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Effects of intergenic single nucleotide polymorphisms in the fast myosin heavy chain cluster on muscle fiber characteristics and meat quality in Berkshire pigs

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ABSTRACT

The heterogeneity of myosin heavy chain (MyHC) isoforms is closely related to muscle fiber characteristics, and meat quality in pigs. The fast MyHC gene cluster on porcine chromosome 12 contains *MYH2*, *MYH1*, and *MYH4*, which encode three fast MyHC isoforms expressed in adult skeletal muscle. Here, we identified four intergenic single nucleotide polymorphisms (SNPs) in the gene cluster. Of these, SNP1 and SNP2, which were located near *MYH2*, were genotyped in 199 Berkshire pigs. SNP1 were significantly associated with the total fiber number ($P = 0.046$) and intramuscular fat contents ($P = 0.041$), and SNP2 had significant effects on type I fiber number/area compositions ($P = 0.039$ and $P = 0.041$, respectively), water holding capacity (drip loss, $P = 0.045$; cooking loss, $P = 0.001$), and meat color ($P = 0.003$). Taken together, our results suggest that the intergenic SNPs near *MYH2* can affect muscle fiber formation and meat quality by modifying the regulatory elements of this gene cluster in pigs.

1. Introduction

Myosins are fundamental myofibrillar proteins found in muscle fibers, and are involved in muscle contraction (Schiaffino & Reggiani, 1996). Myosin molecules comprise two homodimerized heavy chains, two regulatory light chains, and two essential light chains (Weiss & Leinwand, 1996). In particular, the myosin heavy chains (MyHCs) containing the ATP-binding and the actin-binding domains have a major influence on the ATPase activity of myosin, and consequently on the contractile properties of muscle. In mammals, there are various MyHC isoforms with different ATPase activities in skeletal muscle (Lefaucheur et al., 1998). In pigs, four MyHC isoforms (I, IIa, IIx, and IIb) have been identified in adult skeletal muscle (Chang & Fernandes, 1997; Lefaucheur et al., 1998). They are classified into the slow MyHC isoform (type I) and the fast isoforms (type IIa, IIx, and IIb) based on their ATPase activities and maximum contractile velocities, which increase in the order type I, IIa, IIx, IIb (Galler, Schmitt, & Pette, 1994). The heterogeneity of MyHC isoforms in muscle fibers corresponds to histochemical muscle fiber types, which are classified into slow/oxidative (type I), fast/glycolytic (type IIb), and intermediate (type IIa) (Choi, Ryu, & Kim, 2007). Previous studies have revealed a considerable association of muscle fiber type composition with pork quality traits due to the effects on the postmortem metabolic rate in conversion of muscle to meat (Ryu & Kim, 2005).

Porcine MyHC isoforms are each encoded by a separate gene (Sun, Da Costa, & Chang, 2003). *MYH2*, *MYH1*, and *MYH4* encode the fast MyHC isoforms IIa, IIx, and IIb, respectively, and are sequentially located in a cluster on porcine chromosome (SSC) 12 (Davoli et al., 2002). The clustered genomic structure of the fast MyHC isoform genes is completely conserved in humans (chromosome 17) and mice (chromosome 11). In addition, fast MyHC genes show temporal expression patterns that correspond to their order in the cluster during prenatal muscle growth in humans, mice, and pigs (Sun et al., 2003). Notably, *longissimus dorsi* (LD) muscle, which is major skeletal muscles in pigs, contain predominantly fast isoforms, and the relative expression levels of fast MyHC genes are closely related to the variation in muscle properties among breeds and individuals (Gunawan et al., 2007; Wimmers et al., 2008). In addition, the syntenic genomic cluster region is contained within quantitative trait loci (QTLs) for meat quality (Luo et al., 2012; Xiong et al., 2015), and the dynamics of fast MyHC gene expression are closely related to variation of meat quality traits such as muscle pH, meat color, and water holding capacity (Choi et al., 2007; Kang et al., 2011).

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The coding sequences of the fast MyHC genes are very highly conserved between and within species, and there were few reports of polymorphism in the porcine fast MyHC genes (Chikuni, Tanabe, Muroya, & Nakajima, 2001). Therefore, the possibility of the regulatory DNA element in noncoding region for the orientation of MyHC isoforms was suggested by previous study (Da Costa & Chang, 2005), and it may explain expressional and morphological diversity of the fast MyHC. The objective of this study was to identify polymorphisms in the intergenic region of the fast MyHC gene cluster on SSC 12 and to investigate their effects on muscle fiber characteristics and meat quality traits in Berkshire pigs.

2. Materials and methods

2.1. Sequencing and polymorphism identification

To identify polymorphisms in the fast MyHC cluster and its flanking regions on SSC 12, specific primer sets (Table S1) and breed-specific DNA pools from Berkshire, Duroc, Landrace, and Yorkshire pigs were used for polymerase chain reaction (PCR) amplification. The purified PCR products were directly sequenced using an ABI 3730xl DNA Analyzer (Applied Biosystems, Foster City, CA), and polymorphisms were identified based on the resulting sequence alignments by SeqMan software (DNASTAR, Madison, WI, USA). The identified polymorphisms were named according to the Human Genome Variation Society's nomenclature guidelines.

2.2. Animals and muscle samples

A total of 199 Berkshire pigs (90 castrated males and 109 females) were genotyped in this study. Pigs with the same feeding conditions in different pens of the same farm were slaughtered according to a standard protocol, and all experimental procedures were approved by the Ministry of Agriculture, Food and Rural Affairs of Republic of Korea. At 45 min postmortem, the *longissimus dorsi* (LD) muscle at 8th to 9th *thoracic vertebrae* were used for muscle pH and muscle fiber characteristics measurement. After 24 h of chilling at 4 °C, the LD muscles (the 10th to 13th *thoracic vertebrae*) were sampled for other trait measurements. The backfat thickness was measured at the 11th and last *thoracic vertebrae*, and the mean value was used in this study. The loin eye area (LEA) was measured at the last rib.

2.3. Muscle fiber characteristics measurement

The sampled LD muscle at 45 min postmortem were cut into $0.5 \times 0.5 \times 1.0$ cm pieces, and promptly frozen by liquid nitrogen. Serial transverse muscle sections (10 μm) of each sample were obtained using cryostat (CM1 850, Leica, Germany) at −20 °C, and mounted on slide glasses. To classify the fiber types, myofibrillar adenosine triphosphatase staining methods (Brooke & Kaiser, 1970) were used in this study. Next, the histochemical images of all samples were analyzed by optical image analysis program (Image-Pro Plus, Media Cybernetics, L.P., USA). Approximately 600 fibers per sample, which were free from tissue disruption and freeze damage, were evaluated and classified into fiber types of I, IIa, IIb. The cross-sectional area (CSA) of the muscle fiber was expressed as the ratio of the total area of muscle fiber measured to the total number of fibers counted. Fiber density was estimated from the mean number of fibers per mm^2, and then the total number of muscle fibers was calculated as the LEA multiplied by the fiber density.

2.4. Meat quality measurement

Muscle pH at 45 min postmortem was measured directly on the carcass at 8th to 9th *thoracic vertebrae* using spear-type portable pH meter (HM-17MX, Toadkk, Japan). Following 24 h of chilling at 4 °C, the LD muscles were taken to evaluate other meat quality traits. To evaluate drip loss and cooking loss, the meat samples were freshly cut and immediately weighed (initial weights). The samples for drip loss were suspended in an inflated bag at 4 °C for 48 h, and weighed after being taken from the bag and gently blotted drying (final weight). The samples for cooking loss were place in polyethylene bag, and cooked in a boiling water bath to a central internal temperature of 71 °C. In endpoint temperature, the cooled sample at 4 °C until equilibration were taken from the bag, blotted dry, and weighed (final weight). Both drip loss and cooking loss were expressed as a percentage of the initial sample weight. The meat colors (lightness, redness and yellowness) were measured by a Minolta chromameter (CR-300, Minolta Camera Co., Japan) using LD muscle samples placed in a 4 °C cold room for 30 min to expose their surfaces to air (for bloom). The CR-300 apparatus contains a pulsed xenon lamp in the measuring head and was calibrated against the calibration plate supplied by the manufacturer. The aperture size was 8 mm. The illuminant was C and the standard observer position was 2°. Only the light that reflects perpendicularly to the specimen surface was collected for color analysis by the optical-fiber cable. After triplicate measurements, the average values were expressed as Commission Internationale de l'Eclairage (C.I.E., 1978) lightness (L^*), redness (a^*) and yellowness (b^*). The meat color and marbling score for fresh pork according to the National Pork Producers Council (NPPC) standards (2000) were assessed by the panel test for the samples exposed to air at 4 °C for 30 min for complete bloom. A total of 16 panelists (20–40 ages, 7 females and 9 males) were participated. Three preliminary trials were performed for panel orientation and training. During this time, the lead investigator served as the panel leader and provided guidance to panelists on the scale and ensured consistency. A total of 10 sessions were performed with 15 to 20 samples per session.

Intramuscular muscle fat (IMF) content was determined using the Soxhlet diethyl ether extraction method (AOAC, 2000) and was expressed as the percentage of wet muscle tissue weight (Lee et al., 2010).

2.5. Genotyping of fast MyHC single nucleotide polymorphisms (SNPs)

Genomic DNA was extracted from the LD muscle of each pig using a commercial DNA isolation kit (G-DEX IIb; Intronbio, Sungnam, Korea). The restriction enzymes *Pst*I and *Hpy*CH4V, which recognize SNP1 and SNP2, respectively, were identified using a free online tool

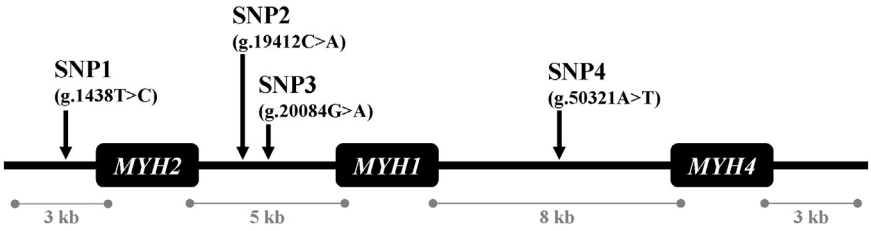

Fig. 1. Identified single nucleotide polymorphisms (SNPs) in the fast myosin heavy chain gene cluster on chromosome 12 in pigs. Genes are represented by filled boxes, and the gray lines represent the directly sequenced regions. The arrows indicate SNP location, and the SNP positions refer to U11771.1 for SNP1 and CR896583.2 for the others. The figure is not drawn to scale.