Journal of Animal and Veterinary Advances 9 (4): 705-709, 2010
ISSN: 1680-5593

Association of the MYPN Gene Polymorphisms with Meat Quality in Commercial Pigs

[1]L.W. Zhai, [2]L.X. Wang, [3]W.L. Zhou and [1]C.D. Wang
[1]Department of Animal Genetics and Breeding, College of Animal Science and Technology,
China Agricultural University, Beijing 100193, China
[2]Institute of Animal Sciences, Chinese Academy of Agricultural Sciences,
Beijing 100193, China
[3]Tianjing Ninghe State Breeding Pig Farm, Tianjin 301504, China

Abstract: This study investigated the polymorphism of MYPN gene, which codes the Myopalladin (Myop) protein, being chosen as a candidate gene for meat quality. Three SNPs were detected in the 3 UTR of MYPN. Association analysis of the MYPN genotype with meat quality of longissmus muscles was conducted in several western meat producing breeds, including Yorkshires (Y), Landraces (L) and L×Y pigs (LY). This study showed that some of the meat quality traits exhibited significant difference in different breeds and different genotypes. The meat pH value, color (Opto-star value) and tenderness (shear force) of Yorkshires were significantly higher than those of Landrace and L×Y pigs; on the other hand, L×Y pigs had significantly higher conductivity and intramuscular fat content and water-hold capacity was significantly higher for Landrace than Yorkshires and L×Y pigs. All the three detected SNPs were shown to be associated with meat color and tenderness, Whereas none showed association with conductivity. The pH value, tenderness and intramuscular fat content were associated to two SNPs, respectively, water-hold capacity was associated to only one SNP. This study suggests that MYPN gene can be investigated as a candidate gene of meat quality in the farther reseach.

Key words: Pig, meat quality, MYPN gene, polymorphisms, pool sequencing, PCR-RFLP

INTRODUCTION

Now-a-days, the marketing, production and researchers lay more stress upon the edible quality of pork meat (Alonso *et al*., 2009) to satisfy the developing consumer demands for more excellent flavor, healthy and nutritional meat products (Davoli and Braglia, 2007). Meat quality is a complex trait, which can be estimated refer to a series of traits such as the compositional, visual and sensory traits of a carcass and its retail cuts (Cameron, 1990).

The narrowly defined meat quality is assessed according to physical and chemical factors such as pH, colours, Water-Holding Capacity (WHC), tenderness, tenacity, conductivity and Intramuscular Fat content (IMF) (Davoli and Braglia, 2007; Simek *et al*., 2004). However, these measurements of meat quality are always too expensive, difficult and only possible after slaughter, so that most of these traits are difficult to be genetically evaluated and improved in traditional strategies. Fortunately, molecular technology has become a powerful method for identifying the underlying genetics of meat quality and improving the traits through Marker Assistant Selection (MAS). Several genes, such as Ryanodine receptor (RYR1), Rendment Napole (RN), Calpastatin (CAST), have been reported to be associated with the pork meat quality (Ciobanu *et al*., 2001, 2004; Fujii *et al*., 1991; Milan *et al*., 2000).

The physiologicalstudies for muscle fiber composition provide effective materials to detect the genes and underlying functional mutation affecting meat quality.

The myopalladin (MYOP) protein coded by Myopalladin (MYPN) gene is a 145 kDa sarcomeric protein, which plays important roles in Z-line and I-band protein assemblies of sarcomere (Bang *et al*., 2001). Polymorphism analysis of partial 3 UTR of MYPN in different porcine breeds has previously suggested a putative effect of MYPN on meat production (Davoli *et al*., 2003).

The aim of this study is to investigate the polymorphisms of 3 UTR of MYPN gene in commercial pig breeds including Landrace (L), Yorkshire (Y) and L×Y cross breed. An association analysis was conducted to evaluate the possible effect of MYPN on meat-quality traits.

Corresponding Author: Chuduan Wang, Department of Animal Genetics and Breeding, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China

MATERIALS AND METHODS

Animals and sampling: The carcasses of 102 market weighted gilts from three different breeds were analyzed in this research, including 31 Yorkshires (Y), 34 Landraces (L) and 37 L×Y pigs (LY). All animals were raised in one testing farm with uniform feeding treatments. All the pigs were feed for about 180 days and were slaughtered when they weighed approximately 110 kg. The samples for meat quality measurements were removed from the 7th thoracic vertebra to the last lumbar vertebra of muscle longissimus dorsi on the left side of the carcasses. Ear tissue was sampled for DNA extract.

Genotype analysis: Genomic DNA was extracted from ear tissue samples using genomic DNA pure tissue kit (Tiangen Biotech Co. Ltd., Beijing, China). Primers were designed using Oligo 6.0 and primer introduced restriction analysis software available on line (http://cedar.genetics.soton.ac.uk/public_html/primer2.html) according to the sequence of EMBL acc. No. of AJ560657 for the fragments of MYPN. The PCR primers of MYPN are as followed: MYPN-F(5'-TCTCCCTGGTGAATCTGGAG-3') and MYPN-R(5'-AGGAACAGGGAATGTGCATC-3'), amplified a 253 bp fragment of the 3'-untranslated region. The PCR was carried out in a total volume of 25 μL including 100-150 ng of template DNA, 10 pmol of each primer, 250 μM dNTPs, 2.5 μL 10×PCR buffer (with MgCl2) and 1.5 U Taq polymerase (Tiangen Biotech Co. Ltd., Beijing, China). PCR was performed in a MJ Research PTC-200 Thermal Cycler (BIO-RAD Co. Ltd., USA) under the following conditions: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min and extension at 72°C for 1 min with a final extension step at 72°C for 7 min.

Each 34 pigs PCR products were pooled and sequenced and then SNPs were identified by sequence traces. For individual genotyping, the PCR products were digested with 2 U Sph 1, Csp45 1 (TOYOBO CO. Ltd., Japan) and Afa 1 (TaKaRa Bio Group, Japan) for at 37°C for 4 h, respectively and analyzed on 3% agarose gels.

Meat quality measurements: The meat quality data of pigs were collected in 24 h after slaughtered. Traits were mensurated as meat quality included the pH value, meat conductivity, meat color, Water-Hold Capacity (WHC), tenderness and Intramuscular Fat content (IMF). Meat pH value, conductivity and color were measured using a star series equipment (Rudolf Matthaeus Company, Germany) at 45 min post-mortem (pm) in longissimus dorsi at the 13-14th ribs (Wimmers *et al*., 2007). The WHC was determined follow the Grau and Hamm (1953) filter paper press method. Tenderness of meat was obtained using the TA.XTplus Texture Analyser (StableMicroSystem CO. Ltd., GB). The IMF was quantified by a near infrared technique Foodscan (Foss CO. Ltd., Sweden) and the value was expressed as the weight percentage of wet muscle tissue.

Association analysis: General Linear Model (GLM, SAS 8.0) was conducted to determine the associations between molecular mutations and the meat quality traits measured considering the breed effect. The data were presented as probability values and least squares means±SEM (standard error of the mean). The significant differences of least squares means were tested with Duncan's multiple range tests and a $p \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Identification and analysis of single nucleotide polymorphisms: A preliminary pool sequencing of the 253 bp fragment was taken after PCR. And the analysis revealed 3 SNPs at position 231, 298 and 318 within the 3 UTR of MYPN (EMBL acc. no. of the cDNA clone sequence AJ560657), which can be distinguished by Sph 1 (G/A), Csp45 1 (G/T) and Afa 1 (T/C), respectively. The results of digestion for these SNPs were shown in Table 1, every position can be distinguished to three genotypes. All the alleles are present in 3 pig breeds. The genotype frequencies of SNP231, SNP298 and SNP318 in different pig breeds are shown in Table 2. In the current research, three SNPs on 3 UTR of MYPN gene were found in different pig breeds. Two of these three SNPs, SNP298 and SNP318, are corresponded to pre-existing assignments by Davoli *et al*. (2003). The SNP231 is peculiar in the animal group. The results of genotype frequencies analysis suggested that different pig breeds have been segregated from each other, even though

Table 1: The results of digestion by different enzyme

SNP	SNP231				SNP298			SNP318	
Position	231				298			318	
Mutation	G/A				G/T			T/C	
Enzyme	Sph 1				Csp45 1			Afa 1	
Genotype	AA	AG	GG	TT	GT	GG	CC	CT	TT
Bands[1]	182 /71	253 (182/71)	253	136 /117	253 (136/117)	253	154 /99	253 (154/99)	253

[1]Result bands from the digestion of correponding enzyme

Table 2: Genotype frequency at the MYPN loci in different breeds

	SNP231			SNP298			SNP318		
Breeds	AA	AG	GG	GG	GT	TT	CC	CT	TT
Y	0.166	0.668	0.166	0.341	0.496	0.163	0.353	0.224	0.423
L	0.389	0.500	0.111	0.044	0.739	0.217	0.381	0.381	0.238
LY	0.327	0.449	0.224	0.299	0.522	0.179	0.108	0.600	0.292
Total	0.319	0.486	0.195	0.219	0.604	0.177	0.174	0.511	0.315

Table 3: Results of association analysis for meat quality traits with different breeds and three SNPs

Traits	pH	Meat color	Water-hold capacity	Tenderness	IMF
Breed					
L	5.86±0.04^{A}	72.27±0.97^{Aa}	0.41±0.005^{A}	54.85±1.41^{A}	1.26±0.04^{Aa}
LY	5.61±0.02^{B}	69.42±0.59^{Ab}	0.37±0.003^{B}	58.04±0.85^{a}	1.47±0.02^{B}
Y	6.58±0.07^{C}	77.30±1.80^{B}	0.36±0.009^{B}	63.96±2.60^{Bb}	1.10±0.07^{Ab}
p-value	<0.001**	<0.001**	<0.001**	0.0029**	<0.001**
SNP231					
AA	5.98±0.04^{A}	71.22±0.96^{Aa}	0.37±0.004^{a}	60.47±1.53^{a}	1.26±0.04
AG	5.97±0.03^{A}	73.88±0.71^{B}	0.38±0.003	57.31±1.13^{b}	1.27±0.03^{a}
GG	6.22±0.04^{B}	73.76±1.09^{b}	0.39±0.005^{b}	56.41±1.73^{b}	1.17±0.05^{b}
p-value	<0.001**	0.0231*	0.0569	0.0705	0.1155
SNP298					
GG	6.04±0.05	73.69±1.07^{a}	0.39±0.005^{a}	53.78±1.66^{A}	1.21±0.04^{A}
GT	6.02±0.03	73.66±0.66^{A}	0.38±0.003^{b}	58.30±1.02^{B}	1.23±0.03^{A}
TT	5.97±0.06	70.23±1.24^{Bb}	0.38±0.006	59.71±1.93^{B}	1.40±0.05^{B}
p-value	0.6471	0.0208*	0.0520	0.0093**	0.0041**
SNP318					
CC	6.00±0.05	70.23±1.05^{A}	0.38±0.005^{a}	60.17±1.65^{A}	1.41±0.04^{A}
CT	5.91±0.04^{A}	74.79±0.82^{B}	0.37±0.004^{Ab}	60.33±1.29^{A}	1.25±0.03^{Ba}
TT	6.11±0.04^{B}	73.82±0.79^{B}	0.39±0.004^{B}	54.74±1.23^{B}	1.16±0.03^{Bb}
p-value	0.003**	0.0011**	0.0143*	0.0012**	<0.001**

The data were presented as least squares means±SEM (standard error of the mean) and p-value, ^{a,b}Means within a row with no common superscript differ significantly ($p<0.05$), ^{A,B}Means within a row with no common superscript differ significantly ($p<0.01$), *$p<0.05$; **$p<0.01$

they were raised under the same feeding treatments that might be a result of selection pressure. Yorkshires and Landrace are both western pig breeds famous for their higher rate of growth and muscularity, however, they are used as different parent lines in the crossbreeding.

Meat quality and association of SNPs and breeds: The traits, least squares mean values, standard deviations and genotypes of different breeds are shown in Table 3. An association study was performed on 3 SNPs and 3 breeds for meat quality traits and p-values are also listed in Table 3 and some of the differences ($p<0.01$) between different genotypes are as shown in Fig. 1. All the traits among the breeds were significantly different. The pH value, meat color and tenderness were significantly higher for Yorkshires than Landrace and L×Y pigs ($p<0.01$), on the other hand, L×Y pigs had significantly higher conductivity and IMF ($p<0.01$) and water-hold capacity was significantly higher for Landrace than Yorkshires and L×Y pigs ($p<0.01$). Three SNPs all revealed significantly association with meat color and the p-value were 0.0231, 0.0208 and 0.0011, respectively. SNP231 and SNP318 showed association at $p<0.01$ with pH value. SNP298 and SNP318 were both significantly associated with tenderness and IMF at $p<0.01$. And SNP318 showed significantly associtation with WHC at $p<0.05$.

The traits involved in the research are physical and chemical properties of muscle, muscle fiber as the foundation component of muscle, its character also might impact the meat quality and the genes affect the characters of muscle fibers could be researched as the candidate gene.

Myopalladin is a structural component of muscle, which plays conserved signaling roles in the sarcomere assembly (Ma and Wang, 2002) and is suggested to fasten the nebulin of skeletal muscle and nebulette of cardiac muscle to α-actinin at the Z-line and effect muscle gene expression (Bang *et al*., 2001).

Hypothesized MYPN might affect on carcass traits (Davoli *et al*., 2003) and located MYPN gene on SSC14 of porcine (Rohrer and Keele, 1998). MYPN revealed association with muscularity traits in Duroc and is remarkable for meat quality traits (Wimmers *et al*., 2007).

In the current research, 3 SNPs of MYPN gene all revealed significantly association with meat quality traits. The polymorphisms analyzed are non-functional mutations, however the results suggested that 3 UTR as the main component of gene expression regulation may play an important role in affecting the phenotypes of traits. In this study, pig breeds exhibited significantly association with almost all meat quality traits measured.

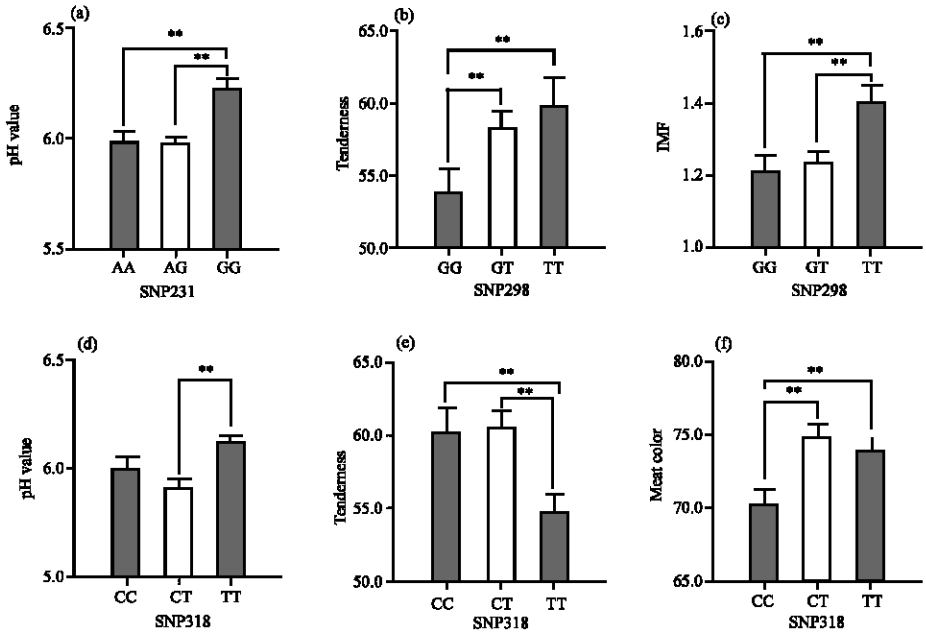

Fig. 1: Crucial results profiles of association analysis. The histograms indicate the least squares means of corresponding SNP genotypes to the trait marked in the vertical axes with error bars for SEM. Link lines marked by stars indicate the results of Duncan's multiple range tests. **p<0.01

The results implyed that genetic background is one of the most important influences of meat quality. As a result, there must be some genes or QTLs concerned with the form of meat quality traits.

CONCLUSION

This study analyzed the partial 3 UTR of MYPN gene and the results showed the presence of three SNPs. The SNPs were associated with several meat quality traits of 102 pigs from three different breeds and we also analyzed the meat quality differences between pig breeds.

The results supplyed valuable information to the evaluation of MYPN gene as a candidate gene of meat quality and encourage performing further study of candidate genes, which might reveal association to meat quality.

ACKNOWLEDGEMENT

The research was supported by the National Key Technologies R and D Program (No. 2008BADB2B11) and partly supported by National Key Technologies R and D Program (No. 2006BAD01A08).

REFERENCES

Alonso, V., M.M. Campo, S. Espanol, P. Roncales and J.A. Beltran, 2009. Effect of crossbreeding and gender on meat quality and fatty acid composition in pork. Meat. Sci., 81: 209-217.

Bang, M.L., R.E. Mudry, A.S. McElhinny, K. Trombitas and A.J. Geach *et al.*, 2001. Myopalladin, a novel 145-kilodalton sarcomeric protein with multiple roles in Z-disc and I-band protein assemblies. J. Cell Biol., 153: 413-428.

Cameron, N.D., 1990. Genetic and phenotypic parameters for carcass traits, meat and eating quality traits in pigs. Livest. Prod. Sci., 26: 119-135.

Ciobanu, D., J. Bastiaansen, M. Malek, J. Helm, J. Woollard, G. Plastow and M. Rothschild, 2001. Evidence for new alleles in the protein kinase adenosine monophosphate-activated {gamma}3-subunit gene associated with low glycogen content in pig skeletal muscle and improved meat quality. Genetics, 159: 1151-1162.

Ciobanu, D.C., J.W.M. Bastiaansen, S.M. Lonergan, H. Thomsen, J.C.M. Dekkers, G.S. Plastow and M.F. Rothschild, 2004. New alleles in calpastatin gene are associated with meat quality traits in pigs. J. Anim. Sci., 82: 2829-2839.

Davoli, R. and S. Braglia, 2007. Molecular approaches in pig breeding to improve meat quality. Brief. Funct. Genomic. Proteomic., 6: 313-321.

Davoli, R., S. Braglia, B. Lama, L. Fontanesi, L. Buttazzoni, C. Baiocco and V. Russo, 2003. Mapping, identification of polymorphisms and analysis of allele frequencies in the porcine skeletal muscle myopalladin and titin genes. Cytogenet. Genome. Res., 102: 152-156.

Fujii, J., K. Otsu, F. Zorzato, S. de Leon and V.K. Khanna *et al*., 1991. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science, 253: 448-451.

Grau, R. and R. Hamm, 1953. Eine einfache Methode zur Bestimmung der Wasserbindung im Muskel. Naturwissenschaften, 40: 29-30.

Ma, K. and K. Wang, 2002. Interaction of nebulin SH3 domain with titin PEVK and myopalladin: Implications for the signaling and assembly role of titin and nebulin. FEBS. Lett., 532: 273-278.

Milan, D., J.T. Jeon, C. Looft, V. Amarger and A. Robic *et al*., 2000. A mutation in PRKAG3 associated with excess glycogen content in pig skeletal muscle. Science, 288: 1248-1251.

Rohrer, G.A. and J.W. Keele, 1998. Identification of quantitative trait loci affecting carcass composition in swine: II. Muscling and wholesale product yield traits. J. Anim. Sci., 76: 2255-2262.

Simek, J., M. Grolichov, I. Steinhauserov and L. Steinhauser, 2004. Carcass and meat quality of selected final hybrids of pigs in the Czech Republic. Meat. Sci., 66: 383-386.

Wimmers, K., E. Murani, M.F. Te Pas, K.C. Chang and R. Davoli *et al*., 2007. Associations of functional candidate genes derived from gene-expression profiles of prenatal porcine muscle tissue with meat quality and muscle deposition. Anim. Genet., 38: 474-484.