Asian-Aust. J. Anim. Sci.
Vol. 21, No. 1 : 11 - 18
January 2008

www.ajas.info

Gene Microarray Analysis for Porcine Adipose Tissue: Comparison of Gene Expression between Chinese Xiang Pig and Large White

W. Guo[1,2,4], S. H. Wang[1], H. J. Cao[2], K. Xu[1], J. Zhang[1], Z. L. Du[1], W. Lu[1], J. D. Feng[1]
N. Li[1,*], C. H. Wu[2] and L. Zhang[3]

[1]State Key Laboratory for Agrobiotechnology, China Agricultural University, Beijing 100094, China

ABSTRACT : We created a cDNA microarray representing approximately 3,500 pig genes for functional genomic studies. The array elements were selected from 6,494 cDNA clones identified in a large-scale expressed sequence tag (EST) project. These cDNA clones came from normalized and subtracted porcine adipose tissue cDNA libraries. Sequence similarity searches of the 3,426 ESTs represented on the array using BLASTN identified 2,790 (81.4%) as putative human orthologs, with the remainder consisting of "novel" genes or highly divergent orthologs. We used the gene microarray to profile transcripts expressed by adipose tissue of fatty Chinese Xiang pig (XP) and muscley Large White (LW). Microarray analysis of RNA extracted from adipose tissue of fatty XP and muscley LW identified 81 genes that were differently expressed two fold or more. Transcriptional differences of four of these genes, adipocyte fatty acid binding protein (*aP2*), stearyl-CoA desaturase (*SCD*), sterol regulatory element binding transcription factor 1 (*SREBF1*) and lipoprotein lipase (*LPL*) were confirmed using SYBR Green quantitative RT-PCR technology. Our results showed that high expression of *SCD* and *SREBF1* may be one of the reasons that larger fat deposits are observed in the XP. In addition, our findings also illustrate the potential power of microarrays for understanding the molecular mechanisms of porcine development, disease resistance, nutrition, fertility and production traits. (**Key Words :** EST (Expressed Sequence Tag), cDNA Microarray, Adipose Tissue, Xiang Pig, Large White, SYBR Green Quantitative RT-PCR)

INTRODUCTION

Gene expression microarrays are a powerful and flexible new tool in molecular biology (Brown and Botstein., 1999) and permit the simultaneous measurement of transcription levels of thousands of genes in different physiological states of an organism tissue or cell (Schena et al., 1996; Shalon et al., 1996). When we utilize information from annotated genome or EST sequencing projects, we can construct microarrays in the most efficient manner and which provides a realistic representation of the system under study (Adams et al., 1991; Band et al., 2000). Recently, evaluation of transcript levels using microarray technology has led to new insights in animal breeding, breed comparison, infectious disease, animal development and evolution and nutrition and so on (White et al., 1999; Ly et al., 2000).

We have constructed a pig fat tissue cDNA library for EST sequencing and these ESTs provide a resource for comparative and functional genome analysis. For the present work, we incorporated a subset of these ESTs into cDNA microarrays representing ~3,500 genes. Because LW also known as Yorkshire is a breed from north of England and is referred as representative muscly pig, however Chinese XP is referred as fatty big and deposited more fat than LW, we profiled transcription difference between adipose tissues of fatty Chinese XP and muscly LW by the cDNA microarray.. These experiments allow us to gain insight into tissue-specific expression patterns in lipid biosynthesis and metabolism processes in different breeds.

MATERIALS AND METHODS

EST clustering and annotation

From among 6,494 high quality sequences obtained from normalized porcine adipose tissues cDNA libraries,

* Corresponding Author: N. Li. Tel: +86-10-6273-3904, Fax: +86-10-6273-3904, E-mail: ninglbau@public3.bta.net.cn
[2] College of Animal Science and Technology, China Agricultural University, Beijing 100094, China.
[3] National Engineering Research Center for Beijing Biochip Technology, Beijing 102206, China.
[4] University of Wisconsin-Madison, Madison, WI, 53706, USA.
Received April 22, 2006; Accepted November 1, 2006

3,426 mostly non-redundant genes were chosen for production of a cDNA microarray. Sequence data for most of ESTs used for construction of the arrays are available in GenBank. The ESTs representing distinct genes were identified by sequence similarity searches against human UniGene sequences (Oct. 15, 2004 release) using BLASTN. An E value of e^{-5} was used as a threshold for identification of putative orthologs with 95% identity (Band et al., 2000). A total of 2,790/3,426 (81.4%) of the ESTs had BLAST hits with an E value $<e^{-5}$. The ESTs with no significant similarity (E value $>e^{-5}$) to UniGene sequences were clustered using PHRAP (Ewing et al., 1998) in order to avoid selecting duplicates from among those sequence clusters with no database match. Finally, all remaining singlets were added to this set in order to obtain the final listing of sequences to be rearrayed from the source plates. Final annotations (after printing and prior to analysis) were made by sequence similarity searches, subtracting ESTs with hits after each search. In order these were: BLASTN against non-redundant GenBank (Oct. 12 2004 release), BLASTN against dbEST (E value $<1e^{-10}$). Finally, we found that 492 ESTs (14.4%) had no annotations and 289 ESTs (8.4%) had no significant similarity (E value $>e^{-5}$) to target sequences in any of the databases. Many of these sequences are likely to represent highly divergent and novel genes that may play a significant role in specific adaptations or phenotypes. Microarray data in this research have been deposited with the NCBI Gene Expression Omnibus data repository (http://www.ncbi.nlm.nih.gov/geo/) under accession numbers GPL2628 or GSM64185.

Design and creation of cDNA microarrays

Creation of the arrays was essentially as described by Brown and Botstein (Brown and Botstein, 1999). Plasmid inserts were amplified by PCR using T3 (5'-GTA AAACGACGGCCAGT-3') and T7 (5'-CAGGAAACAG CTATGAC-3') flanking vector primers in 50 μl volume reactions. Amplifications were performed in a thermal cycler (PE Amp9700, Perkin-Elmer, USA). PCR reaction mixes contained 5 μl 10× Taq polymerase buffer solution, 50 pmol of each primer, 4 μl 10 mM dNTPs, and 1 U Taq polymerase. An initial 5 min denaturation at 95°C was followed by 35 cycles of 1 min denaturation at 95°C, 1 min annealing at 55°C and 1 min elongation at 72°C. The reaction ended with an additional incubation of 5 min at 72°C. PCR products were analyzed on 1.0% agarose gels. Samples containing multiple bands or weak products were reamplified. PCR products were precipitated with isopropanol and redissolved in 50% dimethyl sulphoxide (DMSO). A commercial OmniGrid™ microarrayer (Genomic Instrumentation Services, Inc, San Carlos, CA) was employed to print all spots on Telechem Superamine slides (Arrayit, Sunnyvale, CA) and to verify reproducibility each spot was arrayed three replicates. Control samples included the expressed pig genes encoding glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), ribosomal protein L24 (*RPL24*), eukaryotic translation elongation factor 1 alpha 1 (*EEF1A1*), glutathione peroxidase 1 (*GPX1*), and peptidylprolyl isomerase A (cyclophilin A) (*PPIA*). Additional, control samples including three different Arabidopsis thaliana DNA fragments (GenBank accession numbers AC004146, AC007661 and U09332) and 12 spots of DMSO were also arrayed. The centre-to-centre distance between two adjacent spots was 225 μm. After printing, the slides were baked for 1 h at 80°C and stored dry at room temperature until use.

Animal sampling and RNA preparation

Four hogs, two XPs and two LWs and four sows, two XPs and two LWs, were housed in breed groups in Nutrition and Metabolism Laboratory at the China Agriculture University (CAU). They were fed the same diets for the fattening period of one month. At the time of biopsy, the average age of the animals was 145 days for XP and 149 days for LW. The mean live weights were 45±7 kg for XP and 90±13 kg for LW, because Chinese XP is small pig, the body weight of XP for 145 days corresponds to that of LW for 149 days. The mean backfat depths were 2.618± 0.393 cm and 1.964±0.270 cm for XP and LW, respectively. About 1 g biopsies were taken from backfat adipose tissues of XP and LW. Adipose tissue samples were washed in sterile water, snap frozen in liquid nitrogen and stored at -80°C. Then total RNA was extracted with TRIZOL reagent (*Invitrogen*, Gaithersburg, MD) and further purified with an RNeasy mini kit (Qiagen, Valencia, CA) according to the manufacture's instructions. The RNA quality was assessed by formaldehyde agarose gel electrophoresis and was quantitated spectrophotometrically.

RNA labeling and hybridization

Fluorescent dye (cy5 and cy3-dCTP) labeled DNA was produced through Eberwine's linear RNA amplification method and subsequent enzymatic reaction. In detail, 5 μg of total RNA was pooled from four individual animals within the same breed and then double-stranded cDNA containing the T7 RNA polymerase promoter sequence (5'-AAACG ACGGC CAGTG AATTG T AATA CGACT CACTA TAGGC GC-3') was synthesized using a TaKaRa cDNA Synthesis System Kit according to manufacturer's recommended protocol (TaKaRa, Dalian, China). A T7-OligodT primer (5'- AAACG ACGGC CAGTG AATTG TAATA CGACT CACTA TAGGC GC TT TTT TTT TTT TTT TTTV -3') was used in place of the poly T primer provided in the kit.

After completion of ds cDNA synthesis, the product was purified with a PCR Purification Kit (Qiagen), and the final

Table 1. Primer sequences used in RT-PCR validation

Transcript	Forward primer	Reverse primer
aP2	ACATGAAAGAAGTGGGAGTG	CCACCACCAACTTATCATCT
SCD	AAGGAACTAGAAGGCTGCTC	TGTAGAGCAGCAGCCATCAC
SREBF1	CAAGGCCATCGACTACATTC	TCCTCCACCTCAGTCTTCAC
LPL	GAAGACACAGTTGAGGACAC	GGACATTGTTCGGAGGATAG
RPL24	AGGTCGAGCTGTGCAGTTTC	CAAGGGATGCACCAGTTATG

cDNA was eluted in 60 μl Elution Buffer. Half of the eluted cDNA product was vaccumed to 8 μl and subjected to an *in-vitro* transcription reaction in 20 μl of reaction volume using T7 RiboMAX Express Large Scale RNA Production System (Promega, Madison, WI). The reaction proceeded for 3 h at 37°C and the amplified RNA (aRNA) was purified with RNeasy Mini kit (Qiagen). Since DNA can be manipulated in a more straightforward manner than RNA, some researchers choose to label DNA in subsequent reverse transcription reaction instead of labeling RNA directly *in vitro* transcription (Gomes et al., 2003). We adopted a cDNA labeling approach with a Klenow enzyme after reverse transcription. The labeling strategy using the Klenow enzyme has been introduced into probe labeling in microarray technology by other researchers (Smith et al., 2003). We found the Klenow enzyme possesses high labeling efficiency and the labeled DNAs have shorter lengths (100-400 bp), facilitating the hybridization procedure. Briefly, 1 μg of aRNA was mixed with 2 μg of random hexamer, denatured at 70°C for 5 min and cooled on ice. Then 4 μl of first strand buffer, 2 μl of 0.1 M DTT, 1 μl 10 mM dNTP, and 1.5 μl SuperScript II (*Invitrogen*) were added. Tubes were incubated at 25°C for 10 min then at 42°C for 60 min. The products were purified using a PCR purification kit (Qiagen) and vaccumed to 10 μl. cDNA was mixed with 2 μg random nonamer, heated to 95°C for 3 min and snap cooled on ice. 10×buffer, dNTP and Cy5-dCTP or Cy3-dCTP (Amersham Pharmacia Biotech, Piscataway, NJ) were added at final concentration of 120 μM for dATP, dGTP, dTTP, and 60 μM dCTP and 40 μM Cy-dye respectively. Klenow enzyme (1 μl, Takara) was added and reaction was performed at 37°C for 60 min. The labeled DNA was purified with a PCR purification kit (Qiagen), resuspended in elution buffer and the O.D. was measured.

Prior to hybridization, the slides were rehydrated over 65°C water for 10 seconds, snap dried on a 100°C heating block for 5 seconds and UV cross-linked at 250 mJ/cm^2. The unattached cDNA fragments were washed off with 0.5% SDS for 15 min at RT and SDS was removed by dipping the slides in anhydrous ethanol for 30 seconds. The slides were spin-dried at 1,000 rpm for 2 min. Then labeled control and test samples were quantitatively adjusted based on the efficiency of Cy-dye incorporation and mixed into 30 μl hybridization solution (3×SSC, 0.2% SDS, 25% formamide and 5×Denhart's). DNA in hybridization solution was denatured at 95°C for 3 min prior to loading on a microarray. The array was hybridized at 42°C overnight and washed with two consecutive washing solutions (0.2% SDS, 2×SSC at 42°C for 5 min, and 0.2% SSC for 5 min at room temperature). Two microarray slides were used for the breed comparison by employing the dye-reverse labeling strategy. Slide one was hybridized with Cy3 (G)-labeled XP and Cy5 (R)-labeled LW from the aRNA preparation. Slide two was the reciprocal dye flip using the same aRNA preparation.

Imaging and data analysis

Arrays were scanned with a ScanArray Express scanner (Parckard Bioscience, Kanata, OT), and obtained images were analyzed with GenePix Pro 4.0 (Axon Instruments, Foster City, CA). A space and intensity-dependent normalization based on a LOWESS program (Yang et al., 2002) was employed here. For each pair of samples, each gene was represented triplicate on each slide, and the experiments were performed in duplicate by dye swap, producing 6 data points, so that we determined the differently expressed genes based on the statistical program student *t-test*. Genes whose ratio lay outside the 95% confidence interval were determined to be significantly differently expressed genes.

Quantitative real-time PCR

In order to confirm transcriptional differences observed on the microarray, SYBR Green quantitative RT-PCR assays were developed for measuring differential expression for four candidate genes. The four genes for microarray data validation are *aP2*, *SCD*, *SREBF1* and *LPL*. The housekeeping gene tested is ribosomal protein large 24 (*RPL24*). Primers sequences were as follows (Table 1).

One microgram of total RNA from each individual animal was used to synthesize single-stranded cDNA with 500 ng oligo-dT primer, denatured at 70°C for 10 min and cooled on ice. Then 4 μl of 5× first strand buffer, 2 μl of 0.1 M DTT, 1 μl 10 mM dNTP, and 1 μl Superscript II (*Invitrogen*) were added to 20 μl total reaction volume. Tubes were incubated at 25°C for 10 min then at 42°C for 60 min. Quantitative Real-Time PCR was carried out on an ABI 5,700 PRISM® Sequence Detection System (SDS) machine. For each individual reaction, a total 20 μl volume contained 2 μl 10× SYBR green buffer; 1 μl cDNA; 0.5 μl

Table 2. Genes showing 2-fold or greater expression between fatty Xiang pig and Muscly Large White (3,426 gene array)[1]

Gene classification	Gene	Inducation (↑) or repression (↓) fold	Gene description
Lipid metabolism (20)			
NM_018677	*ACAS2*	2.1↓	acetyl-Coenzyme A synthetase 2
M27606	*CNPase*	2.0↓	2',3'-cyclic-nucleotide 3'-phosphohydrolase
S74803	*CII-3*	2.0↑	CII-3=succinate-ubiquinone oxidoreductase complex II
L06665	*complex III*	2.0↑	mitochondrial ubiquinol-cytochrome *c* reductase
NM_016987	*Acly*	2.1↑	ATP citrate lyase
AF102872	*aP2*	2.1↑	adipocyte fatty acid binding protein
NM_016160	*HSD-2*	2.2↑	amyloid precursor protein homolog HSD-2
Z46376	*HK2*	2.3↑	hexokinase II
AF061742	*retSDR1*	2.3↑	retinal short-chain dehydrogenase/reductase
L77567	*CTP*	2.5↑	mitochondrial citrate transport protein
X63213	*CI-B12*	5.6↑	ubiquinone oxidoreductase complex
Z97186	*SCD*	2.0↑	stearyl-CoA desaturase
NM_004176	*SREBF1*	2.4↑	sterol regulatory element binding transcription factor 1
X62984	*LPL*	2.2↑	lipoprotein lipase
AJ005458	*PP2Cβ*	2.2↑	protein phosphatase 2C beta
Y16039	*A-FABP*	2.6↑	fatty acid-binding protein
BC001305	*ELOVL6*	2.1↑	ELOVL6,elongation of long chain fatty acids
C95061	*FAS*	3.3↑	fatty acid synthase
NM_021814	*ELOVL5*	5.2↑	ELOVL5,elongation of long chain fatty acids
D10040	*LACS*	2.1↑	mRNA for long-chain acyl-CoA synthetase
Cell and organism defence (3)			
X82321	*TSA*	2.1↑	thiol-specific antioxidant proteins
J00658	*α-globin*	3.3↓	rabbit alpha-globin
S69189	*AOX*	2.3↑	peroxisomal acyl-coenzyme A oxidase
Protein synthesis (3)			
L10124	*SP-A*	2.1↑	surfactant protein A
NM_000972	*RPL7A*	2.0↓	ribosomal protein L7a
Z29555	*RPL3*	2.0↓	ribosomal protein L3
Cell signal and transduction (9)			
AB019792	*Endozepine*	2.0↑	endozepine gene
U14588	*Paxillin*	2.0↑	a focal adhesion protein
X56933	*APAS*	2.0↑	alternative polyadenylation signals
AF169286	*PTPLB*	2.5↑	protein tyrosine phosphatase-like protein
NM_000163	*GHR*	2.1↑	growth hormone receptor
Z54280	*RYR1*	2.0↑	The skeletal muscle ryanodine receptor
NM_014748	*SNX17*	4.0↑	sorting nexin 17 (SNX17), mRNA
BC029439	*ZNF258*	2.2↓	zinc finger protein 258, mRNA
NM_016429	*COPZ2*	2.0↑	coatomer protein complex, subunit zeta 2

[1] Sixteen ESTs with no annotation and five ESTs that represent novel genes were induced or repressed by two-fold or greater in adipose tissue of fatty Xiang pig as compared to that of muscly Large White.

25 pmol/μl each primer; 2.4 μl (25 mM) $MgCl_2$ Solution; 1.6 μl dNTP Blend (2.5 mM each dATP, dCTP, dGTP, 5.0 mM dUTP); 0.2 μl (1 u/μl) Amp Erase® UNG; 0.1 μl (5 u/μl) Ampli Taq Gold™ (Applied Biosystems, Foster City, CA, USA) and 10.7 μl ddH_2O. The PCR protocol consisted of denaturation at 50°C for 2 min, then 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min and 72°C for 30 s.

All reactions were carried out in triplicate (XP1, XP2, XP3 and LW1, LW2, LW3) and threshold cycle (Ct) scores were averaged for subsequent calculations of relative expression values. Standard curves for target and reference genes were constructed using 10-fold serial dilutions of adipose tissue cDNA. Relative amounts of four transcripts were calculated by comparison with the standard curves. Data were normalized to the swine housekeeping gene *RPL24* standard. Relative expression for adipose tissue of fatty XP compared to that of muscly LW was determined by the following calculation, as described in the Applied Biosytems users bulletin on Relative Quantitation of Gene Expression (Schmittgen et al., 2000):

Table 3. Relative expression of the four genes by Q-PCR and the microarray results of them

Gene	Relative expression				MR[2]
	XP1/LW1*	XP2/LW2*	XP3/LW3*	XPA/LWA[1]	
aP2	2.30	2.20	3.30	2.60	2.10
SCD	2.00	2.00	2.00	2.00	2.00
SREBF1	3.21	9.42	5.37	6.00	2.40
LPL	2.10	3.74	2.00	2.63	2.20

* The mRNA expression in adipose tissue of three XP (1, 2, 3) and three LW (1, 2, 3) individuals.
[1] The average of mRNA relative expression between XP and LW. [2] The microarray results.

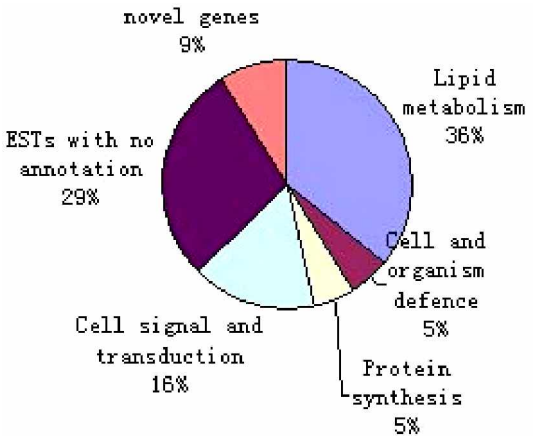

Figure 1. Distribution of genes differently expressed by comparing fatty Xiang pig with Large White. Genes related to lipid metabolism represented the highest proportion, which took up 36% of all genes differently expressed.

$$\text{Rel Exp} = 2^{-\Delta Ct}$$

Where ΔCt = (Ct Target-Ct RPL24)$_{XP}$-(Ct Target-Ct RPL24)$_{LW}$

RESULTS AND DISCUSSION

Gene expression monitoring for adipose tissue

Because cDNA microarray technique is mainly used to detect the differential gene expression between treated and control sample or between two different breeds or between different developmental period and so on, a 3,426 gene prototype microarray was used to explore transcriptional differences between adipose tissues of fatty XP and muscly LW at the gene expression level. Fifty-six different genes of the total 3,426 genes on the array were differentially expressed two-fold or greater in XP or LW. Among these genes differentially expressed (Table 2), twenty are involved in lipid metabolism function; three are genes associated with cell organism and defence; three are relative with protein synthesis; nine are monitored to be relative with cell signal transduction. Other genes induced or repressed by more than two-fold are ESTs without annotations and novel genes.

SYBR Green quantitative RT-PCR verification of array result

In order to validate the data from microarray experiment study and extend the analysis to individual animal samples. SYBR Green RT-PCR was used to confirm the four genes; *aP2*, *SCD*, *SREBF1* and *LPL*, selected from microarray experiment results. The mRNA expression levels of candidate genes were then normalized to the *RPL24* mRNA expression levels in the same sample. Direct comparison of SYBR Green RT-PCR results and cDNA microarray results from the same RNA samples is listed in Table 3. The results of Table 3 were obtained by using equation mentioned above. From Table 3 it is showed that although the mRNA expression levels for the four candidate genes indicated some variations between individuals within the same breed, these data from quantitative RT-PCR in the four genes are highly consistent with those from cDNA microarray analysis. However, additional experiments are required to determine accurately the correlation between transcriptional differences detected using these two methods.

Effects on genes related to lipid metabolism

In the current study, it was noted that genes involved in lipid metabolism represented the highest proportion of expression difference genes identified (Figure 1), which indicated that phenotype difference of the two breeds agreed with gene level difference of them. If these conclusions about the cellular content of the adipose biopsies are correct, then it is reasonable to speculate around the biochemical implications of differential gene expression. Two genes *SCD* and *SREBF1*, which the result validated by quantitative RT-PCR was in accord with that by microarray analysis, were highly expressed in fatty XP. *SCD* was involved in the biosynthesis of oleate (18:1) and palmitoleate (16:1) and is particularly sensitive to *SREBP-1c* regulation in humans and rats (Shimomura et al., 1998; Tabor et al., 1998, 1999; Ntambi, 1999). *SCD* is a microsomal fatty acid modifying enzyme that catalyzes the introduction of the *cis* double bond between carbons 9 and 10 of saturated fatty acyl-CoA substrates, resulting in the production of monounsaturated fatty acids (Enoch et al., 1976) and is the enzyme responsible for conversion of

saturated fatty acids into monounsaturated fatty acids (MUFA) in mammalian adipocytes. Monounsaturated fatty acids have also been implicated as mediators of signal transduction and cellular differentiation (Zhang et al., 1999). *SCD* may also fulfill a larger regulatory role in carcass composition, as suggested by recent knockdown studies in *ob/ob* mice (Cohen et al., 2002; Ntambi et al., 2002). The higher expression of *SCD* in XP relative to backfat biopsies indicates that it can be a candidate gene for genetic variation in fatty acid composition.

SREBF1, also known as sterol-regulatory element binding protein 1 (*SREBP1*) (Miserez et al., 1997), is a transcription factor with important roles in adipogenesis, insulin sensitivity, and fatty acid homeostasis (Spiegelman, 1998) and involved in cholesterol and fatty acid metabolism in mammalian cells (Brown and Botstein, 1999; Osborne, 2000; Kim et al., 2002). *SREBP1* appears to mediate part of the transcriptional effects of insulin (Kim et al., 1998, 1996; Foretz et al., 1999) and in the fatty acid biosynthetic pathway, increases the mRNAs encoding acetyl-CoA carboxylase, fatty acid synthetase, and stearoyl-CoA desaturase. The discovery that insulin stimulates the transcription of the *SREBP1* gene readily explains how insulin exerts a global stimulatory effect on lipogenesis (Shimomura et al., 1999). In addition, cholesterol rich-diets induce *SREBP1* gene expression and maturation, as has been suggested by researchers (Shimomura et al., 1998; Kim et al., 1999; Jing et al., 1999; Xu et al., 2001). Elevated expression of *SREBP1* gene in fatty XP is consistent with enhanced adipogenesis.

LPL plays a central role in lipoprotein metabolism. *LPL* hydrolyzes triglycerides (*TGs*) in chylomicrons and *VLDL*, thereby generating free fatty acids that enter either storage or oxidative pathways. *LPL* also contributes to the exchange of lipids and apoproteins between different lipoprotein classes, thus affecting size and composition not only of TG-rich lipoproteins but also of low and high density lipoproteins (*LDL* and *HDL*) (Eisenberg, 1984; Goldberg, 1996). In addition to its lipolytic function, it has a "bridging function" for receptor-mediated lipoprotein uptake (Chappell et al., 1993; Salinelli et al., 1996; Seo and St Clair, 1997). *LPL*, like *SCD*, is expressed late in adipogenesis, and so increased gene expression would be consistent with more adipocytes, though the hypothesis of more active adipocytes cannot be excluded by these data.

AP2 or FABP are intracellular transporters that deliver fatty acids either to the sites of fat storage or to the sites of energy production (Veerkamp and van Moerkerk, 1993; Glatz et al., 1993) as well as in differentiation (Rump et al., 1996). The observation of higher expression of this gene in the fatty XP samples can be also relative with increased synthetic activity of the cells within the adipose tissues.

ESTs with no annotation and novel genes

By sequence analysis and ESTScan, sixteen clones only have sequence information with no annotation and Genbank accession numbers are separately BE032946, BF190509, R18403, AW315232, BF189734, C94935, AU058662, C94896, AC003663, AL357374, AF147332, X02492, BE013075, BE237259, BF081062 and AW418059. Five clones (DR066115, DR066052, DR066403, DR066357 and DR065991) showed no appreciable similarity to any known genes and they represent novel genes. The sequence from the five clones can be found in http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Search&db=Nucleotide&term=. The XP is Chinese famous breed for its light body weight and taste as animal food. But it is fatty. Comparative studies of the two breeds are important to help identify the genes underlying pork eating quality trait, in addition, they may also have value in the context of animal traceback systems. Our results demonstrate the potential of microarrays for discovery of gene function and metabolism pathways between breeds of farm animals. Importantly, the microarrays provide a means to explore the function of the large number of porcine ESTs with no significant similarity to genome sequence of humans or other species (novel genes and divergent orthologs). Further application of this technology will lead to the understanding of molecular mechanisms involved in development, disease resistance, nutrition, fertility and production traits of pig.

ACKNOWLEDGMENT

We thank Dr. Jamie Wilkenson for the gift housekeeping gene primers (Department of Pathology, University of Cambridge, UK). This work is supported by the National Key Basic Research Development Program (G20000161).

REFERENCES

Adams, M. D., J. M. Kelley, J. D. Gocayne, M. Dubnick, M. H. Polymeropoulos, H. Xiao, C. R. Merril, A. Wu, B. Olde, R. F. Moreno, A. R. KerLavage, W. R. McCombie and J. C. Venter. 1991. Complementary DNA sequencing: Expressed sequence Tags and human genome project. Sci. 252:1651-1656.

Band, M. R., J. H. Larson, M. Rebiez, C. A. Green, D. W. Heyen, J. Donovan, R. Windish, C. Steining, P. Mahyuddin, J. E. Womack and H. A. Lewin. 2000. An ordered comparative map of the cattle and human genomes. Genome Res. 10:1359-1367.

Brown, P. O. and D. Botstein. 1999. Exploring the new world of the genome with DNA microarrays. Nature Genetics. 21:33-37.

Chappell, D. A., G. L. Fry, M. A. Waknitz, L. E. Muhonen, M. W. Pladet, P. H. Iverius and D. K. Strickland. 1993. Lipoprotein lipase induces catabolism of normal triglyceride-rich lipoproteins via the low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor *in vitro*. A process facilitated by cell-surface proteoglycans. J. Biol. Chem.

268:14168-14175.

Cohen, P., M. Miyazaki, N. D. Socci, A. Hagge-Greenberg, W. Liedtke, A. A. Soukas, R. Sharma, L. C. Hudgins, J. M. Ntambi and J. M. Friedman. 2002. Role for Stearoyl-CoA Desaturase-1 in Leptin-Mediated Weight Loss. Sci. 297:240-243.

Eisenberg, S. 1984. High density lipoprotein metabolism. J. Lipid Res. 25:1017-1058.

Enoch, H. G., A. Catala and P. Strittmatter. 1976. Mechanism of rat liver microsomal stearoyl-CoA desaturase: studies of the substrate specificity, enzyme-substrate interactions and the function of lipid. J. Biol. Chem. 251:5095-5103.

Ewing, B., L. Hillier, M. C. Wendl and P. Green. 1998. Base-Valling of automated sequencer traces using PHRED. I. accuracy assessment. Genome Res. 8:175-185.

Foretz, M., C. Guichard, P. Ferre and F. Foufelle. 1999. Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. Proc Natl Acad Sci USA. 96:12737-12742.

Glatz, J. F., M. M. York, D. P. Cistola and G. J. van der Vusse. 1993. Cytoplasmic fatty acid binding protein: significance for intracellular transport of fatty acids and putative role on signal transduction pathways. Prostaglandins Leukot Essent Fatty Acids. 48:33-41.

Goldberg, I. J. 1996. Lipoprotein lipase and lipolysis: central roles in lipoprotein metabolism and atherogenesis. J. Lipid Res. 37:693-707.

Gomes, L. I., R. I. Silva, B. S. Stolf, E. B. Cristo, R. Hirata, F. A. Soares, L. F. Reis, E. J. Neves and A. F. Carvalho. 2003. Comparative analysis of amplified and nonamplified RNA for hybridization in cDNA microarray. Anal Biochem. 321(2):244-251.

Jing, X., M. T. Nakamura, H. P. Cho and S. D. Clarke. 1999. Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acid. J. Biol. Chem. 274:23577-23583.

Kim, H. J., M. Miyazaki, W. C. Man and J. M. Ntambi. 2002. Sterol regulatory element-binding proteins (SREBPs) as regulators of lipid metabolism: polyunsaturated fatty acids oppose cholesterol-mediated induction of SREBP-1 maturation. Ann NY Acad Sci. 967:34-42.

Kim, H-J., M. Takahashi and O. Ezaki. 1999. Fish oil feeding decreases mature sterol regulatory element-binding protein 1 by down-regulation of SREBP-1c mRNA in mouse liver. A possible mechanism for down-regulation of lipogenic enzyme mRNAs. J. Biol. Chem. 274:25892-25898.

Kim, J. B. and B. M. Spiegelman. 1996. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. Genes Dev. 10:1096-1107.

Kim, J. B., H. M. Wright, M. Wright and B. M. Spiegelman. 1998. ADD1/SREBP1 activates PPAR gamma through the production of endogenous ligand. Proc. Natl. Acad. Sci. USA. 95:4333-4337.

Ly, D. H., D. J. Lockhart, R. A. Lerner and P. G. Schultz. 2000. Mitotic misregulation and human aging. Sci. 287:2486-2492.

Miserez, A. R., G. Q. Cao, L. C. Probst and H. H. Hobbs. 1997. Structure of the Human Gene Encoding Sterol Regulatory Element Binding Protein 2 (*SREBF2*). Genomics. 40(1):31-40.

Ntambi, J. M. 1999. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. J. Lipid Res. 40:1549-1558.

Ntambi, J. M., M. Miyazaki, J. P. Stoehr, H. Lan, C. M. Kendziorski, B. S. Yandell, Y. Song, P. Cohen, J. M. Friedman and A. D. Attie. 2002. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. Proc. Natl. Acad. Sci. USA. 99(17):11482-11486.

Osborne, T. F. 2000. Sterol regulatory element-binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action. J. Biol. Chem. 275:32379-32382.

Rump, R., C. Buhlmann, T. Borchers and F. Spener. 1996. Differentiation dependent expression of heart type fatty acid-binding protein in C2C12 muscle cells. Eur. J. Cell Biol. 69:135-142.

Salinelli, S., J. Y. Lo, M. P. Mims, E. Zsigmond, L. C. Smith and L. Chan. 1996. Structure Function Relationship of Lipoprotein Lipase-mediated Enhancement of Very Low Density Lipoprotein Binding and Catabolism by the Low Density Lipoprotein Receptor. J. Biol. Chem. 271:21906-21913.

Schena, M., D. Shalon, R. Heller, A. Chai, P. O. Brown and R. W. Davis. 1996. Parallel human genome analysis: Microarray-based expression monitoring of 1,000 genes. Proc. Natl. Acad. Sci. USA. 93:10614-10619.

Schmittgen, T. D., B. A. Zakrajsek, A. G. Mills, V. Gorn, M. J. Singer and M. W. Reed. 2000. Quantitative reverse transcription-polymerase chain reaction to study mRNA decay: comparison of endpoint and real-time methods. Anal Biochem. 285:194-204.

Seo, T. and R. W. St Clair. 1997. Heparan sulfate proteoglycans mediate internalization and degradation of beta-VLDL and promote cholesterol accumulation by pigeon macrophages. J. Lipid Res. 38:765-779.

Shalon, D., S. J. Smith and P. O. Brown. 1996. A DNA microarray system for analyzing complex DNA samples usiong two-color fluorescent probe hybridization. Genome Res. 6:639-645.

Shimomura, I.,Y. Bashmakov, S. Ikemoto, J. D. Horton, M. S. Brown and J. L. Goldstein. 1999. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. Proc. Natl. Acad. Sci. USA. 96:13656-13661.

Shimomura, I., H. Shimano, B. S. Korn, Y. Bashmakov and J. D. Horton. 1998. Nuclear sterol regulatory element-binding proteins activate genes responsible for the entire program of unsaturated fatty acid biosynthesis in transgenic mouse liver. J. Biol. Chem. 273:35299-35306.

Smith, L., P. Underhill, C. Pritchard, Z. Tymowska-Lalanne, S. Abdul-Hussein, H. Hilton, L. Winchester, D. Williams, T. Freeman, S. Webb and A. Greenfield. 2003. Single primer amplification (SPA) of cDNA for microarray expression analysis. Nucleic Acids Res. 31(3):e9.

Spiegelman, B. M. 1998. PPAR-γ: adipogenic regulator and thiazolidinedione receptor. Diabetes. 47:507-514.

Tabor, D. E., J. B. Kim, B. M. Spiegelman and P. A. Edwards. 1998. Transcriptional activation of the stearoyl-CoA desaturase 2 gene by sterol regulatory element-binding protein/adipocyte determination and differentiation factor 1. J. Biol. Chem. 273(34):22052-22058.

Tabor, D. E., J. B. Kim, B. M. Spiegelman and P. A. Edwards. 1999. Identification of conserved cis-elements and transcription factors required for sterol-regulated transcription of stearoyl-CoA desaturase 1 and 2. J. Biol. Chem. 274(29):20603-20610.

Veerkamp, J. H. and H. T. van Moerkerk. 1993. Fatty acidbinding protein and its relation to fatty acid oxidation. Mol Cell Biochem. 123:101-106.

White, K. P., S. A. Rifkin, P. Hurban and D. S. Hogness. 1999. Microarray analysis of Drosophila development during metamorphosis. Sci. 286:2179-2184.

Xu, J. M., J. H. Teran-Garcia, Y. Park, M. T. Nakamura and S. D. Clarke. 2001. Polyunsaturated fatty acids suppress hepatic sterol regulatory element-binding protein expression by accelerating transcript decay. J. Biol. Chem. 276:9800-9807.

Yang, Y. H., S. Dudoit, P. Luu, D. M. Lin, V. Peng, J. Ngai and T. P. Speed. 2002. Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. Nucleic Acids Res. 30(4):e15.

Zhang, L., L. Ge, S. Parimoo, K. Stenn and S. M. Prouty. 1999. Human stearoyl-CoA desaturase: alternative transcripts generated from a single gene by usage of tandem polyadenylation sites. Biochem. J. 340:255-264.