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Chromosome Localization Analysis of Genes Strongly Expressed in Human Visceral Adipose Tissue

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To understand fully the physiologic functions of visceral adipose tissue and to provide a basis for the identification of novel genes related to obesity and insulin resistance, the gene expression profiling of human visceral adipose tissue was established by using cDNA array. The characterization and chromosome localization of 400 expressed sequence tags (ESTs) strongly expressed in visceral adipose tissue were analyzed by searching PubMed, UniGene, the Human Genome Draft Database, and Location Data Base. Two hundred eighty-nine clones were classified into known genes among the 400 ESTs strongly expressed in the tissue. Among them, <20% have been previously reported to be expressed in adipose tissue. The chromosome localization of 389 ESTs strongly expressed in visceral adipose tissue showed that their relative abundance was significantly increased on chromosomes 1, 16, 19, 20, and 22 compared with the expected distribution of the same number of random genes. The intrachromosome distribution of the genes strongly expressed in visceral adipose tissue was concentrated in certain regions, such as 1p36.2-1p36.3, 6p21.3-6p22.1, 19p13.3 and 19q13.1. Among them, the region of 1p36.2-1p36.3 appeared to be specific for visceral adipose tissue. Interestingly, some genes playing an important role in the pathogenesis of insulin signal transduction and adipocyte differentiation, such as tumor necrosis factor-α and its receptors; CCAAT/enhancer-binding protein-α; and phosphoinositide-3-kinase, regulatory subunit, polypeptide 2 (p85β), were also localized in the concentrated regions, which may provide clues to identifying novel genes closely related to adipocyte function with potential pathophysiologic implications.

Key Words: Visceral adipose; cDNA array; gene expression; chromosome distribution.

Introduction

Adipose tissue has long been considered a passive and an inactive fat storage tissue. However, research in the past decade has demonstrated that adipose tissue plays an important role in energy regulation via endocrine, paracrine, and autocrine signals *(1)*. Application of DNA array to sc adipose tissue has resulted in identifying that the strongly expressed genes in sc adipose tissue showed an uneven distribution throughout the genome *(2)*. However, obese individuals with most of their body fat stored in visceral adipose tissue generally suffer from more adverse metabolic consequences than equally overweight subjects with fat stored predominantly at sc sites. The increased visceral adiposity has been shown to be closely associated with different components of the metabolic syndrome and is an important predictor for increased morbidity and mortality from diabetes, certain kinds of cancer, and coronary heart disease *(3)*. To understand fully the physiologic functions of visceral adipose tissue and to lay a foundation for searching for novel genes related to obesity and insulin resistance, we have developed a cDNA array representing 16,359 clusters to profile the gene expression patterns of human visceral adipose tissue (unpublished data). The aim of the present work was to focus on the strongly expressed genes in visceral adipose tissue and analyze their chromosome distribution.

Results

Functional Classification of Known Genes Strongly Expressed in Visceral Adipose Tissue

Two hundred eight-nine clones were classified into known genes among the 400 expressed sequence tags (ESTs) strongly expressed in the visceral adipose tissue, and the other 111 clones were known ESTs. The known genes were grouped according to their putative functions *(4)*: cell division (G1), cell signaling/communication (G2), cell structure/motility (G3), cell/organism defense (G4), gene expression (G5), protein expression (G6), metabolism (G7), and unclassified genes (G8), and the proportions of functional classification showed that G1, G2, G3, G4, G5, G6, G7, and G8 were 5.2, 22.1, 5.9, 9.0, 7.3, 5.5, 14.9, and 30.1%, respectively (Fig. 1). The proportions of functional classification were similar to those of the sc adipose tissue *(2)*. Among the

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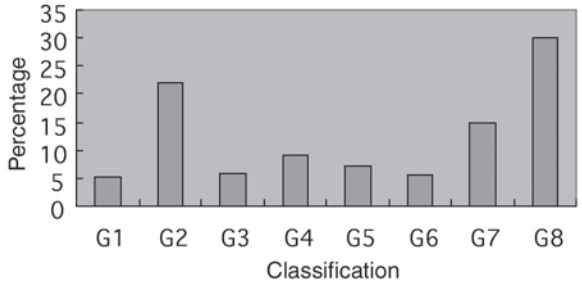

Fig. 1. Functional classification and respective proportions of 289 known genes strongly expressed in visceral adipose tissue.

289 strongly expressed known genes, <20% have previously been reported to be expressed in visceral adipose tissue as determined by UniGene or MEDLINE searches (Table 1).

Chromosome Localization of Genes Strongly Expressed in Visceral Adipose Tissue

Among the 400 ESTs strongly expressed in adipose tissue, 389 genes or ESTs were localized on certain regions of chromosomes. The chromosome distribution of these 389 genes was compared with the expected chromosome distribution of genes based on the assumption that all genes are evenly distributed throughout the genome. Table 2 shows that the relative abundance of these 389 genes was significantly increased on chromosomes 1, 16, 19, 20, and 22 compared with the expected distribution of the same number of random genes. The intrachromosome distribution of the genes strongly expressed in adipose tissue was concentrated in certain regions; for example, 1p36.2-1p36.3 contained nine genes with high expression in visceral adipose tissue. The following regions, 6p21.3–6p22.1, 19p13.3, 19q13.1, also contain a significantly greater number of genes expressed in visceral adipose tissue compared with the expected distribution of genes (Fig. 2). Interestingly, some genes play an important role in the pathogenesis of insulin resistance and adipocyte differentiation were also localized in the concentrated regions. For example, tumor necrosis factor (TNF) receptor TNFRSF14 (shown in Table 1, rank order #49) and TNFRSF1B (#389) were both mapped to 1p36. CCAAT/enhancer-binding protein alpha (CEBPA) (#349) gene was mapped to 19q13.12.

Discussion

Adipocytes are highly specialized cells that play critical roles in energy regulation and homeostasis. Their primary and best-known role is to store energy in the form of triglyceride when energy intake exceeds energy expenditure and to release it in the form of free fatty acids during starvation *(1)*. However, adipose tissue has attracted much attention since the discovery of leptin in the mid-1990s *(5)*. The increased visceral adiposity has been shown to be closely

Table 1
List of 289 Known Genes Strongly Expressed in Visceral Adipose Tissue

Rank order	Description	Previously reported in adipose tissue[a]
	Cell Division	
52	Subtelomeric repeat sequence	n
83	DEC1	y
123	THW	n
129	Ionizing radiation resistance conferring protein	y
159	p53-regulated PA26-T3 nuclear protein	n
170	Cyclin G1	n
201	TD26	n
211	pim-2 protooncogene homolog	n
222	Palmitoylated erythrocyte membrane protein	n
338	Similar to polymerase, δ4	n
341	ST15	n
352	IEF 7442	y
356	OTK27	n
359	Cathepsin Z precursor	y
	Cell Signaling/Communication	
1	Insulin-like growth factor–binding protein	y
2	Myotubularin-related protein 6	n
3	DAP12	n
8	Asialoglycoprotein receptor 2	n
11	ERK activator kinase	y
19	EDF-1 protein	n
23	rhoG	n
27	C-reactive protein	n
33	Tumor necrosis factor–inducible (TSG-6) mRNA fragment	n
35	Matrix Gla protein	y
49	TNFRSF14	n
50	Helix-loop-helix basic phosphoprotein	n
62	Synaptobrevin 2	y
88	Antioxidant enzyme AOE37-2	n
93	E4BP4	y
100	PPP1R5	n
106	Capping enzyme	n
108	HGF activator-like protein	n
109	KIAA0787	n
112	DKFZp434N024	n
113	Hepatocyte growth factor–like protein	n
134	Phosphorylase-kinase, β-subunit	n
137	Secretory carrier membrane protein 3	n
139	Similar to small G proteins especially RAP-2A	n
141	Follistatin-like 3	n
146	Era GTPase A protein	n
174	Transferrin receptor 2α	n
187	Complement component C1q receptor	y
188	Signalosome subunit 2	n
196	Tax interaction protein 1	n
198	Putative chloride channel	n
202	Histidine-rich glycoprotein	n

Rank order	Description	Previously reported in adipose tissue[a]
203	Megakaryocyt-stimulating factor	n
208	Endothelin 3	n
213	Chromogranin B	n
223	Cyclooxygenase-2	n
227	Vesicle-associated membrane protein 4	n
232	VAMP-associated protein B	n
234	Protein kinase Cζ	y
243	Thrombospondin	y
252	Integrin α4-subunit	n
254	Protein serine threonine kinase Clk4	n
255	KIAA0006	n
265	CD27BP	n
267	Reticulocalbin 1	n
271	Aryl hydrocarbon receptor-interacting protein	n
273	Prepro form of corticotropin-releasing factor	n
281	TB1	n
296	Ectonucleoside triphosphate diphosphohydrolase 6	n
301	Protein phosphatase 1γ	n
310	Connective tissue growth factor	y
329	KIAA0879	n
339	Phosphatidylinositol 3-kinase catalytic subunit p110δ	y
342	Leucine-rich repeat interacting protein 2	n
360	M130 antigen extracellular variant	n
369	S100 calcium-binding protein A8	n
374	c-myc-binding protein	y
380	Thyroid hormone–binding protein	y
387	Calcium-binding protein S100P	n
389	Tumor necrosis factor receptor	y
398	Calcium and DAG-regulated guanine nucleotide exchange factor II	n
	Cell Structure/Motility	
30	Vascular smooth muscle α-actin	n
79	Tenascin-R	n
94	RCK	n
110	Skeletal β-tropomyosin	n
126	β-spectrin III	n
149	Elastin	n
163	Brain-type clathrin light-chain b	y
195	KIAA0762	n
250	Cytoskeletal γ-actin	y
282	Type IV collagen α (2) chain	n
295	Lysosomal-associated multitransmembrane protein	y
299	Neurofilament subunit M	n
340	Nonmuscle-type cofilin	y
368	Ninjurin1	y
392	β-Tubulin class III isotype	n
399	Microtubule-associated protein 1B	y
	Cell/Organism Defense	
10	DORA	n
17	Lectin, galactoside-binding, soluble, 2	n
55	Complement factor H	y
56	Putative carboxylesterase	n
63	H factor-like 3	n
72	MHC class II HLA-DR-β	n
80	Butyrophilin	n
89	Proline-rich protein	n
102	Cyclophilin-40	n
116	Prion protein	y
131	Rearranged κ Ig subgroup V κ IV	n
153	Ig J chain	y
158	Solute carrier family 25, member 13	n
172	Properdin	n
176	Complement C1r	n
199	Factor XI	n
224	DKFZp564C246	n
242	High-affinity IgE receptor α-subunit	n
283	Tapasin	n
298	HLA-DMB	n
318	FAA	n
327	Ig rearranged γ chain	y
333	DOWN 16	n
334	Afamin	n
350	Rearranged Ig λ light chain	y
393	Ig superfamily, member 4	n
	Gene Expression	
73	DKFZp434L151	n
85	Low molecular mass ubiquinone-binding protein	y
120	ZNF198 protein	n
132	Pleckstrin homology, Sec7 and coiled/coil domains, binding protein	n
135	Transcription factor (E2A)	n
140	RNA polymerase II–associated protein RAP74	n
148	Brain-expressed ring finger protein	n
150	RNA helicase A	n
185	FUSE-binding protein 3	n
186	Krueppel family zinc finger protein	n
193	RAP30 subunit of transcription initiation factor RAP30/74	n
206	Transcription factor (CBFB)	n
214	Autoantigen DFS70	n
218	Nuclear factor κB DNA-binding subunit	n
219	Polymerase (RNA) II polypeptide C	n
220	KRAB–zinc finger protein SZF1-2	n
246	Zinc finger protein 9	y
292	Apobec-1-binding protein 1	n
300	T-cell leukemia virus enhancer factor	n
349	CCAAT/enhancer-binding protein α	y
	Protein Expression	
54	CST3 cystatin C	y
65	34-kDa Mov34 homolog	n
66	Interleukin-1β convertase	n
90	Carboxypeptidase E	n
103	Elongation factor 2	y
144	p18	n

Rank order	Description	Previously reported in adipose tissue[a]
162	Ribosomal protein L21	y
178	Proteasome subunit HsC10-II	y
221	Cathepsin D	y
231	Tissue inhibitor of metalloproteinases	y
236	Epsilon COP	n
309	Proteasome subunit MECl-1	n
314	Ribosomal protein S15a	y
358	Ubiquitin	y
388	Proteasome subunit HsC7-I	n
	Metabolism	
14	Preapolipoprotein CIII	n
15	Fructose-1,6-biphosphatase	y
16	5,10-Methenyltetrahydrofolate synthetase	n
20	Methylmalonyl-CoA mutase	n
24	Protein phosphatase 2A B'α1 regulatory subunit	n
25	Apolipoprotein H	n
38	Formiminotransferase cyclodeaminase	n
39	Gastric H,K-ATPase catalytic subunit	n
70	Apolipoprotein M	n
81	Tyrosylprotein sulfotransferase-2	n
104	L-Iditol-2 dehydrogenase	n
124	RING4	y
125	Glycogen synthase 2	n
142	Cytochrome-*bc*-1 complex core protein II	n
152	Protein C inhibitor	n
154	Na/PO_4 cotransporter homolog	n
161	Aldehyde dehydrogenase 12	n
166	Enyol-CoA: hydratase 3-hydroxyacyl-CoA dehydrogenase	n
169	Electron transfer flavoprotein α-subunit	n
184	Lysyl hydroxylase	n
204	Glucose 6-phosphate translocase	n
225	Neutrophil cytochrome-*b* light chain p22 phagocyte b-cytochrome	n
239	Prolyl 4-hydroxylase α-subunit	n
240	*N*-acetylgalactosaminidase	n
247	Glucuronyltransferase I	y
253	Acyl-CoA dehydrogenase	n
279	Nuclear-encoded mitochondrial NADH-ubiquinone reductase 24-kDa subunit	n
289	ADH4 class II alcohol dehydrogenase	n
293	ATP synthase α-subunit	y
297	DKFZp564A202	n
319	10-Formyltetrahydrofolate dehydrogenase	n
322	CMP-*N*-acetylneuraminic acid hydroxylase	n
323	Mitochondrial ATP synthase c-subunit	y
325	Aortic carboxypeptidase-like protein ACLP	n
332	Cytochrome oxidase subunit VIIa-H precursor	n
336	Phospholipase D2	y
337	Liver fatty acid–binding protein	y
355	Proton-ATPase-like	n
357	Mitochondrial carrier protein ARALAR1	n
363	Red cell–type low molecular weight acid phosphatase	n
366	ACAT related gene product 1	n
381	Peroxisomal short-chain alcohol dehydrogenase	n
385	Methylthioadenosine phosphorylase	n
	Unclassified	
7	Clone 23859 mRNA sequence	n
21	PEP19	n
28	FLJ22736 fis	n
36	C1orf21	n
41	ET	n
43	KIAA1104	n
45	KIAA0773	n
46	KIAA0793	n
48	KIAA0640	n
58	KIAA0781	n
60	BM-020	n
61	YAP65	n
68	DJ1042K10.2	n
71	KIAA0158	n
76	KIAA1009	n
84	KIAA1004	n
86	Ceroid-lipofuscinosis, neuronal 5	n
91	SEC14L	n
96	FLJ00118	n
105	FLJ22482 fis	n
107	DKFZp434N185	n
115	KIAA0805	n
117	Brain-specific angiogenesis inhibitor 2	n
118	I3	n
119	PRO0903	n
122	HBV-associated factor	n
127	DKFZp547O0510	y
128	FLJ10846	n
138	FLJ10704	y
143	KIAA0307	n
156	DKFZp564L2016	n
160	CG018	n
167	NPAT	n
171	FLJ10761	n
182	KIAA0191	n
183	KIAA1037	n
189	KIAA1536	y
200	KIAA0475	n
205	KIAA0564	n
212	DKFZp434D0935	n
217	FLJ20517	n
233	NPD009	n
235	DKFZp564H2023	n
244	FLJ22195	y
245	FLJ00061	n
248	Integral membrane protein 2A	n
249	KIAA0097	n
251	DKFZp586N0819	n
256	DKFZp564L1916	y
263	GL014	n

Rank order	Description	Previously reported in adipose tissue[a]
266	DKFZp564E1363	y
270	KIAA1333	n
272	MGC19595	n
275	Sex comb on midleg-like 1	n
276	Claudin 14	n
277	FLJ20515	n
278	Syndecan 4	n
284	Leucine-rich protein	n
285	IPW	n
290	HSPCO34	y
291	FLJ22965	n
308	KIAA1586	n
313	KIAA0411	n
315	HSPC117	n
320	Mariner1 transposase	n
324	Nef-associated factor 1 α	y
328	REC8	n
343	FLJ21213	n
344	EDRF	n
346	GrepE-like protein cochaperone	n
347	DKFZp586I0324	n
351	TR2/D15	n
353	KIAA0926	n
354	KIAA1097	n
362	KIAA0764	n
364	Katanin p80 subunit	n
370	KIAA0943	n
371	BCL9	n
377	Dishevelled 1	n
384	CDA018	n
390	HSPC240	n
396	Similar to likely ortholog of yeast ARV1	n
397	PRO2577	n
400	Ly-9	n

[a]Determined by searching UniGene (www.ncbi.nlm.nih.gov/UniGene) and PubMed (www.ncbi.nlm.nih.gov/PubMed). n, no; y, yes.

associated with different components of the metabolic syndrome. To provide a catalog of genes expressed in visceral adipose tissue and information about their functions, the gene expression profiling of human visceral adipose tissue was established by using cDNA array. The results demonstrated for the first time that many kinds of secreted proteins, receptors, and transcription factors were identified to be expressed in visceral adipose tissue (unpublished data). To confirm our cDNA array results, the visceral adipose tissues of 11 nonobese subjects were obtained. Three genes—leptin, nonmuscle type cofilin, and nuclear receptor LXR-α—were selected to verify whether they are expressed in the visceral adipose tissues of different subjects. The results showed that they are all expressed in the selected adipose tissues (Fig. 3). In addition, seven secreted proteins were selected and verified to be expressed in 3T3-L1 adipocytes with reverse transcriptase polymerase chain reaction (RT-PCR) (Fig. 4). Here, we have focused on the genes strongly expressed in visceral adipose tissue as determined by the cDNA array.

Among the 400 ESTs strongly expressed in the visceral adipose tissue, 289 clones were classified into known genes and more than 80% of them have not previously been reported to be expressed in visceral adipose tissue as determined by UniGene or MEDLINE searches (Table 1). The proportion is significantly higher than that observed in sc adipose tissue (50%) (2). The difference may be owing to the fact that most of the spotted clones on the membrane in the present study were obtained from our own laboratory. However, the rank of genes in this work should not be interpreted as an absolute rank of their expression in visceral adipose tissue considering the limitation of the range of target clones. When grouped according to their putative functions, the proportions of the known genes were similar to those reported in sc adipose tissue *(2)*. However, the proportion of unclassified genes in our study (30.1%) was higher than that observed in sc adipose tissue (19.8%), probably owing to the fact that lots of novel genes were cloned during the period 2000–2001, but their functions remain unknown. In addition, the second largest proportion of known genes expressed in both visceral (22.1%) and sc adipose tissue (19.8%) *(2)* was cell signaling/communication genes, demonstrating that adipose tissue is no longer a passive and inactive fat storage tissue, but an active endocrine organ with complex signal communications.

In the present work, the gene with the highest signal on the membrane was insulin-like growth factor–binding protein 1 (IGFBP-1). It has been reported that transgenic mice with overexpressed IGFBP-1 are hyperinsulinemic in the first week of life, while in adult age, the pancreatic insulin content is reduced, insulin resistance is demonstrable in skeletal muscle, and fasting hyperglycemia develops *(6)*. In isolated adipocytes from the transgenic mice, the stimulatory effect of insulin-like growth factor-1 (IGF-1) on 2-de-oxy-[^{3}H]-glucose uptake was reduced *(6)*. These observations suggest that visceral adipose tissue–derived IGFBP-1 might play a potential role in the pathogenesis of insulin resistance and type 2 diabetes. In addition, adipogenesis is impaired in IGFBP-1 transgenic mice, indicating that IGF-1 has a critical role in the proliferation and differentiation of adipocyte precursors *(7)*, which suggests that IGFBP-1 strongly expressed in visceral adipose tissue might play a regulatory role in this process. Among the strongly expressed genes, CEBPA (#349) was one of the most important transcription factors in adipocyte differentiation *(8)*. Furthermore, several genes involved in lipid or glucose metabolism, such as preapolipoprotein CIII (#14), fructose-1,6-biphosphatase (#15), apolipoprotein H (#25), apolipoprotein M (#70), glucose 6-phosphate translocase (#204), phospholipase D2 (#336), and liver fatty acid–binding protein (#337) were found to be strongly expressed in visceral adipose tissue, demon-

Table 2
Chromosome Localization of 389 Genes Strongly Expressed in Visceral Adipose Tissue

Chromosome	Number of genes		Ratio (observed/expected)	Reported ratio of sc adipose tissue[a]	χ^2
	Observed	Expected			
	54	32	1.69	0.90	15.13[b]
2	19	31	0.61	1.15	4.65[c]
3	24	26	0.92	0.66	0.15
4	15	25	0.60	0.52	4.00[d]
5	14	24	0.58	1.14	4.17[d]
6	29	22	1.32	0.84	2.23
7	16	21	0.76	0.83	1.19
8	10	19	0.53	0.84	4.26[d]
9	14	18	0.78	0.81	0.89
10	11	18	0.61	0.81	2.72
11	25	17	1.47	1.54	3.76
12	13	12	1.08	1.00	0.08
13q	12	11	1.09	0.48	0.09
14q	10	11	0.91	1.54	0.09
15q	12	12	1.00	1.07	0.00
16	20	11	1.82	1.44	7.36[c]
17	16	10	1.60	1.54	3.60
18	5	8	0.63	0.28	1.13
19	21	9	2.33	2.49	16.00[b]
20	16	9	1.78	0.65	5.44[d]
21q	6	5	1.20	0.93	0.20
22q	11	5	2.20	2.93	7.20[c]
x	16	20	0.80	1.00	0.80

[a]Reported by Gabrielsson et al. *(2)* shown for comparison.
[b]$p < 0.001$.
[c]$p < 0.01$.
[d]$p < 0.05$.

strating the important functions of visceral adipose tissue in metabolic regulation.

Analysis of tissue-specific expression on genomic maps may reveal the chromosome regions with an increased transcriptional activity, which may provide clues to the positional cloning of disease genes. Previous studies have indicated that portions of chromosome regions contain an overrepresentation of tissue-specific genes in muscle *(9)* and sc adipose tissue *(2)*, and this led us to analyze the chromosome distribution of genes strongly expressed in visceral adipose tissue. Our results demonstrated that there were more than twice the expected number of genes expressed on chromosomes 19 and 22. We also found that the observed number of genes was significantly increased on chromosomes 1, 16, and 20 compared with the expected distribution of the same number of random genes, suggesting that the transcriptional activity was increased on these chromosomes. However, the increased gene density on these chromosomes does not seem to be specific for visceral adipose tissue; a similar distribution was found for 30,075 distinct gene markers *(10)*.

The intrachromosomal distribution of genes expressed at high levels in visceral adipose tissue has been investigated in the present work. On chromosome 1, a 15-Mb fragment (1p36.2-1p36.3) contained nine genes with strong expression in visceral adipose tissue. On chromosome 6, the number of observed genes was similar to the expected number (29 vs 22). However, on a cytogenetic map, 11 genes strongly expressed in visceral adipose tissue were mapped to the region 6p21.3-6p22.1. Both the 19p13.3 and 19q13.1 regions contained a significantly greater number of genes expressed in visceral adipose tissue compared with the expected distribution of genes. These regions, except 1p36.2-1p36.3, have previously been shown to contain a high gene density (www.ncbi.nlm.nih.gov/genemap99) *(9)*. These results are a little different from those obtained in sc adipose tissue *(2)*. It is likely that the differences may result from the different target genes or may be caused by the different locations of the adipose tissue. Although the physical size of these regions is quite long, a high density of genes on a cytogenetic map may be of importance, since euchromatic and heterochromatic regions definitely have a functional significance in relation to gene expression *(2)*. To our interest, some genes, playing an important role in insulin signal transduction and adipocyte differentiation, were mapped to the concentrated

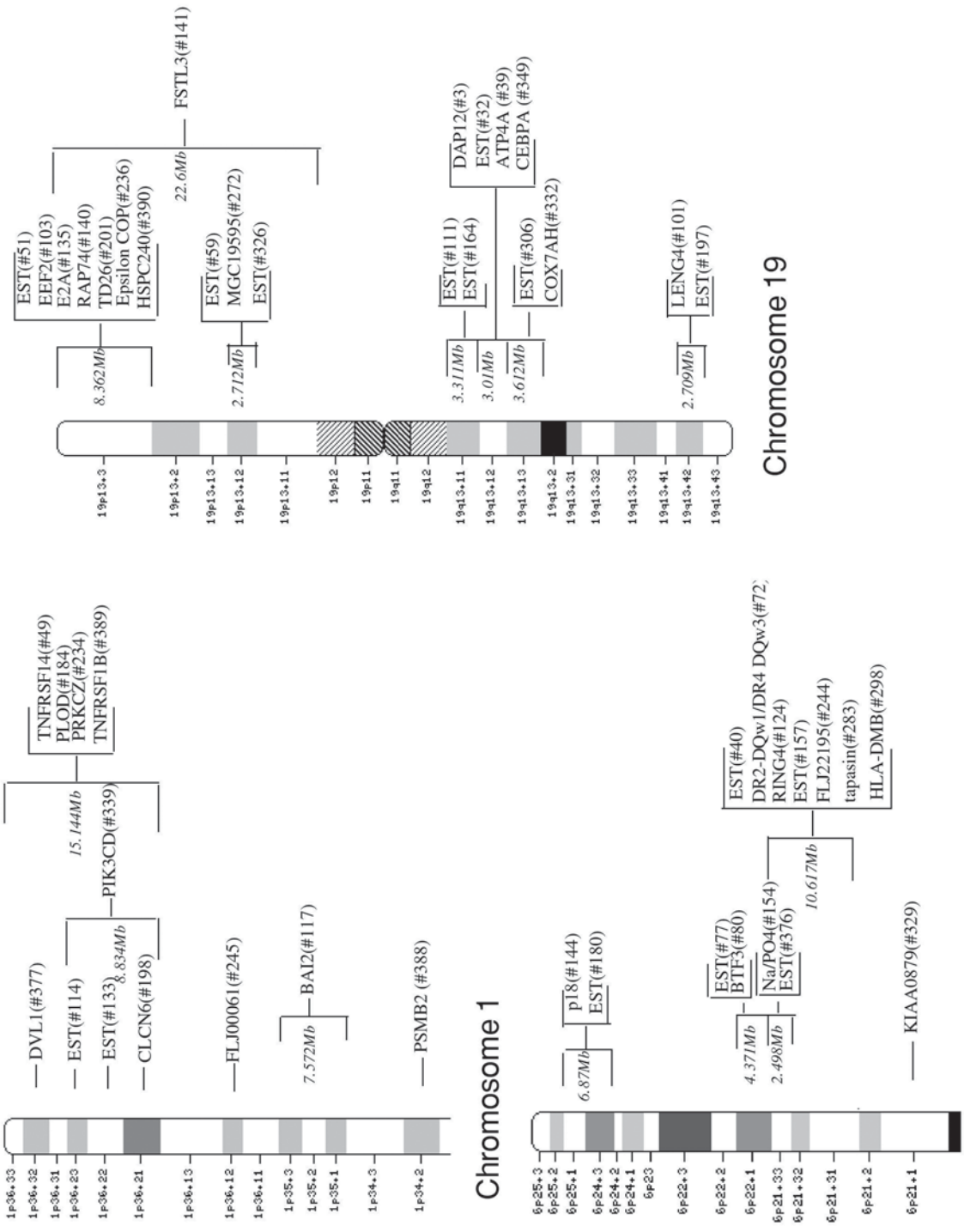

Fig. 2. Distribution of genes strongly expressed in human visceral adipose tissue on chromosomes 1, 6, and 19, showing the regions (1p36.2–1p36.3, 6p21.3–6p22.1, 19p13.3, 19q13.1) where the number of expressed genes was significantly higher than expected.

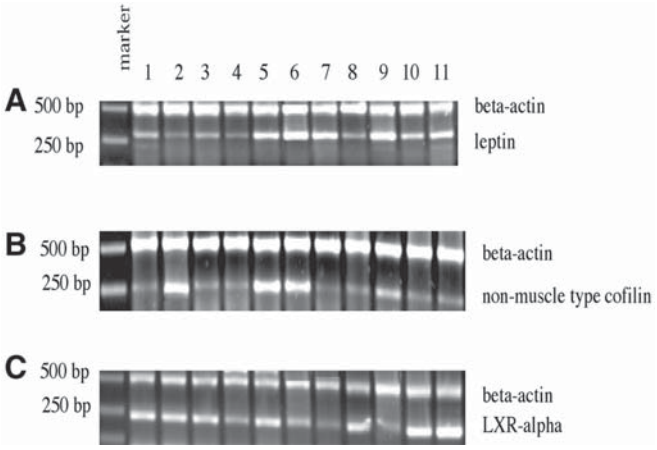

Fig. 3. Identification of leptin (**A**), non-muscle-type cofilin (**B**), and LXR-alpha (**C**) in visceral adipose tissues of nonobese subjects with semiquantitative RT-PCR. Lanes 1–4: postmenopausal females; 5–6: premenopausal females; 7–11: males.

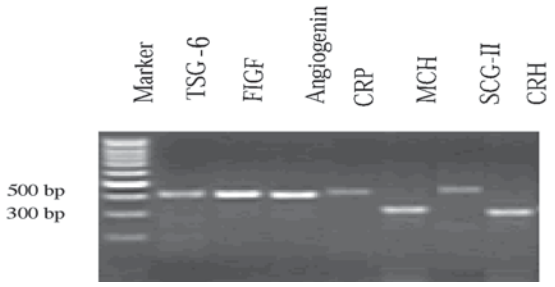

Fig. 4. Seven secreted proteins expressed in visceral adipose tissue were verified using 3T3-L1 adipocytes with RT-PCR. The marker was a 100-bp DNA ladder.

regions. For instance, TNF-α gene was localized on 6p21.3 and TNF receptor TNFRSF14 (#49) and TNFRSF1B (#389) were both mapped to 1p36. CEBPA (#349) gene was mapped to 19q13.12; phosphoinositide-3-kinase, regulatory subunit, polypeptide 2 (p85β) was mapped to 19q13.2-q13.4; and insulin receptor gene was localized on 19p13, suggesting that these concentrated regions might contain other important genes closely related to adipocyte differentiation and physiologic or pathophysiologic functions from an evolutional point of view. It has been reported that adipose tissue–derived TNF-α plays an important role in obesity-related insulin resistance *(11)*, and CEBPA is a key transcription factor in regulating adipocyte differentiation *(8)*. Phosphoinositide-3-kinase is an important molecule in the insulin signal transduction. However, we could not determine the expression levels of TNF-α and p85β, because there were no corresponding EST clones on the membrane in this study.

It is very interesting to indicate that the region of 1p36.3-1p36.23 may contain multiple susceptibility genes in Chinese Han families with type 2 diabetes, as demonstrated by genomewide scanning *(12)*. Moreover, the region of 1p36.2-1p36.3, containing a significantly greater number of genes strongly expressed in visceral adipose tissue than that of expected, appears to be specific for visceral adipose tissue. The increased visceral adiposity has been shown to be closely associated with different components of the metabolic syndrome and is an important predictor for increased morbidity and mortality from diabetes. The findings in the present work suggest that some of these high expression genes localized on the concentrated region (1p36.2-1p36.3) might be related to adipocyte function with some potential pathophysiologic implications.

Research into the genetics of human obesity is continuing at a rapid pace, with the goal focused on the identification of specific causative genes. Although the reported results from the genome scan suggest the existence of a few genes with substantial effects on obesity *(13)*, the large number of genetic loci likely to be involved means that many of these genes on their own may account for only a small portion of the total phenotypic variance. Recently, the gene expression profiling of human visceral adipose tissue was established by using cDNA array for the first time (unpublished data). Among the 400 strongly expressed genes, known genes with unknown function and known ESTs account for about 50%, and <20% of the strongly expressed known genes have been previously reported to be expressed in adipose tissue. These findings may contribute to the extension of our knowledge about the functions of visceral adipose tissue. Some chromosome regions containing an overrepresentation of strongly expressed genes in visceral adipose tissue may provide clues for searching novel genes related to obesity and insulin resistance.

Materials and Methods

Construction of cDNA Array

In recent years, our laboratory has established the gene expression profilings of the hypothalamus-pituitary-adrenal axis *(14)*, CD34(+) hematopoietic stem/progenitor cells *(15)*, liver, and hepatocellular carcinoma *(16)* by using ESTs. In total, 99,621 ESTs were obtained and assembled into clusters. cDNA clones used as the targets of the array were mainly taken from our own EST libraries (www.chgc.sh.cn), and only a few clones were purchased from Research Genetics (Huntsville, AL). A cDNA array was assembled with 16,359 cDNA clones representing the same number of independent cDNA clusters. All cDNA fragments were amplified and verified by gel electrophoresis. The average length of the cDNA fragments was ~1 kb. PCR products were precipitated in isopropanol, redissolved in 10 μL of denaturing buffer (1.5 *M* NaCl, 0.5 *M* NaOH), and spotted on 8 × 12 cm Hybond-N nylon membranes (Amersham Pharmacia, Buckinghamshire, UK) using an arrayer (BioRobotics, Cambridge, UK). Each spot carried ~100 nL in volume and was 0.4 mm in diameter, and each cDNA fragment was placed

in two different spots (double-offset). Lambda phage and pUC18 vector DNA were spotted as negative controls *(17)*.

Hybridization Intramembrane Control

Eight housekeeping genes encoding ribosomal protein S9 (RPS9), β-actin (ACTB), glyceraldehyde-3-phosphate dehydrogenase, hypoxanthine phosphoribosyltransferase 1, M_r 23,000 highly basic protein (RPL3A), ubiquitin C, phopholipase A2, and ubiquitin thiolesterase (UCHL1) were evenly distributed, and each was spotted on an 8 × 12 cm array in 12 places as an intramembrane control. Hybridization data were considered invalid if among the 12 spots representing the same gene the intensity of the darkest spot exceeded 1.5-fold of the weakest spot *(17)*.

RNA Extraction and Probe Preparation

Abdominal omental adipose tissue was obtained from a nonobese subject (female, 59 yr old) while undergoing elective abdominal surgery. Total RNA was extracted using standard Trizol RNA isolation protocol (Life Technologies, Grand Island, NY). Approximately 10 μg of total RNA was labeled in a reverse transcription reaction in the presence of 100 μCi of [α-^{33}P] deoxycytosine 5'-triphosphate (DuPont-NEN, Boston, MA) using superscript II reverse transcriptase (Gibco-BRL).

Hybridization and Image Processing

Prehybridization was carried out in 10 mL of prehybridization solution (6X saline sodium citrate [SSC], 0.5% sodium dodecyl sulfate [SDS], 5X Denhardt's, 100 μg/mL of denatured salmon sperm DNA, 0.5 μg/mL of Cot-1 DNA, and 0.5 μg/mL of polydA) at 68ºC for 3 h. Overnight hybridization was carried out with the ^{33}P-labeled cDNA in the same condition. The membrane was washed three times at 68ºC with 2X SSC/1% SDS for 30 min, followed by 0.1X SSC/0.5% SDS at 68ºC for 15 min. The membrane was exposed on a PhosphorImager screen (Molecular Dynamics, Sunnyvale, CA) for 48 h. Radioactive intensity of each spot was linearly digitalized to 65,500 gray grade in a pixel size of 50 μm in an Image Reader and recorded using ImageQuant and Array Vision 5.1 (Molecular Dynamics). Normalization among arrays was based on the sum of background-subtracted signals from all genes on the membrane. To decrease experimental error, hybridizations were carried out two times, and the average gray levels of the four spots representing the same gene were calculated.

Data Analysis with Bioinformatics

The 400 EST clones with the highest signals on the membrane were further analyzed with bioinformatics. Known genes and known ESTs were differentiated with Blast software. The known genes were divided into eight categories according to their putative functions. Chromosome localization of identified genes was determined by searching the UniGene database (www.ncbi.nlm.nih.gov/UniGene), OMIM database (www.ncbi.nlm.nih.gov/omim), and Human Genome Draft (www.ncbi.nlm.nih.gov/Genome). The putative function of identified genes was determined by searching the PubMed database (www.ncbi.nlm.nih.gov/PubMed). The physical distance between two radiation hybrid markers was calculated based on information obtained from the Location Data Base (http://cedar.gene-tics.soton.ac.uk/pub) *(18)*.

Statistical Analysis

The observed chromosomal distribution of the genes expressed at high levels in visceral 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 χ^2 test with one degree of freedom *(2,10)*.

3T3-L1 Cell Line Culture

3T3-L1 fibroblasts were grown and differentiated into adipocytes in 35-mm culture dishes. Cells were grown to confluence in Dulbecco's minimal essential medium (MEM) containing 25 mmol/L of glucose and 10% calf serum at 37ºC in a humidified atmosphere containing 5% CO_2. Two days after confluence, cells were placed in MEM containing 25 mmol/L of glucose, 0.5 mmol/L of isobutylmethylxanthine, 1 μmol/L of dexamethasone, 10 μmol/L of insulin, and 10% fetal bovine serum (FBS) for 3 d and then in MEM containing 25 mmol/L of glucose, 10 μg/mL of insulin, and 10% FBS for 2 d. Thereafter, cells were maintained in and refed every 2 d with MEM, 25 mmol/L of glucose, and 10% FBS until they were used in experiments 10–14 d after the start of treatment, when from 90 to 95% of the cells exhibited adipocyte phenotype *(19)*.

RT-PCR and Semiquantitative RT-PCR

To confirm our cDNA array results, the visceral adipose tissue of 11 nonobese subjects (4 postmenopausal females, 2 premenopausal females, and 5 males) was obtained. Total RNA of the adipose tissues or 3T3-L1 adipocytes was extracted as previously described. Seven secreted proteins were selected and verified to be expressed in 3T3-L1 adipocytes with RT-PCR. Three genes, leptin, nonmuscle-type cofilin, and nuclear receptor LXR-α, were selected to verify whether they were expressed in different visceral adipose tissues with semiquantitative RT-PCR. For RT-PCR or semiquantitative RT-PCR analysis, first-strand cDNAs were synthesized from total RNA (1–2 μg) of the visceral adipose tissues or 3T3-L1 adipocytes using oligo-dT (Promega, Madison, WI). The resulting cDNAs were amplified by RT-PCR using the specific primers (Table 3). Human β-actin was used as internal control in the semiquantitative RT-PCR reactions. PCR conditions were as follows: 1 cycle of 94ºC, 3 min; 30 cycles of 94ºC, 30 s, 52–58ºC, 45 s, 72ºC, 45 s; and 1 cycle of 72ºC, 10 min.

Table 3
Primer Sequences and Product Size of Genes Used in RT-PCR or Semiquantitative RT-PCR

Gene name[a]	Forward primer	Reverse primer	Product size (bp)	Annealing temperature (°C)
m-TSG-6	TGGTCGTCCTCCTTTGCTTATG	AGACACCACCACACTCCTTTGC	414	58
m-FIGF	AAATGTCTTCCGGTGTGGAGG	TACAGACGCACTCACAGCGATC	410	58
m-Angiogenin	TTGTTCTTGATCTTCGTGCTGG	ATGACTCATCGAAGTGGACCG	403	58
m-CRP	TGGAAGCAGAGTCAAAGAAGCC	TCACATCTCCGATGTCTCCCAC	414	58
m-MCH	CAAAAATGATGAGAGCGGCTTC	ACCAGCAGGTATCAGACTTGCC	312	58
m-SCG-II	TCGTGGTATGGTAGAGGCCTTG	TTTGCTCACCTTGGCCAGTCTC	436	58
m-CRH	GCAGAGCAGTTAGCTCAGCAAG	TGCACATAGAAACTAAGCGTGAAC	306	58
h-leptin	GCTGATGCTTTGCTTCAAATCC	GGTAATTTTGGCCTTGCTTGG	300	52
h-cofilin	GTGCCCTCTCCTTTTCGTTTCC	AGCATCTTGACAAAGGTGGCG	252	58
h-LXR-α	AACCCCATCTTCGAGTTCTCC	TCACCAGTTTCATTAGCATCCG	229	56
h-β-actin	TCCATCATGAAGTGTGACGTGG	TGTGCAATCAAAGTCCTCGG	516	52–58

[a]m, mouse; h, human.

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