identify a novel SNP, the *Hsp92II* polymorphic loci, an adenine to cytosine shift resulting in a methionine (ATG) to leucine (CTG) amino acid substitu-

Table 2 - Association of haplotypes in the *MYF*5 gene with the phenotype value of partial meat quality traits in population 2 (n = 172), consisting of 50 Meishan, 78 Yorkshire x Meishan and 44 Meishan x Yorkshire pigs. Within-trait (*i.e.* within the same row) significant differences between the genotype classes are indicated by superscripts, lower case at $p < 0.05$ and upper case at $p < 0.01$.

	Genotype* (Least square means ± standard error)				
Traits	AA/CC	AA/CD	AA/DD	AB/CD	AB/DD
Drip Loss Rate (DLR, %)	5.979 ± 0.520	6.536 ± 0.229^{a}	5.953 ± 0.161^{b}	6.059 ± 0.546	6.425 ± 0.416
Water Holding Capacity (WHC, %)	91.777 ± 0.691	91.091 ± 0.304^{a}	91.871 ± 0.214^{b}	91.772 ± 0.725	91.242 ± 0.552
BF Meat Color Value (MCV2)	17.144 ± 0.215	17.257 ± 0.095	17.317 ± 0.067	16.943 ± 0.226^{a}	17.540 ± 0.172^{b}
BF marbling score (MM2, on a 1 to 5 scale)	4.142 ± 0.030	4.131 ± 0.013^{A}	4.176 ± 0.009^{B}	4.086 ± 0.031^{Aa}	4.164 ± 0.024^{b}
Intramuscular fat percentage (IMF, %)	4.716 ± 0.202^{Aa}	4.082 ± 0.089^{B}	4.140 ± 0.062^{B}	4.016 ± 0.212^{b}	4.101 ± 0.161^{b}
Water moisture content (WM, %)	72.654 ± 0.223^{Aa}	73.288 ± 0.098^{B}	73.228 ± 0.069^{b}	73.646 ± 0.234^{B}	73.343 ± 0.179^{b}

tion in exon 1 of the *MYF*5 gene and investigated this polymorphism and the previously reported intron 1 *HinfI* polymorphism (te Pas *et al.*, 1999) in two populations (n = 324).

Te Pas *et al.* (1999) investigated the *HinfI* polymorphic site in the first intron of the *MYF*5 gene and found that the A-allele predominated in the all pig breeds tested (including those tested by us), which is supported by our results (Table 1). However, Te Pas *et al.* (1999) found no genotypically associated differences for any of the traits investigated (birth weight, weight at slaughter, growth rate, meat weight and subcutaneous fat thickness) in 1216 Yorkshire pigs and concluded that the porcine *MYF*5 gene lacks a significant causal mutation affecting these traits, or that the linkage phase of the *MYF5/HinfI* polymorphism is not in phase with the *MYF*5 causal mutation. However, Cieslak *et al.* (2002) analyzed the *MYF*5/*HinfI* locus in 333 unrelated (Pietrain, Zlotnicka Spotted, Polish Landrace, Pietrain (Pietrain Zlotnicka Spotted), Pietrain (Polish Large White Polish Landrace) and Dutch Large White Dutch Landrace) pigs with an equal proportion of young female pigs (gilts) and castrated male (barrows) pigs and found that gilts with the TT *RYR1* genotype and AA or AB genotype at the *MYF*5/*HinfI* locus had a significantly higher loin eye area and carcass meat content than pigs without this genotype. Thus, it is still difficult to evaluate the effect of the *MYF*5/*HinfI* polymorphism and the different results may depend on the pig breed investigated and statistical model used.

Traditionally, one single nucleotide polymorphism (SNP) is used for genotyping and association analysis. However, using haplotypes, which are specific combinations of nucleotides on the same chromosome, will provide more information on the complex relationship between DNA variation and phenotypes than any single SNP can provide (Stephens *et al.*, 2001; Grindflek *et al.*, 2004). Thus, we used the haplotype information to evaluate the relationship between *MYF*5 polymorphisms and meat quality traits. Our results showed that two haplotypes, AA/CD and AA/DD, were highly frequent in population 2 and showed significant difference in their effects on drip loss rate (DLR) ($p < 0.05$) and water holding capacity (WHC) ($p < 0.05$). This supports the results of Carmo *et al.* (2005), who found that *MYF*5 gene allelic variants had a significant effect on DLR, cooking properties and total cooking loss in a divergent F2 pig population (n = 359) of Brazilian Piau boars and commercial white females and that an insertion (I) variation in the *MYF*5 gene is associated with water-holding capacity in the same population. In our work, we also found that there were significant differences in population 2 between the different haplotypes in respect of the *biceps femoris* meat color value (MCV2, $p < 0.05$), *biceps femoris* marbling score (MM2, $p < 0.05$), *longissimus dorsi* intramuscular fat percentage (IMF, $p < 0.01$) and *longissimus dorsi* moisture content (WM, $p < 0.01$). Similarly, the differences for MM2 ($p < 0.01$), IMF ($p < 0.01$) and WM ($p < 0.01$) were also found among different *MYF5/Hsp92II* genotypes in population 2 (data not shown). Thus, the mutation (*MYF5/Hsp92II*) in coding regions could be responsible for changes in muscle protein structure or function and lead to changes in meat quality. Of course, the mutation is also possible to link to a quantitative trait locus (QTL) or loci. Because *MYF*5 has been localized to the *Sus scrofa* chromosome 5 (SSC5) (Soumillion *et al.*, 1997) and a meat quality QTL has been mapped between the *MYF*5 and SW967 regions of this chromosome in the W x M family (Lee *et al.*, 2003). In addition, the insulin-like growth factor I (*IGFI*) and *MYF6* genes have been located on the same chromosome as the *MYF*5 gene (Wintero *et al.*, 1994; Vykoukalova *et al.*, 2003).

To better assess the real impact of the effects of the *MYF*5 gene on meat quality, further investigations are needed to confirm our results, such as an appropriate area for research being the possible effects of other genes in linkage disequilibrium with the *MYF*5 SNPs.

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