Expression Differences of the PRKAG3 Gene in Skeletal Muscle of Different Pig Breeds at Different Growth Stages and Its Relationship with Meat Quality (Postprint)

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Abstract

This study aimed to compare the differential expression of the PRKAG3 gene in skeletal muscle across different growth stages in different pig breeds and to investigate the relationship between the PRKAG3 gene and meat quality. A total of 17 Hampshire castrated male pigs and 16 Landrace × Duroc castrated male pigs, weighing approximately 15 kg, were selected and fed the same diet. When their body weights reached 20 kg and 50 kg, five pigs from each breed were slaughtered; when the body weight reached 100 kg, seven Hampshire and six Landrace × Duroc pigs were slaughtered. After slaughter at each growth stage, skeletal muscle pH, muscle glycogen content, and PRKAG3 gene expression levels were measured, and at the 100 kg stage, meat quality traits were also determined post-slaughter. The results showed: 1) The expression level of the PRKAG3 gene in skeletal muscle of Landrace × Duroc pigs was higher than that of Hampshire pigs at all growth stages, particularly at the 100 kg stage, where the expression level in Landrace × Duroc pigs was 6.81-fold that of Hampshire pigs ($P<0.05$). The PRKAG3 gene expression levels in skeletal muscle of both Landrace × Duroc and Hampshire pigs increased with body weight; however, the difference among growth stages was not significant in Hampshire pigs ($P>0.05$), whereas in Landrace × Duroc pigs, expression at the 100 kg stage was significantly higher than at the 20 kg and 50 kg stages ($P<0.05$). 2) Meat quality differed between Hampshire and Landrace × Duroc pigs. Drip loss and water loss rate were significantly higher in Hampshire pigs than in Landrace × Duroc pigs ($P<0.05$), while cooking yield and b* value were highly significantly lower ($P<0.01$), and shear force and pH2 (pH at 24 h postmortem) were significantly lower ($P<0.05$). Compared with Landrace × Duroc pigs, Hampshire pigs had higher muscle glycogen content ($P>0.05$). 3) The correlation between PRKAG3 gene expression level in pig skeletal muscle and meat quality

exhibited a breed effect. In Hampshire pigs, PRKAG3 gene expression level in skeletal muscle was positively correlated with drip loss, negatively correlated with cooking yield, and significantly negatively correlated with pH2 ($P<0.05$). In Landrace × Duroc pigs, PRKAG3 gene expression level was positively correlated with drip loss and water loss rate, and negatively correlated with pH2. These results indicate that the PRKAG3 gene in pig skeletal muscle exhibits breed- and growth stage-specific expression differences; the expression level of the PRKAG3 gene in pig skeletal muscle is correlated with meat quality traits, particularly with pH2, showing a negative correlation.

Full Text

The Expression Difference of PRKAG3 Gene in Skeletal Muscle at Different Growth Stages for Different Breeds of Pigs and the Relationships Between PRKAG3 Gene Expression Level and Meat Quality

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Abstract

This study was conducted to compare the expression difference of PRKAG3 gene in skeletal muscle at different growth stages for different breeds of pigs, and to investigate the relationships between PRKAG3 gene expression level and meat quality. Seventeen Hampshire barrows and sixteen Landrace × Saba (LS) barrows weighing approximately 15 kg were selected and fed the same diet. When body weight reached 20 kg and 50 kg, five pigs from each breed were slaughtered. At 100 kg body weight, seven Hampshire pigs and six LS pigs were slaughtered. Post-slaughter measurements included skeletal muscle pH, muscle glycogen content, and PRKAG3 gene expression level at each growth stage, with additional meat quality traits determined at the 100 kg stage. The results showed: 1) At all growth stages, PRKAG3 gene expression level in skeletal muscle of LS pigs was higher than that of Hampshire pigs, particularly at the 100 kg stage, where it was 6.81 times higher ($P<0.05$). PRKAG3 gene expression level in skeletal muscle increased with body weight in both breeds, though the difference among growth stages was not significant for Hampshire pigs ($P>0.05$). For LS pigs, expression at the 100 kg stage was significantly higher than at the 20 kg and 50 kg stages ($P<0.05$). 2) Meat quality differed between Hampshire and LS pigs. Hampshire pigs exhibited significantly higher drip loss and water loss rate ($P<0.05$), significantly lower cooked meat percentage and b* value ($P<0.01$), and significantly lower shear force and pH_2 (pH at 24 h post-slaughter)

compared to LS pigs (P<0.05). Hampshire pigs also had higher muscle glycogen content than LS pigs (P>0.05). 3) The correlation between PRKAG3 gene expression level in skeletal muscle and meat quality showed breed-specific effects. In Hampshire pigs, PRKAG3 expression level was positively correlated with drip loss, negatively correlated with cooked meat percentage, and significantly negatively correlated with pH_2 (P<0.05). In LS pigs, PRKAG3 expression level was positively correlated with drip loss and water loss rate, and negatively correlated with pH_2. These results indicate that PRKAG3 gene expression in porcine skeletal muscle varies by breed and growth stage, and its expression level correlates with meat quality traits, particularly showing a negative correlation with pH_2.

Keywords: pigs; PRKAG3 gene; expression difference; meat quality; correlation

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Adenosine monophosphate-activated protein kinase (AMPK) is a protein kinase activated by AMP that participates in nutritional metabolism regulation and plays a crucial role in glucose metabolism [1]. Studies in mice have shown that AMPK activation can inhibit glycogen synthase activity through phosphorylation, reduce glycogen synthesis rate, and cause glucose transporter 4 (GLUT-4) translocation from intracellular compartments to the plasma membrane, promoting glucose uptake by muscle and thereby increasing skeletal muscle glycogen content [2], which may subsequently affect meat quality. AMPK consists of catalytic subunit α, regulatory subunit β, and γ subunit, with α and β having two isoforms each, and γ having three isoforms: $ $1, $ $2, and $ $3. The PRKAG3 gene encodes the AMPK $ $3subunitandisspecificallyexpressedinskeletalmuscle$[3].$Milanetal.$[4]$reportedthatamutationatthe$200thcod mutation adjacent to Arg200 in the PRKAG3 gene has opposite effects to the RN gene (Rendement Napole gene, also known as the acid meat gene), reducing skeletal muscle glycogen content and thus improving meat quality. These findings suggest that PRKAG3 may be a major gene affecting meat quality traits. However, no studies have yet reported on the relationship between PRKAG3 gene expression level and meat quality.

This experiment investigated the differential expression of PRKAG3 gene in skeletal muscle of Hampshire and LS (Landrace × Saba) pigs at different growth stages and its relationship with meat quality, which holds significant importance for meat quality research at the molecular level.

1.1 Experimental Design

A single-factor experimental design was employed. Seventeen Hampshire barrows and sixteen LS barrows weighing approximately 15 kg were selected (33 pigs total). Both breeds were housed under identical environmental conditions and fed the same diet formula. When body weight reached 20 kg and 50 kg, five pigs from each breed with similar body weights were selected for slaughter

to determine skeletal muscle pH, muscle glycogen content, and PRKAG3 gene expression level at each developmental stage. At 100 kg body weight, the remaining seven Hampshire pigs and six LS pigs were slaughtered. In addition to pH, glycogen content, and PRKAG3 expression, meat quality traits were also determined at this final stage.

1.2 Experimental Diets

Experimental diets were formulated according to NRC (2012) standards in three phases: 20–50 kg, 50–80 kg, and 80–100 kg. Both breeds received identical diet formulations, with details provided in Table 1 .

1.3 Animal Management

Experimental pigs were individually housed in single pens from 15 kg to 100 kg body weight. Pelleted feed was provided three times daily (08:00, 13:00, and 18:00) ad libitum with slight excess, and water was freely available. Management conditions were identical for both breeds.

1.4 Sample Collection

At slaughter at 20 kg or 50 kg body weight, two approximately 10 g samples were taken from the longissimus dorsi muscle at the 10th rib—one for pH measurement and one for muscle glycogen content determination. At 100 kg slaughter, three approximately 50 g samples were collected from the same location: one for drip loss determination, one for pH, muscle glycogen content, color, and water loss rate measurements, and one for cooked meat percentage determination. An additional approximately 100 g sample of psoas major muscle was taken for shear force measurement using methods described in reference [6].

Post-slaughter, longissimus dorsi muscle samples at the 10th rib were rapidly dissected, minced, washed twice with phosphate-buffered saline (PBS) treated with 0.1% diethylpyrocarbonate (DEPC), placed in sterilized 1.5 mL EP tubes, rapidly frozen in liquid nitrogen, and stored at -70 °C for PRKAG3 gene expression analysis.

1.5 Index Determination

1.5.1 pH Measurement pH was measured using a specialized meat pH meter following methods described in reference [6]. pH_1 refers to pH at 45 min post-slaughter, while pH_2 refers to pH at 24 h post-slaughter.

1.5.2 Muscle Glycogen Content Determination Muscle glycogen content was determined using a commercial assay kit from Nanjing Jiancheng Bioengineering Institute according to the manufacturer' s instructions. Due to rapid post-slaughter glycolysis of muscle glycogen, measurements were conducted on the second day after slaughter.

1.5.3 Meat Quality Traits Determination Drip loss, color, water loss rate, shear force, and cooked meat percentage were determined using methods described in reference [6].

1.5.4 PRKAG3 Gene Expression Level Determination

1.5.4.1 Total RNA Extraction and Reverse Transcription Thirty milligrams of longissimus dorsi muscle sample were ground into powder with liquid nitrogen and collected in a 1.5 mL EP tube. Total RNA was extracted using the QIAGEN RNeasy Mini Kit according to the manufacturer' s protocol. Extracted total RNA integrity was verified by gel electrophoresis, and purity was assessed by measuring OD values at 260 nm and 280 nm. Reverse transcription was performed using the extracted total RNA as template in a 10 L reaction system containing 0.25 L RNase inhibitor, 2 L $MgCl_2$, 1 L Oligo dT primer, 1 L dNTP Mixture, 4.25 L total RNA, 1 L 10×RT buffer, and 0.5 L reverse transcriptase. The reaction program consisted of 30 °C for 10 min, 42 °C for 2 h, 99 °C for 5 min, and 5 °C for 5 min.

1.5.4.2 Primer and TaqMan Probe Design and Synthesis Primers and probes for PRKAG3 gene and the reference gene β-actin were designed based on gene sequences AF214520 and U07786, respectively, and synthesized by Shanghai GeneCore BioTechnologies Co., Ltd. Primer and probe sequences are listed in Table 2 .

1.5.4.3 PCR Reaction System and Program Real-time fluorescent quantitative PCR was performed using cDNA as template in a total reaction volume of 25 L containing 2 L cDNA, 12.5 L Premix Ex Taq (2×), 0.5 L forward primer, 0.5 L reverse primer, 1 L probe (10 mol/L), 0.5 L ROX Dye II (50×), and 8 L 0.1% DEPC-treated water. The PCR program consisted of 95 °C for 3 min, followed by 40 cycles of 94 °C for 25 s and 60 °C for 30 s, with a final extension at 70 °C for 5 min.

1.5.4.4 Quantitative Analysis Standard curves were constructed before quantitative amplification of target and reference genes. Based on these standard curves, the quantitative PCR instrument automatically calculated copy numbers of PRKAG3 and β-actin genes per milliliter of sample. PRKAG3 gene expression level was expressed as the ratio of PRKAG3 gene copy number to β-actin gene copy number per milliliter of sample.

1.6 Statistical Analysis

SPSS 17.0 statistical software was used for variance analysis and Duncan' s multiple comparison tests for data from different breeds at various developmental stages, t-tests for data from different breeds at the same stage, and correla-

tion analysis between PRKAG3 gene expression level and meat quality traits. Results are expressed as mean ± standard error (mean±SE).

2.1 PRKAG3 Gene Expression Levels in Skeletal Muscle of Different Pig Breeds at Various Growth Stages

PRKAG3 gene expression levels in skeletal muscle of Hampshire and LS pigs at different developmental stages are shown in Table 3 . LS pigs exhibited higher PRKAG3 expression than Hampshire pigs at all developmental stages, with expression at the 100 kg stage significantly higher than in Hampshire pigs ($P<0.05$), representing a 6.81-fold increase. PRKAG3 expression increased with body weight in both breeds, though differences among growth stages were not significant for Hampshire pigs ($P>0.05$). In contrast, LS pigs showed significantly higher expression at the 100 kg stage compared to the 20 kg and 50 kg stages ($P<0.05$).

2.2 Muscle Glycogen Content and pH in Skeletal Muscle of Different Pig Breeds at Various Growth Stages

Muscle glycogen content and pH values in skeletal muscle of Hampshire and LS pigs at different growth stages are presented in Table 4 . At the 20 kg stage, no significant differences were observed in muscle glycogen content or pH_1 between breeds ($P>0.05$), though pH_2 was significantly lower in Hampshire pigs ($P<0.05$). At the 50 kg stage, Hampshire pigs showed significantly higher muscle glycogen content and pH_1 ($P<0.05$) and significantly lower pH_2 ($P<0.01$) compared to LS pigs. At the 100 kg stage, muscle glycogen content and pH_2 were significantly lower in Hampshire pigs ($P<0.05$), while pH_1 did not differ significantly between breeds ($P>0.05$).

2.3 Comparison of Meat Quality Traits Between Different Pig Breeds

Meat quality traits of Hampshire and LS pigs are compared in Table 5 . Hampshire pigs exhibited significantly lower shear force ($P<0.05$), significantly higher drip loss and water loss rate ($P<0.05$), and significantly lower cooked meat percentage and b* value ($P<0.01$) compared to LS pigs. No significant differences were observed in L* value or a* value between breeds ($P>0.05$).

2.4 Correlation Analysis Between PRKAG3 Gene Expression Level in Skeletal Muscle and Meat Quality Traits

Correlation coefficients between PRKAG3 gene expression level in skeletal muscle and meat quality traits are presented in Table 6 . In Hampshire pigs, PRKAG3 expression level was positively correlated with drip loss and b* value, negatively correlated with cooked meat percentage and a* value, and significantly negatively correlated with pH_2 ($P<0.05$). In LS pigs, PRKAG3 expression level was positively correlated with water loss rate and L* value, and negatively correlated with pH_2, drip loss, a* value, and dressing percentage, though

these correlations were not significant (P>0.05). When breed effects were ignored, PRKAG3 expression level in skeletal muscle was significantly negatively correlated with pH_2 (P<0.05), negatively correlated with drip loss and water loss rate, and positively correlated with cooked meat percentage, pH_1, L* value, a* value, and b* value, though these correlations were not significant (P>0.05).

To further elucidate the relationship between PRKAG3 gene expression level and pH_2, expression levels were plotted against pH_2 for each breed, as shown in Figure 1 [Figure 1: see original paper] and Figure 2 [Figure 2: see original paper]. Although the trend of pH_2 variation with PRKAG3 expression differed slightly between breeds, both showed a negative correlation between PRKAG3 expression level and pH_2.

PRKAG3 gene expression levels at various growth stages were also plotted against muscle glycogen content for each breed, as shown in Figure 3 [Figure 3: see original paper] and Figure 4 [Figure 4: see original paper]. Despite inconsistent patterns of muscle glycogen content between breeds, both showed a general nonlinear increase in glycogen content with increasing PRKAG3 expression level.

3.1 Differential Expression of PRKAG3 Gene in Skeletal Muscle of Different Pig Breeds at Various Growth Stages

No previous studies have reported on differential PRKAG3 gene expression in porcine skeletal muscle at different growth stages. Milan et al. [4] demonstrated that the Arg200→Gln200 mutation in the PRKAG3 gene reduces AMPK activity by threefold in Hampshire pigs. Galve et al. [7] reported that the Val199→Ile199 mutation in the PRKAG3 gene increased pH_2 by 0.14 and 0.16 in ham and loin muscles of Duroc × Landrace-Large White crossbred pigs, respectively. However, these studies did not measure PRKAG3 gene expression levels. The present results demonstrate that PRKAG3 expression in skeletal muscle was higher in LS pigs than in Hampshire pigs at all growth stages, particularly at the 100 kg stage, where it was 6.81 times higher. Barnes et al. [8] confirmed through transgenic mouse models that the Val199→Ile199 mutation substantially reduces PRKAG3 gene expression level. Moreover, high glycogen content in skeletal muscle can feedback-inhibit AMPK activity [9]. The current findings also confirmed that muscle glycogen content was significantly higher in Hampshire pigs than in LS pigs at both 50 kg and 100 kg stages, which may explain the lower PRKAG3 expression in Hampshire pigs. Mahlapuu et al. [10] reported that $ 3*isthepredominantAMPK* $ isoform in glycolytic fibers (type IIB fibers) and plays a crucial role in glucose metabolism in white glycolytic fibers. Scheffler et al. [11] demonstrated that the R225Q variant in the PRKAG3 gene results in higher glycogen content in porcine skeletal muscle regardless of AMPK activation. In vitro studies have also shown that the AMPK$ $3 isoform is essential for AICAR-induced glucose transport, a function that cannot be compensated by other γ isoforms [8]. These findings indicate that PRKAG3 gene regulates glucose metabolism in skeletal muscle, particularly in glycolytic

fibers, thereby affecting muscle glycogen content and consequently meat quality. The observation that muscle glycogen content did not differ significantly between breeds at 20 kg and 50 kg stages but differed significantly at 100 kg stage provides support for the similar pattern observed in PRKAG3 expression differences. The reason why PRKAG3 expression increased with body weight in both breeds remains unclear and warrants further investigation.

3.2 Relationship Between PRKAG3 Gene Expression Level and Meat Quality

Previous studies have identified PRKAG3 as a key gene affecting porcine meat quality traits, influencing pH, color, and drip loss [4,12]. Uimari and Sironen [13] reported that variations at positions 24E and 199I in the PRKAG3 gene exon improved pork quality in Yorkshire and Landrace pigs. Milan et al. [4] found that the R200Q mutation in PRKAG3 increased muscle glycogen content by 70% in RN-/RN- (dominant homozygous) and RN-/rn+ (heterozygous) animals, with high glycogen content leading to reduced pH_2, decreased water-holding capacity, and affected meat color and shear force. Since pH is strongly correlated with other meat quality traits [14], PRKAG3 expression level is hypothesized to correlate with meat quality. However, no studies have previously reported on this correlation. The present results show that in Hampshire pigs, PRKAG3 expression level was positively correlated with drip loss and b* value, negatively correlated with cooked meat percentage and a* value, and significantly negatively correlated with pH_2 ($P<0.05$). In LS pigs, PRKAG3 expression level was positively correlated with water loss rate and L* value, and negatively correlated with pH_2, drip loss, a* value, and dressing percentage, though these correlations were not significant. These findings demonstrate breed-specific effects in the correlation between PRKAG3 expression level and meat quality traits. Škrlep et al. [15] also reported that variations R200Q and I199V in the porcine PRKAG3 gene resulted in decreased pH_2 and increased L* value, a* value, b* value, and drip loss.

Enfalt et al. [16] identified three genotypes of the PRKAG3 gene in Landrace × Hampshire crossbred pigs: RN- (Val199→Gln200), rn+ (Val199→Arg200), and rn* (Ile199→Arg200), and compared their effects on meat quality and carcass composition. The results showed that RN- and rn* genotypes had opposite effects: RN- individuals had higher muscle glycogen content, higher lean meat percentage, and lower final pH, while rn* individuals had lower muscle glycogen content, lower lean meat percentage, and higher final pH. Granlund et al. [17] reported that the Ile199→Arg200 variation in Hampshire pigs promoted glucose phosphorylation in longissimus dorsi muscle, enhanced oxidative capacity, and reduced glycolytic capacity and phosphatase activity. However, Riedl et al. [18] later found that the AMPK$ 3$isoformisnotessentialformusclegrowthinpigs.WhileitisestablishedthatAMPK $3 isoform variations control glucose uptake and regulate glycogen synthesis in muscle, thereby affecting muscle glycogen content and meat quality [8], the

specific effects of PRKAG3 gene expression level on meat quality and its underlying mechanisms require further investigation.

Conclusion

PRKAG3 gene expression in porcine skeletal muscle exhibits breed- and growth stage-specific differences, with higher expression in LS pigs than in Hampshire pigs and increasing expression with body weight. PRKAG3 gene expression level correlates with meat quality traits, particularly showing a negative correlation with pH_2.

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Note: Figure translations are in progress. See original paper for figures.