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Research Article

Analysis on the Relationship between an Intronic Polymorphism in *Troponin C* Gene (TNNC1) with Pork Quality Traits

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Abstract: The objective of this study was to investigate the distribution of genotype and analyze the effect of the intronic polymorphism (g.C716G) of TNNC1 gene on meat quality traits of Mong Cai (MC) pig. In total, 118 animals were used for phenotype record and genotyping by PCR-RFLP method. Results showed that the presence of allele "G" was more than twice as frequent as allele "C" at the polymorphic site (0.70vs. 0.30) and the frequency of CC genotype was very low (0.07) compared to CG (0.47) and GG (0.46) genotypes. The pH value at 45 min post-mortem was different ($p<0.01$) among three genotype groups with the lowest found in pigs bearing CC genotype (6.43). Significantly higher compression force value (6.16 kg) found in CC pigs implied that loins from these animals were less tender than those from CG animals. Other parameters of meat quality including drip loss and meat color were not affected by this polymorphism. Moreover, in the analysis of thirty MC pigs, although the relationship between genotypes and muscle fiber type composition was unclear, there was a trend of increasing IIa fiber and decreasing IIb fiber percentage in pigs with GG genotype ($p = 0.059$ and $p = 0.082$, respectively). In conclusion, the SNP examined in TNNC1 gene may be of interest in selection for meat tenderness in MC pigs; however as this is not a causative polymorphism, further studies on the association of TNNC1 with meat quality traits regarding to the interaction with other functional genes in surrounding regions are recommended.

Keywords: Association, meat quality, mong cai pigs, muscle fiber type

INTRODUCTION

Troponin, the central regulatory protein of striated muscle contraction, is a component of thin filaments together with actin and tropomyosin (Hooper and Thuma, 2005). The contractile protein troponin, containing the three subunits troponin I, troponin T and troponin C, plays a role for the concentration of Ca^{2+} and is responsible for striated muscle contraction (Xu *et al*., 2010). Each troponin has isoforms encoded by different genes which are expressed exclusively in different muscle fiber types (Mullen and Barton, 2000). Troponin C (TNNC1) is considered as a temporary bridge contributing to the formation of muscle growth and its expression may influence meat quality traits (Kim *et al*., 2005). Previously, Furukawa and Peter (1971) stated that troponin activity in skeletal muscle of guinea pig is related to three histochemical fibers in such a way that intermediate and red fibers have lower troponin activities than white fibers. Thus, the expression of TNNC1 appeared to have a link with different muscle fiber isoforms and thereby it may have certain effects on meat quality traits. In Viet Nam, Mong Cai is a native pig breed being able to adapt to severe environmental condition, low nutrition diet and is well resistant to diseases. Specially, this breed has been known for its superior meat quality, which is an important characteristic to producers, consumers and processing industry. The objective of this study was to examine the distribution of genotype and analyze the effect of the intronic g.C716G SNP of TNNC1 gene on quality traits and muscle fiber type composition in the loin of MC pigs.

MATERIALS AND METHODS

Animals and sampling: The study was carried out on the castrated male MC pigs that were reared at a state farm in Quang Ninh province, Viet Nam. All animals were fed under similar conditions and they were slaughtered at 197±17 days of age with the live weight of 28.5±6.0 kg. The pigs were fasted 12 h before slaughtering followed a commercial standard procedure and under the supervision of the veterinary service. *Longissimus dorsi* (LD) muscle samples were collected for further measurements and analysis.

Meat quality measurements and muscle fiber typing: Meat color, classified as lightness (*L**), redness

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(*a**) and yellowness (*b**), were determined on a fresh cut surface 24 h post-mortem by using a Minolta Chromameter (CR310, Minolta, Japan). Muscle pH was measured at 45 min ($pH_{45\ min}$) and 24 h (pH_{24}) post-mortem with a Delta-320 pH meter (Mettler Toledo, USA). Drip loss was calculated as the weight loss of a meat sample (40±5 g) placed in a bag at 4°C for 24 (drip $loss_{24}$) and 48 h (drip $loss_{48}$) (Rehfeldt *et al*., 2008). To obtain cooking loss, a loin cube (90±5 g) was taken and stored at 4°C for 24 h and subsequently it was bagged in thin-walled plastic and placed in a water bath at 75°C for 30 min. The bag was subsequently cooled under cold water for 30 min and the cooking loss was expressed as the difference of original and cooked weight (Renaudeau and Mourot, 2007). For compression force values, samples were thawed at 4°C and cut into 10×10 mm cross-sections (with the fiber direction) and six samples for each loin were measured using a TA-XT2*i* Texture Analyzer (Stable Micro Systems Ltd.) (Florowski *et al*., 2006). Chemical composition of LD muscle including dry matter, protein, ether extract and ash content was estimated followed the protocols of AOAC (1998). Muscle fiber typing using real-time RT-PCR has been detailed in Ngu *et al*. (2012). In total, there were 30 MC pigs used for muscle fiber typing.

TNNC1 genotyping: The forward and reverse primer pair used to amplify the 449-bp fragment was 5'-GAGGCTCTGTTGCTGTTTCC-3' and 5'-GAGC AG CTGTCCATGTCAGA-3' (Ngu, 2006). DNA samples from LD muscle were extracted using phenol/ chloroform and samples were genotyped by PCR-RFLP (Restriction Fragment Length Polymorphism) technique with the presence of *Ava*II enzyme. A digestion reaction containing 1 unit of enzyme, 1 μL of 10x restriction buffer and 1 μg of DNA was incubated at 37°C in 15 min for complete digestion. The digested product was checked by electrophoresis on 2% agarose gel.

Statistical analysis: The relationship between TNNC1 genotypes with traits of interest was performed using the least squares method of the GLM procedure in Minitab version 13.20. Factors found to affect the traits such as genotype, boar and sow were used in the model and body weight at slaughter was added as a covariate. The difference between genotypes was tested using Tukey pair wise comparison at the 5% significance level. Data were presented as Least square means±Standard error.

RESULTS

Genotyping and frequency: In MC pig, a SNP (g.C716G) was detected in the intronic region of the TNNC1 gene (GenBank accession No. AY958072) (Fig. 1). The amplified PCR product of 449 bp digested

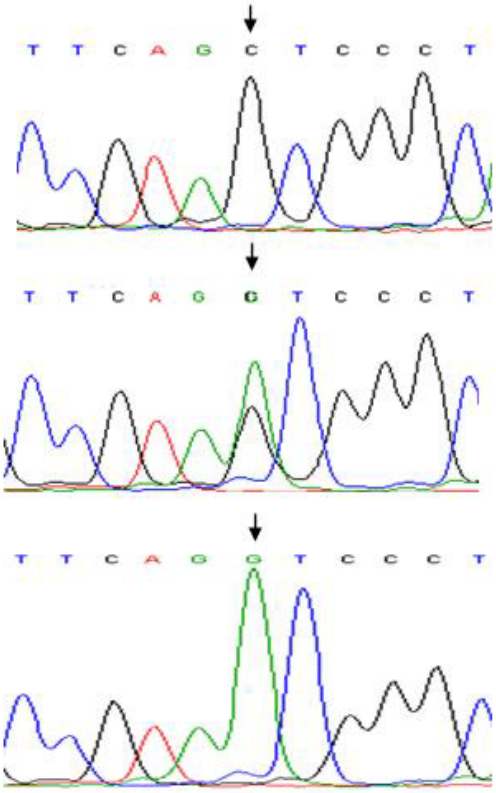

Fig. 1: Sequencing chromatogram showing a mutation (C/G) identified in three individuals

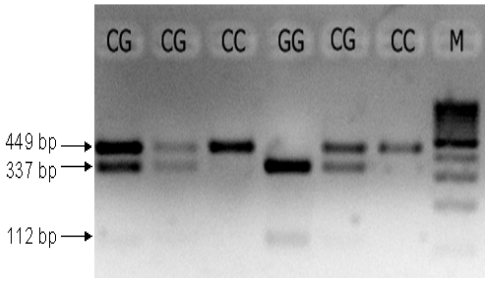

Fig. 2: Electrophoresis on 2% agarose gel of PCR product after digestion with *AVa*II. M: 100 bp DNA ladder, Fermentas

with *Ava*II restriction enzyme resulted in three genotypes of CC (449 bp), CG (449, 337 and 112 bp) and GG (337 and 112 bp) (Fig. 2). At this SNP, the presence of allele "G" was more than twice as frequent as allele "C" (0.70 vs. 0.30); as a result, the distribution of CC genotype was very low (0.07) compared to CG (0.47) and GG (0.46) genotypes (Fig. 3).

Relationship between SNP marker and pork quality parameters: Regarding to meat quality traits, results from Table 1 indicated that the investigated SNP had a significant effect on $pH_{45\ min}$ ($p<0.01$), in which the lowest value was on CC genotype (6.43). Different genotypes also had a relationship with compression

Table 1: Relationship between TNNC1 genotypes with quality and chemical composition of pork loin

	Genotype			
Traits	CC (n = 8)	CG (n = 57)	GG (n = 53)	P
Meat quality				
$pH_{45\ min}$	6.43±0.08[b]	6.65±0.03[a]	6.69±0.03[a]	0.003
pH_{24}	6.17±0.07	6.17±0.03	6.18±0.03	0.956
Drip $loss_{24}$ (%)	2.00±0.32	1.77±0.11	1.99±0.12	0.389
Drip $loss_{48}$ (%)	2.96±0.40	2.53±0.14	2.91±0.15	0.148
Cooking loss	23.7±1.6	23.0±0.6	22.1±0.6	0.481
Compression force (kg)	6.16±0.60[a]	4.64±0.22[b]	5.12±0.24[ab]	0.042
Meat color				
*L** (lightness)	50.2±0.5	49.2±0.2	49.3±0.2	0.215
*a** (redness)	4.2±1.0	6.3±0.4	5.4±0.4	0.068
*b** (yellowness)	9.2±0.5	8.6±0.2	8.8±0.2	0.557
Meat chemical composition (%)				
Dry matter	24.9±0.4	25.7±0.2	25.3±0.2	0.060
Crude protein	22.6±0.4	22.2±1.1	21.9±1.0	0.083
Ether extract	2.49±0.17	2.52±0.07	2.59±0.07	0.682
Ash	0.96±0.13	1.08±0.05	1.01±0.05	0.550

[a,b]: Values in the same row with different superscripts are significantly different ($p<0.05$)

Table 2: Relationship between TNNC1 genotypes with muscle fiber types of pork loin

	Genotype			
Muscle fiber (%)	CC	CG (n = 15)	GG (n = 14)	P
Type I	-	23.1±1.4	21.3±1.4	0.294
Type IIa	-	25.0±1.6	28.8±1.6	0.059
Type IIx	-	39.1±1.7	39.1±1.7	0.989
Type IIb	-	12.8±1.0	10.8±0.9	0.082

-: not available

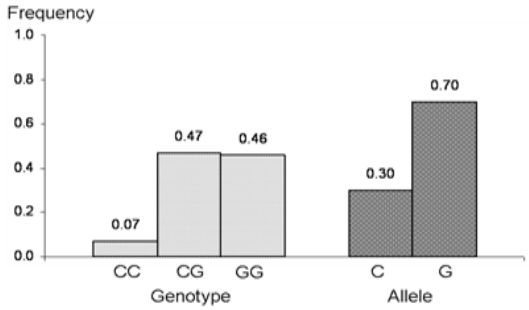

Fig. 3: Genotype and allele frequencies of the C/G SNP in MC population

force; meat from pigs bearing CC genotype was less tender than that from CG and GG genotypes ($p<0.05$). None of significant effects of genotype were found for other meat quality parameters. In addition, meat color was almost the same among three genotype groups except for redness (a^*) value, where the significant level was slightly larger (0.068) than the detected threshold. There was also a trend of difference by genotypes in crude protein content ($p = 0.083$). Finally, the substitution of C/G allele did not lead to a change in muscle fiber type composition though the percentages of IIa and IIb fiber tended to be higher in GG and CG genotypes, respectively ($p = 0.059$) (Table 2).

DISCUSSION

The documentations on the SNP as well as association of TNNC1 with performance traits in pigs are limited. Instead, most of researchers discussed about the role of this gene in clinical setting such as heart failure (Adamcova *et al*., 2006). In the present study, the detected SNP of TNNC1 is located in the intronic region and the relationship of such SNP with observed traits may be explained by the influence of intron on mRNA metabolism including initial transcription, editing and polydenylation of the pre-mRNA, translation and decay of the mRNA product (Le Hir *et al*., 2003). Moreover, there are an increasing number of reports for the role of introns in regulating the expression level of a gene or tissue-specific expression pattern (Greenwood and Kelsoe, 2003; Pagani and Baralle, 2004).

With regards to the distribution of genotype in MC pigs, our study confirmed the previous finding that the number of pigs carrying CC genotype appeared at low frequency (8%) in the population of F2 DUPI (Duroc x Pietrain) while the presence of other two genotypes were almost in similarity (Ngu, 2006). In the association analysis with meat quality traits, the current data additionally agree with those reported by Ngu (2006) that meat color, shear force, drip loss and cooking loss were not affected by the allele substitution; in that study, only conductivity was found to associate with the changing of genotype.

The TNNC1 gene was mapped on SSC 13 where QTL (Quantitative Trait Loci) scan showed evidence of significance at 5% chromosome-wide level with pH_1 and pH_{24} in LD muscle (Wimmers *et al*., 2006). It has been suggested by Metzger (1996) that TnC isoforms may have an effect in conferring pH sensitivity of Ca^{2+}-activated contraction in mammalian fast and slow muscle fibers. It could be hypothesized in this study that the extent of acidic-pH-mediated reduction in Ca^{2+} binding to regulatory binding sites on TnC is dependent on the proportion of muscle fiber; however as these data were not available due to low numbers of pigs bearing CC genotype, a conclusive statement for this difference is open to debate. In addition, significantly

higher compression force value found in CC pigs implied that loins from these animals were less tender than those from CG animals. A lower $pH_{45\ min}$ value in combination with higher compression force made pigs with CC genotype less favorable in breeding selection for meat quality.

Also in our research, thirty pigs were randomly selected for the examination of muscle fiber proportion but out of these there was only one pig with CC genotype, therefore the comparison was drawn only between CG and GG carriers. The result indicated a trend of increasing IIa fiber and decreasing IIb fiber percentage in pigs with GG genotype. Henckel *et al*. (1997) reported the frequency of IIb fiber and intramuscular fat content are positively correlated and thus flavor and tenderness seemed to have a negative relationship with IIa but positive correlation with IIb fibers. Our results confirmed this statement and it is likely that desirable meat in terms of $pH_{45\ min}$ and tenderness in pigs carrying CG genotype could be expected in selection. However, as this is not a causative polymorphism, further studies on the relationship of TNNC1 with meat quality traits with regards to the interaction with other functional genes, i.e., pituitary-specific transcription factor (PIT1) are recommended.

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