

A novel porcine gene-*MGLL*, differentially expressed in the backfat tissues from Meishan and Large White pigs

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Abstract

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. 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 303 amino acids has high homology with the monoglyceride lipase (*MGLL*) of eight species-dog (91%), human (89%), cattle (88%), platypus (83%), mouse (82%), rat (81%), rhesus monkey (80%), and red jungle fowl (70%) – so that it can be defined as swine *MGLL* gene. This gene is structured in seven exons and six introns as revealed by computer-assisted analysis. The tissue expression analysis indicated that the swine *MGLL* gene is differentially expressed in detected tissues. Our experiment suggested that the swine *MGLL* gene might play an important roles in the superabundant fat deposition of Chinese pigs.

Keywords: pig, *MGLL*, mRNA differential display, RACE

Zusammenfassung

Ein neues porcines *MGLL*-Gen, differentiell exprimiert im Rückenspeckgewebe von Meishan und Large-White-Schweinen

Die Differential Display Technik wurde genutzt, um Unterschiede in der Expression im Rückenspeckgewebe von Meishan und Large White Schweinen zu untersuchen. Mittels semi-quantitativer RT-PCR wurde die differentielle Expression eines neuen Transkriptes bestätigt und dessen Sequenz mittels RACE (rapid amplification of cDNA ends) erfasst. Die Nukleotidsequenz zeigt keine Homologie zu bekannten porcinen Genen. Das offene Leseraster kodiert für ein 303 Aminosäurenrestes langes Protein dessen Sequenz Homologie mit der Monoglyceride-Lipase (*MGLL*) von acht Spezies aufweist (Hund 91%, Mensch 89%, Rind 88%, Schnabeltier 83%, Maus 82%, Ratte 81%, Rhesusaffe 80%, Huhn 70%), so dass seine Identität als porcines *MGLL* Gen angenommen werden kann. *In-silico* Analysen weisen für das Gen sieben Exons und sechs Introns aus. Das porcine *MGLL* zeigt gewebespezifische Expressionsmuster. Die Untersuchungen legen nahe, dass das porcine *MGLL* eine bedeutende Rolle in subkutanen Fettdepots spielen könnte.

Schlüsselwörter: Schwein, *MGLL*, mRNA Differential Display, RACE

Introduction

The mRNA differential display, first described by LIANG and PARDEE (1992), is an expeditious and efficient method for identification and characterization of 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. 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 (LIANG *et al.* 1993, YAMAZAKI and SAITO 2002).

Chinese indigenous pig breeds such as Meishan, Erhualian and Tongcheng often have some conspicuous flaws such as superabundant fat and too low grow rate while exotic pig breeds such as Large White, Landrace, Duroc always have lower fat rate, higher lean meat rate and higher grow rate. Therefore, Chinese indigenous pigs are always named fat-type pigs while exotic pigs are always named lean-type pigs (PAN *et al.* 2003). Phenotypic variances are mainly determined by the genetic differences. So that detecting the genetic differences between Chinese indigenous pig breeds and exotic pig breeds or finding out the differentially expressed genes between these two types of pig breeds may serve as a basis for understanding molecular mechanisms of these phenotypic differences.

The present study was carried out with the mRNA differential display technique to identify the differentially expressed genes in the backfat tissues from one Chinese indigenous breed (Meishan) and one exotic pig breed (Large White). We provide here the results on the identification of previously unrecognised porcine gene, *MGLL*, which is differentially expressed in Large White versus Meishan backfat tissues.

Material and methods

Animals and samples collection

Two purebred populations - Large White and Meishan (five male and five female pigs for each breed) were constructed in 2007. These pigs were slaughtered at 180-day-old and the backfat samples of these pigs were collected, immediately frozen in liquid nitrogen and stored at -80°C until isolation of RNA.

RNA reverse transcription and first-strand cDNA synthesis

For each RNA sample, a single reverse transcription reaction was set up. This method was simpler than the multiple cDNA synthesis reactions required for similar methods. Every reverse transcription reaction only required 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 \times Reaction Buffer, 1.25 μl of 10 mM dNTPs, 25 units of rRNasin[®] Ribonuclease Inhibitor (Promega, USA), 200 units of M-MLV 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/EtBr gel (LIU *et al.* 2004).

Differential display PCR

PCR amplification of each reverse transcription product was carried out simultaneously with arbitrary primer (5'-ATT AAC CCT CAC TAA ACA CCA CGT G-3') and oligo (dT) primer (5'-CTT ATG CTG AGT GAT ATC TTT TTT TTT GG-3'). The 25 µl reaction system was: 2.0 µl pooled cDNA, 2.5 µl 2 mM mixed dNTPs, 2.5 µl 10×Taq DNA polymerase buffer, 2.5 µl 25 mM MgCl₂, 2.0 µl 20 µM anchored «T» primer, 2.0 µl 20 µM arbitrary primer, 2.0 units of Taq DNA polymerase (1 U/1 µl) (JINMEI BIOTECH, China), and 9.5 µl sterile water. The PCR was done as follows: 94 °C for 5 min, 40 °C for 5 min, 72 °C for 5 min, 3 cycles, followed by 30 cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 2 min, then 72 °C extension for 10 min, finally 4 °C to terminate reaction.

Non-denaturing polyacrylamide gel electrophoresis and silver stain

A 8 % non-denaturing polyacrylamide gel was prepared by mixing 16 ml 30 % acrylamide stock solution, 6 ml 10×TBE buffer, 37.5 ml sterile water, 400 µl 10 % Ammonium Persulfate and 40 µl TEMED (BioRad, USA). The polyacrylamide gel was then pre-run for 30 min in 1×TBE buffer at 100 V. After that, 4 µl 6×gel loading solution was added to 20 µl PCR product, mixed well and loaded on the polyacrylamide gel; next, the gel was 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 % Na₂Co₃ (with 0.004 % formaldehyde), and reaction was terminated with 3 % acetic acid, then dry the gel (LIU *et al.* 2004).

Re-amplification, cloning and sequencing

Each band of interest was cut from the gel and added to 40 ml water. The gel/water solution was melted at 98 °C for 15 min. The melted solution was then used as the template to perform the re-amplification with DD-PCR primers. Finally, the PCR product was cloned to a T-vector and sequenced.

Semi-quantitative RT-PCR

Semi-quantitative RT-PCR was performed as previously described elsewhere (FEHR *et al.* 2000, DAIGO *et al.* 2003, LIU *et al.* 2005). To eliminate the effect of cDNA concentration, we repeated the RT-PCR four times using 100, 200, 300, 400 and 500ng pooled cDNA as templates, respectively. We selected the housekeeping gene beta-actin (DQ845171) as the internal control. The control primers used were: 5'-TGC TGT CCC TGT ACG CCT CTG-3' (forward primer 1) and 5'-ATG TCC CGC ACG ATC TCC C-3' (reverse primer 1). The PCR product is 220-bp in length. The following expressed sequence tag (EST) 11 specific primers were used to perform the RT-PCR for identification and tissue expression profile analysis: 5'-ACC CAC AGC TCC CAG TCT-3' (forward primer 2) and 5'-GCA GGA AGG GCA AGG TCA-3' (reverse primer 2). The PCR product is 385-bp in length. The 25 µl reaction system was: 2 µl cDNA (100-500ng),

5 pmoles each oligonucleotide primer (forward primer 1 and 2, reverse primer 1 and 2), 2.5µl 2 mmol/l mixed dNTPs, 2.5µl 10×Taq DNA polymerase buffer, 2.5µl 25 mmol/l MgCl₂, 3.0 units of Taq DNA polymerase, and finally add sterile water to volume 25µl. The PCR program initially started with a 94°C denaturation for 4min, followed by 25 cycles of 94°C/50s, 55°C/50s, 72°C/50s, then 72°C extension for 10 min, finally 4°C to terminate the reaction.

5'- and 3'-RACE

5'- and 3'-RACE were performed as the instructions of BD SMART™ RACE cDNA Amplification Kit (BD science, USA). The Gene-Specific Primers (GSPs) were: 3'-RACE GSP: 5'-ACT GGT TCT TGC CAG TCC TGA GTC T-3', 5'-RACE GSP: 5'- TGG ATG CCG AAG CAC ACT TTC AGT C-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 band, nominated as EST 11, later identified as a fragment of the *MGLL* gene, was found to weakly expressed in the backfat of Meishan pigs while was highly expressed in the backfat of Large White pigs as shown in Figure 1.

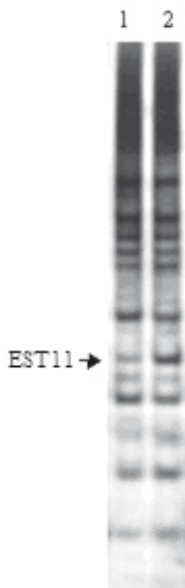


Figure 1

The differential expression analysis of EST 11. The arrow indicates the cDNA profile for the EST11 on a polyacrylamide gel of 8%, stained with silver nitrate.

1 Meishan backfat sample, 2 Large White backfat sample

Differenzielle Genexpressionsanalyse von EST 11. Der Pfeil markiert das cDNA-Profil für EST11 auf einem Polyacrylamidgel von 8%, gefärbt mit Silbernitrat.

1 Meishan Rückenspeckgewebe; 2 Large White Rückenspeckgewebe

Semi-quantitative RT-PCR

The differentially expressed gene band was recovered from gel and the resulting PCR product was 461-bp. Semi-quantitative RT-PCR was then conducted using the EST 11 specific primers and the results are presented in Figure 2.

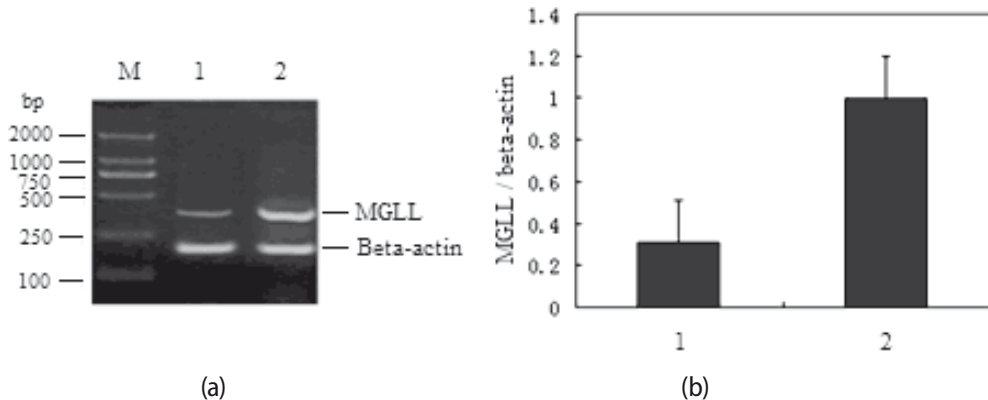


Figure 2

Differential expression analysis of the porcine *MGLL* gene: (a) semi-quantitative RT-PCR identification of the *MGLL* gene; (b) average and standard deviation ($n=5$) of *MGLL* mRNA expression levels relative to beta-actin. 1–Meishan; 2–Large White

Die differentielle Genexpressionsanalyse des MGLL-Gens beim Schwein.

a) semi-quantitative RT-PCR Identifizierung des *MGLL*-Gens b) Durchschnitts- und Standardabweichung ($n=5$) von *MGLL* mRNA Expressionsleveln bezüglich Beta-Actin. 1-Meishan, 2-Large White

Semi-quantitative RT-PCR results indicated that EST11 was highly expressed in the backfat of Large White pigs and weakly expressed in the backfat of Meishan pigs. This also coincided with the result of mRNA differential display.

5'- and 3'-RACE

Through 5'-RACE, one PCR product of ~750 bp was obtained. The 3'-RACE product was ~600 bp. These products were then cloned to T-vector and sequenced. Taken together, a 1 169-bp cDNA complete sequence was finally obtained.

Sequence analysis

The nucleotide sequence analysis using the BLAST software at NCBI server (<http://www.ncbi.nlm.nih.gov/BLAST>) revealed that this gene was not homologous to any of the known porcine genes and it was then deposited into the GenBank database (acc. no. FJ436393). The sequence prediction was carried out using the GenScan software. An open reading frame encoding 303 amino acids was found in this 1 169-bp cDNA sequence. In the predicted results, probability of exon was 0.932, poly-A signal was from 1 093-bp to 1 098-bp (consensus: AAT AAA). The theoretical isoelectric point (pI) and molecular weight (Mw) of this deduced protein of this swine gene was also computed using the Compute pI/Mw Tool (http://www.expasy.org/tools/pi_tool.html). The pI of swine *MGLL* protein was 6.89. The molecular weight of this

Further BLAST analysis of these proteins revealed that swine *MGLL* protein has high homology with the *MGLL* of eight species-dog (XP_856683, 91 %), human (NP_001003794, 89 %), cattle (XP_581556, 88 %), platypus (XP_001506833, 83 %), mouse (NP_035974, 82 %), rat (NP_612511, 81 %), rhesus monkey (XP_001099193, 80 %), and red jungle fowl (XP_414365, 70 %) (Figure 4). To obtain the genomic DNA of *MGLL*, the publicly available pig genome database at the NCBI Pig Genome Resources (<http://www.ncbi.nlm.nih.gov/projects/genome/guide/pig/>) was screened using the full-length cDNA sequence of *MGLL* as a seed. A bacterial artificial chromosome (BAC) clone (*Sus scrofa* chromosome 13 clone CH242-262A3, GenBank acc. no. FP312653) which encompasses entire *MGLL* gene was identified by BLASTGen analysis. The pig gene (nucleotides 83,190-168,079 in the *Sus scrofa* chromosome 13 clone CH242-262A3) is 84,890 bp in length and consists of seven exons. All exon-intron splice junction sequences conform to the GT-AG rule (Figure 5).



Figure 5

The genomic sequence organization representing the ORF of the swine *MGLL* gene.

Gliederung der genomischen Abfolge anhand des offenen Leserasters (open reading frame - ORF) des MGLL-Gens beim Schwein.

Tissue Expression Profile

The RT-PCR analysis of the tissue expression profile was carried out using the tissue cDNAs of one 180-day-old Meishan pig as the templates. The tissue expression analysis indicated that the swine *MGLL* gene is moderately expressed in backfat, muscle and liver, weekly expressed in spleen, heart, lung and kidney, and hardly expressed in and large and small intestine (Figure 6).



Figure 6

Tissue expression profile analysis of the swine *MGLL* gene on the agarose gel of 1% stained with ethidium bromide. The beta-actin expression is the control. 1 small intestine, 2 spleen, 3 muscle, 4 backfat, 5 lung, 6 kidney, 7 heart, 8 large intestine, 9 liver

Profilanalyse der Gewebeexpression des Schweine-MGLL-Gens in mit Ethidiumbromid versetztem Agarosegel von 1%. Die Beta-Actin-Expression dient als Kontrolle. 1 Dünndarm, 2 Milz, 3 Muskel, 4 Rücken fett, 5 Lunge, 6 Niere, 7 Herz, 8 Dickdarm, 9 Leber

Discussion

Monoglyceride lipase (*MGLL*) is an important lipase which functions together with hormone-sensitive lipase to hydrolyze intracellular triglyceride stores in adipocytes and other cells to fatty acids and glycerol. *MGLL* may also complement lipoprotein lipase in completing hydrolysis of monoglycerides resulting from degradation of lipoprotein triglycerides (WALL *et al.* 1997). Up until today, the swine *MGLL* has not been reported.

From the results obtained above, it was found that the *MGLL* gene was differentially expressed in the in the backfat 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.

To the percentage of fat meat, the two divergent pig breeds show the trend of Large White-low, Meishan-high. It is very interesting that the expression of the swine *MGLL* gene in the backfat shows the converse trend of Large White-high, Meishan-low. As we know, monoglyceride lipase is a kind of lipase which can hydrolyze intracellular triglyceride stores in adipocytes and other cells to fatty acids and glycerol. All these evidences above suggested that Large White pigs had much more monoglyceride lipase expressed in the fat tissues than Meishan pigs and the monoglyceride lipase hydrolyzed much more intracellular triglyceride stores in adipocytes. So that, Large White have lower percentage of fat meat than Meishan pigs.

The present study shows that the swine *MGLL* gene also differentially expressed in other tissues through tissue expression profile analysis. As we have not yet studied functions or protein levels, there might be many possible reasons for differential expression of this porcine gene. The best explanation for this under current conditions is that the biological activities related to the mRNA expression of this gene were presented differently in different tissues. This is also deserved to study.

In this experiment the complete cDNA sequence of the swine *MGLL* gene was obtained and it was found that this gene is differentially expressed in the backfat tissues from Meishan and Large White pigs. The results suggested that the swine *MGLL* gene might play an important role in the superabundant fat deposition of Chinese pigs.

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