Gene Expression Profile of Human Skeletal Muscle and Adipose Tissue of Chinese Han Patients with Type 2 Diabetes Mellitus[1]

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Objective To study the differential patterns of gene expression in skeletal muscle and adipose tissue between type 2 diabetes mellitus (T2DM) patients and healthy subjects using DNA microarray analysis. **Methods** T2DM patiens were divided into female group, young male group and old male group. DNA microarray analysis and quantitative real-time PCR were carried out to analyze the relation between gene expressions and T2DM. **Results** The mRNA expression of 298, 578, and 350 genes was changed in the skeletal muscle of diabetes mellitus patients compared with control subjects. The 1320, 1143, and 2847 genes were modified in adipose tissue of the three groups. Among the genes surveyed, the change of 25 and 39 gene transcripts in skeletal muscle and adipose tissue was $\geqslant$2 folds. These differentially expressed genes were classified into 15 categories according to their functions. **Conclusion** New genes are found and T2DM can be prevented or cured.

Key words: Type 2 diabetes mellitus (T2DM); Microarray technology; Skeletal muscle; Adipose tissue

INTRODUCTION

Type 2 diabetes mellitus (T2DM), a metabolic disease involving abnormal regulation of carbohydrate and lipid metabolism by insulin, accounts for about 95% of all cases of diabetes mellitus and increases rapidly due to the epidemic of obesity. T2DM has many complications, including kidney failure, ischemic heart disease, loss of sight, stroke and increased overall mortality. The rapidly increasing diabetes mellitus (DM) is becoming a serious threat to human health in all parts of the world[1]. Although genetic factors are associated with the disease, it has been shown that acquired factors, such as obesity, sedentary life style and hormone excess, also play an important role in its onset. A substantial increase in the prevalence of DM among the migrant individuals moving to a different environment has been reported, suggesting that there is a potential interaction between genetic and environmental factors[2]. A polygenic basis has thus been proposed for the pathogenesis of T2DM. The leading candidate gene causing T2DM remains to be identified. Therefore, identification of genes involved in DM and design of effective strategies for prediction and prevention of the disease and its devastating complications are of critical importance[3].

With the completion of sequencing of human genome, analysis of relative expression levels of these genes has become a major focus in genome

[1]This study was supported by the National High Technology Research and Development Program of China (863 Program No. 2001AA221161) and the National Key Technologies R & D Program of China (No. 2002BA711A05).

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0895-3988/2009
CN 11-2816/Q

research. In diabetes research, profiles of gene expression in critical tissues may provide insight information. DNA microarray, a newly developed high technology, can be used to solve biology puzzle with automated and high throughput features[4]. The efforts to identify the candidate genes of T2DM have been mostly focused on cell culture *in vitro* or animal models[5-6]. Little is known about the transcriptional changes *in vivo*, particularly in skeletal muscle and adipose tissue of DM patients, which are the main sites of insulin-dependent glucose disposal and the major site of insulin resistance in T2DM patients. Under physiological conditions, insulin-stimulated glucose metabolism occurs mainly in skeletal muscle (>80%) and adipose tissue (5%-10%)[7]. Most of the current studies focused on a small number of selected genes and did not give a global view of the altered gene expression in T2DM patients. This study was to identify the differentially expressed genes in skeletal muscle and adipose tissue of T2DM patients and normal subjects in Chinese Han population. This approach we used can examine the mRNA expression levels of several thousand genes simultaneously, identify several potential candidate genes associated with the pathogenesis of T2DM, complement the ongoing genomic linkage and positional cloning analysis of susceptibility genes linked to T2DM by potentially identifying differentially expressed genes located in chromosomal regions.

MATERIALS AND METHODS

Subjects

Fifteen Chinese patients with T2DM were recruited from Peking Union Medical College Hospital, and 10 healthy subjects with no family history of DM served as a control group. The study protocol was reviewed and approved by the Ethics Committee of Peking Union Medical College Hospital according to the Guidelines of the World Medical Association prior to its implementation. Informed consent was obtained after the patients were informed of the nature, purpose and possible risks of the study. The metabolic characteristics of the study population are shown in Table 1. The subjects were classified based on diabetic and nondiabetic manifestations and matched for sex and age to minimize the differences. The patients were divided into female group, young male group and old male group according to their age and sex. Diagnosis of T2DM was made according to World Health Organization (WHO) criteria[8]. Briefly, patients were diagnosed with DM if they met one of the following criteria: treatment with oral hypoglycemic medications, fasting plasma glucose higher than 7.8 mmol/L, and 2-h postchallenge (75 g glucose) glucose higher than 11.1 mmol/L. Other types of DM (e.g., type 1 DM, maturity-onset DM, and mitochondrial DM) were excluded from the study following standard clinical criteria. Patients with high blood pressure, coronary heart disease, or atherosclerosis were also excluded from this study.

Samples of skeletal muscle and adipose tissue obtained at surgical operation were cleaned, rinsed in a sterile 0.9% NaCl solution, immediately frozen in liquid nitrogen, and stored at -80 ℃ for total RNA preparation.

RNA Preparation and Array Hybridization

Total RNA was isolated from frozen skeletal muscle homogenized in buffer RLT using a RNeasy fibrous tissue mini kit (QIAGEN Sciences, Maryland, USA). Since it was difficult to isolate total RNA from fibrous tissues of skeletal muscle, heart and aorta due to the abundance of contractile proteins, connective tissue and collagen, proteinase K was used to remove these proteins. RNeasy lipid tissue mini kit (QIAGEN Sciences, Maryland, USA) was used to isolate total RNA from adipose tissues. mRNA was subsequently isolated using an oligotex mRNA midi kit (QIAGEN Sciences, Maryland, USA). The isolated mRNA (1 μg) was converted to cDNA using reverse transcriptase (Superscript First-Strand Synthesis System: Invitrogen Life Technologies) and labeled with α-^{33}P.

Complementary DNAs (cDNAs) were hybridized overnight at 68 ℃ to the microarray consisting of 18 000 PCR-amplified full-length genes, spotted onto the 0.45-μm-pore-size nylon transfer membranes [Nytran SuPerCharge（SPC），Schleicher & Schuell，Keene, NH] using mechanical microspotting technology (BG600, Biorobotics, UK), and cross-linked by ultraviolet light treatment with a CL1000 ultraviolet cross-linker (UVP, Inc., Upland, Calif.).

Microarray Data Analysis

Microarrays were scanned and quantitatively analyzed using a FLA3000 fluorescent image analyzer (Fuji Photo Film, Tokyo, Japan). The acquired images were analyzed using an array gauge (Fuji Photo Film, Tokyo, Japan) and Microsoft Access software. To control the backgrounds, the signal intensity of each spot was corrected by subtracting the background signals of each matrix.

The genes showing ≥2.0-fold changes (increase or decrease) in T2DM patients compared with control subjects were defined as differentially expressed genes. Hierarchical clustering of these genes in skeletal muscle and adipose tissue was accessed as

previously described[9-10]. Hierarchical clustering is an agglomerative approach in which single expression profiles are joined to form groups, and further joined until the complete process, forming a single hierarchical tree by visualization using "Tree View" (http://genexpress.stanford.edu). The gene cluster data were presented graphically as colored images, and the genes analyzed were arranged in order by the clustering algorithm so that genes with the most similar expression patterns were adjacent to each other.

Quantitative Real-time PCR

Quantitative real-time PCR (Q-RT-PCR) was performed to validate differentially expressed genes. cDNAs were purified using a QIAquick nucleotide removal kit (QIAGEN GmbH, D-40724 Hilden) and subsequently amplified with the specific primers (an initial denaturation at 95 ℃ for 10 min, followed by 40 cycles of PCR amplification, each consisting of denaturation at 95 ℃ for 15 s, annealing and extension at 60 ℃ for 1 min). The primer pairs for each cDNA/EST were designed using Primer Express software (Applied Biosystems, Foster City, CA, USA). Quantitative RT-PCR was performed using the ABI Prism® 7000 sequence detection system and the SYBR Green PCR Master Mix kit (ABI, Warrington, UK). β-actin was used as an internal control for normalization.

Statistical Analysis

All data were expressed as $\bar{x} \pm s$. Statistical significance was determined by Students' *t* test. $P<0.05$ was considered statistically significant.

RESULTS

Blood Chemistry

Plasma glucose and insulin levels in all subjects were measured under fasting condition. The plasma glucose and insulin levels were significantly higher in DM patients than in control subjects (Table 1).

TABLE 1

Characteristics of the Controls and T2DM Patients

	Skeletal Muscle		Adipose Tissue	
	Control	T2DM	Control	T2DM
Subjects (n)	10	15	9	10
Sex	5 F, 5 M	2 F, 13 M	4 F, 5 M	2 F, 8 M
Age (Years)	64.7±11.8	67.9±11.1	65.0±12.5	67.9±8.3
BMI (kg/m^2)	26.2±4.0	26.3±2.4	25.9±4.1	26.4±2.1
Average Glucose (mg/dL)	86.9±12.2	235.1±37.0*	86.6±12.9	247.2±30.2*
Insulin Level (μU/mL)	7.9±2.2	16.5±3.4*	7.8±2.4	16.2±3.3*

Note. T2DM: type 2 diabetes mellitus; F: female; M: male. Data are expressed as $\bar{x} \pm s$. *$P<0.001$ *vs* controls.

Analysis of cDNA Microarray Data

Out of the 18 000 genes and ESTs in skeletal muscle of T2DM patients, the 298, 578, and 350 genes showed ⩾2 fold changes in the female, young and old male groups, respectively. The 1320, 1143, and 2847 genes were differentially expressed in the female, young and old male groups, respectively. The expression levels of the 25 and 39 genes were ⩾2 folds in the skeletal muscle and adipose tissue of the three groups compared with the normal subjects (Tables 2A and 2B).

These differentially expressed genes were involved in various biological processes. Based on the functions of encoding proteins, the genes were classified into 15 categories (including oncogene/tumor-suppressor, iron channel/ transport, cell cycle, cytoskeleton, apoptosis, stress, DNA synthesis/ repair/recombination, DNA binding /transcription, receptor, immune, metabolism, cell signaling/ communication, protein expression, development, cell differentiation) and labeled as HC01-HC15 (Fig. 1). Hierarchical clustering was used to access the differentially expressed genes in skeletal muscle and adipose tissue (Fig. 2).

Q-RT-PCR Verification

Q-RT-PCR was carried out to validate the differentially expressed genes identified in microarray experiments. Using this approach, the changes in mRNA levels of 4 genes (IGF2, RORC, PTPN1, and Pkm2) were quantified (Table 3). The changes detected by RT-PCR confirmed the microarray data.

TABLE 2A

Significant Changes of Gene Expression in Skeletal Muscle of T2DM Patients Compared to Controls

Gene Name	Genbank Number	F T2DM/ Con	MY T2DM/ Con	MO T2DM/ Con
Oncogene/Tumor-suppressor				
Human Pyruvate Kinase, Muscle（PKM2）	NM_002654	↑ 3.18	↑ 4.89	↑ 2.17
Cell cycle				
Human Geminin-DNA Replication Inhibitor	NM_015895	↓ 2.17	↓ 3.33	↑ 2.54
Cytoskeleton				
Human Troponin C2, Fast (TNNC2)	NM_003279	↑ 2.50	↑ 12.60	↓ 2.78
Stress				
Human Growth Arrest and DNA-damage-inducible, Beta (GADD45B)	NM_015675	↓ 2.38	↓ 2.33	↑ 4.75
Human Topoisomerase (DNA) I (TOP I)	NM_003286	↑ 2.10	↑ 3.80	↑ 2.02
DNA Synthesis/Repair/Recombination				
Human Epithelial Membrane Protein 1	NM_001423	↓ 2.50	↓ 3.03	↑ 2.03
DNA Binding/Transcription				
Human SNC73 Protein (SNC73)	AF067420	↓ 3.57	↓ 24.79	↑ 7.99
Human Transcription Factor EC	NM_012252	↓ 2.13	↓ 3.13	↑ 2.23
Immune				
Human Triosephosphate Isomerase 1 Gene (TPI1)	NM_000365	↑ 3.20	↑ 4.03	↑ 2.15
Beta-2-microglobulin (B2M)	NM_004048	↓ 2.22	↓ 3.23	↑ 3.14
Cell Signaling/Communication				
Human Collagen, Type VI, Alpha 1	NM_001848	↑ 2.37	↓ 5.88	↑ 3.53
Thyroid Hormone Receptor Associated Protein 2	NM_015335	↓ 2.13	↓ 2.78	↑ 2.06
Human Protein Tyrosine Phosphatase Type IVA, Member 3	NM_032611	↑ 3.24	↑ 3.58	↑ 2.02
Human Sec13-like Protein (SEC13L)	NM_031216	↑ 5.60	↑ 2.59	↑ 2.89
Metabolism				
Human Hemoglobin, Delta (HBD)	NM_000519	↓ 3.70	↓ 6.25	↓ 3.03
Human Hemoglobin, Beta (HBB)	NM_000518	↓ 3.70	↓ 6.67	↓ 2.86
Human Acyl-Coenzyme A Oxidase 3, Pristanoyl (ACOX3)	NM_003501	↑ 2.18	↑ 3.36	↑ 3.39
Human Hemoglobin, Alpha 1 (HBA1)	NM_000558	↓ 3.13	↓ 3.33	↓ 2.63
Human Spermidine/Spermine N1-acetyltransferase	NM_002970	↓ 2.63	↓ 4.55	↑ 2.45
MAK10 Homolog, Amino-acid N-acetyltransferase Subunit (MAK10)	NM_024635	↑ 2.05	↑ 2.04	↑ 2.60
Protein expression				
Human Eukaryotic Translation Elongation Factor 1 Alpha 1	NM_001402	↓ 2.04	↓ 3.33	↑ 2.12
Cell Differentiation				
Rho GTPase Activating Protein 21	NM_020824	↓ 2.13	↓ 3.70	↑ 2.66
Unclassified				
Human Chromosome 7 Open Reading Frame 23	BC002837	↓ 2.56	↓ 4.00	↑ 2.35
Clone RP11-77F13 in chr.10	AL133327	↑ 2.22	↑ 5.22	↑ 2.52
Human Chromosome 9 Open Reading Frame 85	NM_198394	↓ 2.00	↓ 3.33	↑ 2.05

TABLE 2B

Significant Change of Gene Expression in Adipose Tissue of T2DM patients Compared to Controls

Gene Name	Genbank Number	F T2DM/ Con	MY T2DM/ Con	MO T2DM/ Con
Oncogene/Tumor-suppressor				
Human Cadherin, EGF LAG Seven-pass G-type Receptor 2	NM_001408	↓ 2.00	↓ 2.10	↓ 2.77
Human Integrin, Beta 2, Lymphocyte Function-associated Antigen 1	NM_000211	↓ 9.74	↓ 2.09	↓ 4.47
Human Nuclear Receptor Subfamily 2, Group F, Member 6 (NR2F6)	NM_005234	↓ 3.66	↓ 2.15	↓ 3.67
Human FGFR1 Oncogene Partner (FGFR1OP)	NM_007045	↓ 2.01	↑ 2.51	↓ 2.22
Human Glycoprotein (Transmembrane) nmb	NM_002510	↓ 3.73	↑ 3.28	↓ 2.86
Iron Channel/Transport				
Human Gap Junction Protein, Alpha 1, 43kDa (Connexin 43) (GJA1)	NM_000165	↓ 3.16	↓ 2.24	↓ 2.53
Cytoskeleton				
Human Insulin-Like Growth Factor 2 (Somatomedin A) (IGF2)	NM_000612	↓ 2.04	↓ 2.34	↓ 3.15
Human Dynein 2 Light Intermediate Chain (D2LIC)	NM_016008	↓ 2.21	↓ 2.51	↓ 3.86
Stress				
Human Carboxylesterase1 (Monocyte/Macrophage Serine Esterase 1) (CES1)	NM_001266	↓ 2.03	↑ 2.46	↓ 6.71
DNA Synthesis/Repair/Recombination				
Human Protein Tyrosine Phosphatase, Non-receptor Type 1 (PTPN1)	NM_002827	↑ 2.12	↑ 2.33	↑ 2.60
DNA Binding/Transcription				
Human SEC31-like 2 (*S. cerevisiae*) (SEC31L2)	NM_015490	↓ 2.16	↓ 2.92	↓ 2.31
Human RAR-related Orphan Receptor C (RORC)	NM_005060	↑ 2.63	↑ 2.35	↑ 2.84
Human Early Growth Response 3 (EGR3)	NM_004430	↑ 4.4	↓ 2.1	↓ 2.7
Cell receptor				
Human Benzodiazepine Receptor (Peripheral) Associated Protein1 (BZRAP1)	NM_004758	↓ 2.77	↑ 3.19	↓ 3.86
Human Leukocyte-associated Ig-like Receptor 1 (LAIR1)	NM_002287	↓ 2.24	↓ 2.43	↓ 4.16
Immunity				
Human Immunoglobulin Kappa Constant (IGKC)	BC073779	↑ 9.15	↓ 2.20	↑ 44.18
Human Immunoglobulin Light Chain Variable Domain	U86790	↑ 13.73	↓ 2.55	↑ 96.62
Human Exosome Component 10 (EXOSC10)	NM_002685	↑ 14.85	↓ 2.22	↑ 46.02
Human Junctional Adhesion Molecule 3 (JAM3)	NM_032801	↓ 2.21	↓ 3.13	↓ 4.78
Cell Signaling/Communication				
Human Sprouty Homolog 1, Antagonist of FGF Signaling	NM_005841	↓ 2.37	↓ 2.08	↓ 2.84
Human SMAD, Mothers against DPP Homolog 5	NM_005903	↓ 2.36	↓ 2.24	↓ 7.20
Metabolism				
Human Aldehyde Dehydrogenase 1 Family, Member A1 (ALDH1A1)	NM_000689	↑ 2.09	↑ 4.06	↓ 2.96
Human Alcohol Dehydrogenase IB (class I), Beta Polypeptide (ADH1B)				
Human Monoglyceride Lipase (MGLL)	NM_000668	↑ 2.81	↑ 2.70	↓ 3.79
	NM_007283	↓ 3.32	↑ 2.19	↓ 3.36
Human Acetyl-Coenzyme A Acyltransferase 2 (ACAA2)	NM_006111	↑ 2.29	↑ 2.89	↓ 3.65
Human Procollagen-proline, 2-oxoglutarate 4-dioxygenase (Proline 4-hydroxylase), Alpha Polypeptide II (P4HA2)	NM_004199	↓ 2.07	↑ 3.06	↓ 7.78
Human Inorganic Pyrophosphatase 2 (PPA2)	NM_176869	↓ 3.22	↓ 2.28	↓ 3.24
Protein Expression				
Human Ariadne Homolog, Ubiquitin-conjugating Enzyme E2 Binding Protein,1 (Drosophila) (ARIH1)	NM_005744	↓ 2.09	↓ 2.52	↓ 3.97
Human HLA Class II Region Expressed Gene KE2 (HKE2)	NM_014260	↓ 2.20	↓ 2.04	↓ 6.05
Unclassified				

(to be continued)

(continued)

Gene Name	Genbank Number	F T2DM/ Con	MY T2DM/ Con	MO T2DM/ Con
Human DNA Sequence from Clone RP11-251017 on Chromosome 9	AL137070	↓ 2.04	↓ 2.11	↓ 2.80
Human Chromosome 8, Clone RP11-140B16	AC021305	↓ 3.88	↓ 3.15	↓ 2.95
Human mRNA for KIAA0280 Gene	XM_370635	↓ 2.45	↑ 2.06	↓ 3.00
Human KIAA0676 Protein	NM_198868	↓ 2.01	↑ 2.39	↓ 3.55
Human Stomatin (EPB72)-like 1 (STOML1)	NM_004809	↓ 6.86	↓ 2.08	↓ 8.64
Human PAC Clone RP5-850I1 from 7	AC006009	↓ 2.10	↓ 2.13	↓ 6.80
Human 12 BAC RP11-285E23	AC010205	↓ 6.53	↑ 2.90	↓ 55.48
Human Chromosome 1 Clone RP11-411H5	AC104334	↓ 3.57	↓ 2.72	↓ 16.18
Human 12 BAC RP11-444B24	AC007458	↓ 2.33	↑ 2.26	↓ 3.75
Human 12 BAC RP11-370I10	AC024257	↓ 2.62	↓ 2.27	↓ 2.61

Note. F T2DM/Con, T2DM patients in female group *vs* controls; MY T2DM/Con, T2DM patients in young male group *vs* controls; MO T2DM/Con, T2DM patients in old male group *vs* controls. ↑ represents up-regulated; ↓ represents down-regulated. $P<0.05$ *vs* controls.

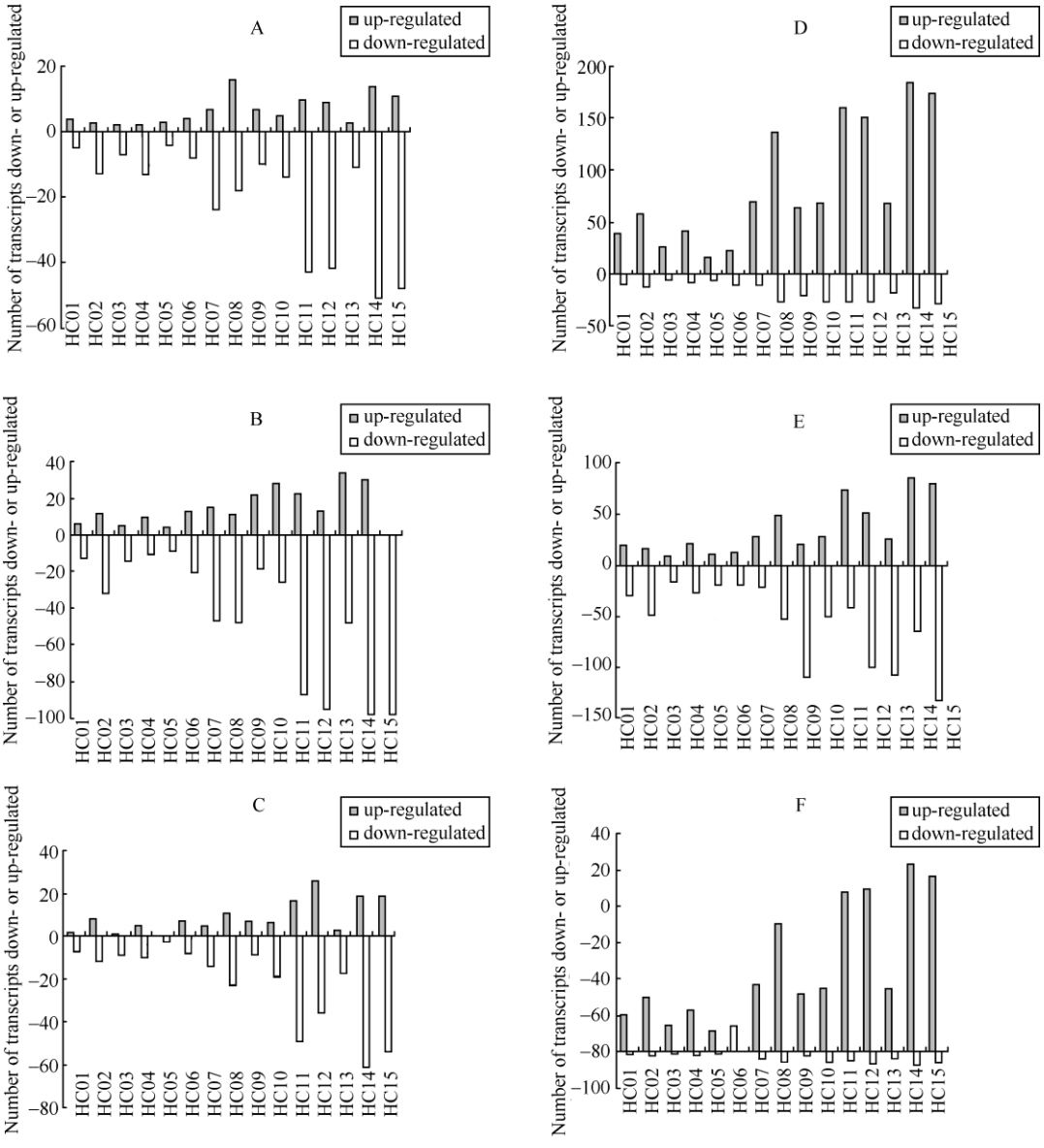

FIG. 1. Number of differentially expressed genes in skeletal muscle of DM patients in female group (A), young male group (B), old male group (C), and in adipose tissue of DM patients in female group (D), young male group (E), old male group (F).

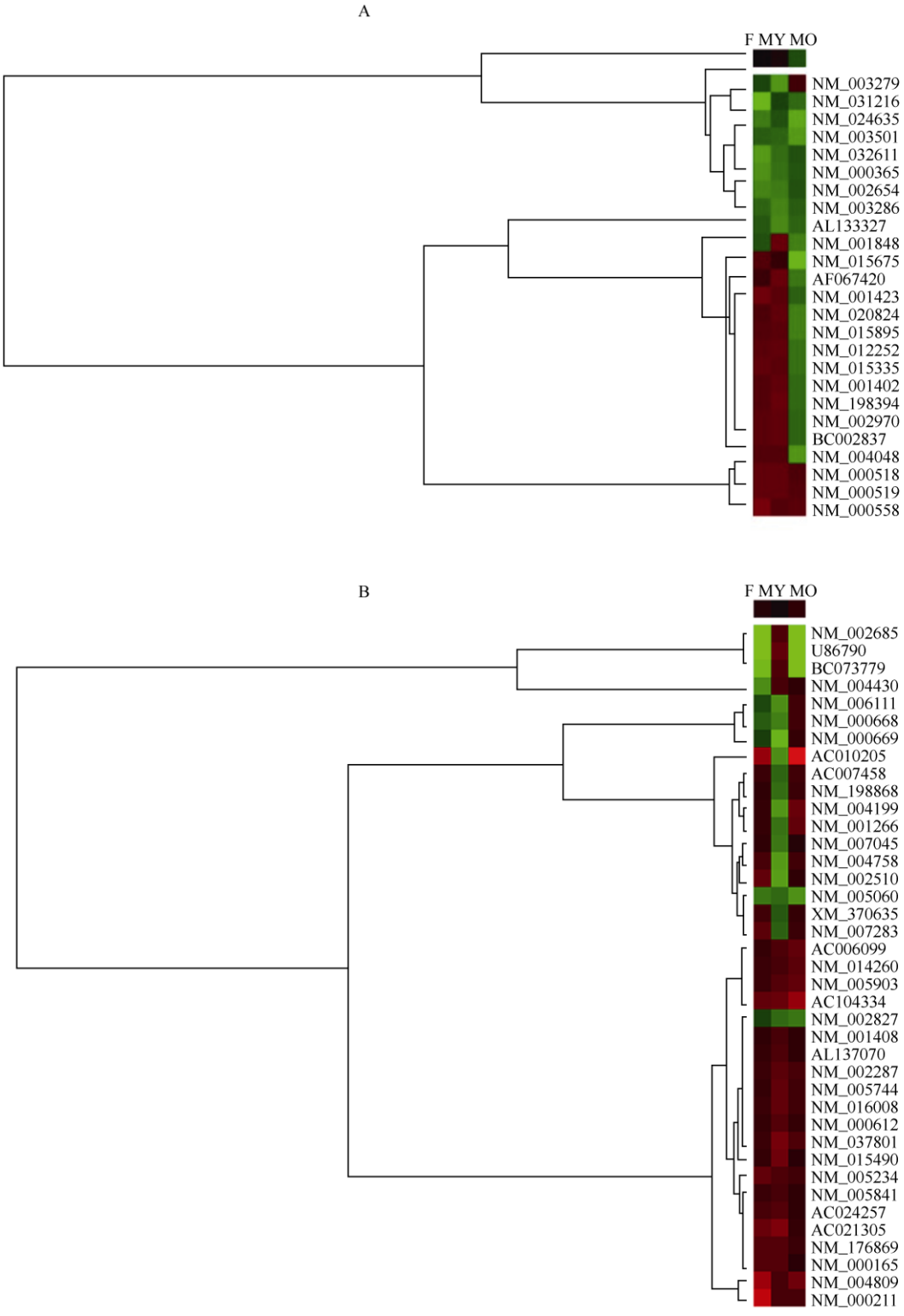

FIG. 2. Cluster analysis of differentially expressed genes in skeletal muscle and adipose tissue of T2DM patients in comparison with controls showing hierarchical clustering of differentially expressed genes in skeletal muscle of T2DM patients (A) in adipose tissue of T2DM patients (B). Genes down-regulated in T2DM patients are indicated in *red*, genes up-regulated in T2DM patients are indicated in *green*. F, MY, and MO represent ration of gene expression between the control and T2DM patients in the female, young and old male groups respectively.

TABLE 3

Comparison of Microarray Results with Q-RT PCR Results ($\bar{x} \pm s$)

Gene	Female		Young Male		Old Male	
	Microarray	RT-PCR	Microarray	RT-PCR	Microarray	RT-PCR
IGF2	0.49	0.42±0.05	0.43	0.40±0.06	0.32	0.29±0.04
RORC	2.6	2.3±0.09	2.4	2±0.09	2.8	3.1±0.17
PTPN1	2.1	2±0.11	2.3	2.1±0.14	2.6	3.3±0.22
Pkm2	3.2	3.1±0.16	4.9	5.5±0.37	2.2	2.0±0.11

Note. Microarray and RT-PCR protocols are outlined in the Methods section. Results are expressed as fold change of 4 selected genes in T2DM patients compared with controls. There was no statistical significance in fold change of genes using the two methods (P>0.05).

DISCUSSION

Adipose tissue (WAT) is considered as a multifunctional organ. In addition to the central role of lipid (energy) storage, it has a major endocrine function secreting several hormones (e.g., leptin and adiponectin) and different protein factors and signals collectively referred to as adipokines with fatty acids and other lipid moieties. There is evidence that adipose tissue dysregulation plays an important role in the development of metabolic disorders like insulin-resistance, DM and their associated complications. Skeletal muscle is the major site of glucose utilization and peripheral insulin action in response to nutritional challenges. It has been shown that dysregulation of fatty acid and lipid metabolism influences insulin signaling at various levels, thus leading to impaired glucose tolerance, decreased fatty acid oxidation and glycogen synthesis, and eventually insulin resistance and T2DM. Therefore, we studied the changes of gene expression profiles in adipose tissue and muscle of T2DM patients compared with normal individuals.

In this study, DNA microarray was performed to identify the differential gene expression profiles in skeletal muscle and adipose tissue of T2DM patients and normal subjects. Fifteen patients with T2DM were selected and 10 healthy subjects with no family history of DM served as a control group. The T2DM patients were divided into female group, young male group and old male group according to their age and sex. The analysis of gene expression profiles showed that the expression levels of the 25 and 39 genes were $\geqslant$ 2-fold higher (upregulation or downregulation) in three groups of T2DM patients. The down-regulated genes might represent the candidate genes responsible for some of the preventable chronic complications of T2DM. In contrast, the up-regulated genes might represent the candidate genes responsible for the pathogenesis of T2DM. These differentially expressed genes could be classified into 15 categories according to their functions. Various genes, biological processes and signaling pathways took part in the development of T2DM.

In the adipose tissue, insulin-like growth factor 2 (IGF2), RAR-related orphan receptor C (RORC), and protein tyrosine phosphatase non-receptor type 1(PTPN1) showed consistent changes in T2DM patients, suggesting that these genes participate in the development of T2DM.

IGF2 plays a key role in fetal growth and development[11], and is associated with lipid metabolism and body weight regulation. Low levels of circulating IGF2 are associated with an increased risk of weight gain and obesity in a population with normal glucose tolerance[12]. Meanwhile, variations in the IGF2 gene are associated with overfeeding-induced metabolic changes and decreased insulin sensitivity[13]. One of the mechanisms underlying the participation of IGF2 in metabolism is to change the glucose transporter gene expression and the serum glucose levels.

The protein encoded by RORC, a DNA-binding transcription factor and a member of the NR1 subfamily of nuclear hormone receptors, has been mapped to a well replicated T2DM susceptible region, 1q21-q23 region. RORC is considered as a positional and functional candidate for T2DM based on its expression pattern in adipose tissue, skeletal muscle, pancreatic islets and liver[14], as well as its potential to form heterodimers with peroxisome-proliferator activated receptor gamma. The protein encoded by PTPN1 is a member of the protein tyrosine phosphatase (PTP) family and acts as a negative regulator of insulin signaling by dephosphorylating the phosphotryosine residues of insulin receptor kinase[15-16]. Genomic variation in PTPN1 is

associated with insulin sensitivity contributing to central fat and metabolic syndrome traits[17]. A study in PTPN1-deficient mice suggested that PTPN1 plays a major role in negative feedback of insulin sensitivity and fuel metabolism, and can be used as a potential therapeutic target for the treatment of T2DM[18].

In our study, the expression of IGF2 was down-regulated, while RORC and PTPN1 were up-regulated in adipose tissue of T2DM patients compared with the controls, indicating that these differentially expressed genes are correlated with the etiology of T2DM.

Muscle is the major site for insulin action, and T2DM is characterized by muscle insulin resistance. In our study, M2-type pyruvate kinase (PKM2) was up-regulated in skeletal muscle of T2DM patients compared with the controls. The protein encoded by this gene is a pyruvate kinase (PK), a key regulatory glycolytic enzyme that catalyzes the transfer of a phosphoryl group from phosphoenolpyruvate (PEP) to ADP, produces ATP and pyruvate. There are 4 distinct PK isoenzymes (M1, M2, L, and R) in mammals, which are expressed in a tissue-specific manner and can be activated by Fru-1, 6-bisphosphate (Fru-1, 6-P2). There are two major forms of Pkm2 in cells: a highly active tetramer and a less active dimer. High glucose levels can accelerate the conversion of dimeric form into tetrameric form, thus increasing the enzymatic activity of PKM2[19-20]. The up-regulation of PKM2 in skeletal muscle of T2DM patients can be regarded as a compensatory mechanism underlying T2DM in response to high glucose levels. We demonstrated, in a previous study, that pantothenate kinase 4 (PANK4) is directly inter-reacted with PKM2, and over-expression of PANK4 increases PKM2 activity by conversing dimer into tetramer [21], thus modulating glucose metabolism.

Most of the differentially expressed genes in our study belong to the categories of metabolism and cell signaling/communication. Triosephosphate Isomerase 1 (TPI1) is a glycolytic enzyme that catalyzes the conversion of D-glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. Carboxylesterase 1 (CES1) hydrolyzes aromatic and aliphatic esters and is necessary for cellular cholesterol esterification. Monoglyceride lipase (MGLL) catalyzes the last step of TG hydrolysis and releases glycerol and FFA. Spermidine/spermine N1-acetyltransferase (SAT) is a rate-limiting enzyme in the catabolic pathway of polyamine metabolism. Acetyl-coenzyme A acyltransferase 2 (ACAA2) is one of the five types of thiolase in mammalian tissues and catalyzes the last step of the mitochondrial fatty acid beta-oxidation spiral. Aldehyde dehydrogenase 1 family, member A1 (ALDH1A1) and alcohol dehydrogenase 1B (ADH1B) are oxidative and dehydrogenic enzymes in the pathway of alcohol metabolism. Eukaryotic translation elongation factor1 alpha1 (EEF1A1) is involved in the binding of aminoacyl-tRNAs to 80S ribosomes and GTP is hydrolyzed into GDP during this process. Sprouty homolog 1 (SPRY1) participates in the signaling pathway of Jak/Stat, phosphorylated SPRY proteins bound to the adaptor protein Grb2 and inhibits the recruitment of Grb2-Sos complex to Frs2 or Shp2.

There is evidence that production of adipokines by adipose tissue is dys-regulated in obesity and is associated with insulin resistance, metabolic syndrome and T2DM. Since these metabolic and vascular pathological conditions are associated with chronic low-grade inflammation, it is not surprising to see changes in genes involved in stress and immune responses. In the present study, growth arrest and DNA damage-inducible gene beta, GADD45B, a stress-sensitive regulator of the NF-κB signaling pathway, was down-regulated in skeletal muscle of T2DM patients compared with the controls. One mechanism by which NF-κB protects cells is to down-regulate the JNK cascade through the transcriptional activation of GADD45B[22]. Meanwhile, in adipose tissue, a positive regulator of I-κ B/NF-κ B cascade, gap junction protein, alpha 1 (GJA1), is down-regulated in T2DM patients compared with controls[23].

Considering the fact that the pathogenesis of DM is related with sex and age, T2DM patients and normal controls in our study were grouped according to their age and sex (Tables 2A and 2B). Previous works characterizing the large-scale patterns of gene expression in human tissues are limited. The influence of age, sex, and strength training (ST) on large-scale gene expression patterns in vastus lateralis muscle biopsies has been studied using high-density cDNA microarray, showing that sex has the strongest influence on muscle gene expression, while age and ST contribute to different gene expressions, suggesting that in the analysis of skeletal muscle gene expression, sex, age, and habitual physical activity must be addressed, with sex being the most critical variable[24].

In summary, the 25 and 39 gene transcripts involved in a variety of functions are altered in skeletal muscle and adipose tissue of T2DM patients. IGF2, RORC, and PTPN1 in adipose tissue, and PKM2 in muscle, are of particular interest. Genes involved in metabolism and stress/immune responses are also worthy to be further investigated.

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(*Received November* 21, 2008 *Accepted May* 25, 2009)