Isolation and Cloning of Porcine SLC27A2 Gene and Detection of Its Polymorphism Associated with Growth and Carcass Traits

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ABSTRACT : The protein encoded by SLC27A2 gene is an isozyme of long-chain fatty-acid-coenzyme A ligase family, and it converts free long-chain fatty acids into fatty acyl-CoA esters, and thereby plays a key role in lipid biosynthesis and fatty acid degradation. In the present study, SLC27A2 located on human chromosome 15 was selected as candidate gene and we isolated and cloned partial fragments of mRNA sequence and genomic fragments of porcine SLC27A2 gene. The coding region of the gene as determined by alignments shared 90% and 82% identity with human and mouse cDNAs, respectively. Detection in LargeWhite and Meishan breeds showed that a single nucleotide polymorphism (SNP) (A→G) existed in exon 7, which caused corresponding amino acid changed for encoding. In LargeWhite pigs it encoded for Val while in Meishan pigs it encoded for Ile, so we developed the PCR-RFLP genotype method for detection of this polymorphism. Association study in 135 F2 reference family indicated that significant correlation existed between the polymorphism and growth and carcass traits. (Key Words : Solute Carrier Family 27, Member 2, Pig, Polymorphism)

INTRODUCTION

In recent years marked assisted selection (MAS) has been playing an important part in animal breeding, using halothane gene (Fuji et al., 1991; Hamilton et al., 2000; Krzecio et al., 2005) and Rendement Npole gene (Hamilton et al., 2000; Andersson, 2003) as molecular marker in pig breeding great profits have been made, which also accelerated pig breeding greatly. As the basis of MAS, candidate gene method is easy and efficient to identify the molecular marker, for example, Niu et al. (2006) select the OVGP1 gene as the candidate gene for reproduction traits. This study selected SLC27A2 located on human chromosome 15 as candidate gene. The protein encode by SLC27A2 gene is an isozyme of long-chain fatty-acid-coenzyme A ligase family, and this enzyme activates long-chain, branched-chain and very-long-chain fatty acids (VLCFA) containing 22 or more carbons to their CoA derivatives. Based on its important function Wakui et al. (1998) mapped the SLC27A2 gene to human chromosome 15q21.2 by fluorescence in situ hybridization, and he deposited the nucleotide sequence of human SLC27A2 gene in GenBank (D88308) (1998). Also Uchiyama et al. cloned cDNAs encoding rat SLC27A2 gene (1996). The predicted human and rat proteins each have 620 amino acids and share 82% sequence identity. Northern blot analysis revealed that the SLC27A2 gene is expressed only in rat liver and kidney (1996). In mouse the highest levels of SLC27A2 activity in liver and kidney was found (Berger et al., 1998; Heinzer et al., 2002; Mootha et al., 2003). Studies show that SLC27A2 is expressed in major organs of fatty acid metabolism, such as adipose tissue, liver, heart, and kidney, indicating its function for traits related to fat and growth (Schaffer and Lodish, 1994; Hirsch et al., 1998). Thus the SLC27A2 gene may be a promising candidate gene for the traits related to fat metabolism. The objective of this study was to examine this gene as candidate gene for the production traits in Large White and Meishan pig resource population.

MATERIALS AND METHODS

Sample collection and total RNA extraction

The pig resource family used in this study was...
established by mating three Large White boars to seven Meishan sows. Five males and twenty-three females in the F₁ generation were selected for intercrossing randomly. 135 F₂ individuals as reference family (Zuo et al., 2003; 2005) were slaughtered for test. The pigs in reference family were characterized for their weight at 170 days of age (test weight) and backfat thickness (adjusted to a common body weight, i.e. test backfat). Carcass traits and meat quality traits were recorded according to the method of Xiong and Deng (1999). 40 LargeWhite pigs and 45 Meishan pigs were from Jinpin pig station of Huazhong Agricultural University.

Liver tissues samples from LargeWhite and Meishan were collected from 180 days pigs slaughtered in August, 2002. For each breed, total RNA was extracted using the Trizol reagent total RNA extraction kit (GIBCO, USA) and pooled, and the total RNA sample was treated with DNase I (Biostar International, Toronto, Canada), and 2 µl template. PCR was run in the GeneAmp PCR system 9600 (Perkin-Elmer Co., Norwalk, CT, USA) thermocycler as follows: initial denaturation at 94°C for 4 min, 35 cycles of 94°C for 50 s, optimal temperature for 50 s, 72°C for 45 s, and a final extension time of 10 min at 72°C. The amplified fragments were cloned into the pGEM-T vector (TaKaRa, Dalian, China) and were sequenced using standard M13 primers.

RNA reverse transcription and first-strand cDNA synthesis

For each RNA sample, a single reverse transcription reaction was set up. Every reverse transcription reaction used 4 µg of total RNA per sample. In a sterile RNase-free microcentrifuge tube, 0.5 µg of the oligo (dT) 15 primer per microgram of the mRNA sample was added in a total volume of 15 µl with water. The tube was heated to 70°C for 5 min to melt the secondary structure within the template, and then cooled immediately on ice to prevent the secondary structure from reforming. After that, the tube was spun briefly to collect the solution at the bottom. The following components were added to the annealed primer/template in the order: 5 µl of M-MLV 5µl reaction buffer, 1.25 µl of 10 mM dNTPs, 25 units of rRNasin ribonuclease inhibitor (Promega, USA), 200 units of MMLV RT (Promega, USA), nuclease-free water to a final volume of 25 µl and mixed gently by flicking the tube. The tube was incubated for 60 min at 37°C, and then the efficiency of reverse transcription was checked on 1% agarose EB gel.

Amplification, cloning and sequencing

A number of porcine ESTS were initially identified using the human cDNA sequence (NM_003645) by running a BLASTN search against the GENBANK ‘EST others databases. These ESTs were collected and then assembled into one contig. From this contig, primer SLC1 and primer SLC2 were designed using Primer 5.0 software (http://www.premierbiosoft.com) to amplify cDNA sequences. Then primer SLC3 was designed to amplify genomic sequence. The primers were listed in Table 1.

Table 1. Primers used for amplification of porcine SLC27A2 gene

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer symbol</th>
<th>Primer sequence (5'-3') (F-R)</th>
<th>Annealing temperature</th>
<th>Amplification purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC27A2</td>
<td>SLC1</td>
<td>F: TGTTGCGTGTGGGAAGAGTTA R: GACAGCCATAAACAATTACAC</td>
<td>53.5°C</td>
<td>cDNA fragments amplification</td>
</tr>
<tr>
<td>SLC2</td>
<td>F: CCGAGAAAGACTCAGACAG R: GGGTAGGCTCCCTCATGTTC</td>
<td>54°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLC3</td>
<td>F: GCAGAAAAACTAATCTTAC R: GATCTCCACTGTTGAAATA</td>
<td>54°C</td>
<td>PCR-RFLP genotyping</td>
<td></td>
</tr>
</tbody>
</table>

PCR-RFLP, Non-denaturing polyacrylamide gel electrophoresis and silver stain

According to sequences analysis results, we detected restriction enzyme AaII site in exon 7 of SLC27A2 gene. For the PCR-RFLP assays, 8.5 µl of primer SLC3 PCR products were digested with 5 U AaII (TaKaRa) in 1× digestion buffer with 1×BSA added in a total volume of 10 µl. Following digestion for 4 h at 37°C, digested products were separated by 8% PAGE (PolyAcrylamide Gel Electrophoresis, PAGE) in 1×TBE buffer with silver staining. A 8% non-denaturing polyacrylamide gel was prepared by mixing 16 ml 30% acrylamide stock solution, 6
10× TBE buffer, 37.5 ml sterile water, 400 µl 10% ammonium persulfate and 40 µl TEMED (BioRad, USA). The polyacrylamide gel run in 1× TBE buffer at 100 V until the xylene cyanol reached the bottom of the gel. Finally, silver stain was done as follows: the gel was fixed with 10% ethanol for 10 min, washed with 1% HNO3 for 10 min, stained for 15 min using 0.2% AgNO3, rinsed in distilled water three times, 3 min each time, developed in 3% NaCarbonate (with 0.004% formaldehyde), and reaction was terminated with 3% acetic acid, then the gel was dried.

**Statistical analysis**

The association between genotype and production traits was performed with the least square method (GLM procedure, SAS version 8.0). Both additive and dominance effects were also estimated using REG procedure of SAS version 8.0, where the additive effect was denoted as -1,0 and 1 for AA, AB and BB, respectively, and the dominance effect represented as 1, -1 and 1 for AA, AB and BB, respectively (Liu, 1998). The model used to analyze the data was assumed to be:

\[ Y_{ijk} = \mu + S_i + F_j + G_k + b_{ijk}X_{ijk} + e_{ijk} \]

Where, \( Y_{ijk} \) is the observation of the trait; \( \mu \) is the overall mean; \( S_i \) is the effect of its sex; \( F_j \) is the effect of its family; \( G_k \) is the effect of its genotype; \( b_{ijk} \) is the regression coefficient of the slaughter age and \( e_{ijk} \) is the random residual.

**RESULTS**

**Amplification, cloning and sequencing**

Products of primer SLC1, SLC2 and SLC3 were 414 bp length, 604 bp length (Figure 1) and 117 bp (Figure 2) length respectively.

The sequences of primer SLC1 and SLC2 were assembled into a 787 bp length cDNA sequence. Analysis showed that the sequence included 729-bp protein-coding region that was composed of exons 6, 7, 8, 9 and parts of exons 5 and 10 of SLC27A2 mRNA, and 242 amino acids were encoded in the region. The coding region of the contig as determined by alignments shares 90% and 82% identity with human and mouse cDNAs, respectively, which showed high similarity of the three species indicating that the SLC27A2 gene has been evolutionarily conserved to some extent. Analysis in LargeWhite and Meishan breeds showed that a SNP (A→G) existed in exon 7, which caused...
corresponding amino acid changed. In LargeWhite breed it encoded for Val while in Meishan breed it encoded for Ile.

The result of detection by PCR-AatII-RFLP genotype method is as Figure 3.

Distribution of porcine SLC27A2 AatII-RFLP genotypes in 40 LargeWhite, 45 Meishan and 135 F2 pig population was listed in Table 2.

In Meishan breed allele A (frequency: 1) was the single genotype while in LargeWhite breed allele B (frequency: 88.75%) was the main genotype which showed great genotype frequency difference in the two purebreds.

Analysis of the association of genotype value about growth and carcass traits

SAS package was conducted for statistical analyses on production traits on F2 offspring. The result was listed in Table 3.

Highly significant associations of the genotype with lean meat percentage (p = 0.0097, 0.0146 respectively), average skin thickness (p = 0.0063, 0.0020 respectively), birth weight (p = 0.0043), and four significant associations with dressing percentage (p = 0.0119, 0.0186 respectively), fat percentage (p = 0.0423), backfat at 6-7th thorax (p = 0.0276), backfat at thorax-waist (p = 0.0288) were found, while no significance was found on other production traits or any of the meat quality traits.

Result showed that allele A was associated with increases in the trait values. Pigs with AA genotype have more birth weight (+0.106), backfat at thorax-waist (+0.350), backfat at 6-7th thorax (+0.402), and fat percentage (+2.934) than pigs with BB genotype, while pigs with AA genotypes have less dressing percentage (-3.226), lean meat percentage (-3.201), and average skin thickness (-0.102) than pigs with BB genotype.

Table 2. Distribution of porcine SLC27A2 AatII-RFLP genotype

<table>
<thead>
<tr>
<th>Breed</th>
<th>Number</th>
<th>Genotype AA</th>
<th>Genotype AB</th>
<th>Genotype BB</th>
<th>A frequency</th>
<th>B frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>LargeWhite</td>
<td>40</td>
<td>0</td>
<td>9</td>
<td>31</td>
<td>0.1125</td>
<td>0.8875</td>
</tr>
<tr>
<td>Meishan</td>
<td>45</td>
<td>45</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>F2</td>
<td>135</td>
<td>59</td>
<td>61</td>
<td>15</td>
<td>0.6630</td>
<td>0.3370</td>
</tr>
</tbody>
</table>

Table 3. Association between SLC27A2 genotype and production traits

<table>
<thead>
<tr>
<th>Traits</th>
<th>SLC27A2 genotype (µ±SE)</th>
<th>Effect (µ±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AB</td>
</tr>
<tr>
<td>Dressing percentage (%)</td>
<td>70.28±±0.556e</td>
<td>70.59±±0.5377a</td>
</tr>
<tr>
<td>Carcass weight (kg)</td>
<td>61.76±±0.472</td>
<td>61.98±±0.456</td>
</tr>
<tr>
<td>Lean meat percentage (%)</td>
<td>51.80±±0.582aa</td>
<td>53.95±±0.560b</td>
</tr>
<tr>
<td>Fat Meat Percentage (%)</td>
<td>20.04±±0.6299a</td>
<td>18.50±±0.608ab</td>
</tr>
<tr>
<td>Backfat at 6-7th Thorax (cm)</td>
<td>2.56±±0.079a</td>
<td>2.42±±0.077ab</td>
</tr>
<tr>
<td>Backfat at Thorax-Waist (cm)</td>
<td>1.89±±0.070b</td>
<td>1.77±±0.067ab</td>
</tr>
<tr>
<td>Average skin thickness (cm)</td>
<td>0.35±±0.016b</td>
<td>0.34±±0.016a</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1.42±±0.028ab</td>
<td>1.49±±0.027b</td>
</tr>
</tbody>
</table>

1 Overall mean values (±SE).
2 Letters denoting significant difference between groups: a,b * p<0.05, significant association; a,b, * p<0.01, highly significant association.

DISCUSSION

In the present study, we selected SLC27A2 gene as candidate gene according to its important function, and isolated porcine SLC27A2 gene fragments with corresponding knowledge of known SLC27A2 gene of human, rat and mouse by bioinformatics way. This proved to be a rapid and effective method to obtain genomic sequence of candidate genes, which might be developed as molecular marker to serve in pig breeding.

GenBank BLAST analysis revealed that the gene was not homologous to any of the known porcine genes, so the gene was a novel porcine gene. The polymorphism analyzed found in exon 7 causes amino acid change, which may be directly related to functional variation. According to the association result, if we increase A frequency, birth weight could be improved, while increasing B frequency could greatly improve carcass quality-lower backfat at thorax-waist, backfat at 6-7th thorax, and fat percentage; higher dressing percentage, lean meat percentage, and average skin thickness. This may provide important reference in pig molecular breeding. According to the results obtained in the study, porcine SLC27A2 gene was supposed to be a candidate gene or closely associated with the major gene or QTLs affecting production traits. However, further investigation is required among more populations of pigs to verify whether the substitution could be utilized as a genetic marker in MAS programs. Next we will continue the research to this novel gene and strive to obtain the complete sequence of this gene, and then do the functional analysis.

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