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RESEARCH ARTICLE

# Identification of genome regions and important variations associated with expression level of *MYH* genes and its correlation with meat quality traits in pigs

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**Abstract** Meat quality is one of the most important economic traits in pig breeding. It has been reported that the composition of type II muscle fibers is correlated with meat quality in pigs. Type II muscle fibers contain three isoforms, IIa, IIb and IIx, which contain specific myofibrillar proteins MYH2, MYH4 and MYH1, respectively. In this study, the expression levels of *MYH1*, *MYH2*, and *MYH4* genes in the Longissimus thoracis (LT) muscle were measured in 114 Yorkshire pigs. Further, the correlations between the expression level of *MYH* genes and the meat quality traits of intramuscular fat, drip loss, water-holding capacity and postmortem pH values were analyzed. The results showed that the expression level of *MYH2* was positively correlated with the intramuscular fat ($R = 0.20$, $P < 0.05$). The expression levels of *MHY1* and *MYH4* were negatively correlated with the pH at 24 h post mortem ($pH_{24h}$ ; $R = –0.28$, $P < 0.01$; $R = –0.25$, $P < 0.01$, respectively). Besides, the 60K SNP chip was used for genotyping the individuals. Genome wide association analysis indicated that 15 SNPs were significantly associated with the expression levels of these three *MYH* genes. The results indicated the expression levels of *MYH1*, *MYH2* and *MYH4* genes could be useful markers for improvement of meat quality in pigs.

**Keywords** pig, IMF, pH, *MYH1*, *MYH2*, *MYH4*

## 1 Introduction

Skeletal muscle fibers are classified as slow twitch (type I or red muscle) and fast twitch (type II or white muscle) fibers. Type I fibers are rich in mitochondria, which also called slow oxidative muscle fibers. Type II fibers can be further differentiated into 3 isoforms: type IIa is called fast oxidative type, type IIb\IIx are called fast glycolytic type. These four skeletal muscle types contain their unique myofibrillar proteins MYH1 (type IIx), MYH2 (type IIa), MYH4 (type IIb) and MYH7 (type I)[1,2]. They belong to MyHC (myosin heavy chain) family, and are located on porcine chromosome 12.

It is generally accepted that the composition of muscle fiber is related to meat quality. The increase of type I fibers can cause postmortem pH decrease, and improve water holding capacity of skeletal muscle in the pig[3]. In contrast, type IIB fibers have been found to be closely related to paleness, toughness and low water holding capacity of skeletal muscle, which could also affect meat quality of pigs[4,5]. IMF and postmortem pH value could affect meat flavor and juiciness. Previous studies indicated that the heart type FABP (*H-FABP*) gene and the adipocyte type FABP (*A-FABP*) gene are candidate genes for the IMF trait. The *H-FABP* gene is predominantly expressed in cardiac and skeletal muscle cells. It can influence the long-chain fatty acid oxidation and uptake, as well as energy homeostasis in skeletal muscle tissues[6,7]. Moreover, *A-FABP* can play a critical role in the balance of lipolysis and lipogenesis, which was important for the deposition of IMF[8,9]. In addition to IMF, pH is another important factor for palatability that affects pork quality. It is accepted that the pH is influenced by slaughter procedure as well as genetics. As reported, two major genes related to pig meat pH value have been identified: the Ryanodine receptor 1 (*RYR1*)[10] and the *PRKAG3* gene[11]. In addition to these single genes effects, some quantitative trait loci (QTL) for meat pH on pig chromosome 2 have also been identified (pig QTL database, PigQTLdb)[12–14].

Previous studies have found different fiber composition

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among muscle types. However, few studies have shown the relationship between the expression of type II *MYH* genes and meat quality traits. In this study, we investigated the correlation between the expression level of *MYH1*, *MYH2* and *MYH4* genes and meat quality traits in Yorkshire pigs. It was found that the expression level of *MYH2* gene was positively correlated with the IMF, and *MHY1* and *MYH4* genes were negatively correlated with $pH_{24h}$. Additionally, genome wide association analysis of *MYHs* expression levels was conducted, and 15 potential candidate SNPs were found to be related to the expression levels of *MYH* genes. This study provided new evidence for the correlation between meat quality traits and the expression levels of *MYH* genes.

## 2 Materials and methods

### 2.1 Animals and tissues

The 114 castrated purebred Yorkshire boars were slaughtered. The IMF content, water-holding capacity (WHC), drip loss (DLS), 45 min and 24 h postmortem pH value ($pH_{45min}$ and $pH_{24h}$) traits of the 114 Yorkshire castrated boars were measured as previously described[15]. The Longissimus thoracis (LT) muscle samples were collected, immediately after slaughtered and stored at −80°C for RNA isolation.

### 2.2 Total RNA isolation and cDNA synthesis

Total RNAs were isolated using an RNA isolation reagent (Takara RNAiso Plus, Tokyo) according to the manufacturer's protocol. The RNA integrity was checked using denaturing gel electrophoresis and the RNA concentration was measured with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham). Total RNAs were reverse-transcribed using a RevertAidTM First Strand cDNA Synthesis Kit (Fermentas, Lithuania). Oligonucleotide primers for *MYH1* and *MYH2* genes were derived from the literature[16] and the primers for *MYH4* gene were designed using Oligo 7 software (Wojciech Rychlik, Colorado). Primer sequences and PCR conditions are listed in Table 1.

### 2.3 Quantitative real-time PCR

The fragments of *MYH* genes used for qPCR were cloned and inserted into the pMD18-T vector (Takara, Tokyo). Then, the plasmids were extracted and the concentration was measured using a Beckman DU-640 spectrophotometer. Finally, the standard curves for the qPCR of these *MHY* genes were established by using plasmids serially diluted from $10^{-1}$ to $10^{-8}$ times. Quantitative Real-Time PCR was conducted on a Bio-Rad CFX384 Real-Time System, using SYBR Green PCR Master Mix (Bio-Rad, California) according to the instruction manual. Reactions were done in triplicate in 384 well plates. Each well (total volume of 10 μL) contained 1 μL cDNA with the following reagents: 5 μL 2 × SYBR Green PCR Master Mixture, 0.2 μL forward and reverse primers and 3.6 μL RNase-free $H_2O$. Samples were pre-incubated at 95°C for 5 min followed by 45 PCR amplification cycles (denaturation: 95°C for 30 s, annealing: 60°C for 30 s, elongation: 72°C for 20 s). A dissociation curve was generated at the end of the last cycle via collecting the fluorescence data from 65°C to 95°C.

The copy number of the *MYH* genes was calculated using the formulae:

$$\begin{aligned}&\text{Plasmid copies(number per microgram)}\\&=6.02\times10^{23}\text{(copies per mol)}\\&\times\text{plasmid concentration}\left(\text{g}\cdot\mu\text{L}^{-1}\right)\\&/\text{the molecular weight of plasmid}\left(\text{g}\cdot\text{mol}^{-1}\right)\end{aligned}$$

$$\lg C=(C_t-b)/a$$

$$\text{CP}=10^{(\lg C\times\text{CP of plasmid})}$$

where lg$C$ is the log of the gene copy numbers, $C_t$ is the threshold cycle, and a and b are the slope and intercept of the standard curve; CP is the copy number of the *MYH* genes.

### 2.4 60K SNP genotyping

Genomic DNA extraction from ear tissue was performed with a TIANamp Genomic DNA Kit (Tiangen, Beijing,

**Table 1** List of oligonucleotide primers of *MYH* genes

| Gene | Primer symbol | Primer sequence 5′–3′ | Product length/bp | $T_m$/°C |
|---|---|---|---|---|
| *MYH1* | MYH1-F | AGAAGATCAACTGAGTGAACT | 149 | 58 |
| | MYH1-R | AGAGCTGAGAAACTAACGTG | | |
| *MYH2* | MYH2-F | GCTGAGCGAGCTGAAATCC | 137 | 60 |
| | MYH2-R | ACTGAGACACCAGAGCTTCT | | |
| *MYH4* | MYH4-F | ACCTCTCCAAGTTCCGCAAG | 172 | 59 |
| | MYH4-R | TGTGCATTTCTTTGGTCACTTT | | |

China) following the instruction manual. Illumina 60 K Porcine SNP Beadchip (Illumina, Inc., San Diego, CA, USA) was used for genotyping, which contains 61177 SNP sites across the whole genome. Quality control was assessed with SNP call rate > 90%, minor allele frequency > 0.03, and the final SNP sets included 33759 SNPs for genome-wide association analysis.

### 2.5 Statistical analysis

Analysis of variance was performed on the data (SAS, 2002). Simple correlation coefficient analysis was used to analyze the relationship between the *MYHs* and meet quality traits. GWAS was performed using GAPIT software to identify the candidate SNPs for the expression level of *MYHs*[17], and the threshold of empirical *P* value was set to $1 \times 10^{-3}$ for each phenotype.

## 3 Results

### 3.1 Expression level of *MYH* genes in LT muscle

The expression level of the *MYH* genes of 114 LT muscle samples was analyzed using qPCR. The results showed that all the three *MYH* genes were highly expressed in the LT muscle. *MYH4* was the gene with the highest expression and *MYH2* the lowest (Fig. 1).

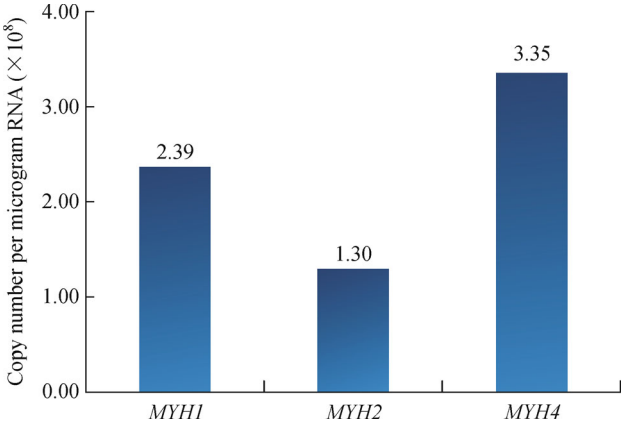


**Fig. 1** The expression level of *MYH1*, *MYH2* and *MYH4* genes in LT muscle in 114 Yorkshire pigs

### 3.2 Correlation between *MYH* genes expression and meat quality traits

The expression level of *MYH2* gene was positively correlated with the IMF content ($R = 0.20$, $P < 0.05$). *MYH1* and *MYH4* genes were negatively correlated with $pH_{24h}$ ($R = -0.28$, $P < 0.01$; $R = -0.25$, $P < 0.01$) (Table 2).

### 3.3 Genome-wide association study

After quality control, 33759 SNPs distributed on 19 chromosomes were chosen for GWAS analysis. Fifteen candidate SNPs were significantly ($P < 1 \times 10^{-3}$) associated with the expression level of *MYH* genes (Table 3). Among them, four SNPs were associated with the expression level of *MYH2* gene, five with *MYH1* gene and six with *MYH4* gene. Some important genes that were closely linked with these SNPs were also identified. Among them, the mitochondrial ribosomal protein S9 (*MRPS9*) gene was linked with SNP ASGA0014535, interacting factor homolog (*RIF1*) gene linked with DIAS0001219, inter-alpha-trypsin inhibitor heavy chain 2 (*ITIH2*) gene linked with ASGA0048857, and both centriolar protein (*POC5*) and synaptic vesicle glycoprotein 2C (*SV2C*) genes linked with ASGA0010629 (Table 3).

## 4 Discussion

Meat quality is an important economic trait and it is one of the major research areas in pig breeding. The molecular mechanisms underlying meat quality are largely unknown in pig. In this study, we investigated the correlation between the expression of type II muscle fiber marker genes and meat quality traits. Furthermore, we detected

**Table 2** Correlation coefficients for a matrix of expression levels of *MYH* genes and meat quality traits

| | *MYH1* | *MYH2* | *MYH4* | WHC | $pH_{45min}$ | $pH_{24h}$ | DLS | IMF |
|---|---|---|---|---|---|---|---|---|
| *MYH1* | 1 | 0.61 | 0.96 | −0.16 | 0.11 | −0.28** | −0.17 | 0.01 |
| *MYH2* | | 1 | 0.52 | 0.09 | 0.16 | 0.17 | 0.14 | 0.20* |
| *MYH4* | | | 1 | −0.12 | 0.08 | −0.25** | −0.18 | < 0.01 |
| WHC | | | | 1 | 0.04 | 0.12 | 0.23 | −0.13 |
| $pH_{45min}$ | | | | | 1 | 0.18 | 0.03 | −0.11 |
| $pH_{24h}$ | | | | | | 1 | 0.24 | −0.09 |
| DLS | | | | | | | 1 | −0.29 |
| IMF | | | | | | | | 1 |

Note: WHC, water-holding capacity; $pH_{45min}$ and $pH_{24h}$, pH at 45 min and 24 h; DLS, drip loss within 24 h, IMF, intramuscular fat content; *, $P < 0.05$; **, $P < 0.01$.

**Table 3** SNPs and genes related to the expression levels of *MYH* genes

| Gene | SNP | Chr. | Position | Associated gene | Variant | *P*-value ($\times 10^{-4}$) |
|---|---|---|---|---|---|---|
| *MYH1* | MARC0059864 | 10 | 75940391 | *ENSSSCG00000011153* | Downstream gene | 4.88 |
| | ASGA0071803 | 15 | 156955591 | *ENSSSCG00000016397* | Intron | 5.18 |
| | DIAS0001219 | 11 | 9547194 | *NSSSCG00000009348*<br>*RIF1* | Intergenic | 9.25 |
| | ALGA0059421 | 10 | 61014593 | – | Intergenic | 9.55 |
| | ASGA0048857 | 10 | 69757171 | *ITIH2* | Upstream gene | 9.68 |
| *MYH2* | ASGA0014535 | 3 | 52293293 | *MRPS9* | Intergenic | 4.93 |
| | ALGA0109569 | 3 | 51843143 | *TGFBRAP1* | Intron | 6.42 |
| | MARC0059864 | 10 | 75940391 | *ENSSSCG00000026093* | Downstream gene | 8.25 |
| | ALGA0090727 | 16 | 54349200 | *ENSSSCG00000016979* | Intergenic | 8.45 |
| *MYH4* | ASGA0010629 | 2 | 86507283 | *POC5*<br>*SV2C* | Intergenic | 2.85 |
| | H3GA0011751 | 4 | 8230320 | *ENSSSCG00000027885*<br>*WISP1* | Intron<br>Intergenic | 6.47 |
| | ASGA0101845 | 2 | 88201093 | *TBCA* | Intron | 6.94 |
| | MARC0059864 | 10 | 75940391 | *LARP4B* | Downstream gene | 8.73 |
| | ALGA0084260 | 15 | 21658450 | *ENSSSCG00000024217*<br>*ENSSSCG00000023479* | Intergenic | 9.54 |
| | ASGA0071803 | 15 | 156955591 | *ENSSSCG00000016397*<br>RIF1 | Intron<br>Intergenic | 9.77 |

candidate SNPs and genes that are associated with the expression of *MYH* genes using porcine 60K SNP chip.

*MYH* genes are the markers of the muscle fibers and studies indicated that muscle fiber composition was correlated with the meat quality traits of IMF, drip loss, pH and meat color[5]. The correlation between the expression of *MYH* genes and meat quality traits was largely unknown. In this study, we investigated the relationship between the expression of type II muscle fibers' marker genes and meat quality traits. The expression of *MYH2* gene was found to be positively correlated to IMF. It has been reported that the expression of *MYH2* gene is positively related with IMF in Laiwu pigs and Duroc[18], which is consistent with the findings reported here. Also, the expression of *MYH1* and *MYH4* genes were found to be negatively correlated with $pH_{24h}$. One previous study indicated that the higher pH value was due to the lower percentage of type IIb fibers in the Berkshire pigs[19]. Also, increasing the proportion of type IIb muscle fibers has been shown to decrease postmortem pH in pigs[4]. These results indicated that the proportion of type IIb fibers was negatively correlated with pH value in pigs. Therefore, our study was in accordance with these previous studies of muscle fiber composition, meat quality and pH value. These results indicated that the expression of *MYH* genes may be markers of meat quality traits.

In this study, 15 SNPs were found to be associated with the expression levels of *MYH* genes. For the *MYH1* gene, one SNP was located upstream of the *RIF1* gene. As reported, RAP1 could specifically control actin organization and myosin II activity[20], and *RIF1* deficiency led to failure in embryonic development[21]. Thus, *RIF* may be important for the activity of the myosin heavy chain gene and it may be a candidate gene for *MYH* expression levels. For the *MYH2* gene, one SNP was closely linked with the *MRPS9* gene. It has been reported that MRPS9 is a member of the mammalian mitochondrial ribosomal proteins family, which promotes protein synthesis within the mitochondrion[22]. It is known that one of the main differences between type I and type II muscle fibers is the content of mitochondria. Thus the *MRPS9* gene may affect the muscle fiber type through regulating the expression of *MYH2*. One SNP associated with the expression of *MYH4* gene was linked with the *SV2A* gene. It has been shown that SV2A is a synaptic vesicle protein, which is selectively localized in motor nerve terminals on slow (type I and partial type IIA) muscle fibers, and its expression decreased in the fast motoneurons during the first postnatal week[23]. Thus, SV2A may be a marker of myosin heavy chain. These genes closely linked with the candidate SNPs are important in the regulation of the expression of *MYH* genes. These results indicated that the SNPs and the expression level of *MYH* genes could be useful markers for meat quality traits in pigs.

## 5 Conclusions

This study found that expression levels of *MYH1*, *MYH2* and *MYH4* genes were significantly correlated with meat quality traits including IMF and pH value. Additionally, SNPs associated with *MYH* expression levels were detected along with the functional genes linked with these SNPs. These results indicate the expression levels of *MYH1*, *MYH2* and *MYH4* genes could be useful markers for improvement of meat quality in pigs.

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All applicable institutional and national guidelines for the care and use of animals were followed.

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