MOLECULAR & CELLULAR TOXICOLOGY, Vol. 2, No. 4, 266-272, December 2006

Gene Expression Profile in Carpal Tunnel Syndrome Patients

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Accepted 7 December 2006

Abstract

Carpal tunnel syndrome (CTS) is one of the most common disorders by under pressure of the median nerve at the wrist in these days. However, pathological mechanism of CTS is unknown. We carried out this study to identify the changes of gene expression and to evaluate possible mechanism in CTS. 120 CTS patients and 30 control patients were included in this study. Patients with a history of diabetes, hypertension, thyroid diseases, and arthritis were excluded. CTS patients were divided to three experimental groups-Mild, Moderate, and Severe group-according to elecrodiagnosis. Radioactive cDNA microarrays (Nylon membrane including 1,152 genes) were used to examine the difference of gene expression profile in CTS. We identified up-regulated genes by more than 2.0 value of z-ratio, and down-regulated genes by less than -2.0 value of z-ratio. 20 genes such as the ITGAL, ITGAM, PECAM1, VIL2, TGFBR2, RAB7, RNF5 and NFKB1 were up-regulated, and 28 genes such as PRG5, CASP8, CDH1, IGFBP5, CBX3, HREV107, PIN, and WINT2 were down-regulated. These genes were related with TGF beta signaling pathway, NF-Kb signaling pathway, antiapoptotic pathway and T cell receptor signaling pathway. However, there were no differences in gene expression profiles according to severities of symptoms. We suggest that CTS could be related with proinflammatory mechanism and antiapoptotic mechanism.

Keywords: Carpal tunnel syndrome, Gene expression, Proinflammatory, Antiapoptosis

Carpal tunnel syndrome (CTS) is one of the most common disorders by under pressure of the median nerve at the wrist in these days[1]. CTS is a condition characterized by pain, neurologic symptoms and functional limitation of the hand[2]. It has been known that it could be developed according to repeated using of wrist and could be treated almost completely by medical surgery.

The modern understanding of carpal tunnel syndrome has evolved from a variety of clinical and pathologic observation. Fibrosis of the subsynovial connective tissue is the most characteristic histopathologic finding in patients with carpal tunnel syndrome[3].

The diagnosis of carpal tunnel syndrome was based on both clinical and electrophysiological examination in all cases[4]. CTS can categorize according to the severity of median nerve damage. -Mild, moderate and severe.

There were a markedly higher number of fibroblasts with expression of transforming growth factor-β RI (TGF-βI) in the experimental group than control group. The increase in TGF-βI expression in the fibroblasts that this process leads to scarring and fibrosis and may thus plays a role in the etiology of carpal tunnel syndrome[5].

There are some reference, in connection with children and CTS. CTS is probably due to a combination of excessive lysosomal storage in the connective tissue of the flexor retinaculum and a distorted anatomy because of underlying bone dysplasia. According to this study, CTS is not only adulthood disorder but also childhood. CTS were related with a genetic condition[6].

As recent evidence has shown that chronic nerve compression (CNC) such as CTS induces a permeability change in neural vasculature. CNC provides a slow, sustained stimulus for macrophage recruitment, which may be responsible for the up-regulation of immunological NOS (iNOS) gene expression[7].

In this way, although there are many studies of CTS symptom, but pathological mechanism is not well-known. It is known that gene expression profiling using cDNA microarray is a useful first step toward understanding the functions of these known or novel genes[8]. Therefore, we carried out this study to identify the changes of gene expression and to evaluate possible mechanism in CTS.

Gene Expression Profile in CTS Patients

Using the cDNA microarray, we found that among 1,152 genes on the array membrane. Gene expression profiles of interest were up-regulated or down-regulated in experimental group when compared with control group. In these data, there were non-significantly data between symptoms, so we compared between control groups, mild and sever CTS groups.

The up- and down-regulated genes are listed in Table 2 and 3. Gene expression profiles showed that 36 genes were up-regulated in CTS patients group like as Notch (Drosophila) homolog 4, transforming growth factor and member RAS oncogene family. Gene expression profiles showed that 50 genes were down-regulated in CTS patients group like as spectrin alpha non-erythrocytic 1, p53-responsive gene 5 and caspase 8.

To show the relationships between the controls with experimental group, we used a hierarchical clustering and visualized up- and down-regulated genes (Figs. 1, 2). The expression level of gene can confirm in color,

Table 1. Average of control and patient's age.

	Control (n=2)	Patients		Significance
		Mild (n=10)	Severe (n=5)	
Age	51.5±0.7	48.2±8.0	53.0±4.3	N.S.

N.S.: Non-significant

Table 2. Up-regulated gene expression.

Up-regulated gene	Z-ratio	
	Mild	Severe
Notch (Drosophila) homolog 4 (NOTCH4)	2.72	2.52
transforming growth factor, beta receptor II (70-80 kD) (TGFBR2)	2.74	2.29
ring finger protein 5 (RNF5)	3.01	2.16
RAB5B, member RAS oncogene family (RAB5B)	2.35	2.13
cytochrome P450, 51 (lanosterol 14-alpha-demethylase) (CYP51)	−0.93	4.72
synaptosomal-associated protein, 23 kD (SNAP23)	−0.92	4.32
integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide) (ITGAL)	−1.09	2.78
solute carrier family 6 (neurotransmitter transporter, betaine/GABA), member 12 (SLC6A12)	1.22	2.68
CD24 antigen (small cell lung carcinoma cluster 4 antigen) (CD24)	−1.17	2.66
platelet/endothelial cell adhesion molecule (CD31 antigen) (PECAM1)	−1.13	2.58
general transcription factor IIF, polypeptide 1 (74 kD subunit) (GTF2F1)	0.66	2.55
villin 2 (ezrin) (VIL2)	−2.29	2.54
potassium voltage-gated channel, Shab-related subfamily, member 1 (KCNB1)	1.88	2.52
protein kinase C substrate 80K-H (PRKCSH)	0.96	2.42
syndecan binding protein (syntenin) (SDCBP)	0.34	2.32
nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105) (NFKB1)	0.80	2.22
cytochrome P450, subfamily IVA, polypeptide 11 (CYP4A11)	1.37	2.21
ribosomal protein, mitochondrial, S12 (RPMS12)	0.37	2.20
proprotein convertase subtilisin/kexin type 2 (PCSK2)	0.14	2.10
ubiquinol-cytochrome c reductase (6.4 kD) subunit (UQCR)	1.16	2.09
Human guanine nucleotide-binding regulatory protein (Go-alpha) gene	4.95	−1.45
insulin-like growth factor 2 receptor (IGF2R)	3.93	1.20
protein kinase C, beta 1 (PRKCB1)	3.45	−0.21
integrin, alpha M (complement component receptor 3, alpha; also known as CD11b (p170), macrophage antigen alpha polypeptide) (ITGAM)	3.27	0.26
recoverin (RCV1)	3.18	−1.27
caspase 4, apoptosis-related cysteine protease (CASP4)	3.06	−0.53
insulin-like growth factor 2 (somatomedin A) (IGF2)	2.94	1.53
dihydropyrimidinase (DPYS)	2.77	−0.58
RAB7, member RAS oncogene family (RAB7)	2.72	−0.50
mitogen-activated protein kinase kinase 4 (MAP2K4)	2.60	0.95
somatostatin (SST)	2.37	−0.26
EphA1 (EPHA1)	2.37	1.14
inositol 1,3,4-triphosphate 5/6 kinase (ITPK1)	2.32	0.24
prion protein (p27-30) (Creutzfeld-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia) (PRNP)	2.26	0.85
adrenergic, beta-2-, receptor, surface (ADRB2)	2.03	1.49
cytochrome P450, subfamily IIJ (arachidonic acid epoxygenase) polypeptide 2 (CYP2J2)	2.00	1.99

Table 3. Down-regulated gene expression.

Down-regulated gene	Z-ratio	
	Mild	Severe
mitogen-activated protein kinase kinase 3 (MAP2K3)	−3.75	−5.08
spectrin, alpha, non-erythrocytic 1 (alpha-fodrin) (SPTAN1)	−4.33	−4.83
ADP-ribosylation factor-like 3 (ARL3)	−3.82	−4.56
ubiquitin-conjugating enzyme E2I (homologous to yeast UBC9) (UBE2I)	−3.74	−4.05
p53-responsive gene 5 (PRG5)	−3.91	−3.93
integrin-linked kinase (ILK)	−2.63	−3.88
Homo sapiens clone 24703 beta-tubulin mRNA, complete cds	−2.73	−3.36
regulator of G-protein signalling 13 (RGS13)	−3.08	−3.13
syntaxin 5A (STX5A)	−2.51	−2.95
carboxypeptidase E (CPE)	−2.34	−2.73
ATPase, Na^+/K^+ transporting, alpha 2 (+) polypeptide (ATP1A2)	−2.61	−2.68
dynein, cytoplasmic, intermediate polypeptide 1 (DNCI1)	−2.71	−2.67
chloride intracellular channel 1 (CLIC1)	−2.05	−2.60
upstream transcription factor 2, c-fos interacting (USF2)	−2.47	−2.56
serine threonine protein kinase (KIAA0137)	−2.15	−2.43
Human DNA sequence from clone 1189B24 on chromosome Xq25-26.3	−2.45	−2.17
similar to rat HREV107 (HREV107)	−2.92	−2.03
guanine nucleotide binding protein-like 1 (GNL1)	−2.27	−2.02
protease, serine, 2 (trypsin 2) (PRSS2)	−1.81	−4.45
chromobox homolog 3 (Drosophila HP1 gamma) (CBX3)	−0.93	−2.70
glutamate receptor, ionotropic, AMPA 2 (GRIA2)	−1.57	−2.45
phosphatase and tensin homolog (mutated in multiple advanced cancers 1) (PTEN)	−1.17	−2.40
protein kinase, Y-linked (PRKY)	−1.11	−2.39
cadherin 1, E-cadherin (epithelial) (CDH1)	−0.53	−2.31
serine/threonine kinase 2 (STK2)	−0.83	−2.30
caspase 8, apoptosis-related cysteine protease (CASP8)	−1.71	−2.17
Rab9 effector p40 (RAB9P40)	−1.44	−2.13
insulin-like growth factor binding protein 5 (IGFBP5)	−0.70	−2.08
platelet-derived growth factor receptor, beta polypeptide (PDGFRB)	−1.27	−2.01
Human DNA sequence from clone 366N23 on chromosome 6q27	−5.33	−0.04
Rho GDP dissociation inhibitor (GDI) beta (ARHGDIB)	−3.97	−0.55
mannosidase, alpha, class 1A, member 1 (MAN1A1)	−3.48	0.91
ubiquitin-conjugating enzyme E2D 3 (homologous to yeast UBC4/5) (UBE2D3)	−3.39	−1.30
dynein, heavy chain beta-like (DNHBL)	−3.24	−1.17
insulin-like growth factor binding protein 2 (36kD) (IGFBP2)	−3.08	−1.62
collagen, type VI, alpha 3 (COL6A3)	−2.69	−0.30
wingless-type MMTV integration site family member 2 (WNT2)	−2.59	−1.71
chromosome 11 open reading frame 8 (C11ORF8)	−2.48	−1.08
guanine nucleotide binding protein (G protein), alpha z polypeptide (GNAZ)	−2.48	1.27
protein phosphatase 1, catalytic subunit, gamma isoform (PPP1CC)	−2.42	−1.38
v-akt murine thymoma viral oncogene homolog 1 (AKT1)	−2.38	−1.89
villin 2 (ezrin) (VIL2)	−2.29	2.54
cadherin 17, LI cadherin (liver-intestine) (CDH17)	−2.29	−1.15
ATP-binding cassette, sub-family B (MDR/TAP), member 2 (ABCB2)	−2.23	1.19
intercellular adhesion molecule 2 (ICAM2)	−2.20	−1.53
golgi SNAP receptor complex member 1 (GOSR1)	−2.17	−1.17
profilin 1 (PFN1)	−2.16	−0.37
dynein, cytoplasmic, light polypeptide (PIN)	−2.10	−1.00
guanine nucleotide binding protein (G protein), alpha transducing activity polypeptide 1 (GNAT1)	−2.06	−1.01
Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog (FGR)	−2.04	−1.29

relative to its median expression level across all samples. Red represented expression greater than the mean, green represents expression less than the mean and black represented the median expression level.

Discussion

Carpal tunnel syndrome is the most common entrapment neuropathy[5]. Although there are many stud-

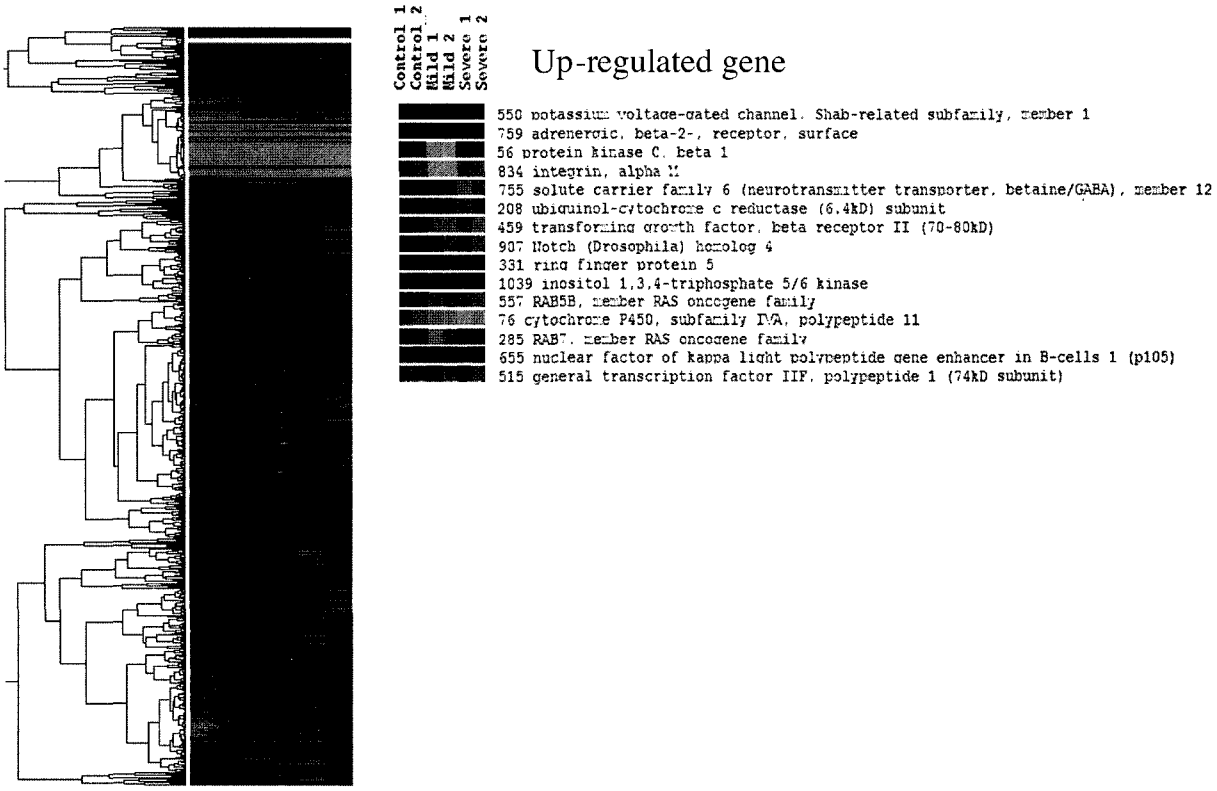

Fig. 1. Up-regulated gene between Control and CTS patients.

ies of CTS symptom, but pathological mechanism is not well-known. In these days, scientists are researching about CTS with amylodosis that is genetic disorder[9-11]. According to theses researches, CTS is related with genetic history and family history. Therefore, we suggested that CTS could be connected with gene expression. Accordingly, we identify the changes of gene expression and to evaluate possible mechanism in CTS using cDNA microarray.

Interleukins and other inflammatory factors have been implicated in the development of CTS[12]. Several researchers detected several macrophage-like cells around local blood vessels as well as invading the synovial tissues of CTS, and discovered that there cells were positive for IL-1 and IL-6[13]. More recently, several adhension molecules on macrophage-like cell in local areas of CTS[14]. As well as the findings of the present study, suggest that bioactive substances such as cytokines induce selling of the flexor tenosynovium in the absence of inflammatory cell infiltration[15]. These results suggest that cytokines and adhension molecules play an important role in the development of CTS[12]. In our data, cell adhension and inflammatory genes such as TGFBR2, RAB5B, CD24 and NFKB1 were up-regulated, and apoptotic genes such as PRG5 were down-regulated in CTS patients. From these results, we suggested that down-regulation of PGR5 means, cell apoptotic reaction is decreased. So, because of several adhension and anti-apoptotic molecules, median nerve could be pressed from around tissue.

In conclusion, through the cDNA microarray, pro-inflammatory genes and antiapoptotic genes are up-regulated in CTS patients. We suggest that CTS could be related with proinflammatory mechanism and antiapoptotic mechanism.

Methods

Subjects

120 CTS patients and 30 healthy controls patients were included in this study. They had undergone an annual health examination at Korea University Hospital (Seoul, South Korea) from December 1, 2004 to September 30, 2006. All subjects were women who were 35 to 61 years of age and had exhibited symptoms for more than 12 months. They were completed a questionnaire including medical history of diabetes, hypertension, thyroid diseases, and arth-

Fig. 2. Down-regulated gene between Control and CTS patients.

ritis. Also, the symptoms were recorded by electromyography (EMG: Counterpoint MK2, Dantec, Skovlunde, Denmark)[16]. The electrodiagnostic criteria are as follows, and the results of the electrodiagnosis itself were categorized according to the severity of median nerve damage[17].

1. More than a 4.0-msec motor latency
2. More than a 3.0-msec motor latency
3. Less than a 20-μV sensory amplitude
4. More than a 1.5 ratio of median to ulnar motor latency
5. More than a 1.2 ratio of median to ulnar sensory latency
6. Less than a 0.6 ratio of median to ulnar sensory amplitude

Blood Sample Preparation

Blood samples, 3-5 mL of heparinized whole blood, were collected by vein puncture from each human subject and were centrifuged to isolate the buffy coat and plasma at 3,500 × g for 15 minutes. We stored these samples in −70°C deep freezer until RNA extraction.

Human cDNA Microarray

A blood cDNA microarry was derived principally from a commercially available master set of approximately 15,000 human verified-sequences (Research Genetics, Inc,m Huntcville, AL). The 15,000 human cDNA clone set was sorted for a list of genes (1,152 elements) representing families such as differentiation, development, proliferation, transformation, cell cycle progression, immune response, transcription and translation factors, oncogenes, and molecules involved in cell growth and maintenance. PCR amplified cDNAs were spotted on nylon membranes. The general methodology of arraying is based on the procedure of DeRisi *et al.*[18].

Hybridization

cDNA microarrays were pre-hybridized in hybridization buffer containing 4.0 mL Microhyb (Invitrogen,

Calsbad, CA, USA), 10 μL of 10 mg/mL human Cot 1 DNA (Invitrogen, Calsbad, CA, USA), and 10 μL of 8 mg/mL poly dA (Invitrogen, Calsbad, CA, USA). Both Cot 1 and poly dA were denatured at 95°C for 5 min prior to use. After 4 h of pre-hybridization at 42 °C, approximately 10^7 cpm/mL of heat-denatured (95 °C, 5 min) probes were added and incubation continued for 17 h at 42°C. Hybridized arrays were washed three times in 2X SCC and 0.1% SDS for 15 min at room temperature.

RNA Preparation and cDNA Radiolabeling

Frozen whole bloods in human were transferred to 900 μL of TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was isolated using TRIzol reagent. After homogenization with TRIzol, 0.24 mL chloroform was added for each 0.7 mL of TRIzol usage, and the sample was centrifuged at 12,000 × g, 4°C for 15 minutes. Then, the aqueous phase was transferred into a new tube and 0.5 mL of isopropyl alcohol was added. After centrifugation at 12,000 × g, 4°C for 10 minutes, the RNA was precipitated as a white pellet at the bottom of the tube. The RNA pellets in 75% ethanol were washed and centrifuged at 7,500 × g, 4 °C for 5 minutes. The TNA pellets were dried and dissolved in DEPC treated water (Invitrogen, Calsbad, CA, USA) and incubated for 10 minutes at 65°C. The total RNA was quantified using the NanoDropND-1,000 Spectrophotometer (NanoDrop, Montchanin, USA).

After quantification, 2-3 μg of total RNAs prepared from blood were used for each sample for adjustment of different diagnosis. To synthesize 33P-labeled cDNAs, quantified RNA were labeld in a reverse transcription reaction containing 8 μL of 5X first standard PCR buffer (Invitrogen, Milano, Italy), 4 μL of 24-mer poly dT primer (Invitrogrn, Carlsbad, CA, USA), 4 μL of dNTP excluding dCTP (Invitrogrn, Carlsbad, CA, USA), 4 μL of 0.1 M DTT (Invitrogen, Milano, Italy), 1 μL of RNaseOUT (LIT), 1 μL of DEPC water and 5 μL of 3,000 Ci/mmol a-33P dCTP to a final Volume of 20 μL. Two μL of M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA, USA) was then added and the samples were incubated for 30min at 42°C, followed by the addition of 2 μL of M-MLV reverse transcriptase and another 30min at 42°C. 2.5 μL of 0.5 M EDTA was added to chelate divalent cations. After the addition of 5 μL 0.1 M NaOH, the samples were incubated at 65°C for 30 min to hydrolyze remaining RNA. Following the addition of 12.5 μL of 1 M Tris HCl (pH 8.0), the samples were purified using purification columns (Bio-rad, Hercules, CA, USA). After purification, each sample are put in 4 mL of hybrid solution and reacted with nylon membrane during 24 hr.

Image Scanning

The microarrays were exposed to phosphorimager screens for 1-5 days, and the screens were then scanned in a FLA-8000 (Fuji Photo Film Co., Japan) at 50 μm resolution.

Data Analysis

Microarray images were trimmed and rotated for further analysis using L-Processor system (Fuji Photo Film Co., Japan). Gene expression of each microarray was captures by the intensity of each spot produced by radioactive isotopes. Pixels per spot were counted by Arraygauge (Fuji Photo Co., Japan) and exported to Microsoft Excel (Microsoft, Seattle, WA, USA).

Statistical Methods

Data analysis of microarray was used by Microsoft Excel (Microsoft, Seattle, WA, USA). To normalize each membrane or each gene across membranes, we used global normalization basic methods, which calculate the mean or median of the signal intensities of each individual experimental data set and then calculate the mean of the means (or grand mean) for all of the included experiments. Each individual data set is then mathematically adjusted such that the mean of that data set equals the calculated grand mean. Raw intensity values obtained from the previous step were exported to EXCEL and normalized with Z transformation by subtracting with each average of gene intensity and dividing with each S.D. Raw intensity data for each experiment is $\log_{10}$ transformed and then used for the calculation of Z scores. Z score transformation statistics have been used in comparing experimental and control group gene expression differences by microarray[8]. Z value, Z difference and Z ratio are calculated according to the formula:

$$Z\ value_{(gene1)} = \log_{10}[raw\ intensity_{(gene1)}] - \log_{10}[mean\ raw\ intensity_{(all\ genes)}] / S.D.\ \log_{10}[raw\ intensity_{(all\ genes)}]$$

$$Z\ difference_{(gene1)} = Z_{(gene1,\ array1)} - Z_{(gene2,\ array2)}$$

$$Z\ ratio_{(gene1)} = Z\ difference_{(gene1)} / S.D._{(Z\ difference\ all\ genes)}$$

Hierarchical clustering was determined using software programs developed at Stanford University[19]. And cluster analysis was performed on Z-transformed microarray data by using two programs available as shareware from Michael Eisen's laboratory (http://rana.lbl.gov). Clustering of changed in gene expression was determined by using public domain cluster based on pairwise complete-linkage cluster

analysis. Gene expression raw data, log values and Z scores were averaged by using the mean ± S.D.

Acknowledgements

We thank Dr. Yoon S. Cho-Chung (Cellular Biochemistry Section, BasicResearch Laboratory, CCR, NCI, NIH, Bethesda, MD) and Dr Kevin G. Becker (DNA Array Unit, NIA, NIH, Baltimore, MD) for valuable advices on cDNA microarray. This study was partially supported by grant of the Korea Health 21 R&D Project, Ministry of Health and Welfare, R. O. K (Hmp-00-GN-01-0002 & 03-PJ10-PG13-GD01-0002). This work was partially supported by the Korea Science and Engineering Foundation (KOSEF) grant funded by the Korea Government (MOST) (No. R13-2003-016-03002-0 & No. M20502240001-06L0224-00111).

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