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ARTICLE

Effects of a Novel p.A41P Mutation in the Swine *Myogenic factor 5 (MYF5)* Gene on Protein Stabilizing, Muscle Fiber Characteristics and Meat Quality

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Abstract *Myogenic factor 5 (MYF5)* plays an important role in regulating skeletal muscle fiber characteristics, consequently affecting meat production and quality. We identified a novel p.A41P mutation in exon1 of the porcine *MYF5* gene by direct sequencing. The mutation was predicted to be destabilizing in protein structure based on the resultant amino acid substitution. We estimated the significant substitution effect of p.A41P on the energy stabilization of Myf5 protein structure. Then, we demonstrated that the mutation in Yorkshire population significantly affected muscle fiber type I composition ($p<0.05$), loin-eye area of lean meat content ($p<0.05$) and filter-fluid uptake of meat quality ($p<0.01$). Furthermore, dominant effects significantly influenced total muscle fiber number ($p<0.05$). This study suggests that the novel p.A41P mutation in porcine *MYF5* may be a valuable genetic marker to affect the muscle fiber characteristics and consequently improve meat production quality and quantity.

Keywords *Myogenic factor 5 (MYF5)*, single nucleotide polymorphism (SNP), muscle fiber characteristics, lean meat content, meat quality

Introduction

Myogenic regulatory factor (MRF) genes encode highly conserved basic helix-loop-helix proteins that control the embryonic muscle development process (Olson, 1990). *Myogenic factor 5 (MYF5)* encodes a MRF named myogenic factor 5 (Atchley et al., 1994). Along with *MYOD1*, *MYF5* expression is induced in myoblasts and is important to the regulation of myogenic proliferation and differentiation into myofibers (Montarras et al., 1991). Disruption in mice of the *MYF5* locus, but not of the *MYOD1*,

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leads to a delayed and reduced myogenesis (Braun et al., 1992a). The porcine *MYF5* was previously mapped to chromosome 5 (Soumillion et al., 1997), and it comprises 3 exons; 500, 76 and 191 bps long (Te Pas et al., 1999). This gene likely function in formation of muscle fiber characteristics, and has been considered a candidate gene for lean meat production and meat quality (da Silva Carmo et al., 2005; te Pas and Visscher, 1994). Therefore, the aim of the current study was to find the novel genetic marker for lean meat production and meat quality, according to functional validation via protein stabilizing changes and association analysis between polymorphism of porcine *MYF5* gene and the related traits.

Materials and Methods

Identification of p.A41P mutation in *MYF5*

Direct sequencing analysis was performed to identify the novel non-synonymous single nucleotide polymorphism (nsSNP) mutations in the porcine *MYF5* gene. Oligonucleotide primers for the sequencing analysis were designed with forward (5'-TGCGGTGGGATATGCTAATA-3') and reverse primers (5'-CTCTGGTTGGGGTTAGTCGT-3') based on published sequence data (GenBank ID. Y17154.1). A conventional polymerase chain reaction (PCR) amplification that produces a 600-bp fragment was conducted as follows: After heating at 95°C for 10 min, then 35 cycles were adapted for denaturation at 95°C for 1 min, annealing at 60°C for 1 min, and polymerization at 72°C for 1 min. The amplified PCR products were purified with QIAquick PCR purification kit (Qiagen, Inc., Venlo, Netherlands) and bidirectionally sequenced on an ABI 3730 automated sequencer with Big-Dye terminator cycle sequencing reagents (Applied Biosystems, Foster City, CA, USA).

Validations of p.A41P mutation effects on protein stabilizing and phenotypes

To investigate the substitution effect on the energy stabilizing of protein structure via nsSNP mutation, we used the molecular modeling package and protein design program in the Discovery Studio (DS) 4.0 (Accelrys Inc., San Diego, CA, USA) to estimate the amino acid substitution effect on model structure (Spassov and Yan, 2013).

The p.A41P mutation was genotyped on 429 Yorkshire pigs by the RFLP analysis along with the *Hha*I restriction enzyme. The Yorkshire population was chosen randomly from a single farm and slaughtered across an average of 188.9±20.45 days, following standard guidelines from the Korean grading service for animal products. Backfat thickness was measured at the 11th and last thoracic vertebrae. The mean of these 2 measurements was used as the backfat thickness value. The loin eye area was measured at the level of the last rib. Carcasses were chilled at 4°C for 24 h, after which the *longissimus dorsi* (LD) muscle was obtained to evaluate muscle fiber characteristics and meat quality traits. As we previously described in details (Kim et al., 2009), muscle fibre characteristics were estimated using the myosin ATPase activities via histochemical analysis and meat quality traits were tested by pH values at 45 min (pH_{45min}), drip loss, filter paper fluid uptake (FFU) and lightness (L*). The association analysis of the mutation with both sets of traits was conducted by the GLM procedure in SAS (ver. 9.3, SAS Institute) as following the model: $y_{ijklm}=\mu+M_i+S_j+B_k+b_1Sday_l+e_{ijklm}$, where y_{ijklm} denotes the observed traits, μ is the overall population mean, and M_i and S_j are the fixed effects of the i^{th} genotype and j^{th} sex. B_k is a random effect for the k^{th} batch of slaughter, b_1Sday_l is a covariance regression coefficient for the day of slaughter, and e_{ijklm} is the random residual error. Multiple comparisons of the least-square means between genotypes were performed using the SAS PDIFF option with a Tukey-Kramer adjustment.

Results and Discussion

We identified a novel non-synonymous single nucleotide polymorphism (nsSNP, p.A41P via g.1121G>C) in the exon 1

region of Yorkshire pig *MYF5* by direct sequencing (Fig. 1a). This amino acid substitution mutation (p.A41P) was located in the exposed loop of the Myf5 protein's basic helix-loop-helix (bHLH) domain, rather than in the DNA-binding interface (Fig. 1b). Efficient DNA binding of Myf5 requires dimerization with another Myf5 (Winter et al., 1992). Therefore, the model structure for Myf5 bHLH domain-DNA binding complex was constructed upon a dimeric Myf5 complex. The substitution effect of p.A41P on the entropy term was predicted to be significantly destabilizing ($\Delta\Delta G_{mut}$>0.5 kcal/mol) via an increase of 2.18 kcal/mol in mutation energy compared with wild-type. Transcriptional activation via *MYF5* requires activation of domains found in the amino- and carboxyl-terminal ends of the *MYF5* peptide, along with the highly conserved bHLH domain encoded by exon 1 (Braun et al., 1992a; Braun et al., 1992b; Winter et al., 1992). Therefore, we supposed that the newly identified non-synonymous mutation in *MYF5* gene may lead to destabilizing of Myf5 bHLH domain leading to structural change in DNA binding complex. It presumably could be a trigger of transcriptional activation of downstream target genes of *MYF5* and consequently effect phenotypic changes.

In this study, we used 429 Yorkshire pigs as a study population to validate the mutation effects on measured phenotypes

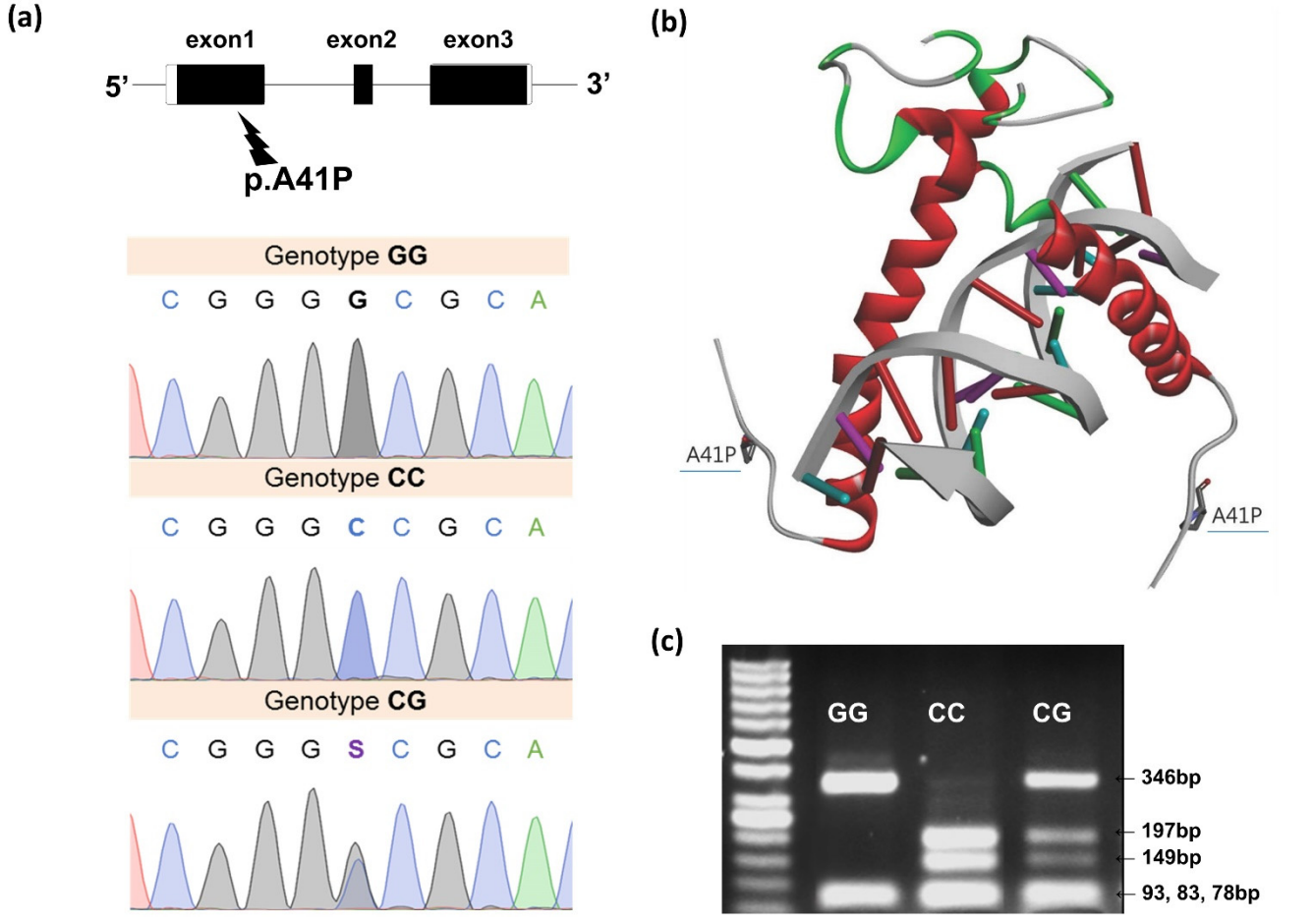

Fig. 1. (a) Identification of p.A41P mutation in the exon 1 region of *MYF5* gene on chromosome 5 in Yorkshire pigs by direct sequencing: GG, homozygote for proline (P) substitution; CC, homozygote for alanine (A) wild-type; and CG, heterozygote. (b) Stereoview of model structure for Myf5 bHLH domain-DNA complex, drawn as a ribbon by using the molecular modeling package in the Discovery Studio (DS) 4.0 (Accelrys Inc., San Diego, CA, USA). (c) The 600 bp PCR product was digested with *Hha*I (New England Biolabs, MA, USA), resulting in products 345, 93, 83 and 78 bp (genotype GG). The homozygote C allele (CC) resulted in product 197, 149, 93, 83 and 78 bp. The heterozygote (GC) resulted in 346, 197, 149, 93, 83 and 78 bp. The 93 bp, 83 and 78 bp fragments were shown as one band. The DNA fragments were separated on 3% agarose gels in 1× Tris-borate-EDTA buffer at 100 V for 30 min. *MYF5*, *myogenic factor 5*.

such as muscle fiber characteristics and meat quality traits. The basic statistics (i.e., number of measurements per trait, means, standard deviations, minimum and maximum) for every measured trait were given in the Table 1. The p.A41P locus was genotyped for all the animals (Fig. 1c), and showed significant difference in their genotype frequencies from Hardy-Weinberg equilibrium (Table 2). Then, the association analysis revealed the significant effects of the p.A41P genotypes on the muscle fiber characteristics, lean meat production, and meat quality traits (Table 3). The p.A41P mutation was significantly associated with muscle fiber type I composition in both area and number ($p<0.05$). In mouse, *MYF5* plays a role in the upregulation and activation of the developmental myosin heavy chain genes (Beylkin et al., 2006). Moreover, a previous study reported a SNP in porcine *MYF5* has an influence on fast-twitch oxidative fiber contents of *longissimus lumborum* muscle (Klosowska et al., 2004). Therefore, it leads us to suggest that the novel nsSNP in exon 1 of *MYF5* could have an effect on the formation of muscle fiber types.

Additionally, we observed that the p.A41P mutation was significantly associated with loin-eye area ($p<0.05$) and filter-fluid uptake ($p<0.01$). Variations in porcine *MYF5* were reported to be associated with meat quality traits especially including moisture content of LD muscle and water holding capacity (da Silva Carmo et al., 2005; Liu et al., 2008; Liu et al., 2007). *MYF5* gene is located at SSC5q25 (Čepica et al., 1999), and mapped near the drip loss quantitative trait loci (QTL) regions (Jennen et al., 2007). Moreover, another SNP in *MYF5* has been reported to be associated with lean meat content (Verner et

Table 1. Summary statistics for measured traits in 429 Yorkshire pigs

Traits	N	Mean	SD	Min	Max
Muscle fiber characteristics					
Total fiber number ($\times 10^3$)	429	1,165	260	523	2,159
Fiber number per unit area (/mm^2)	429	242.0	34.9	149.0	368.0
CSA of fibers (μm^2)	429	4,219	622	2,718	6,691
Fiber number composition (%)					
Type I	429	9.23	3.98	1.23	30.00
Type IIa	429	13.97	5.17	0.85	37.91
Type IIb	429	76.80	6.51	48.01	92.12
Fiber area composition (%)					
Type I	429	6.94	2.85	1.35	16.47
Type IIa	429	8.31	3.34	0.58	17.85
Type IIb	429	84.75	4.58	70.94	95.72
Lean meat production					
Loin-eye area (cm^2)	429	48.17	8.29	24.59	73.43
Backfat thickness (mm)	429	20.99	5.82	6.00	36.00
Meat quality					
pH_{45min}	428	6.13	0.28	5.34	6.94
Drip loss (%)	429	3.35	1.98	0.57	13.31
Filter-fluid uptake (mg)	429	28.19	15.83	5.10	99.30
Lightness (L*)	429	46.41	2.82	33.47	54.27

CSA, cross sectional area.

Table 2. Genotype distribution of p.A41P mutation in 429 Yorkshire pigs

Genotype count		MAF	Het	χ^2
Total	429			
CC	29	0.331	0.527	15.41
CG	226			
GG	174			

MAF, minor allele frequency; Het, heterozygosity; χ^2, statistic for Hardy-Weinberg equilibrium.

Table 3. Effects of p.A41P mutation in *myogenic factor 5 (MYF5)* on muscle fiber characteristics and economic traits in 429 Yorkshire pigs

Traits	p.A41P Genotype			Additive	Dominant	Significance		
	CC (29)	CG (226)	GG (174)			G	A	D
Muscle fiber characteristics								
Total fiber number ($\times 10^3$)	1,169±41.2	1,226±18.4	1,177±20.5	–8.906	105.2	†	ns	*
Fiber number per unit area (/mm^2)	234.6±6.36	242.5±2.85	241.6±3.17	–7.061	8.748	ns	ns	ns
CSA of fibers (μm^2)	4,392±114.2	4,215±51.1	4,217±56.9	174.5	–179.9	ns	ns	ns
Fiber number composition (%)								
Type I	11.03±0.76^X	9.17±0.34^Y	9.03±0.38^Y	2.006	–1.717	*	*	†
Type IIa	14.65±0.95	14.70±0.42	15.17±0.47	–0.515	–0.430	ns	ns	ns
Type IIb	74.32±1.18	76.13±0.53	75.81±0.59	–1.490	2.145	ns	ns	ns
Fiber area composition (%)								
Type I	8.08±0.55^X	6.75±0.25^Y	6.76±0.27^Y	1.323	–1.330	*	*	†
Type IIa	8.84±0.61	8.77±0.27	9.09±0.31	–0.250	–0.396	ns	ns	ns
Type IIb	83.08±0.84	84.48±0.37	84.15±0.42	–1.075	1.726	ns	ns	ns
Lean meat production								
Loin–eye area (cm^2)	49.76±1.24^{XY}	50.59±0.55^X	48.68±0.62^Y	1.079	2.739	*	*	†
Backfat thickness (mm)	22.28±0.79	21.00±0.35	20.91±0.39	1.362	–1.189	ns	ns	ns
Meat quality								
pH_{45min}	6.12±0.05	6.14±0.02	6.16±0.02	–0.037	–0.008	ns	ns	ns
Drip loss (%)	3.56±0.37	3.33±0.17	3.02±0.19	0.545	0.087	ns	ns	ns
Filter–fluid uptake (mg)	30.41±2.74^X	27.75±1.22^X	23.58±1.37^Y	6.837	1.500	**	*	ns
Lightness (L*)	46.67±0.49	46.13±0.22	46.19±0.24	0.478	–0.598	ns	ns	ns

Different superscript letters (X and Y) indicate significant differences between genotypes at $p<0.05$.
Significance levels for genotype (G), additive (A), and dominant (D) effects: ns, not significant; † $p<0.10$; * $p<0.05$; ** $p<0.01$.
CSA, cross sectional area.

al., 2007). Our results showed that additive genetic effects were significant and in line with the results of genotype associations ($p<0.05$). Moreover, dominant effects significantly influenced total muscle fiber number ($p<0.05$), while the influence on muscle fiber type I composition and loin-eye area were trended to near significance ($p<0.10$). The number of muscle fibers at birth in piglets was regulated by *MRF* genes (Handel and Stickland, 1987), and low birth weight was associated with impaired pre-natal muscle development (Foxcroft et al., 2006). In addition, piglets with low birth weights had less lean meat content (Gondret et al., 2005; Paredes et al., 2013; Rehfeldt et al., 2008). Taken together, the novel p.A41P

mutation in porcine *MYF5* had impact on the muscle fiber formation and thus lean meat content.

Conclusion

Overall, the novel non-synonymous SNP (p.A41P via g.1121G>C) in the exon 1 region of *MYF5* was predicted to destabilize the protein structure, and had impact on the muscle fiber formation and thus lean meat content. Based on these findings, we suggest that the p.A41P mutation could be a meaningful marker for muscle fiber regulation and to choice favourable pork when it applies as a potential target in the porcine breeding program.

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