New Evidence of Alleles (V199I and G52S) at the PRKAG3 (RN) Locus Affecting Pork Meat Quality

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ABSTRACT: The porcine PRKAG3 (RN) gene encodes the regulatory gamma subunit of adenosine monophosphate-activated protein kinase (AMPK), which is a good candidate gene affecting meat quality. In this study, the effects of two missense mutations A595G (Ile199Val) and G154A (Gly52Ser) in porcine PRKAG3 gene on meat quality traits were studied in M. Longissimus dorsi (LD), M. Semispinalis capitis (SC) and M. Biceps femoris (BF) from different populations of 326 pigs. The PRKAG3 alleles 199I, 199IV, 52S and 52G were identified with PCR-RFLPs and all genotypes - 199I/199I, 199I/199V, 199V/199V, 52S/52S, 52S/52G and 52G/52G - were found. The frequency of V allele was larger than that of I allele in all populations. I allele frequency was zero in Chinese Meishan pigs (population D) especially. G allele frequency was larger than that of S allele in all populations except Large White (population A). Both variations at the PRKAG3 locus significantly affected these meat quality traits. The pork meat quality has not previously been established in Meishan or crosses thereof. The results suggested that generally pH of LD, SC and BF was higher in Meishan pigs than that in other populations. Moreover, Meishan pigs showed higher water-holding capacity and intramuscular fat (IMF), lower water content and water loss percentage compared to other populations in terms of the two variations. The results present here supply new evidence that alleles V199I and G52S at the PRKAG3 locus affect pork meat quality and provide useful information on pork production.

(Key Words: Pork Meat Quality, PRKAG3, I199V Allele, G52S Allele, Different Populations)

INTRODUCTION

Hampshire pigs were first described as characteristic of meat with low pH, high glycogen content and low technological yield by Monin and Sellier (1985). The RN (from the French “Rendement Napole”) gene, a dominant major gene in the Hampshire breed, was later found to be associated with “Hampshire effect” (Le Roy et al., 1990; Fernandez et al., 1992). The dominant RN mutation, which causes high glycogen content in skeletal muscle, was found to be a nonconservative substitution (R200Q) at the Protein Kinase Adenosine Monophosphate-Activated γ3-Subunit (PRKAG3) Gene, and RN mutation (200Q) has been found only in Hampshire or its crossbred pigs but not in other pig breeds (Milan et al., 2000; Ciobanu et al., 2001). The PRKAG3 gene encodes a muscle-specific isoform of the regulatory gamma subunit of adenosine monophosphate-activated protein kinase (AMPK). AMPK is activated by an increase in the ratio of adenosine monophosphate (AMP) to adenosine triphosphate (ATP) and thereby plays a key role of regulating energy metabolism in eukaryotic cells. AMPK can also inactivate glycogen synthase which is the key regulatory enzyme of glycogen synthesis by phosphorylation (Hardie and Carling, 1997; Milan et al., 2000).

Low protein content (Monin et al., 1992; Estrade et al., 1993) and a higher degree of protein denaturation (Lundström et al., 1996) are present in RN carriers (200Q) compared with non-carriers. Le Roy et al. (2000) suggested that the RN allele is associated with a high level of glycogen and low pH, and lower sensory tenderness in meat from animals which are homozygous and heterozygous regarding the RN allele than that from non-carriers. However, most studies by trained expert panels or instrumental methods implied the opposite result that greater tenderness in meat is found in RN allele carriers than in non-carriers of the RN allele. (Lundström et al.,
1996; Jonsäll et al., 2000, 2001; Miller et al., 2000; van Laack et al., 2001; Josell et al., 2003b). No difference was found in sensory tenderness or juiciness between the RN carriers and non-carriers by Lundström et al. (1998).

In addition, new alleles found on the RN locus could have an important effect on meat quality. New alleles V199I and G52S were detected by Milan et al. (2000) and Ciobanu et al. (2001). In the five pig breeds Landrace, Large White, Berkshire, Duroc and Duroc Synthetic, higher pH 24 hours post-mortem and lower levels of glycogen, lactate and glycolytic potential were found in pigs with the 199I allele (Ciobanu et al., 2001). It was revealed that the influence of the 199V allele resembled that of 199I on sensory parameters in a Swedish Hampshire × Finnish Landrace pig population (Josell et al., 2003a). The 199I-200R allele gives higher ultimate pH and, in female pigs, produces a lower glycogen (Lindahl et al., 2004a, b). The G52S substitution significantly (p<0.05) affects only two traits (ham pH and loin Minolta L) in across-line analysis, and within-line analysis showed significant associations for loin Minolta color scores for just the Duroc Synthetic population (Ciobanu et al., 2001).

It is well documented that the Chinese Meishan pig has super reproduction traits. However, the relationship between meat quality and variations of the RN gene has not been established in Meishan or crosses thereof. The aim of this study was to detect associations between polymorphisms (I199V and G52S) and meat quality traits such as pH, intramuscular fat rate, water-holding capacity and water content in different pig populations including Meishan and crosses thereof as well as other populations.

**MATERIALS AND METHODS**

**Animals**

The 326 pigs were chosen from three different pureblood breed, Large White (LW), Landrace (L) and Chinese breed Meishan (MS); and four crossbreed, LW×L, L×LW, LW× MS and MS×LW, reference families founded by Key Laboratory of Swine Genetics and Breeding, Ministry of Agriculture, Huazhong Agricultural University. The breed compositions of these populations were: A (LW, n = 34), B (L, n = 45), C (LW, L, LW×L and L×LW, n = 144), D (MS, n = 54) and E (LW×MS and MS×LW, n = 128) (Tables 1 and 2). All pigs were transported for approximately 10 minutes and rested approximately 1h prior to stunning and exsanguination at the slaughterhouse of Agricultural Ministry Key Laboratory of Swine Genetics and Breeding. Muscle samples (M. Longissimus dorsi, LD; M. Semispinalis capitis; SC. Biceps femoris, BF) were obtained.

**pH and meat chemical content**

The pH was measured (Mettler Toledo Instrument Ltd, Shanghai, P. R. China) at LD, SC and BF 45 min post mortem. Water holding capacity, water loss percentage, water content and IMF content were analyzed as described previously (Xiong et al., 1999).

**Determination of genotypes**

Two alleles I199V and G52S of the PRKAG3 gene were identified with a DNA test using the PCR-restriction fragment length polymorphism (PCR-RFLP) method described by Ciobanu et al. (2001).
Statistical analysis

The associations between each allele polymorphism of PRKAG3 and meat quality traits were tested using mixed-model procedures (SAS procedure MIXED; SAS Institute), which included the fixed effects of genotype (G), sex of animals (S), slaughter day (D) and the random effects of family (F). The procedure was described as follows: $Y_{ijkl} = \mu + G_i + S_j + F_k + D_l + e_{ijkl}$. Some populations for which genetic background was close were pooled for a cross-population (population C and population E) analysis for both variations. Breed was added as a fixed effect for Population C and E. The two-way interaction between genotype and breed was included when significant. Similar analytical methods were used respectively by Kim et al. (2000), Ciobanu et al. (2001), Lindahl (2004a, b) and Chen et al. (2005). In addition, bodyweight at slaughter date was added as a covariate for IMF analysis. Significant differences between least squares means (LSM) were evaluated using the option Pdiff.

To estimate the effects of interaction between the two allelic variations, another model: $Y = \mu + B + S + F + D + G_1 + G_2 + G_1 \times G_2 + e$ was used in Population C. In this model, G1 and G2 represent alleles I/V and G/S, respectively, and letters B, S, F and D are as described above. In addition, bodyweight at slaughter date was added as a covariate for IMF analysis.

RESULTS

Genotype frequencies of I199V, G52S of PRKAG3 gene in five populations

Genotypes of I199V and G52S are shown in Figure 1 and Figure 2. The frequencies of different genotypes are shown in Table 1 and Table 2. For I199V in each population, frequency of allele I was lower than that of allele V and allele I frequency was zero in MS (population D), the Chinese pig breed. For G52S, frequency of allele S was lower than that of allele G in all populations except population A. Genotype SS (n = 1) was found in MS (population D).

pH

Significant differences were found in all three traits (pH in LD, SC, BF) in different populations. The LS mean of genotype SS in population D was not shown in the table because only one pig was carrying the SS genotype for the G52S mutation (Table 3).

For I199V, genotype IV showed the highest pH in LD compared with genotype II and VV in population C (p<0.05, difference between IV and VV was highly significant). The highest pH of genotype IV in BF was also shown in populations A (p<0.1 for difference between IV and VV) and C (p<0.05). Lower pH of IV in BF was shown in Population B (p<0.05). The highest pH of genotype IV in SC was found in populations A and C, although it was not statistically significant.

For G52S, a higher pH of genotype SG in LD and BF was shown in Population E (p<0.05). Higher pH of genotype SG and a lower pH of genotype GG in SC were found in populations B (p<0.05) and C (p<0.05).

Generally, Meishan breed pigs (population D) showed higher pH in each muscle than other populations for a given genotype.

Water

Significant differences in water loss percentage, water-
Table 3. Association results between the variation I199V, G52S of PRKAG3 gene and pH of LD, BF and SC in five populations

<table>
<thead>
<tr>
<th>pH</th>
<th>LD Water loss percentage (%)</th>
<th>LD Water-holding capacity (%)</th>
<th>SC Water content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>II</td>
<td>IV</td>
<td>VV</td>
</tr>
<tr>
<td>Population A</td>
<td>6.22±0.07</td>
<td>6.37±0.05</td>
<td>6.32±0.04</td>
</tr>
<tr>
<td>Population B</td>
<td>6.36±0.07</td>
<td>6.27±0.03</td>
<td>6.41±0.05</td>
</tr>
<tr>
<td>Population C</td>
<td>6.25±0.07a</td>
<td>6.40±0.03a</td>
<td>6.31±0.01a</td>
</tr>
<tr>
<td>Population D</td>
<td>6.49±0.02</td>
<td>6.47±0.02</td>
<td>6.57±0.01</td>
</tr>
<tr>
<td>Population E</td>
<td>6.33±0.03</td>
<td>6.40±0.01</td>
<td>6.45±0.03a</td>
</tr>
</tbody>
</table>

Table 4. Association results between the variations I199V, G52S of PRKAG3 gene and Water loss percentage (%), Water-holding capacity (%) and Water content (%) in five populations

<table>
<thead>
<tr>
<th>Water</th>
<th>LD Water loss percentage (%)</th>
<th>LD Water-holding capacity (%)</th>
<th>SC Water content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>II</td>
<td>IV</td>
<td>VV</td>
</tr>
<tr>
<td>Population A</td>
<td>8.89±0.22</td>
<td>6.31±0.66</td>
<td>7.13±0.49</td>
</tr>
<tr>
<td>Population B</td>
<td>6.77±0.87</td>
<td>7.56±0.31</td>
<td>90.88±1.15</td>
</tr>
<tr>
<td>Population C</td>
<td>9.12±0.80a</td>
<td>6.83±0.37</td>
<td>7.33±0.16</td>
</tr>
<tr>
<td>Population D</td>
<td>6.49±0.03</td>
<td>6.57±0.02</td>
<td>92.53±0.09</td>
</tr>
<tr>
<td>Population E</td>
<td>6.40±0.01a</td>
<td>6.32±0.04a</td>
<td>6.53±0.01a</td>
</tr>
</tbody>
</table>

Table 4. Association results between the variations I199V, G52S of PRKAG3 gene and Water loss percentage (%), Water-holding capacity (%) and Water content (%) in five populations

| Population A | 7.07±0.48 | 6.34±0.68a | 8.88±0.92a | 90.58±0.63 | 91.52±0.89a | 88.20±1.21a | 75.04±0.15 | 74.84±0.22 | 75.00±0.29 |
| Population B | 6.56±0.62 | 7.72±0.32 | 91.22±0.82 | 89.66±0.43 | 74.63±0.20 | 74.69±0.10 |
| Population C | 7.13±0.44 | 6.98±0.25 | 7.59±0.22 | 90.53±0.58 | 90.69±0.33 | 89.87±0.28 | 75.28±0.16a | 75.03±0.09 | 74.87±0.08a |
| Population D | 5.43±0.06 | 92.51±0.09 | 72.56±0.12 |
| Population E | 6.48±0.17 | 6.23±0.46 | 91.18±0.22 | 91.54±0.61 | 73.46±0.06 | 73.62±0.17 |

For I199V, water loss percentage was significantly highest in LD of genotype II and lowest for genotype IV in populations A (p<0.05) and C (p<0.01). The water-holding capacity in LD was significantly highest for genotype IV and the lowest for genotype II in populations A (p<0.05) and C (p<0.01). For G52S, water loss percentage in LD of genotype GG was the highest and that of genotype SG was the lowest in populations A (p<0.05) and B (p=0.1). Water-holding capacity in LD of genotype GG was the lowest and that of genotype SG was the highest in populations A (p=0.05) and B (p=0.1). Significant (p<0.05) difference of water content was only observed in population C, in which the value of genotype SS was the highest and that of genotype GG was the lowest. Generally, Meishan pigs showed higher water-holding capacity in LD, lower water content and water loss percentage than other populations for a given genotype. IMF

For I199V, significantly lower IMF in LD of genotype IV compared with genotype VV was found in population E (p<0.05), while there was an opposite result in population B (p<0.01) (Table 5). For G52S, the highest IMF in LD of genotype GG was observed in populations A (p<0.1 for difference between SS and GG) and C (p=0.01). Meishan pigs showed a higher IMF value than other populations for a given genotype.

Interaction effects between two allelic variations

The interaction between the two allelic variations significantly affected pH in LD (p<0.05) and BF (p<0.05) (Table 6). The highest pH in LD, BF and SC was shown in Genotype IVSG. The highest water loss percentage and the lowest water-holding capacity was shown in I1GG.

DISCUSSION

The Chinese pig breed Meishan is well known for
### Table 5. Association results between the variations I199V, G52S of PRKAG3 gene and LD IMF (%) in five populations

<table>
<thead>
<tr>
<th>Genotype</th>
<th>IMF (%)</th>
<th>LD pH</th>
<th>BF pH</th>
<th>SC pH</th>
<th>LD WLP</th>
<th>LD WHC</th>
<th>LD WC</th>
<th>LD IMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population A</td>
<td>2.04±0.13</td>
<td>1.97±0.13</td>
<td>1.80±0.07</td>
<td>1.78±0.07</td>
<td>1.96±0.09</td>
<td>2.04±0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population B</td>
<td>2.38±0.12b</td>
<td>1.92±0.04b</td>
<td>1.85±0.10</td>
<td>2.00±0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population C</td>
<td>1.97±0.13</td>
<td>1.90±0.06</td>
<td>1.80±0.03</td>
<td>1.66±0.07b</td>
<td>1.81±0.04</td>
<td>1.88±0.04b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population D</td>
<td>5.25±0.11</td>
<td></td>
<td></td>
<td></td>
<td>5.26±0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population E</td>
<td>3.43±0.12a</td>
<td>3.75±0.05a</td>
<td>3.73±0.05</td>
<td>3.48±0.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All estimates are least-squares means±standard error. Estimates with the same subscript are significantly different. *Represents p<0.05. **Represents p<0.01. LD is the abbreviation of *M. Longissimus dorsi*. IMF is the abbreviation of intramuscular fat.

The results suggested that Meishan breed pigs (population D) showed higher pH (in LD, SC and BF), water-holding capacity in LD and IMF, lower water content and water loss percentage than other populations for the same genotype carried.

The *RN* gene is related to pig chromosome 15 (Milan et al., 1995; Loof et al., 1996; Mariani et al., 1996). Further studies showed that the *PRKAG3* (*RN*) gene was located between the markers SW1683 and SW1983 (Ciobanu et al., 2001) on SSC15 (Milan et al., 2000). The bacterial artificial chromosome library and contig in the porcine chromosome 15 (Milan et al., 2000). Jeon et al. (2001) suggested the high degree conservative linkage order in the *RN* region of the porcine map compared with the human transcript map, and identified *PRKAG3* as the causative gene for the *RN* phenotype. Based on this information, the dominant *RN* mutation was found to be a nonconservative substitution (R200Q) at the *PRKAG3* gene, and allele 200Q has been found only in Hampshire or its crossbred pigs but not in other pig breeds, though the alleles 200R, 199I and 199V were found in many pig breeds including the Hampshire breed (Milan et al., 2000; Ciobanu et al., 2001).

In addition, new alleles 52G and 52S of the *PRKAG3* gene were observed in several commercial populations such as Landrace, Large White, Berkshire and Duroc pig breeds etc. (Ciobanu et al., 2001).

The *PRKAG3* gene encodes the regulatory gamma subunit of adenosine monophosphate-activated protein kinase (AMPK) (Milan et al., 2000). Beta and gamma regulatory subunits of the AMPK are essential for kinase activity (Hardie and Carling, 1997). Cheung et al. (2000) suggested that the allostery AMP-binding site may involve both the gamma- and alpha-subunits of the AMPK complex, and proposed that the heterotrimeric complex may be predominantly inactive without interaction between the gamma- and alpha-subunits in the absence of AMP.

Ciobanu et al. (2001) revealed that the I199V substitution may play a role in the activity of AMPK based on the evidence of alignment information, the proposed model of the regulation of the AMPK complex and the presence of the R200Q site nearby I199V. The I199V substitution is located in the first and most conserved domain (Ciobanu et al., 2001) of four cystathionine beta-synthase (CBS) domains in the *PRKAG3* gene (Milan et al., 2000). Ponting (1997) suggested that the CBS domains are involved in cytoplasmic targeting. Bateman (1997) revealed that CBS domains play a role in protein-protein interaction and/or regulation of protein activity.

Besides the evidence and hypotheses above, Ciobanu et al. (2001) hypothesized that the amino acid change might also influence the structure and activity of the enzyme, resulting in the observed effect of the G52S substitution.
although the molecular structure of the AMPK complex has not been resolved.

In our study, both I199V and G52S variations significantly affected most of the traits analyzed in four populations. Ciobanu et al. (2001) suggested that I199V substitution also significantly affected glycogen and lactate content, glycolytic potential measures and some of the meat quality traits associated with these measures, while G52S only significantly affected ham pH and loin Minolta L (the latter was not analyzed in our study). The 52G allele is a favorable one in Berkshire×Yorkshire F1 in terms of meat quality, while in a cross-line analysis, a different favorable allele was obtained (Ciobanu et al., 2001). In our study, for water loss percentage (%) and water-holding capacity (%), the 52S was favorable, while for IMF, 52G was favorable. For I199V, the allele 199V was favorable for water loss percentage (%) and water-holding capacity (%). In addition, the results of interaction effects also indicated the highest water loss percentage and the lowest water-holding capacity on genotype IIGG.

In population C, it was revealed that both allelic variations affected pH significantly. I199V affected pH in LD and BF (p<0.05), and G52S affected pH in LD, BF and SC (p<0.05) for association analyses of individual variations. The heterozygote pigs showed highest values of pH for both allelic variations, which implied that a dominant effect is the main effect for pH. For interaction effects between the two variations, genotype IVSG also showed highest pH in LD, BF and SC.

In this paper, two variations, I199V and G52S, of candidate gene PRKAG3 can explain significant differences for pH in LD, BF and SC, water content, IMF, water-holding capacity and water loss percentage in four populations of pigs. These results illustrate the potential value of Marker Assisted Selection (MAS) with candidate genes in the livestock industry.

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