

Original Article

A Novel Porcine Gene – *SLC9A3R2*, Differentially Expressed in the Longissimus Muscle Tissues from Meishan and Large White Pigs

(pig / *SLC9A3R2* / mRNA differential display / RACE)

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Abstract. The mRNA differential display technique was performed to investigate the differences of gene expression in the longissimus muscle tissues from Meishan and Large White pigs. One novel gene that was differentially expressed was identified through semi-quantitative RT-PCR and the cDNA complete sequence was then obtained using the rapid amplification of cDNA ends (RACE) method. The nucleotide sequence of the gene is not homologous to any of the known porcine genes. The sequence prediction analysis revealed that the open reading frame of this gene encodes a protein of 337 amino acids that has high homology with the solute carrier family 9 isoform 3 regulator 2 isoform a of four species – human (92 %), mouse (91 %), rat (90 %), rabbit (87 %) – so that this gene can be defined as swine *SLC9A3R2* gene. The phylogenetic tree analysis revealed that the swine *SLC9A3R2* gene has the closest genetic relationship with the *SLC9A3R2* gene of human. This gene is structured in seven exons and six introns as revealed by

computer-assisted analysis. The tissue expression analysis indicated that the swine *SLC9A3R2* gene is differentially expressed in different tissues. Our experiment is the first to establish the primary foundation for further research on the swine *SLC9A3R2* gene.

Introduction

mRNA differential display first described by Liang and Pardee (1992) is a fast and efficient method for isolating and characterizing altered gene expression in different cell types. It was statistically shown that 80–120 primer combinations would be sufficient to cover all the transcript populations in the cell (Liang et al., 1993). This technique possesses the following advantages over other similar techniques: it is based on simple and established methods, more than two samples can be compared simultaneously, and only a small amount of starting material is needed (Yamazaki and Saito, 2002).

Chinese indigenous pig breeds such as Meishan, Erhualian and Tongcheng often have valuable traits such as disease resistance, high fertility, good maternal qualities, unique product qualities, longevity, and adaptation to harsh conditions. Exotic pig breeds such as Large White, Landrace, Duroc possess good achievements in growth rate, high lean rate; especially, the introduced pigs have higher lean meat rate and food conversion efficiency, whereas Chinese indigenous pigs have more fat deposition and superior meat quality (Pan et al., 2003). Phenotypic variances are mainly determined by the genetic differences. This means that detection of the genetic differences between Chinese indigenous pig breeds and exotic pig breeds or determination of the differentially expressed genes between Chinese indigenous pig breeds and exotic pig breeds, which determine these phenotypic variances, is necessary for pig breeders.

Our present study was carried out with the mRNA differential display technique to isolate the differentially expressed genes in the longissimus dorsi muscle tissues

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Abbreviations: BAC – bacterial artificial chromosome, EST – expressed sequence tag, GSPs – gene-specific primers, PCR – polymerase chain reaction, RACE – rapid amplification of cDNA ends, RT-PCR – reverse transcriptase-polymerase chain reaction, *SLC9A3R2* – solute carrier family 9 isoform 3 regulator 2 isoform a.

from one Chinese indigenous pig breed – Meishan pigs – and one exotic pig breed – Large White pigs.

Material and Methods

Sample collection

Large White and Meishan pigs, the two pure-bred populations, were constructed in 2007. All the longissimus dorsi muscle samples were collected from 120-day-old pigs slaughtered in 2008. For each breed, the total RNA was extracted from four male and four female pigs using the Total RNA extraction kit (Gibco, Tulsa, OK). Before the first-strand cDNA synthesis, DNase I treatment of the total RNA was done.

Differential display

The differential display polymerase chain reaction (PCR) amplification of each reverse transcription product was carried out with ten arbitrary primers and nine oligo (dT) primers as previously described (Liu et al., 2004; 2005a). The PCR products were then separated on 8% non-denaturing polyacrylamide gel and displayed using the silver stain described previously (Liu et al., 2004; 2005a).

Semi-quantitative reverse transcriptase-polymerase chain reaction

Semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) was performed as previously described elsewhere (Fehr et al., 2000; Daigo et al., 2003; Liu et al., 2005b). To eliminate the effect of cDNA concentration, we repeated the RT-PCR four times using 100, 200, 300, 400 and 500 ng cDNA as templates, respectively. We selected the housekeeping gene β -actin (*DQ845171*) as the internal control. The control primers used were: 5'-TGCTGTCCCTGTACGCCTCTG-3' (forward primer 1) and 5'-ATGTCCCGCACGATCTCCC-3' (reverse primer 1). The PCR product was 220 bp long. The following expressed sequence tag (EST) or gene-specific primers were used to perform the RT-PCR for identification and tissue expression profile analysis: 5'-CACCCAGGAGCACATGGAA-3' (forward primer 2) and 5'-TGGGACCACCACGGCTTA-3' (reverse primer 2). The PCR product was 429 bp long. The 25 μ l reaction system was: 2 μ l cDNA (100–500 ng), 5 pmoles each oligonucleotide primer (forward primer 1 and 2, reverse primer 1 and 2), 2.5 μ l 2 mmol/l mixed dNTPs, 2.5 μ l 10 \times Taq DNA polymerase buffer, 2.5 μ l 25 mmol/l MgCl₂, 1.0 unit of Taq DNA polymerase, and finally sterile water to a volume of 25 μ l. The PCR program initially started with a 94 °C denaturation for 4 min, followed by 25 cycles of 94 °C/50 s, 56 °C/50 s, 72 °C/50 s, then 72 °C extension for 10 min, finally 4 °C to terminate the reaction.

5'- and 3'-RACE

5'- and 3'-RACE were performed according to the instructions of BD SMART™ RACE cDNA Amplifica-

tion Kit (BD Clontech, Palo Alto, CA). The Gene-Specific Primers (GSPs) were: 3'-RACE GSP: 5'-CACCA-ATGGGACCAGCTCAGCCCAG-3', 5'-RACE GSP: 5'-ACCCTGGTGGCCCTCGCCTTCTCCT-3'.

Sequence analysis

The cDNA sequence prediction was conducted using GenScan software (<http://genes.mit.edu/GENSCAN.html>). The protein prediction and analysis were performed using the Conserved Domain Architecture Retrieval Tool of BLAST at the National Center for Biotechnology Information (NCBI) server (<http://www.ncbi.nlm.nih.gov/BLAST>) and the ClustalW software (<http://www.ebi.ac.uk/clustalw>).

Results

mRNA differential display

From the mRNA differential display, one gene, nominated as Gene 11, was found to be almost not expressed in the longissimus dorsi muscle of Meishan pigs, while it was over-expressed in the longissimus dorsi muscle of Large White pigs as shown in Fig. 1.

Semi-quantitative RT-PCR

The differentially expressed gene band was recovered from the gel and used as the template for re-amplification, which was performed with the corresponding oligo (dT) primer and the arbitrary primers used in the mRNA differential display. The resulting PCR product was 561 bp long. This was in agreement with the result of the mRNA differential display. The purified PCR product was then cloned into the T-vector and the recombinant plasmid was sequenced. Semi-quantitative RT-PCR was then conducted using the EST specific primers and the results are presented in Fig. 2.

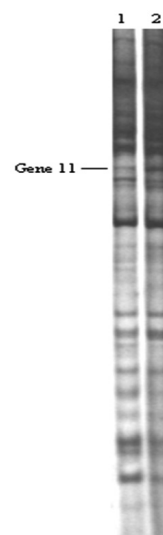


Fig. 1. Differential expression analysis of Gene 11. The arrow indicates the cDNA profile for the gene 11 on 8% polyacrylamide gel stained with silver nitrate. 1 – Meishan; 2 – Large White.

ited into the GenBank database (Accession number: FJ217221). The sequence prediction was carried out using the GenScan software. An open reading frame encoding 337 amino acids was found in the 2102-bp cDNA sequence. In the predicted results, probability of exon was 0.998, that of poly-A signal was from 1599-bp to 1604-bp (consensus: AATAAA). Further BLAST analysis of this protein revealed that this protein has high homology with SLC9A3R2 of four species – human (accession number: NP_001123484, 92 %), mouse (accession number: Q9JHL1; 91 %), rat (accession number: NP_446263; 90 %), and rabbit (accession number: NP_001075576; 87 %) (Fig. 3). The complete cDNA

sequence of this gene and the encoded amino acids are shown in Fig. 4.

From the sequencing and structural results described, this gene can be defined as the swine *SLC9A3R2* gene. Based on the results of the alignment of seven different species of *SLC9A3R2*, a phylogenetic tree was constructed using the ClustalW software (<http://www.ebi.ac.uk/clustalw>), as shown in Fig. 5. The phylogenetic tree analysis revealed that the swine *SLC9A3R2* gene has a closer genetic relationship with the *SLC9A3R2* gene of human than those of mouse, rat and rabbit.

To obtain the genomic DNA of *SLC9A3R2*, the publicly available pig genome database at the NCBI Pig Ge-

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ACCGAGCGGGAGCCGCCGAGGCCGCCGAGGGTCTCCCGCGCCGCTCCCGCGCCCGCCCCAGCGCCCCCGCTGCCGCG
GCGGGCGTGAGCGCCGTGGCCAGCCCGCGGCCGGCCGGTGGGCAGCAGGCGCCATGGCTGCCCGGAGCCGCTGCGGCCG
M A A P E P L R P
CGCCTGTGCCTCCTGGTGC GCGGCGAGCACGGCTACGGCTTCCACCTGCACGCGGAGAAGGGCCCGCGGGCAGTTC
R L C L L V R G E H G Y G F H L H G E K G R R G Q F
ATTTCGGCGCGTGGAGCCGGGTTCCCGGCCGAGGCGGCCGCGCTGCGCGCCGGGGACCGCCTGGTTCGAGGTCAACGGC
I R R V E P G S P A E A A A L R A G D R L V E V N G
GTCAACGTGGAGGGCGAGACGCACCACCAGGTGGTGCAGCGGATCAAGGCTGTGGAGGGCCAGACGCGGCTGCTCGTT
V N V E G E T H H Q V V Q R I K A V E G Q T R L L V
GTGGACAAGGAGACGGACGAGGAGCTCCGCGAGGCGCCAGCTGACTTGCACCGAGGAGATGGCCAGCGAGGGCTGCCG
V D K E T D E E L R R R Q L T C T E E M A Q R G L P
CCTGCCCTTGACCCCTGGGAGCCGAAGGCGGACTGGGCGCCCGCAGGCAGCCTCAGCGGTGAGGCCGGCCAGAAGGAT
P A L D P W E P K A D W A P A G S L S G E A G Q K D
GTCAACGGGCCCCTGAGGGAGCTGCGCCCAAGGCTCTGCCACCTGCGAAAAGGCCCCAGGGTTACGGGTTTAACTTG
V N G P L R E L R P R L C H L R K G P Q G Y G F N L
CACAGCGACAAGTCCCGCCCTGGACAGTACATCCGCTCCGTGGACCCAGGCTCACCTGCTGCCCACTCCGGCCTCCGA
H S D K S R P G Q Y I R S V D P G S P A A H S G L R
GCCCAGGACCGACTCATAGAGGTGAACGGGCAGAATGTGGAGGGGCTGCGGCACGCGGAGGTGGTTGCCTGCATCAAG
A Q D R L I E V N G Q N V E G L R H A E V V A C I K
GCGCGGGAGGACGAGGCCCGGCTGCTGGTGGTGGACCCCGAGACGGATGCGTACTTCAAGCGGCTGCGGGTCACACCC
A R E D E A R L L V V D P E T D A Y F K R L R V T P
ACCCAGGAGCACATGGAAGGTCCACTGCCATCACCTGTCCCAATGGGACCAGCTCAGCCAGCTCAATGGTGGCTCC
T Q E H M E G P L P S P V T N G T S S A Q L N G G S
GTGTGCTCGTCCCGAAGTGACCTGCCCGGCTTAGACAAGGACACTGAGGACAGCAGCACCTGGAAGCGTGACCCTTTC
V C S S R S D L P G L D K D T E D S S T W K R D P F
CAGGAGAGTGGCCTCCACCTGAGCCCCACGGCGGCTGAGGCCAAGGAGAAGGCGAGGGCCACCAGGGTCAACAAGCGG
Q E S G L H L S P T A A E A K E K A R A T R V N K R
GCGCCACAGATGGACTGGAACCGGAAGCGTGAGATCTTCAGCAACTTCTGAGACCCCCACCCTCCGCCGAGCCGCCG
A P Q M D W N R K R E I F S N F *
CTGGTCCCCAGCCGGGCTCCTCTGGGCATGGACCTGGGCCCTTGCCAGAGCGCCCCGAGCCTCAGTGGACTGCAGCGGG
GGCACCTTCGCTCGTAAGCCGTGGTGGTCCCACCACCCCCATGAACCAGCCCGTGCCCCAGTGAGCCCCGTCTGCC
CCTTCCCACGGGGTGTGGGGAGCGGGCAGAGGAAGCCCTGAGACGGGAGGGACAGAGACACCCAGAGAGGTGGGCTG
GGGAGGGGAGGCTGGGGTGACCCGCCAGGCCGGGCCCTTGCTGCTTGCTGCTGGGCTGCTGACTTAAAGGAATTTGTGTTT
TGGCTTTTTTCCAACACGAGCTCTGGCTCCACACATGTTTCCACTTAATACCAGAGCCCCACCCCATCCCCCAGGACG
TGCTCTCTAAATAATTGCAATAAAACAAACCTTTCTCTGCAAACATTTCTCCACCTCCCTCAGCAGCAGCCTTCCCCGA
GGGAGTCCCAGGGCCTTCCAGGGGCAGAGTTCTGGGGCCCCAGGCCGTGTTACAGCAGGCTTCGGGGAGGCTAGGAGCC
CCACGTGGCTATAAGAGCTAAGAGCTGCCACGCTGCTCAGGCCCTGCCCTCCCTGGCGTGGGGGTTGGGTGGGGGCAG
GCCTGGGTTCTTGGTGGGGCCCGAGATGGAGAAGTTCGGCCCTGCCCGAGGAGGGCAGCCTGTAGACAGGCCCTTGTCC
CTGCACGGCAGCCCCAGGAAGTCCCAAGCTCTGGTCTGCTGCTGCTGAGCCGCCCTGAAGATGCCCCACCGACTCC
GCCTTCTTCTGCCCAGAACCGAGCCGACCCGGCATCGTGTCTGCCCTAAAAGCCATAGCGCATGCGCGCCCTCAGAAT
AAATAGGCCTTCTTGTAATAAAAAAAAAAAAAAAAAAAAAA

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Fig. 4. The complete cDNA sequence and encoded amino acids of Gene 11 (GenBank accession number: FJ217221). ATG – start codon; TGA – stop codon

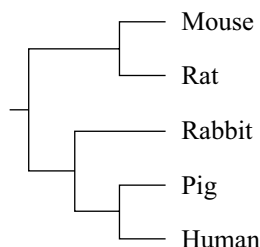


Fig. 5. The phylogenetic tree for five kinds of *SLC9A3R2* from pig, human, mouse, rat and rabbit

nome Resources (<http://www.ncbi.nlm.nih.gov/projects/genome/guide/pig/>) was screened using the full-length cDNA sequence of *SLC9A3R2* as a seed. A bacterial artificial chromosome (BAC) clone (*Sus scrofa* chromosome 3 clone CH242-207G4, GenBank accession no. FP102350), which encompasses the entire *SLC9A3R2* gene, was identified by BLASTGen analysis. The pig gene (nucleotides 37,279–46,667 in the *Sus scrofa* chromosome 3 clone CH242-207G4) is 9,388 bp long and consists of seven exons. All exon-intron splice junction sequences conform to the GT-AG rule (Fig. 6).

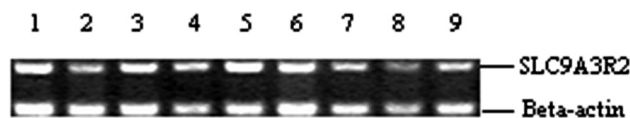
Tissue expression profile

The RT-PCR analysis of the tissue expression profile was carried out using the tissue cDNAs of one adult Meishan×Large White cross pig as the templates. The tissue expression analysis indicated that the swine *SLC9A3R2* gene is highly expressed in the kidney, small intestine, liver and muscle, moderately expressed in the heart, spleen and ovary, and weakly expressed in fat (Fig. 7).

Discussion

NHE3 (*SLC9A3*; MIM 182307) is a sodium/hydrogen exchanger in the brush-border membrane of the proximal tubule, small intestine, and colon that plays a major role in transepithelial sodium absorption. *SLC9A3R2*, as well as *SLC9A3R1* (MIM 604990) and protein kinase A phosphorylation, may play a role in NHE3 regulation (DeMarco et al., 2002; Mahon et al., 2002; Yun et al., 2002; Palmada et al., 2003). To date, the swine *SLC9A3R2* has not been reported.

From the results obtained above, we found that the *SLC9A3R2* gene was differentially expressed in the longissimus dorsi muscle from Meishan and Large White pigs. Meishan is a fat-type pig breed, comprising much more body fat than lean meat or muscle. On the other hand, Large White is a typical lean-type pig breed, presenting the opposite phenotype than that described for the Meishan breed. Concerning the muscle tissue



1 – kidney, 2 – heart, 3 – small intestine, 4 – spleen, 5 – muscle, 6 – liver, 7 – lung, 8 – fat, 9 – ovary

Fig. 7. Tissue expression profile analysis of the swine *SLC9A3R2* gene on 1% agarose gel stained with ethidium bromide. The β -actin expression serves as a control

content, the two divergent pig breeds show the trend of Large White – high, Meishan – low. It is very interesting that the expression of the swine *SLC9A3R2* gene in the longissimus dorsi muscle also shows the trend of Large White – high, Meishan – low. As we know, phenotypic variances are mainly determined by the gene expression differences. Could the swine *SLC9A3R2* gene expression be associated with the development or metabolic processes of longissimus dorsi muscle tissue? This deserves further study.

Using tissue expression profile analysis we also found that the swine *SLC9A3R2* gene is differentially expressed in other tissues as well. Could this gene also be associated with the metabolic processes of these tissues? This should also be further studied.

In this experiment we only obtained the cDNA sequence of the swine *SLC9A3R2* gene and found that this gene is differentially expressed between the Meishan and Large White pigs and differentially expressed in different tissues. Would this gene also be differentially expressed in other pig breeds? To further understand the function of the novel gene, more research based on these primary results is needed.

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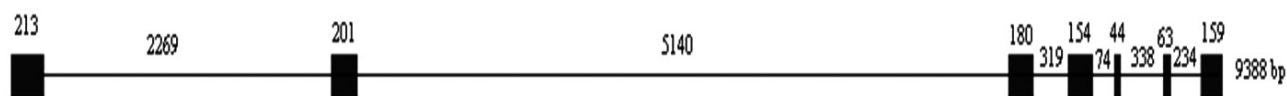


Fig. 6. The genomic sequence organization representing the ORF of the swine *SLC9A3R2* gene

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