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Genetic polymorphisms in *SIRT5* gene and their association with carcass traits in Congjiang Xiang pigs

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ABSTRACT - This study was conducted to investigate the polymorphism of the silence information regulator 5 (*SIRT5*) gene in 103 Congjiang Xiang pigs from Southwest China. We searched for SNP (single nucleotide polymorphism) loci of *SIRT5* gene through sequence alignment and PCR. We obtained nine SNP loci: g.14135 A>C (intron 6), g.14247 C>A (intron 6), g.14305 C>T (exon 7), g.14335 C>T (exon 7), g.16603 T>C (intron 7), g.16613 T>C (intron 7), g.16800 G>A (intron 7), g.16812 C>G (intron 7), and g.16916 A>G (exon 8). Further analysis of SNP genotypes associated with the carcass traits of skin thickness, backfat thickness, and eye muscle area was carried out in pigs. We found that the genotypes g.14305 C>T (CC) and g.16812 C>G (CG) had certain advantages for improving the carcass traits of Congjiang Xiang pigs. The haplotype combination of the *SIRT5* gene that improved skin thickness was H2H3:CCGGCTCA. These results may provide empirical support for molecular-based breeding of carcass traits in Congjiang Xiang pigs.

Keywords: carcass traits, Congjiang Xiang pig, *SIRT5*, SNP

1. Introduction

The Congjiang Xiang pig, as a national Grade II protected livestock breed, is a valuable miniature pig breed primarily farmed in Guizhou Province, China. The breed is famous for its small size, early sexual maturity, adaptability to coarse feed, superior meat quality, low level of genetic diversity, and high level of homozygosity (Ran et al., 2016). Previous studies identified *APOC3*, *APOA5*, *FUT1*, *PHKG1*, and *PRKAG3* as candidate genes for pork quality traits and flavor fatty acid composition and content (Jiang et al., 2005; Ryan et al., 2012; Hui et al., 2013; Zappaterra et al., 2019). In addition, SIRT5 (silence information regulator 5), a member of the Sirtuin family, is involved in the metabolic processes of desuccinylation, demalonylation, and deglutarylation (Simó-Mirabet et al., 2018). It has been demonstrated that SIRT5 regulates the balance of fatty acid oxidation in mice (Rardin et al., 2013). The browning of subcutaneous white fat in SIRT5 knockout mice was impaired, indicating that SIRT5 is also a key factor in the differentiation of brown fat (Shuai et al., 2019). In livestock, SIRT5 has been demonstrated to regulate fat deposition in a Chinese indigenous cattle breed (Deng, 2014). However, little is known about *SIRT5* polymorphism in pigs.

In recent years, studies with Congjiang Xiang pigs have focused on the processes of their testis development, fertility, and germplasm resources (Tang et al., 2018; Meng et al., 2020; Gong et al., 2021). Carcass traits have always been an economic concern in pig breeding. The Congjiang Xiang pig is an economically important species in China, and thus it is worthwhile to improve its carcass traits.

In this study, we identified polymorphism of *SIRT5* and examined its relationship with carcass traits. Therefore, this study aimed to provide a foundation for applying the *SIRT5* gene in the breeding of Congjiang Xiang pigs.

2. Material and Methods

The study was carried out in Guiyang, Guizhou, China (26°63'65" N latitude, 106°75'75" E longitude, and elevation of 1125 m). Research on animals was approved by the institutional committee on animal use (case number: EAE-GZU-2021-T113). The experimental animals were maintained and processed in accordance with institutional guidelines for the care and use of animals.

2.1. Experimental animals and sample collection

One hundred and three blood samples were collected from healthy eight-month-old male Congjiang Xiang pigs, and the carcass traits of these pigs were measured. All pigs were obtained from a commercial farm in Southwest China (Guizhou Province).

2.2. Carcass trait measurement

The skin thickness (from the 6th to the 7th ribs of the right half of the carcass of each pig), backfat thickness (the average thickness of the thickest part of the shoulder, the joint of the thoracolumbar vertebrae, and the joint of the lumbar-sacral vertebrae on the right side of each pig), and eye muscle area (the area of a transverse section of the *longissimus dorsi* muscle at the last rib on the left side of the carcass) were measured using calipers (HOLEX, Germany) after slaughter.

2.3. Acquisition of DNA

Genomic DNA was extracted from each pig blood sample using an Ezup Column Genomic DNA Extraction Kit (Blood) (Sangon, China) and stored at –20 °C.

2.4. Primer design and synthesis

The porcine *SIRT5* gene complete sequence (accession no.: NC_010449.5) was obtained from GenBank (https://www.ncbi.nlm.nih.gov/). Primers were designed using Primer Premier 5.0 (Singh et al., 1998) and synthesized by Sangon Co., Ltd., China. Table 1 lists the primer sequences and product lengths.

Table 1 - Information of primers

Primer name	Primer sequence	Tm (°C)	Production length (bp)
SIRT5-1	F: CTAGGAGCGCCTGAGAACAC R: CAGAGTGGCTTTCTCCAAGG	60	436
SIRT5-2	F: GAGGTGCGTGGCGAGGTTT R: ATCCAGCGTTGCCGTGAGC	59	472
SIRT5-3	F: GATTTTCCAGGGTCCCTCAT R: CACTCCAGGATCGTGTTAAGC	58	505
SIRT5-4	F: AGTCCCTGGTCGCGTAGTAA R: TCCTGTGGTTTGCACTGAAC	58	522
SIRT5-5	F: CTCTGGAGGGAGAACCACTG R: CCTGTCACCCCAGGCTAATA	58	636
SIRT5-6	F: GGCACCTAAAAGCAGGACAA R: AGGGGAAAGGCTTTGAGAAG	58	560
SIRT5-7	F: GCACAACCAGAATGTGAACG R: CGTTTGCTTCCATCAGAACA	57	554
SIRT5-8	F: ATCATCTGTGTGGTGCCTGA R: ACACCTTTACTCCCCCTGCT	60	656

2.5. Sequencing of the *SIRT5* gene

The *SIRT5* gene was characterized from 103 blood samples using the polymerase chain reaction (PCR), which was performed 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 of complementary DNA (cDNA), and 7 μL of double distilled water (ddH_2O). The reaction was pre-denatured for 2 min at 94 °C, followed by 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.

After electrophoresis, the rubber plate was removed, and the gel was placed in an irradiation instrument to observe the target bands. The optimal products were sent to Sangon Co., Ltd. (Shanghai, China), for sequencing, sequence alignment, single nucleotide polymorphism (SNP) analysis, and genotype determination.

2.6. Statistical analysis

The genotype frequency, allele frequency, polymorphism information content (PIC), effective number of alleles (Ne), heterozygosity (He), and homozygosity (Ho) were calculated. The statistical analysis of the associations between the carcass traits and the SNP of the *SIRT5* gene was performed using SPSS Statistics 18. Duncan's method was used for multiple comparisons. A P-value < 0.05 was considered significant. Values are reported as the mean ± standard deviation (SD). Haplotype analysis was performed using SHEsis software (http://analysis.bio-x.cn/myAnalysis.php). The equation for the animal model used was as follows:

$$Y_{ij} = \mu + h_i + e_{ij},$$

in which Y_{ij} is the trait being measured, μ is the population mean, h_i is the effect of genotype or combined haplotype, and e_{ij} is the random error term.

3. Results

3.1. SNP locus mapping

We compared the results of *SIRT5* gene with its gene sequences. DNAMAN software was used for the comparison process. In the comparative analysis, nine SNP were mined, and SNP were present in all of the exonic regions and several of the intronic regions of the *SIRT5* gene in the Congjiang Xiang pig sequences.

Referring to the complete sequence of the porcine *SIRT5* gene (taking the first transcription start site +1), the nine SNP (Figure 1) were named g.14135 A>C (intron 6), g.14247 C>A (intron 6), g.14305 C>T (exon 7), g.14335 C>T (exon 7), g.16603 T>C (intron 7), g.16613 T>C (intron 7), g.16800 G>A (intron 7), g.16812 C>G (intron 7), and g.16916 A>G (exon 8).

3.2. Genetic diversity analysis

The Ne values of g.16603 T>C, g.16613 T>C, and g.16916 A>G loci were close to 2, indicating that the alleles of these three variants were evenly distributed in Congjiang Xiang pigs. The Ne and He values of the g.14135 A>C, g.14247 C>A, g.14305 C>T, and g.16800 G>A loci were low, indicating relatively low genetic variability and insufficient diversity excavation of species resources. The PIC values of these four loci were low polymorphic (PIC < 0.25), indicating that the genetic marker loci provided little genetic information (Table 2).

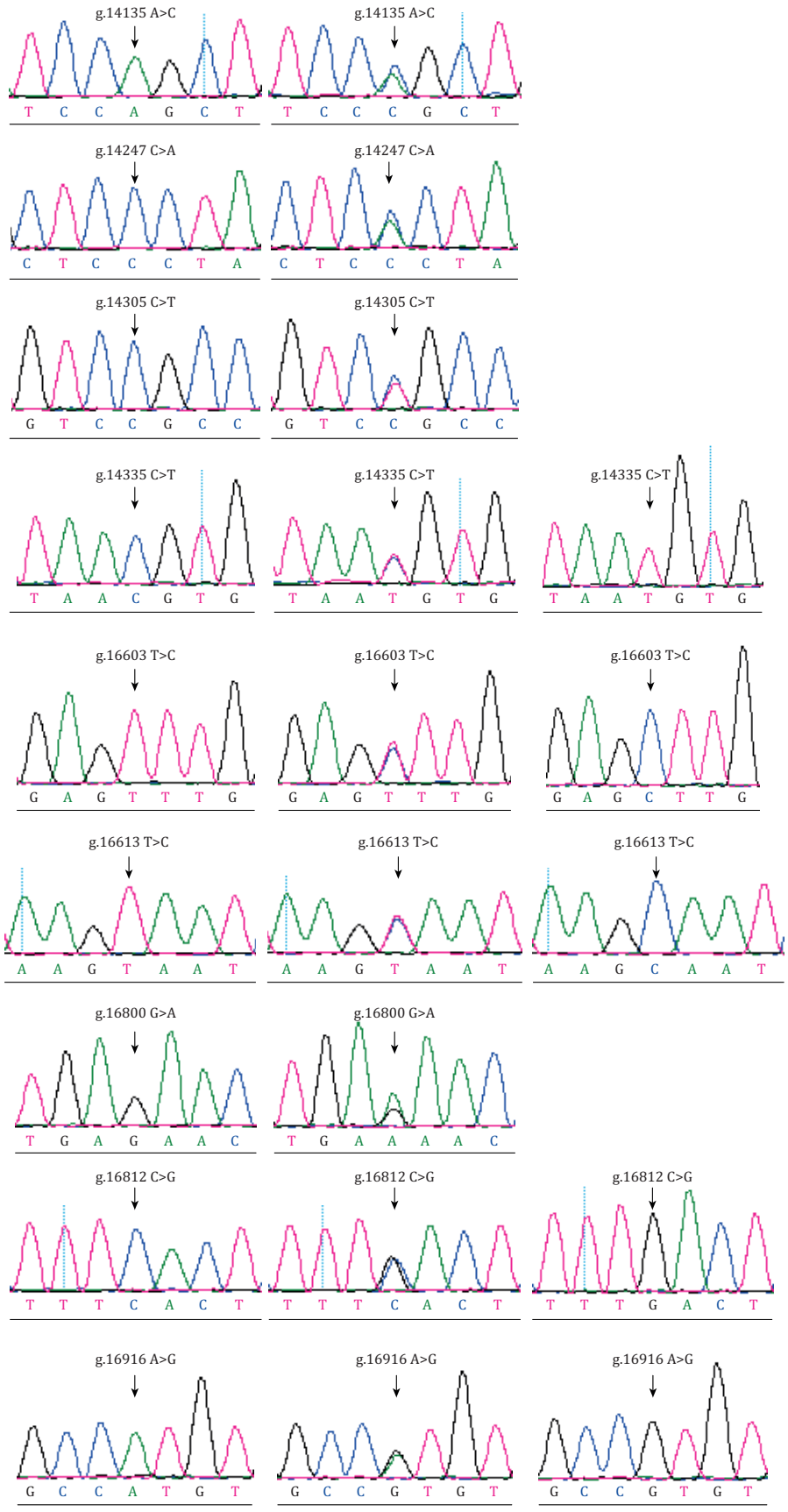

Figure 1 - Sequencing map of *SIRT5* gene SNP in Congjiang Xiang pigs.

Table 2 - Genetic indices of *SIRT5* gene SNP site from Congjiang Xiang pigs

Site	Genotype	Genotype frequency	Allele	Allele frequency	Ne	Ho	He	PIC
	AA	0.8852	A	0.9426				
g.14135 A>C	AC	0.1148			1.1213	0.8918	0.1082	0.1023
	CC	0	C	0.0574				
	AA	0	A	0.0574				
g.14247 C>A	AC	0.1148			1.1213	0.8918	0.1082	0.1023
	CC	0.8852	C	0.9426				
	CC	0.9344	C	0.9672				
g.14305 C>T	CT	0.0656			1.0677	0.9366	0.0634	0.0614
	TT	0	T	0.0328				
	CC	0.4918	C	0.7131				
g.14335 C>T	CT	0.4426			1.6925	0.5908	0.4092	0.3255
	TT	0.0656	T	0.2869				
	CC	0.2917	C	0.5625				
g.16603 T>C	CT	0.5417			1.9692	0.5078	0.4922	0.3711
	TT	0.1667	T	0.4375				
	CC	0.2917	C	0.5625				
g.16613 T>C	CT	0.5417			1.9692	0.5078	0.4922	0.3711
	TT	0.1667	T	0.4375				
	AA	0	A	0.0833				
g.16800 G>A	AG	0.1667			1.1803	0.8472	0.1528	0.1411
	GG	0.8333	G	0.9167				
	CC	0.4792	C	0.6667				
g.16812 C>G	CG	0.3750			1.8000	0.5556	0.4444	0.3457
	GG	0.1458	G	0.3333				
	AA	0.1667	A	0.4167				
g.16916 A>G	AG	0.5000			1.9459	0.5139	0.4861	0.3680
	GG	0.3333	G	0.5833				

SNP - single nucleotide polymorphism; Ne - effective number of alleles; Ho - homozygosity; He - heterozygosity; PIC - polymorphism information content.

3.3. Correlations between *SIRT5* gene variation and traits

The correlations between *SIRT5* variation and traits in the Congjiang Xiang pigs were measured (Table 3). The skin thickness of individuals with the g.14305 C>T locus CC type was higher than that of individuals with the TT genotype by 14.04% ($P<0.05$). The skin thickness and eye muscle area of individuals with the g.16812 C>G locus CG type were greater than those of individuals with the GG type by 28.12 and 17.99%, respectively ($P<0.05$), but the difference in the level of individuals with the CC type was not significant ($P>0.05$).

3.4. Haplotype combination analysis of different SNP loci

There were five haplotypes of four SNP loci (g.14335 C>T, g.16613 T>C, g.16812 C>G, and g.16916 A>G) in 43 Congjiang Xiang pigs, named H1:CCCG, H2:CCGG, H3:CTCA, H4:TCCG, and H5:TCGG, which were analyzed by the SHEsis software. Among these, H2, H3, and H4 were the main haplotypes, with frequencies of 27.8, 44.2, and 17.3%, respectively, while the H1 haplotype had the lowest frequency of 3.6% (Table 4).

There were four haplotype combinations in 43 Congjiang Xiang pigs, H2H2:CCGGCCGG, H2H3:CCGGCTCA, H3H3:CTCACTCA, and H3H4:CTCATCCG. We found that skin thickness associated with H2H3 was superior to the H2H2 haplotype combination (Table 5).

Table 3 - Correlations between *SIRT5* variation loci and traits in the Congjiang Xiang pig

Site	Genotype	Skin thickness (mm)	Backfat thickness (mm)	Eye muscle area (cm^2)
g.14135 A>C	AA	3.89±0.83	21.55±5.86	16.96±3.31
	AC	4.42±1.06	23.88±4.50	17.19±2.91
g.14247 C>A	AC	4.42±1.06	23.88±4.50	17.19±2.91
	CC	3.89±0.83	21.55±5.86	16.96±3.31
g.14305 C>T	CC	3.98±0.88a	22.00±5.81	17.08±3.36
	CT	3.49±0.12b	18.64±3.09	15.73±1.07
g.14335 C>T	CC	3.96±0.79	22.07±6.57	16.28±2.81
	CT	3.99±0.95	21.88±4.80	17.56±3.59
	TT	3.73±1.08	19.82±6.45	18.57±3.38
g.16603 T>C	CC	3.69±0.94	21.68±5.74	16.99±3.51
	CT	3.86±0.74	21.99±5.25	16.87±2.86
	TT	3.99±0.90	24.18±7.51	16.48±3.59
g.16613 T>C	CC	3.69±0.94	21.68±5.74	16.99±3.51
	CT	3.86±0.74	21.99±5.25	16.87±2.86
	TT	3.99±0.90	24.18±7.51	16.48±3.59
g.16800 G>A	AG	3.89±0.67	21.83±6.43	16.99±2.41
	GG	3.83±0.84	22.37±5.67	16.81±3.26
g.16812 C>G	CC	3.81±0.91ab	22.54±6.02	17.00±3.33ab
	CG	4.10±0.68a	22.53±5.86	17.44±2.89a
	GG	3.20±0.44b	20.53±4.70	14.78±2.38b
g.16916 A>G	AA	3.99±0.90	24.18±7.51	16.48±3.59
	AG	3.87±0.76	22.06±5.43	16.60±3.13
	GG	3.71±0.90	21.15±5.85	17.49±3.64

a-b - Means in the same single nucleotide polymorphism site within a column with different letters differ by P<0.05.

Table 4 - Haplotype construction and frequency of four SNP loci of *SIRT5* gene

Haplotype	g.14335 C>T	g.16613 T>C	g.16812 C>G	g.16916 A>G	Frequency (%)
H1	C	C	C	G	3.6
H2	C	C	G	G	27.8
H3	C	T	C	A	44.2
H4	T	C	C	G	17.3
H5	T	C	G	G	7.1

SNP - single nucleotide polymorphism.

Table 5 - Haplotype combination analysis of four SNP loci of the *SIRT5* gene

Haplotype combination	Skin thickness (mm)	Backfat thickness (mm)	Eye muscle area (cm^2)
H2H2	3.18±0.49a	19.81±4.87	14.36±2.76
H2H3	4.03±0.55b	20.25±5.13	16.72±1.52
H3H3	3.99±0.90ab	24.18±7.52	16.48±3.59
H3H4	3.62±0.73ab	20.91±5.60	17.77±4.16

SNP - single nucleotide polymorphism.
a-b - Means in the haplotype combination within a column with different letters differ by P<0.05.

4. Discussion

Recent studies have reported that the *SIRT5* gene regulates the activity of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) through demethylation and further upregulates glycolytic flux (Nishida et al., 2015). SIRT5 also increases the activity of very long-chain acyl-CoA dehydrogenase (VLCAD) by desuccinylation to promote fatty acid β-oxidation and ketone body production (Zhang et al., 2015). Hong et al. (2020) reported that during the differentiation of preadipocytes, SIRT5 inhibited the expression of key genes that promote lipid formation and differentiation in fatty acid biosynthesis and PPAR pathways. SIRT5 plays an important role in biological processes such as maintaining the balance of lipid metabolism and promoting the mobilization of fatty acid oxidation. However, little is known concerning SNP of the *SIRT5* gene in pigs. Our study detected nine SNP loci in the *SIRT5* gene. As mentioned above, SIRT5 significantly inhibits the differentiation of bovine preadipocytes and simultaneously inhibits lipid synthesis and lipid deposition in adipocytes (Hong et al., 2020), and thus we speculated that the nine sites identified in this study may have potential roles in production and breeding of Congjiang Xiang pigs. The genetic diversity of these nine loci was quite different in Congjiang Xiang pigs. We used PIC values as indicators to evaluate the degree of genetic variation in the population. The PIC can be used to classify loci as highly polymorphic (PIC > 0.5), medium polymorphic (PIC between 0.25 and 0.5), and low polymorphic (PIC < 0.25). According to the PIC values, g.14135 A>C, g.14247 C>A, g.14305 C>T, and g.16800 G>A loci were low polymorphic in Congjiang Xiang pigs (PIC < 0.25). We speculate that the reasons for these results may be related to the intensity of artificial selection that may have affected allele frequencies and genotype frequencies (Zhang et al., 2023).

In this study, the g.14305 C>T locus genotype was significantly associated with skin thickness, and CC was the dominant genotype. The g.16812 C>G locus genotype was significantly associated with skin thickness and eye muscle area, and CG was the dominant genotype. Although the g.16812 C>G locus is in the intron and cannot be directly involved in protein coding, it has been demonstrated that mutations in the non-coding region affect processes such as mRNA stabilization, localization, and translation (Kosinska-Selbi et al., 2020). Compared with SNP genotyping, haplotype combination analysis can provide a more comprehensive reference as to functional significance, because the various loci in the haplotype combination will affect each other rather than acting as a simple combination of various genotypes (Wang et al., 2011). We combined the haplotypes of the four SNP loci of the *SIRT5* gene and analyzed their correlations with carcass traits. The results showed that the dominant haplotype combination for skin thickness of the *SIRT5* gene was H2H3:CCGGCTCA.

5. Conclusions

This study identified nine SNP loci through analysis of *SIRT5* gene in Congjiang Xiang pigs. The CC genotype of the g.14305 C>T locus and the CG genotype of the g.16812 C>G locus were the dominant genotypes associated with carcass traits; and H2H3:CCGGCTCA was associated with skin thickness. These dominant genotype and haplotype combinations can be used as a primary reference in the breeding of Congjiang Xiang pigs.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Conceptualization: Zhang, X. **Formal analysis:** Wang, J. **Funding acquisition:** Zhang, J. **Writing – original draft:** Zhao, C.

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