Partial Genome Scale Analysis of Gene Expression in Human Adipose Tissue Using DNA Array

Britt L. Gabrielsson, Björn Carlsson, and Lena M. S. Carlsson

Abstract

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Objective: Large scale analysis of gene expression in adipose tissue provides a basis for the identification of novel candidate genes involved in the pathophysiology of obesity. Our goal was to explore gene expression in human adipose tissue at a partial genome scale using DNA array.

Research Methods and Procedures: Labeled cDNA, derived from human adipose tissue poly(A^+) RNA, was hybridized to a DNA array containing over 18,000 human expressed sequence-tagged (EST) clones. The results were analyzed by database searches.

Results: Homology searches of the 300 EST clones with highest hybridization signals revealed that 145 contained DNA sequences identical to known genes and 79 could be linked to UniGene clusters. Of the 145 identified genes, 136 were nonredundant and subsequently characterized with respect to function and chromosomal localization by searching MEDLINE, UniGene, GeneMap, OMIM, SWISS-PROT, the Genome Database, and the Location Data Base. The identified genes were grouped according to their putative functions; cell/organism defense (9.6%), cell division (5.1%), cell signaling/communication (19.8%), cell structure/motility (12.5%), gene/protein expression (16.9%), metabolism (16.2%), and unclassified (19.8%). Less than 50% of these genes have previously been reported to be expressed in adipose tissue. The chromosomal localization of 268 genes strongly expressed in adipose tissue showed that their relative abundance was significantly increased on chromosomes 11, 19, and 22 compared to the expected distribution of the same number of random genes.

Discussion: Our study resulted in the identification of numerous genes previously not reported to be expressed in adipose tissue. These results suggest that DNA array is a powerful tool in the search for novel regulatory pathways within adipose tissue on a scale that is not possible using conventional methods.

Key words: cDNA, expressed sequence tags, metabolism, putative functions, DNA array

Introduction

Obesity increases rapidly in many societies and plays an important role in the development of chronic health problems, in particular type 2 diabetes, stroke, and heart disease (1). Although dieting and increased physical activity result in reduced weight, it is usually difficult to maintain long-term weight losses (2,3). In the field of obesity research, large efforts are therefore focused on the identification of regulatory systems and potential targets for drug development. Such targets may be found among the genes expressed in adipose tissue. It should also be remembered that, in addition to its function as an energy depot, the adipose tissue plays a dual role in cell-to-cell communication. It is now well established that the adipose tissue is both the target organ for regulatory signals from other parts of the body and at the same time it functions as an active endocrine tissue, which sends out signals to influence fuel metabolism and to modulate physiological processes (4), e.g., sexual maturation, reproduction, and growth. Consequently, expression profiling of adipose tissue may result in the identification of potential drug targets for several clinically important conditions.

The research approach used to identify candidate genes for obesity as well as for other diseases is likely to change

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Research Centre for Endocrinology & Metabolism (RCEM), Department of Internal Medicine, Sahlgrenska University Hospital, Göteborg University, S-41345 Göteborg, Sweden. Address correspondence to Dr. Lena Carlsson, RCEM, Department of Internal Medicine, Gröna Stråket 8, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden. E-mail: lena.carlsson@medic.gu.se

dramatically with the use of DNA arrays (5). A DNA array is a glass slide or a filter on which thousands of probes, consisting of gene-specific oligonucleotides, are attached. The immobilized DNA probes are hybridized to target DNA or RNA in samples, in a manner similar to Southern or Northern blots. High density DNA arrays can be used for many applications, including analysis of gene expression, DNA sequencing, and genotyping. For expression analysis, the arrayed probes can be simultaneously hybridized with radioactively or fluorescently labeled cDNAs, representing the mRNA pool in tissues or cells. Thus, this technique allows the exploration of global gene expression in a specific tissue in a single experiment. For example, using DNA array analysis it is possible to determine which genes are expressed in adipose tissue and, based on their regulation and putative functions, select interesting genes for further studies. As demonstrated in the present study, this will include growth factors, receptors, and transcription factors, many of which have not previously been shown to be expressed in adipose tissue.

The potential of DNA array technology is currently discussed extensively in the scientific literature (5–9), and so far, the number of review articles on this subject greatly exceeds the number of scientific reports. This study is, to our knowledge, the first to address the question of gene expression in human adipose tissue from a genome perspective using DNA array analysis.

Methods and Procedures

Poly(A^+) RNA Isolation

The study was approved by the Ethics Committee of the Medical Faculty, University of Göteborg. Abdominal subcutaneous adipose tissue was obtained from four nonobese subjects (three females, one male; ages 59 to 82 years) undergoing elective abdominal surgery. The tissue samples were rapidly frozen in liquid nitrogen and stored at −70°C until analysis. Total RNA was extracted according to the method of Chomczynski and Sacchi (10) with minor modification (11). Poly(A^+) RNA was isolated from a pool of total RNA from the four subjects using FastTrack 2.0 (Invitrogen, San Diego, CA) following the manufacturer's protocol.

Labeling of cDNA and DNA Orientation Marker

Poly(A^+) RNA (2.5 μg) and 100 pmol of T_{18}(G/A/C)N-primer were denatured at 70°C for 10 minutes and chilled on ice. The following reagents were added in order; Moloney murine leukemia virus-reverse transcription buffer (final concentration 1× buffer); dATP, dGTP, and dTTP (final concentrations, 350 μM); 50 μCi of [^{33}P]dCTP (3000 Ci/mmol; Life Science Products Inc., Boston, MA); and 240 units of Moloney murine leukemia virus-reverse transcription (Promega, Madison, WI), and incubated at 42°C for 1 hour. Unincorporated nucleotides were removed with a G-50 spin column (Amersham Pharmacia Biotech, Bucks, UK). The RNA/DNA hybrids were denatured at 100°C for 2 minutes, and the RNA was hydrolyzed in 0.2 M NaOH. Degraded RNA was removed with G-50 spin columns after neutralization with HCl and Tris-HCl, pH 7.5. Approximately 22% of the [^{33}P]dCTP was incorporated into cDNA.

DNA orientation marker (25 ng; Genome Systems Inc., St Louis, MO) was labeled with [^{33}P]dCTP, using a random oligo-labeling kit (Amersham Pharmacia Biotech).

Hybridization to the Array

A Human Gene Discovery Array (GDA) 1.2 filter (Genome Systems Inc.) was prewashed in two changes of 2× standard sodium citrate (SSC)/0.1% sodium dodecyl sulfate (SDS). The membrane was hybridized according to the manufacturer's instructions. Briefly, prehybridization was performed in 10 ml of hybridization buffer (0.75 M NaCl; 0.1 M Na_2HPO_4/NaH_2PO_4; 0.1% (w/v) $Na_2P_2O_7$; 0.15 M Tris-HCl, pH 7.5; 5× Denhardt's reagent; 2% SDS; 50% formamide; 100 μg of denatured salmon sperm DNA/mL) for 4 hours at 42°C. Hybridization was performed overnight at 42°C in the presence of heat-denatured labeled cDNA (10^6 cpm/mL) and labeled DNA orientation marker (2.5 x 10^5 cpm/mL). The membrane was washed at room temperature with 2× SSC/1% SDS for 15 minutes, followed by two washes with 2× SSC/1% SDS at 68°C for 10 minutes, and two washes with 0.6× SSC/1% SDS at 68°C. The membrane was exposed on a PhosphorImager screen (Molecular Dynamics Inc., Sunny Vale, CA) for 48 hours.

Detection, Quantification, and Identification of Positive Colonies

The Human GDA 1.2 filter (Genome Systems Inc.) is a 22- × 22-cm nylon membrane spotted with duplicates of 18,376 human EST clone-containing bacterial colonies. There are approximately 1×10^8 copies of plasmid per spot on the membrane. The EST clones are chosen from the Integrated Molecular Analysis of Genomes and their Expression (IMAGE) clone collection (12). After hybridization, the signal intensities on the image of the membrane were measured in predefined areas corresponding to the positions of EST clones, using the GDS software (Genome Systems Inc.). EST clones were identified by position on the membrane and pattern of the double spots. The sum of the two hybridization signals for each clone was used for ranking of the EST clones.

EST clone sequences were matched to sequences of characterized genes either via links to the UniGene databank (www.ncbi.nlm.nih.gov/UniGene/index.html) provided by Genome Systems (www.genomesystems.com) or by database homology searches (www.ncbi.nlm.nih.gov/BLAST). Sequences, not identified via UniGene links and that showed more than 98% identity with the EST clone se-

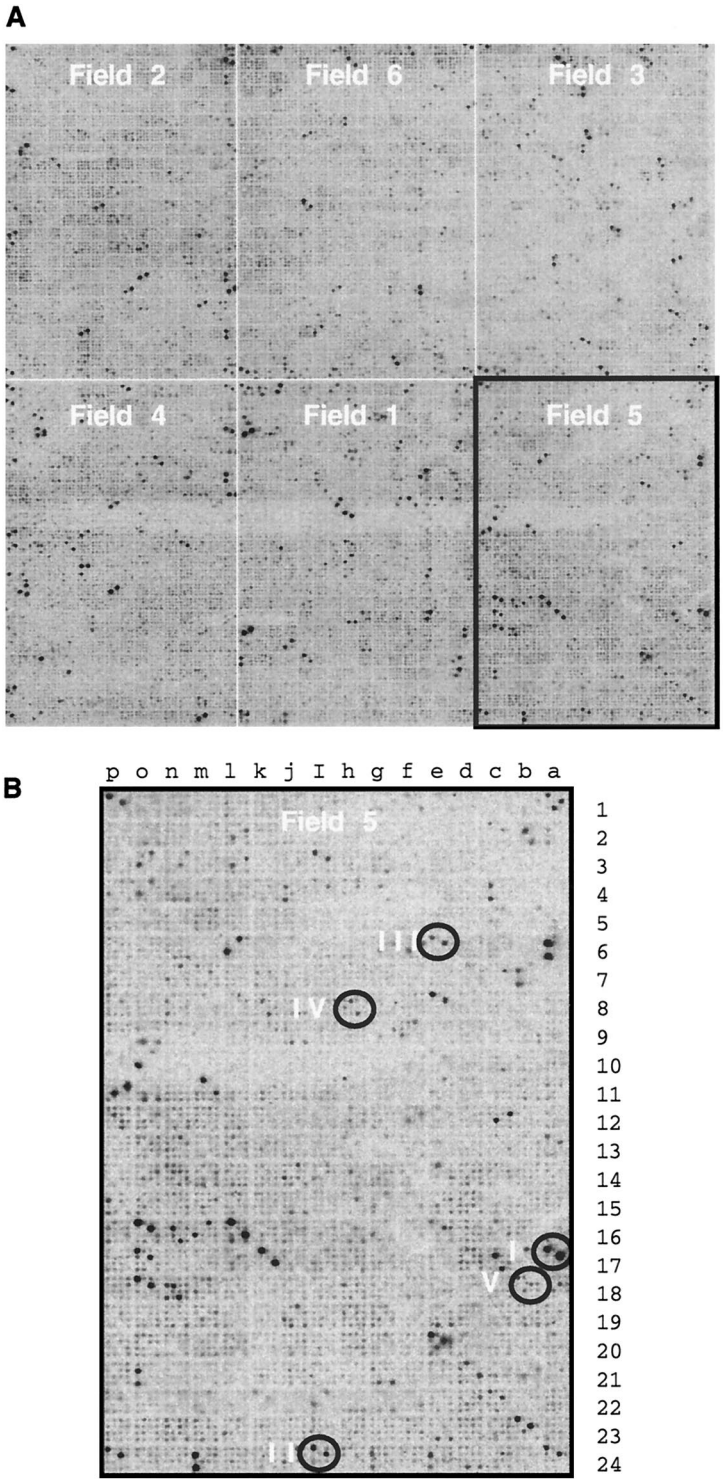

Figure 1. Analysis of gene expression in human adipose tissue by DNA array. ^{33}P-labeled cDNA generated by reverse transcription of mRNA extracted from human adipose tissue was hybridized on a Human GDA 1.2 membrane containing 18,376 human cDNA clones in duplicates. (A) The image of the whole membrane is shown with the six field sections marked. EST clones were identified by a coordinate system in each field as shown in B. Each coordinate corresponds to eight double spotted EST clones in a 4 x 4 grid. The positions in the coordinate system and the pattern of signals in the grid were used for identification of the EST clones. The signal marked I in B was identified through a BLAST search as sulfonylurea receptor 2 and ranked #1 of all signals on the membrane. EST clones marked II, III, and IV were identified as zinc finger protein 35 (rank number #58), aquaporin-chip (#171), carbonic anhydrase III (#249), and cell surface glycoprotein MCAM (#300) using UniGene links.

quence over a minimum of 100 nucleotides, were considered to be identical (13). Chromosomal localization of identified genes was determined using the UniGene database, OMIM database (www.ncbi.nlm.nih.gov/omim), GeneMap'98 (www.ncbi.nlm.nih.gov/genemap98), and the Genome Database (GDB; www.gdb.org). The physical distance between two radiation hybrid markers was calculated based on information obtained from the Location Data Base (LDB; http://cedar.genetics.soton.ac.uk/pub) (14). The putative function of products of identified genes was determined by searching the SWISS-PROT database (expasy.hcuge.ch/sprot) or MEDLINE (www.ncbi.nlm.nih.gov/PubMed) and using the classification into functional groups according to the Human Gene Anatomy project (13).

Analysis of Gene Expression in Adipose Tissue by DNA Array

The DNA array membrane containing 18,376 human EST clones (Human GDA 1.2 filter) was hybridized with ^{33}P-labeled human adipose tissue cDNA, and the signals were visualized using the PhosphorImager (Figure 1). The EST clones were identified by a coordinate system, where each coordinate corresponded to eight double spotted EST clones in a 4 x 4 grid. The position in the coordinate system and the pattern of signals in the grid were used for identification of the EST clones. Signals corresponding to genes coding for the sulfonylurea receptor 2, zinc finger protein 35, aquaporin-chip, carbonic anhydrase III, and cell surface glycoprotein melanoma adhesion molecule (MCAM) are marked as examples of identified genes (Figure 1B).

Each EST clone is represented by two spots on the membrane. The variation of the hybridization signal between the two spots containing the same EST clone is shown in Figure 2. For the 5000 highest-ranking signals, spot-to-spot variation for 95% of the EST clones was within 2 SD of the mean. Thirty-four of the 5000 EST clones showed more than 2-fold variability in signal intensity between the two spots, and five double spots varied more than 5-fold in intensity. Of the genes reported in Table 1 only six had more than 2-fold spot-to-spot variation. These were: #74, collagen IV, alpha 2; #77, NF2; #103, chondromodulin I; #130, TAL1; #155, pregnancy-specific β1-glycoprotein; and #281, γ-actin.

The sum of the two signals for each double spotted EST clone ranged from 260,000 arbitrary units down to 6000 arbitrary units (Figure 3). The hybridization signals decreased rapidly, and the hybridization signal of the 10th-ranking EST clone (#10) was less than half of that of #1, and at #100 the signal was reduced to approximately 12% of the signal of #1. As the signals subsequently started to plateau, we set the limit for identification and further analysis at EST clone #300, where the signals were still clearly distinguishable from background noise by visual inspection of the image of the membrane (Figure 1).

Statistical Analysis

The observed chromosomal distribution of the genes expressed at high levels in adipose tissue was compared with the theoretical distribution of an equal number of genes based on the assumption that genes are evenly distributed throughout the genome. For each chromosome or subregion of a chromosome, the observed distribution of genes was tested for deviation from the expected distribution, by Chi-square test with one degree of freedom.

Results

The 300 EST clones with the highest signals (range, 17,800 to 260,000 arbitrary units) on the membrane after hybridization with the labeled cDNA were further analyzed. Identification of the corresponding genes, using the UniGene data bank or BLAST searches, showed that 145 of the 300 EST clones with highest signal intensity corresponded to known genes, and 136 of these were nonredundant. Of the remaining 155 EST clones, 79 could be linked to UniGene EST clusters and 7 to genomic clones.

In Table 1, 136 genes expressed at high levels in adipose tissue are listed with their individual ranks, EST clone accession number, match accession numbers (GenBank or SWISS-PROT), UniGene accession number, gene symbol, and chromosomal localization. Several genes expected to be expressed in adipose tissue were among the top 300, for

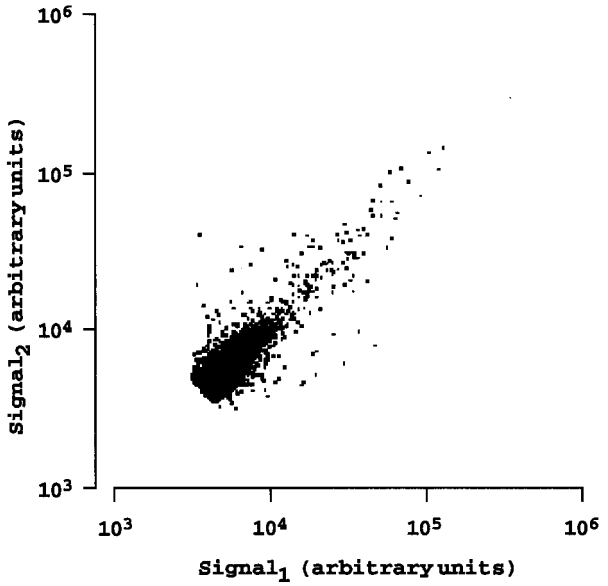

Figure 2. Spot-to-spot variation in hybridization signal for all double spotted EST clones on the DNA array membrane.

Table 1. List of 136 abundant mRNAs expressed in human adipose tissue as analyzed by DNA array

Rank order	Gene/protein	Previously reported in adipose tissue*
Cell/organism defense		
35	IgG lambda light chain	Yes
61	Parvalbumin-α	No
90	Ferritin light chain	Yes
107	Heat shock 70-kDa protein 6	No
124	MHC class I C α2	Yes
140	β2 microglobulin	Yes
152	MHC class I C α2	Yes
156	Complement C2	No
213	Lipopolysaccharide-binding protein	No
221	IgA Cα switch region	Yes
238	MHC class I C α2	Yes
239	SNC73 protein	Yes
247	MHC class I, C	Yes
249	Carbonic anhydrase III	Yes†‡
251	Metallothionein 2A	Yes
297	Protease inhibitor 1, α1 antitrypsin	No
Cell division		
38	Apoptosis inhibitor 5/Aac-11	No
56	Histone H1(0)	No
153	Cell cycle-regulated factor p78	No
160	Su(var)3-9 homolog	No
182	DEC1	Yes
210	Mdm2-E	Yes
Cell signaling/communication		
1	Sulfonylurea receptor 2	Yes†
6	DAB2/DOC-2	Yes
11	Phosphatidic acid phosphatase 2a/ KIAA0052	No
14	RXR-α	Yes
17	3-Phosphoinositide-dependent protein kinase 1	Yes†
25	Thyroptropin receptor	Yes
37	Stimulator of Fe transport	No
46	AE2 anion exchanger	No
66	$GABA_A$ receptor γ2	No
77	Neurofibromin 2	No
88	Proteolipid protein 1	No
91	RanBP2 α/NUP358	No
99	Cadherin 11	No
101	Multidrug-resistance-associated protein 6	No
103	Chondromodulin-I	No
108	FGF-9	No
109	KIAA0671/SOCS-5	Yes
110	Thymosin β4	Yes
121	IGF-BP6	Yes
132	Oligophrenin 1	Yes
145	Guanine nucleotide binding protein-like 1	No
157	Postmeiotic segregation increased 2-like 12	Yes
198	KIAA0472 protein kinase-like	No
202	RanBP3	No
250	PPAR-α	Yes
252	Low density lipoprotein receptor	Yes
291	Cell surface glycoprotein	Yes
298	Protein kinase C	Yes
300	Cell surface glycoprotein	Yes
Cell structure/motility		
29	Myosin light chain/titin	Yes
41	Myosin heavy chain-B	Yes§
51	Highly similar to lambda crystallin	No
74	Collagen IV, α2	Yes
105	Chondroitin/decorin	Yes
114	Homologous mouse Dynein intermediate chain 2	No
122	Crystallin, αB	Yes
142	Glypican-3	No
154	Semaphorin IV	No
168	Kinesin 2	No
176	Inter-α-trypsin inhibitor component II	No
185	Crystallin, αB	Yes
190	Homologous to mouse Mena	No
253	Fibronectin 1	Yes
255	Desmin	No
262	Dystrophin	No
281	Actin, γ	Yes
289	XPR2	Yes
Gene/protein expression		
8	Fragile X mental retardation 1	No
9	AUF-1/hnRNP D	No
13	Similar to mouse NEX-1/MATH-2	No

Table 1. Continued

Rank order	Gene/protein	Previously reported in adipose tissue*
39	Ribosomal protein S4, 40S Y isoform	No
57	Ribosomal protein fau	Yes
58	Zinc finger protein 35	No
75	Ribosomal protein P2	Yes
83	*S*-adenosylhomocysteine hydrolase-like 1	No
98	RNase1, RNase A family	Yes
115	AUF-1/hnRNP D	No
118	Ribosomal protein L31	Yes
130	SCL/Tal-1	No
133	FUS/TLS	No
159	Ribosomal protein P1	Yes
166	Heat shock factor protein I	No
175	Ribosomal protein L23A	Yes
183	Hemoglobin β	Yes
212	Hemoglobin β H	Yes
227	Protein translation factor SUI1 homolog/eIF-2A	Yes
229	AblBP3/Abi-2	No
236	LIM-protein FHL1	Yes
242	Ribosomal protein L11	Yes
246	Ribosomal protein P1	Yes
259	Ribosomal protein L32	Yes
263	Ribosomal protein L9	Yes
278	Zinc finger protein HF.12	No
Metabolism		
4	Aldehyde dedydrogenase	Yes
5	Na/K ATPase α3 chain	No
15	NADH-ubiquinone oxidoreductase, 75 kDa	No
23	Sialyltransferase SThM	No
28	Iduronate sulfate sulfatase	No
31	Glycogen phosphorylase	Yes†¶
55	Glutamine synthase	Yes
63	α-GlucosidaseGAA	No
71	Fatty acid binding protein 4/aP2	Yes
80	CD73 /5′nucleotidase	No
112	Sulfotransferase	No
149	NADH-cyt B5 reductase	No
164	Lipoprotein lipase	Yes
171	Aquaporin-chip	Yes

Table 1. Continued

Rank order	Gene/protein	Previously reported in adipose tissue*
172	Fatty acid binding protein 4/aP2	Yes
191	Ubiquinol-Cyt-C reductase complex core protein I	Yes
192	Arginase type II	No
194	GHighly similar to GAPDH	No
206	Annexin II	Yes
208	Glutathione *S*-transferase	Yes§
258	GAPDH	Yes
273	Alcohol dehydrogenase β chain	Yes
299	Annexin 1	Yes§
Unclassified		
7	KIAA0549	No
20	Pregnancy-specific β1 glycoprotein 7	No
32	Weakly similar to ORF YOR271c [*S. cerevisiae*]	No
47	Weakly similar to K04G11.4 [*C. elegans*]	No
82	Weakly similar to F08G12.1 [*C. elegans*]	No
84	KIAA0286	No
95	Translationally controlled tumor protein	Yes
111	Similar to KIAA0191	No
126	H326	No
134	Similar to guanine nucleotide releasing factor	No
144	KIAA0607	No
147	KIAA0259	No
155	Pregnancy-specific β1 glycoprotein 11	No
178	Weakly similar to CMP-*N*-acetyl-neuraminate-β-1,4-galactoside α2,3-sialyltransferase	No
181	Unknown RNA 23565	No
186	Weakly similar to line-1 protein ORF2	No
207	Weakly similar to KIAA0005	No
230	Weakly similar to Mi-2 protein	No
237	Weakly similar to lactose permease	No
	Weakly similar to RNA-BP	Yes

Table 1. Continued

Rank order	Gene/protein	Previously reported in adipose tissue*
269	TPRD	No
279	Autotaxin-t gene	No
280	KIAA0064	No
283	Insulin-induced protein-1	Yes†
284	Weakly similar to pol/env ORF	No
287	Similar to mouse cell death activator B	No

* Determined through UniGene and MEDLINE searches (www.ncbi.nlm.nih.gov/PubMed).
† Rodent.
‡ 3T3-F442A.
§ 3T3-L1.
¶ Monkey.

example, fatty acid binding protein 4 (FABP4, #71 and #172**,** also known as adipocyte FABP or aP2) and lipoprotein lipase (LPL, #164) (15). However, approximately 50% of the identified genes had previously not been reported to be expressed in adipose tissue as determined by UniGene or MEDLINE searches.

The genes expressed at high level in adipose tissue were tentatively classified into categories depending on their putative functions according to the classification used by the Human Gene Anatomy project (Table 1). Figure 4 shows the relative distribution of the identified genes in the different categories and comparison with a previous study based on random sequencing of adipose tissue cDNA libraries (13).

Because there are indications that genes expressed at high levels in a specific tissue may be selectively concentrated to certain chromosomes or chromosomal regions (16), we examined the chromosomal distribution of genes expressed at high levels in adipose tissue. For this purpose we extended the analysis to include nonredundant genes identified among the 500 EST clones with the highest hybridization signals on the membrane. Database searches (UniGene, GDB, OMIM, and MEDLINE) revealed that 268 of the 500 EST clones had been mapped. The chromosomal distribution of these 268 genes was compared with the expected chromosomal distribution of genes based on the assumption that all genes are evenly distributed throughout the genome. As can be seen in Table 2, there was a wide range in the ratio between the

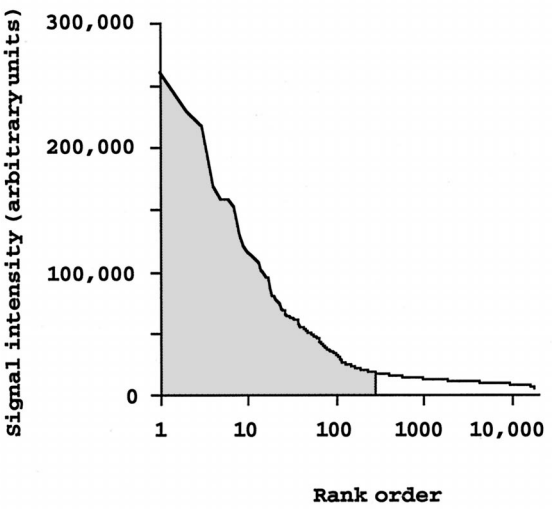

Figure 3. The sum of the two hybridization signals for each EST clone on the DNA array membrane is shown. The shadowed area represents the top 300 ranking EST clones, which were selected for further analysis.

observed chromosomal distribution and the corresponding calculated distribution of the genes on the different chromosomes. Chromosomes 11, 19, and 22 showed significantly higher numbers of expressed genes compared to the expected number of genes. The intrachromosomal distribution of the genes expressed at high levels in adipose tissue was also examined. On chromosome 6, the number of observed genes was similar to the expected number (13 vs. 16). However, on a cytogenetic map, 8 of the 13 genes were clustered around a certain region (6p21.1-6p21.3) on this chromosome, whereas on chromosome 22, the genes active in adipose tissue appeared to be more evenly distributed (Figure 5). In contrast,

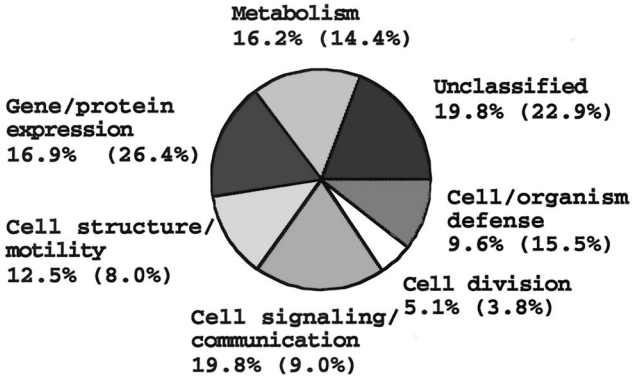

Figure 4. Classification of 136 genes expressed at high levels in adipose tissue according to their functions. For comparison, values based on sequencing of adipose tissue cDNA libraries reported by Adams et al. (13) are shown in parentheses.

Table 2. Chromosomal distribution of 268 genes expressed at high levels in human adipose tissue detected by DNA array

Chromosome	Number of genes: Observed (obs)	Number of genes: Expected* (exp)	Ratio (obs/exp)	Previously reported† (obs/exp)
1	20	22	0.90	1.24
2	25	22	1.15	0.93
3	12	18	0.66	0.99
4	9	17	0.52	0.76
5	19	17	1.14	0.83
6	13	16	0.84	1.09
7	12	14	0.83	0.98
8	11	13	0.84	0.82
9	10	12	0.81	0.90
10	10	12	0.81	1.00
11	19	12	1.54‡	1.28
12	12	12	1.00	1.16
13q	4	8	0.48	0.75
14q	12	8	1.54	1.18
15q	8	8	1.07	1.21
16	12	8	1.44	0.91
17	12	8	1.54	1.44
18	2	7	0.28	0.65
19	14	6	2.49§	1.75
20	4	6	0.65	1.10
21q	3	3	0.93	0.82
22q	11	4	2.93¶	1.38
X	14	14	1.00	0.56

* Based on chromosome size and on assumption of an even distribution of genes throughout the genome.

† Results reported by Deloukas et al. (18) are shown for comparison.

Statistical significances between observed and expected gene distributions, as tested by χ^2 test (1 degree of freedom), are: ‡ $p < 0.05$; § $p < 0.01$; ¶ $p < 0.001$.

when the distribution of the genes on these chromosomes was compared on a physical map, the region on chromosome 6, which contained eight genes with high expression in adipose tissue, was estimated to be more than 15 megabases (Mb). On chromosome 22, a 4-Mb region (22q12.1-22q13.1) contained six genes with high expression in adipose tissue. Both of these regions, 6p21.1-6p21.3 and 22q12.1-22q13.1, contained a significantly higher number of genes expressed in adipose tissue compared to the expected distribution of genes ($p < 0.001$).

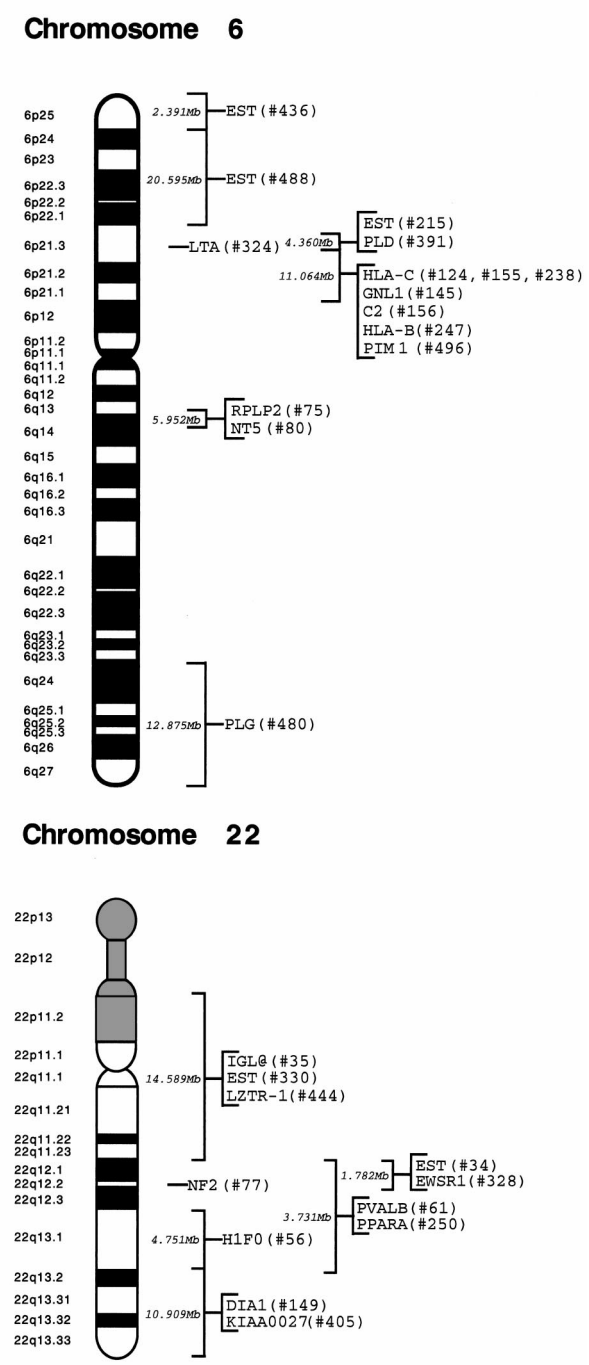

Figure 5. Distribution of genes expressed at high levels in human adipose tissue on chromosomes 6 and 22. Both of these chromosomes contained regions (6p21.1-6p21.3 and 22q12.1-22q13.1) where the number of expressed genes in adipose tissue was significantly higher than expected ($p < 0.001$). Two hundred sixty-eight genes were identified among the 500 highest hybridization signals on the GDA membrane, and their chromosomal localization was determined. Rank order of the signal of the EST clone on the membrane is shown in brackets. The physical distance between two markers was calculated based on information obtained from the Location Data Base.

Discussion

The identification of expressed sequences in the human genome and the development of DNA array-based technologies have opened the possibility to simultaneously measure the expression of thousands of genes. The speed and ease of such analysis is demonstrated here by the screening of approximately 18,000 genes for expression in human adipose tissue. As discussed by Brown and Botstein (9) in a recent review, analysis of gene expression by DNA array allows discovery of things we neither knew nor expected. Thus, in addition to hypothesis-driven research, the technology allows nonhypothesis-driven exploration of gene expression patterns. From a physiological point of view, it is likely that interactions between the different cell types in adipose tissue influence adipocyte function. Our purpose was therefore to explore gene expression in adipose tissue, including all cell types rather than analyzing genes only expressed in adipocytes. In this study we have focused on the genes with highest estimated expression as determined by the DNA array. The fact that approximately 50% of the 136 identified genes with the highest estimated expression had not previously been reported to be expressed in adipose tissue in either man or rodents, clearly illustrates the power of expression analysis by DNA arrays.

As with any new technology, analysis of gene expression by DNA array has to be interpreted with some caution. Our ranking of the genes should not be interpreted as an absolute ranking of gene expression in adipose tissue. In some cases, more than one EST clone corresponding to the same gene was present on the membrane. One example is fatty acid binding protein (FABP/adiposeP2) for which two separate clones resulted in hybridization rankings #71 and #172. Although there is a difference in ranking between the two EST clones, they both display a strong hybridization signal, indicating that it is a highly expressed gene. Furthermore, we have labeled the cDNA with [^{33}P]dCTP during the reverse transcription reaction. It is obvious that, using this strategy, different amounts of radioactivity will be incorporated into short and long cDNAs. End labeling would eliminate this problem, because each cDNA, independent of length, would incorporate the same amount of isotope. However, the use of end labeling would markedly decrease the sensitivity of the assay and increase the amount of required RNA. In this study we could easily detect genes known to be expressed at high or intermediate levels in adipose tissue. For example, lipoprotein lipase was ranked #164 on the membrane, whereas peroxisome proliferator-activated receptor-γ (PPAR-γ) was ranked #9627. This is consistent with the results obtained by the quantitative polymerase chain reaction method, which demonstrated that the expression of lipoprotein lipase is approximately 2000-fold higher than PPAR-γ expression in adipocytes (17). In addition to PPAR-γ, there were also other genes that are known to be expressed in adipocytes, for example leptin, which did not give high hybridization signals on the DNA array. There are several possible causes of the low hybridization signal for leptin. The leptin mRNA contains an internal poly(A) sequence that is known to bind oligo(dT) and compete with the poly(A)-tail during reverse transcription, generating a truncated leptin cDNA (18). Unfortunately, the EST clone used for detection of leptin mRNA corresponds to the most 3′-untranslated sequence of the leptin mRNA and may therefore generate a very low hybridization signal if hybridized with the truncated form of leptin cDNA. In addition, it should be remembered that we have studied adipose tissue, which includes a mixture of cell types. Genes that are specific for adipocytes may therefore have an unexpectedly low hybridization signal on the array due to the fact that the adipocyte mRNA is diluted with mRNA derived from other cell types.

There were also genes with an unexpectedly high level of expression, such as PPAR-α. In this study, PPAR-α had a much higher hybridization signal than PPAR-γ, which is the predominant PPAR isoform in adipocytes. Analysis of the PPAR-α mRNA revealed the presence of an Alu repetitive element (GenBank accession number N46949) that is present in the EST clone on the DNA array, and this may have caused the high hybridization signal.

When grouped according to their putative functions, the distribution of the genes expressed in adipose tissue in this study was similar to that previously determined by random sequencing of cDNA libraries (19). However, the group of genes involved in cell signaling and communication was twice as large as in the study by Adams et al. (13), whereas the groups with genes assigned to gene/protein expression and cell/organism defense were smaller. These differences were not due to variation in the number of novel genes in the groups, because the percentage of genes that had previously been detected in human adipose tissue ranged between 40% and 52% in all groups. Furthermore, it is unlikely that the differences are due to differences in classification of the individual genes, because the majority of genes in our study had been classified by Adams et al. (13), although in other tissues. It is more likely that the differences between the studies may be caused by the difference in methods used. The DNA array method is limited to the genes attached to the membrane, which may skew the distribution of genes detected in each category. Random sequencing of libraries relies on how well the library reflects the expression of individual genes. Classification of genes according to their putative function may not only provide information on the relative proportion of genes involved in a particular cellular function but may also be an important tool for analysis of functional groups of genes. For example, coexpression of a ligand and a receptor may indicate a paracrine/autocrine system, whereas isolated expression of a ligand or a receptor suggests an endocrine mechanism of action. In the analysis of the genes ranked 1

through 300, we were unable to identify coexpression of a ligand and its receptor. However, extending the analysis further there were examples of expression of receptors and their ligands among the first 1000 hits (manuscript in preparation).

Analysis of tissue-specific expression on genomic maps may reveal chromosomal regions with an increased transcriptional activity. A previous study has indicated that five chromosomal regions contain an over-representation of muscle genes (16), and this led us to investigate if gene expression in adipose tissue reflects the gene distribution on the chromosomes. When the distribution of genes expressed at high levels in adipose tissue was compared with the expected number of genes on each chromosome (based on chromosome size and the assumption that genes are evenly distributed throughout the genome), there were more than twice the expected number of genes expressed on chromosomes 11, 19, and 22. The increased gene density on these chromosomes does not seem to be specific for adipose tissue, because a similar distribution was found for 30,075 distinct gene markers (19). Chromosome 6 contained a region (6p21.1-6p21.3) with a cluster of genes expressed at high levels in adipose tissue. This region has previously been shown to contain a high gene density (16) (www.ncbi.nlm.nih.gov/genemap98). Although the physical size of this region is quite large (15.4 Mb), a high density of genes on a cytogenetic map may be of importance, because euchromatic and heterochromatic regions have functional significance in relation to gene expression. Interestingly, the tumor necrosis factor-α (TNF-α) gene also maps to this region (20), and there is experimental support for a possible role of adipose tissue-derived TNF-α in obesity-related insulin resistance (21). However, in this study we could not determine the expression level of the TNF-α gene, because no corresponding EST clone was present on the membrane. On a physical map we identified a region of approximately 4 Mb on chromosome 22 (D22S1144-D22S272) with a higher than expected number of genes expressed at high levels in adipose tissue. This chromosomal region also has a high gene density (www.ncbi.nlm.nih.gov/genemap98).

Traditional methods for analysis of gene expression are very laborious. In contrast, DNA array monitoring rapidly generates a large amount of raw data, but instead creates a challenge with respect to data storage, processing, and interpretation. The databases currently available are extremely useful for retrieving information about a specific gene or a small number of genes. However, querying for large number of genes, which is necessary for analysis of data obtained by high density DNA arrays, becomes extremely time-consuming. Novel databases and software, suitable for analysis of expression profiles, are needed for efficient interpretation of the data. It is also evident that, with the amount of data that soon will be generated by the use of DNA arrays, it will be impossible to publish all the information about gene expression patterns in scientific journals. These problems will be even more obvious in the near future when DNA arrays will include the majority of expressed sequences of the human genome. To enable comparison of expression profiles, searchable databases for expression analysis, similar to those now available for DNA and protein sequences, need to be established.

In conclusion, this first attempt to explore gene expression in human adipose tissue from a global perspective resulted in the identification of numerous genes previously not reported to be expressed in adipose tissue. The 300 genes that we have investigated are highly expressed in adipose tissue, however, for many of them, the function remains to be determined. For example, the gene with the highest ranking on our array, sulfonylurea receptor 2, has previously not been demonstrated in human adipose tissue. However, the expression of this receptor in adipose tissue could have important implications for the effects of sulfonylureas, for which increased body weight is a well known side effect in patients with type 2 diabetes. This demonstrates that exploration of gene expression can provide clues that merit further investigation. In addition, further use of DNA array-based monitoring of gene expression in adipocytes and preadipocytes, in adipose tissue in response to different stimuli, and in adipose tissue obtained from lean and obese subjects is likely to provide important information on the regulation and regulatory role of adipose tissue in health and disease.

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