Polymorphism of Congjiang Pigs FUT1 Gene and Its Association with Carcass and Meat Quality Traits

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Abstract. In order to investigate the effects of the M229 and M307 locus in FUT1 gene on the carcass and meat quality of Congjiang pigs. In this study, PCR-SSCP, PCR-RFLP and direct sequencing methods were used to quickly type the M229 and M307 loci, and then the association between different genotypes and meat traits was analysed. Population genetic structure analysis showed that M229 locus of FUT1 gene was in Hardy-Weinberg equilibrium, with high polymorphism information content, rich genetic diversity and large space for directional breeding. However, the M307 locus had the opposite result, showing a skewed distribution. Correlation analysis showed that the average backfat thickness index of CT and TT genotypes from the M229 locus of FUT1 gene in Congjiang pigs was significantly lower than that of CC (P <0.05). The conclusion of this experiment indicates that the M229 locus of FUT1 gene can be strengthened in Congjiang pigs, which can be used for breeding applications.

1. Introduction
Congjiang pigs, a characteristic miniature local pig breeds of China, is mainly produced in towns and villages such as Zhaibian, Congjiang County, Guizhou, which were classified by the Ministry of Agriculture as national second-level protected breeds in 1993, and it is famous for its characteristics of slow growth, tender meat, early sexual maturity, strong disease resistance, low genetic diversity and high genetic homogeneity [1-3].

ETEC F18 is the major pathogen causing edema and diarrhea of weaned piglet [4]. Researchers have demonstrated that AA genotype from the M307 locus of FUT1 gene was resistant to ETEC F18 through in vitro adhesion tests [5]. Accompanied by the in-depth research on diarrhea resistance in domestic pigs of China, researchers regard the M229 locus of FUT1 gene as a mutation site that regulates edema and diarrhea, and they found the TT genotype was the resistance genotype [6]. For the functional analysis of FUT1 gene, we not only focused on the diarrhea traits of piglets, but also carried out exploration work on reproductive performance. Researchers found that the M307 locus of pig FUT1 gene was related to litter performance indicators, and the litter size of the AA genotype sows was higher than the AG and GG genotypes [7].

In this experiment, Congjiang pigs was used as the research object. We systematically analyzed the polymorphic distribution of the M229 and M307 locus in FUT1 gene, and carried out population
genetic structure analysis and combined phenotypic trait association studies. The purpose of this study is to provide basic data for genetic improvement and disease-resistant breeding of Congjiang pigs.

2. Materials and methods

2.1. Collection of test samples
Sixty-six blood samples of Congjiang pigs were collected from Guiyang Lushengyuan Xiangzhu Ecological Welfare Farm. Pig blood samples were prepared using Ezup column-type genomic DNA extraction reagents produced by Shanghai Biotech Biotechnology (Shanghai) Co., Ltd. The box is mainly for the extraction of genomic DNA from blood, and the sample is stored at -20 ℃ until use.

2.2. Carcass meat quality index determination
In this test, the carcass and meat quality indicators of 66 Congjiang pigs were determined. Routine carcass quality indicators were measured in accordance with the NY / T825-2004 protocol, and cable extraction method (NY / T821-2004) was used to measure the intramuscular fat content of the longest muscle samples.

2.3. Primer design and synthesis
We refer to the full sequence of pig FUT1 gene (accession number: NC_010448), and then we use the obtained data to design primers for amplification using Primer 5.0 software (as shown in Table 1). In this study, the FUT1-1 primer was used to amplify M229 site and FUT1-2 primer was used to amplify the M307 site.

| Name   | Sequence                         | Tm/℃ | Products length/bp | Utilization   |
|--------|----------------------------------|------|--------------------|---------------|
| FUT1-1 | F:CCCTGTGTCCAGACCATAACG         | 61   | 165                | PCR-SSCP      |
|        | R:GTCCCATCTGGTTCCCAAAC          |      |                    |               |
| FUT1-2 | F:CTTCCTGAACGTCTATCAAGACC       | 58   | 421                | PCR-RFLP      |
|        | R:CTTCAGCCAGGGCTCTTTAAG         |      |                    |               |

2.4. PCR amplification reaction
A 25 μL reaction system was used for PCR amplification. The reaction system was as follows: 2 ×Es Taq Master Mix 12.5 μL, ddH2O 8.5 μL, the sample volume of each upstream and downstream primer was 1 μL, and the sample DNA was 2 μL. The conditions of this reaction were: the pre-denaturation duration at 94℃ was 2 min; the denaturation duration at 94℃ was 30 s, the annealing duration was 30 s (see Table 1 for temperature details), and the 72℃ extension duration was 30 s. The experiment went through 35 cycles; the final extension duration at 72℃ was 2 minutes, and the PCR amplification product was stored at 4℃. Finally, the experiment was detected by 1.2% agarose gel electrophoresis, and the gel imaging system was photographed and stored.

2.5. Genotyping of mutation sites
We used the PCR-SSCP method to detect the polymorphism of the M229 locus of the FUT1 gene, photographed with silver nitrate staining, and analyzed the genotypes statistically. PCR-RFLP technology was used to detect the polymorphism of M307 locus of FUT1 gene. We used restriction enzyme Hhal (recognition of GCGC) to amplify PCR products. This experiment was detected by 1.2% agarose gel electrophoresis. Analyze genotypes and send samples for sequencing verification.

2.6. Statistical analysis of data
This experiment used Excel2007 to calculate the population genetic structural parameters. We used SPSS18.0 analysis and statistical software to analyze the correlation between traits. The results were
expressed as "mean ± SD", with P <0.05 as the criterion for determining the significance of the difference.

3. Results and analysis

3.1. Analysis of population genetic structure

As can be seen from Table 2, the dominant genotype of the M229 locus is CT, and the frequency of the C allele is 60.61%. The number of effective alleles was close to 2, indicating that the alleles are more evenly distributed in Congjiang pigs. The polymorphic information content was moderate polymorphism (0.25 <PIC <0.5), indicating that the genetic marker provided more genetic information. The dominant genotype of the M307 locus was GG. The frequency of the G allele was as high as 93.18%. The number of effective alleles was 1.1456, indicating that the alleles are relatively concentrated in this groups. The polymorphic information content was low-level polymorphism (PIC <0.25), indicating that the genetic marker locus provided less genetic information.

Table 2. Population genetic parameters of FUT1 gene M229 and M307 site of Congjiang pigs

| Site | Genotype | Genotype frequency | Allele | Allele frequency | Ne   | Ho   | He   | PIC  |
|------|----------|--------------------|--------|------------------|------|------|------|------|
| M229 | CC       | 0.3636             | C      | 0.6061           | 1.9138 | 0.5225 | 0.4775 | 0.3635 |
|      | CT       | 0.4849             |        |                  |      |      |      |      |
|      | TT       | 0.1515             | T      | 0.3939           |      |      |      |      |
| M307 | AA       | 0                  | A      | 0.0682           | 1.1456 | 0.8729 | 0.1271 | 0.1190 |
|      | AG       | 0.1364             | G      | 0.9318           |      |      |      |      |
|      | GG       | 0.8636             |        |                  |      |      |      |      |

3.2. Association analysis of mutation sites and traits

From Table 3, the thickness backfat and intramuscular fat content of GG genotype individuals from M307 locus of congaiing pigs were higher than AG genotype by 10.53% and 8.12%, but the differences were not significant (P> 0.05). However, the backfat thickness of CC genotype individuals at M229 locus was significantly higher than CT and TT genotypes by 20.99% and 22.30% (P <0.05).

Table 3. Association analysis between M229 and M307 site of FUT1 gene and meat quality on Congjiang pigs

| Site | Genotype | Skin thickness/mm | Backfat thickness/mm | Lion eye area/cm² | Lion muscle depth/mm | Free water% | Intramuscular fat% |
|------|----------|-------------------|----------------------|-------------------|----------------------|-------------|-------------------|
| M229 | CC       | 4.25±0.88         | 24.73±5.45           | 17.05±2.96        | 37.87±3.81           | 73.69±1.41 | 3.04±1.13         |
|      | CT       | 3.79±0.84         | 20.44±4.99           | 16.74±3.37        | 37.32±3.62           | 73.87±0.83 | 3.08±0.71         |
|      | TT       | 3.99±0.77         | 20.22±6.76           | 18.13±3.96        | 36.84±5.29           | 73.59±1.83 | 3.32±1.78         |
| M307 | AG       | 4.02±1.25         | 20.03±3.33           | 16.74±4.28        | 37.17±4.66           | 74.05±0.58 | 3.11±0.54         |
|      | GG       | 3.97±0.80         | 22.14±5.99           | 17.11±3.17        | 37.47±3.86           | 73.72±1.29 | 3.33±1.13         |

4. Discussion

In this test, the M307 locus of FUT1 gene showed a skewed distribution in Congjiang pigs. In other domestic pig species, this locus is no A allele [8]. However, this study found that the frequency of allele A at this site was low, suggesting that congjiang piglets have strong anti-diarrhea ability. We compared the genetic parameters of the M307 and M229 locus, it showed that the M229 locus of FUT1 gene in Congjiang pigs has rich genetic polymorphisms. The researchers explored the M229 locus of FUT1 gene in the Duroc and found that the gene distribution of the M229 locus was CC>
CT > TT, the TT genotype was resistant, and the T allele frequency was 0.2575 [9]. In this test, the frequency of the T allele at the M229 locus of FUT1 gene in Congjiang pigs (0.3939) was higher than that of Duroc, further confirming that the M229 marker locus can be used as a candidate site for anti-diarrhea in Congjiang pigs.

In terms of carcass and meat quality traits, there are few reports about the FUT1 gene. Researchers have analyzed the correlation between the polymorphism of M307 locus in FUT1 gene and the phenotypic value in the offspring of large white pigs and Meishan pigs, and found that the AA genotype was significantly lower than the AG at the rib backfat thickness index [10]. In this test, Congjiang pigs did not detect AA type individuals, but AG type individuals had low backfat thickness. This data confirms that the FUT1 gene M307 locus an allele was helpful for the selection of low backfat thickness and high lean meat traits. At the same time, the research team found that the M229 locus could be used as a key mutation site for regulating the backfat thickness of Congjiang pigs. The backfat thickness of CT and TT genotype was significantly lower than that of CC, indicating that the M229 locus was effective for local pigs.

5. Conclusion
In the genetic improvement of FUT1 gene in Congjiang pigs populations, we should weaken the selection and breeding of M307 locus, and strengthen the homozygous cultivation of TT genotype in M229 locus in order to achieve the double improvement of anti-diarrhea and low backfat thickness performance.

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