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

Wang Tao[1, 2, a], Liu Chang[3, a], Xiong Yuan-zhu[1, *], Deng Chang-yan[1], Zuo Bo[1], Xie Hong-tao[1] and Xu De-quan[1]

[1] Key Laboratory of Swine Genetics and Breeding, Ministry of Agriculture & Key Lab of Agricultural Animal Genetics, Breeding and Reproduction, Ministry of Education, Huazhong Agricultural University, Wuhan, 430070, P. R. China

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 F_2 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.

* Corresponding Author: Xiong Yuan-zhu, Tel: +86-27-87287390, Fax:+86-27-87394184, E-mail: xiongyzh@mail.hzau.edu.cn

[2] Hainan Luoniushan Swine Breeding Center, Haikou, 571100, P. R. China.

[3] Department of Food Engineering, Hebei Normal University of Science & Technology, Qinhuangdao 066600, P. R. China.

[a] These two authors contribute equally to this work.

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MATERIALS AND METHODS

Sample collection and total RNA extraction

The pig resource family used in this study was

Table 1. Primers used for amplification of porcine SLC27A2 gene

Gene	Primer symbol	Primer sequence (5'-3') (F-R)	Annealing temperature	Amplification purpose
SLC27A2	SLC1	F:TGGTGCTGTTGGAAGAGTTA R:GACACGCCATAAACATTCAC	53.5°C	cDNA fragments amplification
	SLC2	F:CGGAGGAAAGACTCAGACAG R:GGGTAGGTCCCTTCATGTTC	54°C	
	SLC3	F:GCAAAATCACAAATCTTAC R:GATCTCCACTGTTGAAATA	54°C	PCR-RFLP genotyping

established by mating three Large White boars to seven Meishan sows. Five males and twenty-three females in the F_1 generation were selected for intercrossing randomly. 135 F_2 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 before continuing with the first-strand cDNA synthesis.

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 5×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% agrose 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.

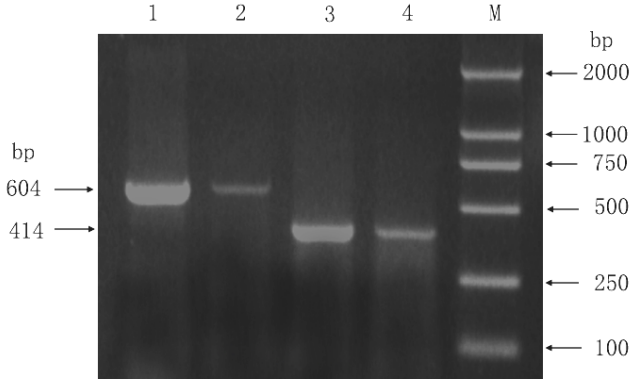
PCR was performed in 25 μl reaction mix containing: 1×PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.4 μmol of each PCR primer, 3 UTaq DNA polymerase (Biostar International, Toronto, Canada), and 2 μl template. PCR was run in the GeneAmp PCR system 9600 (Perkin-Elmer Co., Norwalk, CT, USA) thermocycler 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.

Sequences analysis

With assembly procedure of Sequencher 4.14 software sequences were assembled; multiple sequences blasting was carried out with Cluster W software (http://www.ebi.ac.uk/clusterw/index.html). Exons sizes and borders of human *SLC27A2* gene were predicted by Spidey procedure analysis (http://www.ncbi.nlm.nih.gov/IEB/Research/Ostell/Spidey/), while exons sizes and borders of pig were obtained according to the conservation of splicing sites between human and pig. With DNAclub software restriction enzyme site analysis was done; amino acid sequences analysis was done on website software (http://cn.expasy.org).

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

According to sequences analysis results, we detected restriction enzyme *Aat*II 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 *Aat*II (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



Figrue 1. cDNA amplification results of SLC1 and SLC2 for porcine *SLC27A2* gene. Lane 1, 2: SLC2; lane 3, 4: SLC1. Lane 1, 3: Meishan breed; Lane 2, 4: LargeWhite breed Lane M: DL2000 marker.

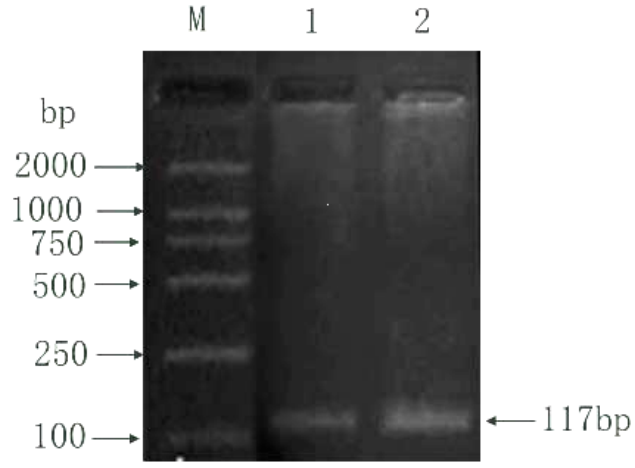


Figure 2. Amplification results of SLC3 for porcine *SLC27A2* gene. Lane M: DL2000 marker; lane 1: LargeWhite breed; lane 2: Meishan breed.

ml 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% HNO_3 for 10 min, stained for 15 min using 0.2% $AgNO_3$, 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

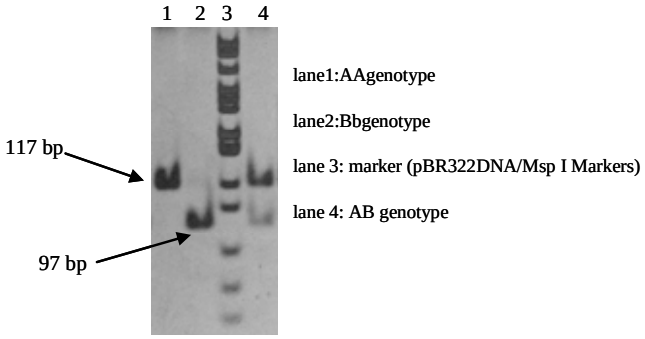


Figure 3. *Aat*II recognition site with three genotypes in F_2 population. The 117-bp（SLC3）PCR product was alternatively split into two fragments of 97 and 20 bp. The 117-bp fragment was A allele, and the 97-bp and 20-bp fragment was B allele respectively.

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; μ 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 have been deposited in the GenBank database, and SLC1 was for LargeWhite breed with accession number AY822678, while SLC2 was for Meishan breed with accession number AY822679.

Sequence analysis and PCR-RFLP

With Sequencher 4.14 software sequences of 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-*Aat*II-RFLP genotype method is as Figure 3.

Distribution of porcine SLC27A2 *Aat*II-RFLP genotypes in 40 LargeWhite, 45 Meishan and 135 F_2 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 F_2 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.

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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Table 2. Distribution of porcine *SLC27A2 Aat*II-RFLP genotype

Breed	Number	Genotype AA	Genotype AB	Genotype BB	A frequency	B frequency
LargeWhite	40	0	9	31	0.1125	0.8875
Meishan	45	45	0	0	1	0
F_2	135	59	61	15	0.6630	0.3370

Table 3. Association between *SLC27A2* genotype and production traits

Traits	SLC27A2 genotype (μ±SE)[1]			Effect (μ±SE)	
	AA	AB	BB	Additive	Dominance
Dressing percentage (%)	70.286±0.556^{a2}	70.595±0.537^{a}	73.512±1.109^{b}	1.613±0.632*	0.652±0.405
carcass weight (kg)	61.761±0.472	61.980±0.456	63.725±0.942	0.982±0.537	0.382±0.344
Lean meat percentage (%)	51.802±0.582^{Aa}	53.957±0.560^{B}	55.003±1.124^{b}	1.601±0.646*	-0.277±0.416
Fat Meat Percentage (%)	20.046±0.629^{a}	18.500±0.608^{ab}	17.112±1.255^{b}	-1.467±0.715*	0.039±0.458
Backfat at 6-7th Thorax (cm)	2.568±0.079^{a}	2.428±0.077^{ab}	2.166±0.158^{b}	-0.201±0.090*	-0.031±0.058
Backfat at Thorax-Waist (cm)	1.895±0.070^{a}	1.775±0.067^{ab}	1.544±0.139^{b}	-0.175±0.079*	-0.028±0.051
Average skin thickness (cm)	0.351±0.016^{A}	0.341±0.016^{A}	0.453±0.032^{B}	0.051±0.018**	0.030±0.012*
Birth weight (kg)	1.423±0.028^{AB}	1.498±0.027^{A}	1.317±0.056^{B}	-0.053±0.032	-0.064±0.020**

[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.

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