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Association of *SIRT5* gene expression with meat quality traits in F1 generation of wild boar × kele pig

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Abstract

The aims of this study were to analyze the coding region sequence of the *SIRT5* gene and to explore the association of *SIRT5* gene expression with meat quality traits in the F1 generation of wild boar × Kele pigs (Yeke F1). In this study, two SNPs (g.5753 A > G, g.16916 A > G) were found in the CDS region of the *SIRT5* that could affect meat quality. *SIRT5* was an unstable hydrophilic protein with a highly conservative structure of the SIR2 functional domain. The expression of *SIRT5* in subcutaneous fat was significantly higher than that of the longissimus dorsi muscle from Yeke F1 pigs ($P < 0.01$). The expression level of *SIRT5* negatively correlated with meat color ($P < 0.01$) and positively correlated with the pH_1 ($P < 0.05$) in longissimus dorsi muscle. Expression of *SIRT5* was positively correlated with meat tenderness, muscle moisture, heptadecanoic acid, elmintic acid and methyl nervonate ($P < 0.01$); however, it was negatively correlated with intramuscular fat content, myristic acid, stearic acid and trans linoleic acid ($P < 0.05$) in subcutaneous fat. This study indicated that *SIRT5* could be used as a candidate gene for regulating intramuscular fat content and flavor indexes of Yeke F1 pigs.

Keywords: *SIRT5*; qRT-PCR; pork quality; fatty acids; correlation analysis.

Practical application: A candidate gene for pork quality.

1 Introduction

Different criteria are used to asses pork quality including the color of the meat, intramuscular fat or marbling, water-holding capacity (or drip loss) and fatty acid composition, all of which play an impotant role in the flavor of the final product. These pork quality traits have been linked to the expression of multiple genes including *PHKG1*(Zappaterra et al., 2019), *PRKAG3*(Ryan et al., 2012), *FUT1*(Jiang et al., 2005), *APOA5* and *APOC3* (Hui et al., 2013). SIRT5 (Silence Information Regulator 5) is part of the Sirtuins family (Lombard et al., 2011; Yao et al., 2014; Simó-Mirabet et al., 2018) and has been associated with dessuccinylation, dipropionylation and glutarylation. Rardin et al. (Rardin et al., 2013) found that SIRT5 regulates the balance of fatty acid oxidation between mitochondria and peroxidase in mouse proximal tubular epithelial cells and participates in fatty acid oxidation in the body. Browning ability of subcutaneous white fat in *SIRT5* knockout mice was impaired, which confirmed that *SIRT5* is also a key factor in the differentiation of brown fat cells (Shuai et al., 2019). It has been demonstrated that *SIRT5* can regulate fat deposition and meat quality traits in Qinchuan cattle (Deng, 2014). However, there is little research on the sequence and function of the *SIRT5* gene in pigs, which is widely expressed in various tissues of Sujiang pigs, especially in the heart and liver (Wang et al., 2019).

Kele pigs are a high-quality breed native to Southwest China and the Guizhou Province, characterized by tolerance to extreme cold, humidity resistance, and stress resistance; the pig is also suitable for the complex breeding environment in Guizhou karst areas (Yang et al., 2019). Increased backfat thickness is one of the disadvantages that restrict Kele pigs from entering the international market. F1 generation commercial pigs were obtained by crossing Kele pigs with wild boars (Yeke F1). These animals maintain the excellent meat quality of Kele pigs as well as the low backfat thickness of wild boars, and their meat has high commercial value.

In view of the role of the *SIRT5* gene in brown fat differentiation, fat deposition and meat quality traits of mammals. To further explore the relationship between the *SIRT5* gene and pork quality performance indicators in Yeke F1 pigs, the coding sequence (CDS) region of the *SIRT5* gene was obtained by cloning and its mutation sites were analyzed. The expression levels of *SIRT5* in the longissimus dorsi muscle and subcutaneous fat of Yeke F1 pigs were detected by qRT-PCR, and their correlation with meat quality traits were analyzed, aiming to provide a foundation for understanding the regulation of the *SIRT5* gene on pork quality and performance in the future.

2 Materials and methods

2.1 Animals and sample collection

Crossbred pigs (Kele pig ♀ × wild boar ♂) with average body weights of 100 to 120 kg at finishing ages of about 300 days were

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collected from local farms (Guizhou younonggu ecology industry co., ltd). All pigs were reared under the same conditions and fed the same commercial diet (Charoen Pokphand Group). Pigs were slaughtered, and then the carcasses were transferred to a cutting room. Longissimus thoracis et lumborum (LTL) muscle samples (n = 5) were collected from the left side of each carcass and used for meat quality analyses. The pH, instrumental color, drip loss, cooking loss, Warner-Bratzler shear force (WBSF), muscle moisture content, and intramuscular fat (IMF) were assesssed on the same sampling day. The samples used for fatty acids were vacuum-packaged and stored at −20 °C until use. The longissimus dorsi and subcutaneous fat for gene cloning was stored at −80 °C.

2.2 pH measurements

pH values of the pork samples were determined using a portable pH meter (Ohaus, USA) with a stainless steel and solidstate probe. Before use, the pH meter was calibrated with pH 4.0 and 7.0 standards according to the manufacturer's instructions, and then the probe was inserted deep into the muscle tissue. The pH values of each sample were measured at 1 h (pH_1) and 24 h (pH_{24}) after slaughter, and the average of three readings was recorded.

2.3 Instrumental color measurements

Instrumental color traits were determined on 3 different locations of the freshly cut surface of each sample within 60 min of blooming using a CR-400 colorimeter (Minolta Camera Co, Japan). Color is expressed according to the Commission International de l'Eclairage (CIE) system and reported as L* (lightness), a* (redness), b* (yellowness).

2.4 Drip loss

Drip loss of the muscle samples was measured following a procedure described in previous study (Fischer, 2007). Briefly, slices of standard thickness (2 cm) wer cut perpendicular to the fiber orientation within 2 h. Each sample was assessed in triplicate. The slices were then suspended in an inflated sealed plastic bag, sealed in a refrigerator at 4 °C, and removed after 48 h. The weight of each sample before dripping (W_1) and after dripping (W_2) was weighed using an electronic analytical balance (Sartorius, German) and recorded. The drip loss of each sample was calculated as (Formula 1):

$$\left(W_1 - W_2\right) / W_1 \times 100\% \quad (1)$$

The drip loss value was the average of three repetitions.

2.5 Cooking loss and Warner-Bratzler shear force value measurements

Cooking loss and WBSF were measured on muscle samples (3 cm thick, approximately 100 g) cooked in a pre-heated water bath until the core temperature reached 70 °C. The samples were then cooled in running water for 30 min to reach a core temperature below 30 °C. Cooking loss was determined by calculating the difference in weight of the muscles before and after cooking and is expressed as percentage of initial weight. The cooking loss value of each sample reported herein is the average of three repetitions.

The same samples were used for WBSF analysis. Three cores of 2 cm in diameter were collected from each sample. WBSF values were obtained by completely cutting through the cores in an Instron Universal Testing Machine (Model 4465, Instron Corp, UK) using a crosshead speed of 200 mm/min and a 40 N load cell.

2.6 Chemical composition

IMF content was measured using a Soxtec2055 automatic Soxhlet extractor (Foss, Denmark). Moisture content in the longissimus dorsi muscles was measured as described by Hoa et al. (Hoa et al., 2019).

2.7 Fatty acid profiles

Fatty acid profiles were determined as described below: Fats in samples were extracted using a solvent mixture of phenol:petroleum ether (1:1, v/v). The extract was methyl esterified using potassium hydroxide-methanol solution (2 mol/L). Fatty acid composition was determined using a gas chromatography/flame ionization detector system (8860, Agilent Technologies, CA, USA). Fatty acids were separated on a DB-FastFAME (30 m × 0.25 mm × 0.25 μM film thickness; Agilent Technologies, CA, USA) at a split ratio of 100:1. Nitrogen was the carrier gas in constant pressure mode at 12.0 psi. A 2 μL aliquot of each sample was injected into the injection port, and the temperatures of the injector and detector were set to 250 °C and 260 °C, respectively. The oven temperature was kept at 80 °C for 30 s, raised to 165 °C at a rate of 40 °C/min, and further increased to 230 °C at a rate of 4 °C/min. The fatty acids in samples were identified by comparing their retention times to those obtained from standard fatty acids. The results are expressed as relative percentage (%) of total fatty acids based on total peak area.

2.8 RNA extraction and reverse transcription

Total RNA was extracted using a RNAiso plus (Takara, Japan) from all samples, and the concentration and OD value (OD260 nm/OD280 nm = 1.8~2.0) were determined using an ultra-micro ultraviolet spectrophotometer (Thermofisher Ultrospec 2100 pro, USA). According to the instructions of the reverse transcription kit (Thermo Scientific RevertAidTM First Strand cDNA Synthesis; Thermofisher, USA), the total RNA of each sample was reverse transcribed, with the following reaction conditions: 42 °C for 60 min and 75 °C for 5 min.

2.9 Primer design and synthesis

According to the GenBank porcine *SIRT5* gene mRNA sequence (accessionNo.: NM_001105308.1), the internal reference gene is the porcine *GAPDH* gene (accessionNo.: NM_001206359.1). CDS region cloning and real-time fluorescent primers (Table 1) were designed using Primer Premier 5.0 and synthesized by Sangon Corporation.

Table 1. Primer information.

Gene	Primer sequence	Annealing temperature/°C	Fragment length/bp	Utilization
SIRT5-KL	F: AACCTGATGCCACCTCTCTGG	60	947	CDS region clone
	R: CGGGACGACTAAGAGACAGGTT			
SIRT5	F: CGTGGTCATCACCCAGAACA	61	108	qRT-PCR
	R: GCTCCACAAGAGGTACACCG			
GAPDH	F: TTGTGATGGGCGTGAACC	61	169	qRT-PCR
	R: GTCTTCTGGGTGGCAGTGAT			

2.10 Cloning and sequencing of the CDS region of SIRT5 gene

PCR was peformed in a 20 μL reaction mixture containing 10 μL of 2 × Taq PCR Master Mix (CW Biotech, China), 1 μL of primers (10 μmol/L), 1 μL cDNA, and 7 μL ddH_2O. The reaction was run through pre-denaturation for 2 min at 94 °C; then run for 35 cycles of denaturation for 30 s at 94 °C, annealing for 10 s at 60 °C, and extension for 30 s at 72 °C; followed by a final extension for 30 s at 72 °C.

The PCR product obtained by the SanPrep column DNA gel recovery kit (Sangon, China) was purified and recovered by gel cutting and ligation with pCR2.1 vector (Thermofisher, USA). The ligation solution was transformed into DH5α (Takara, Japan). The qualified amplification products were sent to Sangon for sequencing.

2.11 Quantitative real-time PCR

A quantitative real-time PCR instrument (Bio-Rad CFX96, USA) was used to detect the expression of the *SIRT5* gene, and the *GAPDH* gene was used as an internal control. Reaction conditions of qRT-PCR were as follows: 94 °C for 2 min; 94 °C for 15 s, 61 °C for 15 s, 72 °C for 30 s, 40 cycles. The amplification system was as follows: 2 × Plus SYBR Real-time PCR mixture (CW Biotech, China) 10 μL, primers 0.5 μL, cDNA 1 μL, and ddH_2O 8 μL; 3 replicates per sample.

2.12 Protein sequence analysis

The physicochemical properties of the protein were analyzed using the Prot param (http://web.expasy.org/protparam/) program in the ExPASy online platform. Smart V6.0 (http://smart.embl-heidelberg.de/) online software was used to analyze certain functional domains of the protein.

2.13 Statistical analysis

Pork quality traits and relative gene expression are expressed as mean ± standard deviation. The relative gene expression obtained by real-time fluorescent quantitative PCR was calculated using the $2^{-\Delta\Delta Ct}$ method. SPSS 18.0 software was used for *t*-test analysis for *SIRT5* relative expression. The correlation between *SIRT5* gene expression and meat quality indicators was analyzed by the *Pearson* correlation coefficient.

3 Results

3.1 Cloning, identification and amino acid sequence analysis of SIRT5 gene from Yeke F1 generation pigs

Electrophoresis on 1.2% agarose gel (Figure 1) yielded a single well-specific cloned fragment (GeneBank No. MT149213), with a size of about 947 bp. The complete sequence of the CDS region of the *SIRT5* gene of the Yeke F1 generation pig was 933 bp, encoding 310 amino acids in total, which was consistent with the expected target fragment size. Moreover, we found that there were nucleotide mutations at g.5753 A > G and g.16916 A > G in Yeke F1 pigs relative to the published sequence in GenBank. g.5753 A > G resulted in the change of lysine (Lys) to arginine (Arg), and the g.16916 A > G site was a synonymous mutation (Figure 2).

According to the analysis of the physical and chemical properties of the protein according to the amino acid sequence of *SIRT5*, the molecular formula of SIRT5 protein was obtained as follows: $C_{1487}H_{2344}N_{440}O_{424}S_{14}$, the molecular mass is 33.6 kDa, theoretical isoelectric point is 8, and instability coefficient is 48.32, it belongs to unstable protein. The total average hydrophilicity is –0.193, indicating that the SIRT5 protein is hydrophilic. Structural and functional domain analysis of SIRT5 protein showed that a highly conservative structural and functional domain SIR2 existed in the SIRT5 protein at positions 58-256 (Figure 3).

3.2 Expression of SIRT5 gene in longissimus dorsi muscle and subcutaneous fat of Yeke F1 generation pigs

With *GAPDH* as the internal reference gene, the relative expression of the *SIRT5* gene in the longissimus dorsi of Yeke F1 generation pigs was 1.42 ± 0.35, and the relative expression in subcutaneous fat was 30.06 ± 9.77. The expression level of the *SIRT5* gene in the subcutaneous fat tissue of the Yeke F1 generation pig is extremely significantly higher than that of the longissimus dorsi ($P < 0.01$) (Figure 4).

3.3 Correlation analysis between SIRT5 gene expression level and meat quality performance indicators

The measurement results of the pork quality index of Yeke F1 generation are shown in Tables 2 and 3. Among them, the muscle moisture content of the Yeke F1 generation pig was 71.67%, and the intramuscular fat was 3.26%. Among the fatty acid composition indexes, the polyunsaturated fatty acid was 11.933%. The correlation analysis between *SIRT5* gene expression level and

meat quality performance indicators is shown in Tables 2 and 3. Increased *SIRT5* gene expression in the longissimus dorsi muscle was significantly correlated to the pH_1 value ($R^2 = 0.946$, $P < 0.05$) but significantly negatively correlated with L^*($R^2 = -0.994$), a^*($R^2 = -0.994$), and b^* ($R^2 = -0.991$) ($P < 0.01$). The expression level in subcutaneous fat was negatively correlated with shear force ($R^2 = -0.891$), muscle water ($R^2 = -0.89$), and intramuscular fat content ($R^2 = -0.843$) ($P < 0.05$). The expression level of the *SIRT5* gene in Yeke F1 longissimus dorsi muscle tissue is not related to fatty acid composition indexes, but the expression level of *SIRT5* in subcutaneous fat is significantly negatively correlated with myristic acid ($R^2 = -0.906$), stearic acid ($R^2 = -0.953$) and Trans-linoleic acid ($R^2 = -0.889$) ($P < 0.05$), and significantly positively correlated with heptadecanoic acid ($R^2 = 0.985$), elmic acid ($R^2 = 0.918$) and methyl nervate ($R^2 = -0.936$) ($P < 0.05$).

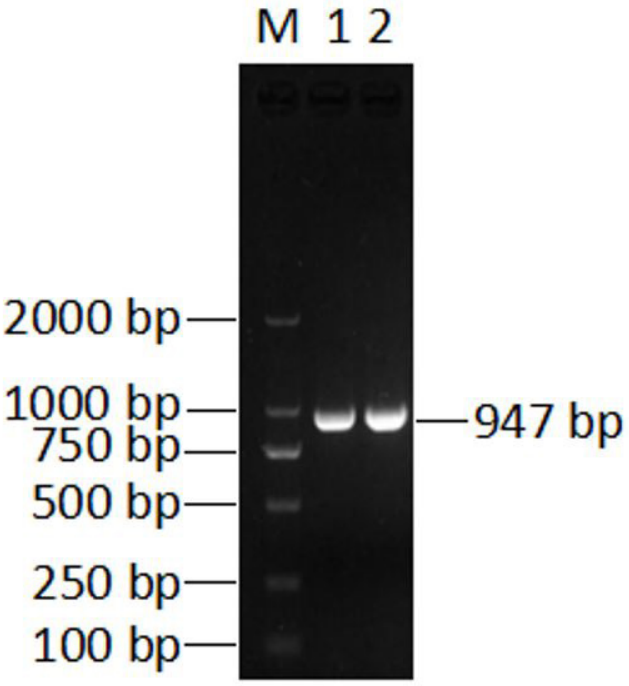

Figure 1. Amplification of *SIRT5* gene in Yeke F1 pigs (M: DL2000 DNA marker; 1-2: Yeke F1 Generation).

4 Discussion

The SIRT gene family plays a critical role in the energy metabolism of the animal body, and the absence of *SIRT5* can result in disorders in several metabolic pathways. Studies have shown that the *SIRT5* gene can regulate fat deposition and meat quality in Qinchuan cattle (Gui, 2015). Comparing the sequence of the CDS region of the *SIRT5* gene of the Yeke F1 generation pig with the Duroc pig revealed a mutation site (g.5753 A > G) in the core protein domain of the Yeke F1 pig *SIRT5* gene. When the SNP site occurs, mutations can cause changes in the amino acid sequence, affect the structure and function of the protein, and change the phenotypic traits of pig production. This study found that the expression levels of *SIRT5* mRNA in the subcutaneous fat of Yeke F1 pigs was significantly higher than that in the longissimus dorsi muscle, indicating that the gene was expressed in subcutaneous fat with high white fat content in Yeke F1 pigs. The expression levels of SIRT5 in muscle tissue with high brown fat content is lower. It is speculated that this gene may be related to changes in the color of the pig fat and the process of pig fat deposition or fatty acid metabolism, which is consistent with previous reports in mice (Shuai et al., 2019).

To verify the correlation between the *SIRT5* gene and pork quality traits, the correlation between the expression of the gene in the longissimus dorsi muscle, subcutaneous fat and the meat quality traits was analyzed. The *SIRT5* gene expression level in the longissimus dorsi muscle correlates with meat color (L*, a*, b*) and pH_1, indicating that *SIRT5* can affect meat color and pH in muscle containing brown fat. Morever, *SIRT5* gene expression was

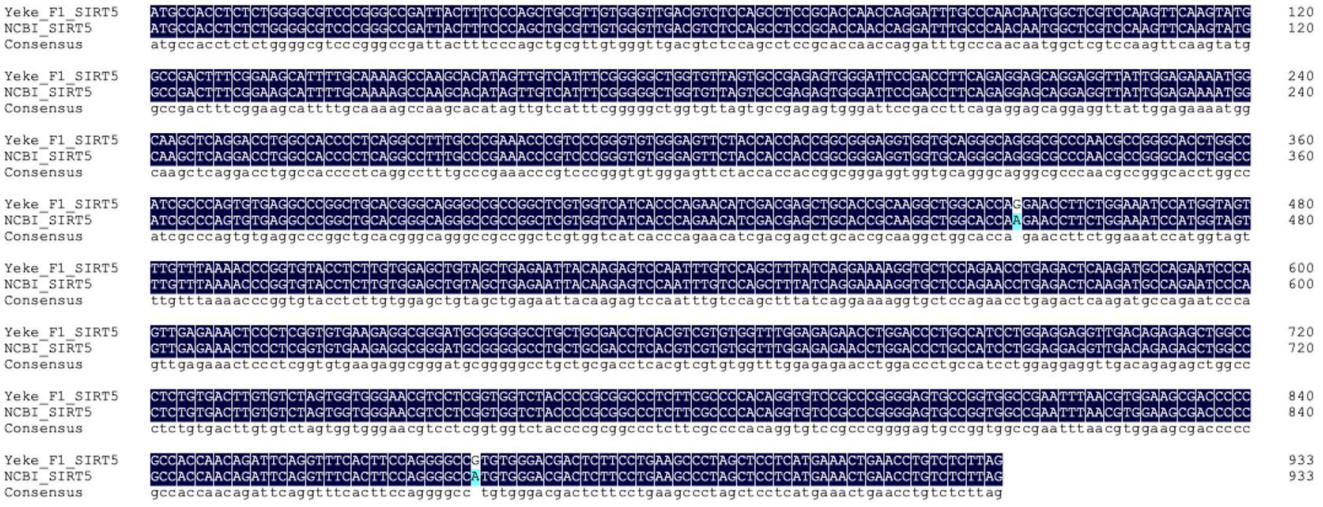

Figure 2. Sequence alignment diagram of *SIRT5* gene sequence with public sequence in NCBI database.

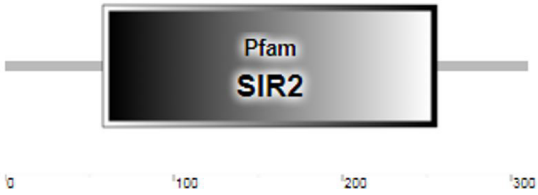

Figure 3. Prediction of the structural domain of *SIRT5* protein in pigs.

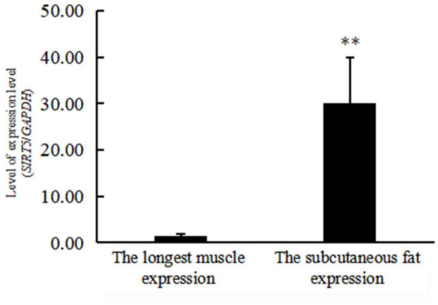

Figure 4. Expression analysis in the longest muscle and subcutaneous fat tissue of the *SIRT5* gene in pigs. * indicates significant difference ($P < 0.05$); ** indicates extremely significant difference ($P < 0.01$).

positively associated with WBSF and muscle moisture content, and negatively associated with IMF in subcutaneous fat, which indicates that *SIRT5* in subcutaneous fat can indirectly affect intramuscular fat deposition. This finding is consistent with the results that SIRT5 protein expression is negatively correlated with the abdominal fat content of Tongcheng pigs (Jin et al., 2009).

Fatty acids play an essential role in the composition of intramuscular fat. Fatty acids are carboxylic acids with a long aliphatic chain, which are divided into saturated fatty acids (SFA) and unsaturated fatty acids (UFA) (Khan et al., 2015). Results showed the expression of the *SIRT5* gene in subcutaneous fat tissue to be correlated with four SFAs (C14:0, C17:0, C18:0 and C22:0), indicating that *SIRT5* gene may influence the prone to oxidative rancidity of pig fat, affecting meat quality and shelf life(Nong et al., 2019). Increased levels of UFA improve meat quality, flavor and nutritional value. Many UFAs are precursors of meat flavor and have an essential impact on the formation of unique flavors of different breeds of pigs (Zhang et al., 2018). UFAs are divided into monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). PUFA react to produce flavoring agents (including esters, ketones, and alcohols) when heated and react with other substances to determine meat flavor

Table 2. Pork quality traits and siginificant association of *SIRT5* gene expression with pork quality traits index in Yeke F1 pigs.

Traits		Value	Longussimus dorsi	Subcutaneous fat
PH_1		6.27 ± 0.47	0.946*	0.470
PH_{24}		5.61 ± 0.65	−0.418	0.351
Instrumental color	L*	46.16 ± 5.96	−0.994**	−0.516
	a*	7.86 ± 1.53	−0.994**	−0.584
	b*	4.73 ± 1.65	−0.991**	−0.49
	Drip loss/%	3.36 ± 0.37	−0.631	0.123
	Cooking loss/%	58.68 ± 0.69	0.554	0.69
	WBSF/kg·f	6.81 ± 2.94	0.624	0.891*
	Moisture content/%	71.68 ± 0.70	0.578	0.889*
	IMF/%	3.26 ± 0.94	−0.262	−0.843*

* Indicates significant correlation ($P < 0.05$). ** Indicates extremely significant correlation ($P < 0.01$).

Table 3. Fatty acid index content and siginificant association of *SIRT5* gene expression with fatty acid index in Yeke F1 pigs.

Fatty acid name	Content (%)	Longus dorsi	Subcutaneous fat
Myristic acid (c14:0)	1.250 ± 0.168	−0.389	−0.906*
Palmitic acid (c16:0)	24.760 ± 0.685	0.029	−0.685
XXVII carbonic acid (C17:0)	0.133 ± 0.014	0.638	0.985**
Stearic acid (C18:0)	11.015 ± 0.724	−0.466	−0.953*
Eicosanoic acid (c20:0)	0.227 ± 0.021	−0.034	−0.786
Elm Acid (C22:0)	0.184 ± 0.035	0.275	0.918*
Palmitoleic acid (c16:1)	3.665 ± 0.131	−0.148	0.172
Heptadecanoic acid (c17:1)	0.272 ± 0.434	−0.352	−0.768
Trans Oleic Acid (C18:1n9t)	0.248 ± 0.069	−0.142	−0.561
Oleic Acid (C18:1n9c)	45.825 ± 0.662	0.752	0.524
Eicosapentaenoic acid (C20:1n9)	0.240 ± 0.037	0.423	0.102
Methyl nervonate (C24:1n9)	0.246 ± 0.060	0.317	0.935*
Trans-Linoleic Acid (C18:2n6t)	0.065 ± 0.053	−0.565	−0.889*
Linoleic acid (c18:2nc6c)	8.882 ± 1.093	0.088	0.824
Eicosadienoic acid (c20:2)	0.351 ± 0.046	−0.310	−0.425
α-Linolenic Acid (C18:3n3)	0.632 ± 0.300	0.402	0.663
Linolenic acid (C18:3n6)	0.188 ± 0.330	−0.291	−0.737
Eicosatrienoic acid (C20:3n3)	0.042 ± 0.013	−0.424	−0.376
Arachidonic acid (C20:4n6)	1.652 ± 0.597	−0.068	0.699
Docosapentaenoic acid (c22:5)	0.122 ± 0.039	−0.294	0.549
Saturated fatty acid (SFA)	37.570 ± 1.481	−0.247	−0.866
Monounsaturated fatty acid (MUFA)	50.497 ± 0.467	0.752	0.124
Polyunsaturated fatty acids (PUFA)	11.933 ± 1.539	0.009	0.796

* Indicates significant correlation ($P < 0.05$). ** Indicates extremely significant correlation ($P < 0.01$).

(Zhao et al., 2017). Cameron et al. (Cameron et al., 2000) found that the specific types of fatty acids affect flavor. Specifically, C18:2n6, C20:4n6 and C22:4n6 correlate with improved meat flavor. This showed that the expression of the *SIRT5* gene in subcutaneous fat tissue showed a significant negative correlation with C18:2n6, indicating that the low expression level of the *SIRT5* gene in pigs can enhance the flavor of meat; however, the specific regulation mechanism still needs to be further explored.

5 Conclusion

This study successfully cloned the 933 bp sequence of the *SIRT5* gene CDS region from Yeke F1 generation pigs, which encodes 310 amino acids. There are two base mutation sites, of which the g.5753 A > G is a missense mutation, and the SNP site is in the core domain of the SIRT5 protein. The expression level of the *SIRT5* gene in the longissimus dorsi muscle and subcutaneous fat tissue of Yeke F1 pigs correlates with improved meat quality traits. High expression of the *SIRT5* gene was negatively corelated with IMF content and fatty acid composition in subcutaneous fat.

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