

Molecular Profile of Peripheral Blood Mononuclear Cells from Patients with Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a chronic inflammatory arthritis. Currently, diagnosis of RA may take several weeks, and factors used to predict a poor prognosis are not always reliable. Gene expression in RA may consist of a unique signature. Gene expression analysis has been applied to synovial tissue to define molecularly distinct forms of RA; however, expression analysis of tissue taken from a synovial joint is invasive and clinically impractical. Recent studies have demonstrated that unique gene expression changes can be identified in peripheral blood mononuclear cells (PBMCs) from patients with cancer, multiple sclerosis, and lupus. To identify RA disease-related genes, we performed a global gene expression analysis. RNA from PBMCs of 9 RA patients and 13 normal volunteers was analyzed on an oligonucleotide array. Compared with normal PBMCs, 330 transcripts were differentially expressed in RA. The differentially regulated genes belong to diverse functional classes and include genes involved in calcium binding, chaperones, cytokines, transcription, translation, signal transduction, extracellular matrix, integral to plasma membrane, integral to intracellular membrane, mitochondrial, ribosomal, structural, enzymes, and proteases. A *k*-nearest neighbor analysis identified 29 transcripts that were preferentially expressed in RA. Ten genes with increased expression in RA PBMCs compared with controls mapped to a RA susceptibility locus, 6p21.3. These results suggest that analysis of RA PBMCs at the molecular level may provide a set of candidate genes that could yield an easily accessible gene signature to aid in early diagnosis and treatment.

Online address: <http://www.molmed.org>

doi: 10.2119/2006-000056.Edwards

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease causing synovial joint damage, disability, and a shortened life expectancy (1,2). An awareness of the destructive potential of RA has led to more aggressive use of disease-modifying anti-rheumatic drugs (DMARDs) (3) and the development of immune therapies targeted to molecules and cells important

in the pathogenesis of RA. These include the TNF inhibitors infliximab, etanercept, and adalimumab (4). Synovial joint damage occurs early in the disease course, and many patients demonstrate erosions within a few months after becoming symptomatic (5). Recent evidence suggests that early aggressive therapy (infliximab and methotrexate) yields greater benefit than similar therapy after failure

of other drugs (6-8). To initiate early aggressive therapy requires reliable and rapid determination of diagnosis and prognosis. In addition, factors used to predict a poor prognosis, including sex, age of onset, multiple joint involvement, rheumatoid factor, and the presence of the shared epitope of HLA-DR4, are not always reliable (9-12).

Gene expression profiling may allow early diagnosis, aid in identifying factors that predict poor prognosis, and help focus early, aggressive, and expensive therapy to those that would benefit the most. Expression analysis of tissues taken at the site of disease within a synovial joint is invasive and impractical on a routine basis. However, recent studies have demonstrated unique gene expression changes in peripheral blood mononuclear cells (PBMCs) from pa-

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Submitted July 20, 2006; Accepted for publication December 7, 2006.

tients with cancer, multiple sclerosis, and lupus (13-17). In this study, a genome-wide scan of PBMCs from normal volunteers and RA PBMCs was performed using oligonucleotide arrays representing 6800 human genes to explore gene expression in the PBMCs of individuals with RA.

MATERIALS AND METHODS

Patient Selection

Patients with RA, defined by American College of Rheumatology (ACR) criteria (18), were identified in a rheumatology clinic with approval from the local research ethics committee. Demographic data including age, sex, and time since diagnosis were collected. A tender joint count (TJC 0-28), swollen joint count (JC 0-28), patient's best global assessment (visual analog scale), and erythrocyte sedimentation rate (ESR) were performed to calculate a 28-joint disease activity score (DAS28). The presence of rheumatoid factor (RF) and the use of DMARDs were recorded. Blood was also collected from healthy volunteers with no previous diagnosis of RA or other chronic inflammatory diseases.

Isolation of RNA and Preparation of Labeled Hybridization Solutions

An 8-mL sample of venous blood was collected into CPT Vacutainer cell purification tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and refrigerated immediately. Samples were immediately transferred to the laboratory, and PBMCs from the 9 RA and 13 normal volunteers were separated according to the manufacturer's recommendations. Briefly, the tube was centrifuged at 1500g (2700 rpm) at room temperature, and PBMCs were isolated before being washed twice in PBS. Total RNA was extracted using the RNeasy minikit (Qiagen, Valencia, CA, USA). For each sample, 2 µg total RNA was used to generate cDNA as described (19). RNA quality was determined by observing distinct 28S and 18S ribosomal bands on an agarose gel. First-strand cDNA synthesis was performed under

the following buffer conditions: 1× 1st-strand buffer (Invitrogen Life Technologies, Carlsbad, CA, USA), 10 mM DTT (Gibco/Invitrogen), 500 µM of each dNTP (Invitrogen Life Technologies), 400 units Superscript RT II (Invitrogen Life Technologies), and 40 units RNase inhibitor (Ambion, Austin, TX, USA). The reaction proceeded at 47°C for 1 h. Second-strand cDNA was synthesized with the addition of the following reagents at the final concentrations listed: 1× 2nd-strand buffer (Invitrogen Life Technologies), an additional 200 µM of each dNTP (Invitrogen Life Technologies), 40 units *E. coli* DNA polymerase I (Invitrogen Life Technologies), 2 units *E. coli* RNaseH (Invitrogen Life Technologies), and 10 units *E. coli* DNA ligase. The reaction proceeded at 15°C for 2 h; during the last 5 min of this reaction, 6 units T4 DNA polymerase (New England Biolabs, Beverly, MA, USA) was added. The resulting double-stranded cDNA was purified with the use of BioMag carboxyl-terminated particles as follows: 0.2 mg BioMag particles (Polysciences, Warrington, PA, USA) were equilibrated by washing three times with 0.5 M EDTA and resuspended at a concentration of 22.2 mg/mL in 0.5 M EDTA. The double-stranded cDNA reaction was diluted to a final concentration of 10% PEG/1.25 M NaCl, and the bead suspension was added to a final bead concentration of 0.614 mg/mL. The reaction was incubated at room temperature for 10 min. The cDNA/bead complexes were washed with 300 µL of 70% ethanol, the ethanol was removed, and the tubes were allowed to air dry. The cDNA was eluted with the addition of 20 µL of 10 mM Tris-acetate, pH 7.8, and incubated for 2 to 5 min, and the cDNA-containing supernatant was removed.

Purified double stranded cDNA (10 µL) was added to an in vitro transcription (IVT) solution which contained 1× IVT buffer (Ambion), 5000 units T7 RNA polymerase (Epicentre Technologies, Madison, WI, USA), 3mM GTP, 1.5 mM ATP, 1.2 mM CTP, and 1.2 mM UTP (Amersham/Pharmacia), 0.4 mM

each bio-16 UTP and bio-11 CTP (Enzo Diagnostics, Farmingdale, NY, USA), and 80 units RNase inhibitor (Ambion). The reaction proceeded at 37°C for 16 h. Labeled RNA was purified with the use of an RNeasy kit (Qiagen). The RNA yield was quantified by measuring absorbance at 260 nm.

Hybridization to Affymetrix Microarrays and Detection of Fluorescence

Eleven in vitro synthesized transcripts from segments of bacterial genes were included in each hybridization reaction to generate a global standard curve to normalize the oligonucleotide microarrays to each other and estimate the sensitivity of the arrays (20). Purified biotinylated cRNA (10 µg) was hybridized to oligonucleotide arrays comprised of 6937 human gene qualifiers (human FL6800 array P/N900183, Affymetrix, Santa Clara, CA).

Raw fluorescent intensity values were collected and reduced with GeneChip v3.2 software (Affymetrix) as described (Affymetrix GeneChip Analysis Suite User Guide). This determined the probability of each gene qualifier represented on the array being absent, present, or marginal, as well as calculating a specific hybridization intensity value, or average difference, for each transcript. The relative abundances of the 11 bacterial control cRNA transcripts ranged from 1:300,000 (3 ppm) to 1:1000 (1000 ppm) stated in terms of the number of control transcripts per total transcripts. As determined by the signal response from these control transcripts, the sensitivity of detection of the arrays ranged between ~1:300,000 and 1:100,000 copies/million. The average difference for each transcript was normalized to frequency values as described (20).

Transcripts designated absent in all samples were excluded from the analysis; 3295 (49%) of the transcripts remained. Further analysis of the processed data was performed with GeneSpring version 7.1 (Agilent Technologies, Redwood City, CA, USA). To

identify transcripts that were increased in the RA samples compared with controls, >50% (at least 5 of 9) of the samples had to be called present, with a frequency of 10 ppm or greater, and have a change in expression, relative to the average expression of the controls, of at least 2-fold. The resulting data set had 324 gene qualifiers. To find gene qualifiers whose expression was decreased, a list was generated of gene qualifiers from normal samples that were called present with a frequency of ≥ 10 ppm. The resulting list was filtered for an average decrease in expression, relative to the controls, of at least 2-fold in the disease samples. Six gene qualifiers met these criteria; 330 transcripts were used for the analyses. Annotation for each gene was determined based on GO, Entrez Gene, PubMed, and literature searches.

Statistical and Clustering Analyses

An unsupervised hierarchical clustering was performed on the 330 genes to group the samples on the basis of similarity of their expression profiles (21). Statistically significant differences in expression were determined using Welch ANOVA (22) coupled with two different multiple testing corrections. The Benjamini and Hochberg false discovery rate (FDR) (23) was applied with a P value < 0.05 , with 326 genes passing this criterion. The Bonferroni family-wise error rate (FWER) (24,25) was applied with a P value < 0.05 , with 189 gene qualifiers passing this criterion. Finally, a class prediction using the k -nearest neighbor method (26) was applied to the filtered data to determine which genes had the highest discrimination between normal and RA samples.

RESULTS

Characteristics of the RA patients used in the study, including demographics, disease activity scores, and DMARD use, are illustrated in Table 1. In total, 324 transcripts increased by at least two-fold between the RA and control subjects, and six transcripts decreased by at least two-fold between the RA and control subjects (Table 2).

Table 1. Characteristics of RA patients including demographics, disease activity scores, and DMARD use.

ID	Age, years	Sex	RF titer	Erosions	DMARD use	DAS
RA1	61	F	—	+	Prednisolone 5 mg/d, methotrexate 15 mg/wk	3.9
RA2	66	F	1/5120	+	Prednisolone 7.5 mg/d, methotrexate 20 mg/wk, sulfasalazine 500 mg BD	4.5
RA3	46	F	1/640	+	Methotrexate 15 mg/wk, hydroxychloroquine 200 mg BD	3.5
RA4	52	F	1/640	—	Methotrexate 12.5 mg/wk	3.6
RA5	55	F	1/320	+	Methotrexate 12.5 mg/wk, hydroxychloroquine 200 mg BD	3.3
RA6	35	F	1/2560	+	—	3.0
RA7	74	M	1/320	+	Methotrexate 7.5 mg/wk	3.5
RA8	77	M	1/20400	+	Prednisolone 7.5 mg/d, methotrexate 17.5 mg/wk	3.2
RA 9	49	F	1/1250	+	Methotrexate 10 mg/wk	2.9

Unsupervised Clustering

An unsupervised clustering analysis was performed on the 330 genes that passed the initial filtration, based on a hierarchical correlation coefficient algorithm (21). Samples were grouped based on similarity of expression. The resulting dendrogram describes the sample relationships by grouping the RA samples and controls by their expression patterns (Figure 1). Figure 1A depicts a region where expression levels in the RA samples were increased compared with the normal samples. This analysis suggests that there are significant differences in the gene expression of RA and control samples.

ANOVA Analysis

To minimize the inclusion of genes not related to the disease state, several statistical approaches were used. The 330 transcripts that passed the initial filtration (Table 2) were subjected to a Student t test and a Welch ANOVA with two multiple testing corrections (22). To control for a proportion of genes that may appear in the analysis by chance, an FDR was calculated set to a threshold of 5%. This analysis defines a proportion of the genes that are expected to occur by chance relative to the total number of transcripts identified; 326 transcripts

were called significant with this analysis (Table 2). In addition, the more stringent Bonferroni FWER using a P value cutoff of 0.05 was also performed, with 189 transcripts passing this analysis (Table 2).

Class Prediction

A k -nearest neighbor analysis was performed to identify a gene set that may distinguish the RA samples from normals. The prediction strength was evaluated using the 330 genes shown in Table 2. A list of predictor genes was assembled using the k -nearest neighbor method (26) to organize genes based on normalized expression levels. Cross-validation analyses comparing each sample to the model generated by the remaining samples were used to optimize the analysis parameters. This resulted in a number of neighbors value of 6 with a decision cutoff P value of 0.2 to predict expression patterns in RA vs. controls. Twenty-nine transcripts comprise the prediction gene set. The 29 prediction transcripts were grouped based on a hierarchical correlation to show the relationships (Figure 2).

Characterization of the RA Disease-Related Genes

The 330 differentially regulated transcripts were categorized into functional groups and are presented as the average

Table 2. Differentially regulated transcripts.

Symbol	Name	GenBank acc. no.	Map	Freq RA	Freq control	% disease samples, $\geq 2x$ or $\leq 2x$	Fold change ^a	t test (freq)	ANOVA – FDR	ANOVA – FWER	Class predictor gene: strength
Calcium Binding											
ANXA11	Annexin A11	L19605	10q23	97.6 ± 9.1	39.1 ± 1.8	89%	2.5 ± 0.2	2.8E-02	1.5E-05	6.0E-04	1.954
S100A4	S100 calcium binding protein A4	M80563	1q21	213.2 ± 19.9	87.6 ± 7.8	89%	2.4 ± 0.2	3.2E-02	5.3E-05	5.3E-03	
S100A12	S100 calcium binding protein A12	D83657	1q21	65.4 ± 14.0	22.8 ± 4.8	67%	2.6 ± 0.8	5.2E-01	3.3E-03	—	
CALM3	Calmodulin 3	J04046	19q13.2-q13.3	29.6 ± 1.8	13.8 ± 1.0	67%	2.1 ± 0.1	6.9E-02	4.7E-06	1.1E-04	
CACYBP	Calcylin binding protein	BC001431	1q24-q25	179.2 ± 23.4	72.2 ± 8.3	67%	2.3 ± 0.5	1.1E-01	4.0E-04	—	
Chaperone											
HYOU1	Hypoxia up-regulated 1	U65785	11q23.1-q23.3	33.7 ± 3.6	11.4 ± 0.7	78%	3.0 ± 0.3	4.2E-02	2.0E-05	1.1E-03	
NAP1L4	Nucleosome assembly protein 1-like 4	U77456	11p15.5	26.6 ± 3.6	9.8 ± 0.8	78%	2.7 ± 0.4	5.7E-02	1.9E-04	3.3E-02	
TTC1	Tetratricopeptide repeat domain 1	U46570	5q32-q33.2	41.7 ± 4.7	16.1 ± 1.3	67%	2.6 ± 0.3	2.9E-02	5.3E-05	5.4E-03	
DNAJC7	DnaJ (Hsp40) homolog, subfamily C, member 7	U46571	17q11.2	17.8 ± 2.3	6.5 ± 0.5	67%	2.7 ± 0.3	1.6E-01	1.0E-04	1.2E-02	
Cytokine/chemokine											
IGF2	Insulin growth factor 2 gene, intron 7	S73149		34.1 ± 4.9	8.9 ± 0.7	89%	3.8 ± 0.5	8.0E-02	1.9E-05	1.0E-03	1.874
CSF1	Human macrophage-specific colony-stimulating factor	M11296	1p21-p13	28.0 ± 3.6	9.6 ± 0.7	78%	2.9 ± 0.4	9.6E-02	4.3E-05	3.7E-03	
CCL5	Chemokine (C-C motif) ligand 5	M21121	17q11.2-q12	156.8 ± 14.9	53.7 ± 3.7	78%	2.9 ± 0.3	2.7E-02	2.5E-05	1.5E-03	
CCL22	Chemokine (C-C motif) ligand 22	U83239	16q13	31.2 ± 5.0	11.2 ± 0.9	67%	2.8 ± 0.4	1.3E-01	4.4E-04	—	
CCL22 (duplicate)	Chemokine (C-C motif) ligand 22	U83171	16q13	15.2 ± 2.1	6.2 ± 0.2	67%	2.5 ± 0.3	1.8E-02	6.3E-04	—	
TGFB1	Transforming growth factor, β 1	M38449	19q13.2	26.6 ± 4.0	10.1 ± 1.1	67%	2.4 ± 0.6	7.2E-03	1.3E-03	—	
MLN	Motilin	X15393	6p21.3	13.4 ± 1.8	6.0 ± 0.4	67%	2.2 ± 0.3	9.5E-02	8.9E-04	—	
PF4	Platelet factor 4 (chemokine (C-X-C motif) ligand 4)	M25897	4q12-q21	278.3 ± 14.2	136.5 ± 13.7	56%	2.0 ± 0.1	1.2E-01	1.2E-05	4.7E-04	
IL7R	Interleukin 7 receptor	M29696	5p13	24.0 ± 10.8	30.2 ± 9.6	67%	-2.3 ± 1.0	1.3E-01	—	—	
DNA binding											
HMG1	High-mobility group box 1	D63874	13q12	83.2 ± 9.3	27.3 ± 4.3	78%	3.0 ± 0.3	2.5E-01	3.3E-05	2.6E-03	
MKRN4	Ring zinc-finger protein	U41315	Xp21.1	15.0 ± 1.9	5.8 ± 0.3	67%	2.6 ± 0.3	1.3E-01	3.1E-04	—	
HIST2H2AA	Histone 2, H2aa	L19779	1q21.3	71.9 ± 8.2	32.9 ± 3.0	56%	2.2 ± 0.2	5.2E-02	6.6E-05	7.2E-03	
DBP1	Damage-specific DNA binding protein 1	U32986	11q12-q13	24.7 ± 3.3	11.0 ± 0.6	67%	2.0 ± 0.5	6.2E-02	1.5E-03	—	
Enzyme											
LIZ	Lysozyme	M21119	12q14.3	53.0 ± 27.2	69.2 ± 20.0	67%	-6.6 ± 2.2	8.0E-02	—	—	
AGPAT1	1-acylglycerol-3-phosphate O-acyltransferase 1	U56417	6p21.3	26.3 ± 2.7	7.2 ± 0.4	89%	3.7 ± 0.4	6.6E-02	3.1E-05	2.3E-03	2.006
DIA1	NADH-cytochrome b5 reductase	M28713		33.3 ± 3.4	8.9 ± 0.5	100%	3.7 ± 0.4	5.1E-02	2.5E-06	3.7E-05	2.462
KIAA0220	Pi-3-kinase-related kinase SMG-1-like	D86974	16p12.2	239.2 ± 33.3	73.4 ± 8.9	78%	3.3 ± 0.5	1.5E-02	1.2E-04	1.6E-02	
GSTZ1	Glutathione transferase zeta 1	U86529	14q24.3	25.6 ± 3.3	7.8 ± 0.6	78%	3.3 ± 0.4	1.0E-01	2.5E-05	1.5E-03	
PYGB	Phosphorylase, glycogen	U47025	20p11.2-p11.1	25.2 ± 2.7	7.8 ± 0.4	89%	3.2 ± 0.3	5.1E-02	1.3E-04	1.9E-02	

Continued

Table 2. Continued

SAT	Spermidine/spermine N1-acetyltransferase	U40369	Xp22.1	37.7 ± 4.0	12.3 ± 2.0	89%	3.1 ± 0.3	4.1E-01	1.9E-05	9.6E-04
UROD	Uroporphyrinogen decarboxylase	X89267	1p34	42.7 ± 6.2	14.2 ± 1.1	78%	3.0 ± 0.4	1.1E-01	2.1E-04	3.7E-02
GPI	Glucose phosphate isomerase	K03515	19q13.1	35.8 ± 4.5	12.1 ± 1.0	67%	3.0 ± 0.4	2.3E-02	3.3E-05	2.6E-03
GSTO1	Glutathione S-transferase omega 1	U90313	10q25.1	43.2 ± 4.2	14.3 ± 1.5	89%	3.0 ± 0.3	8.9E-02	5.4E-06	1.4E-04
DDX11	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11	U75968	12p11	20.6 ± 2.8	7.1 ± 0.6	100%	2.9 ± 0.4	6.3E-02	1.5E-05	6.7E-04
IMPDH1	IMP (inosine monophosphate) dehydrogenase 1	J05272	7q31.3-q32	31.4 ± 4.7	10.9 ± 0.9	78%	2.9 ± 0.4	3.7E-02	2.3E-04	4.3E-02
PIB5PA	Phosphatidylinositol (4,5) biphosphate 5-phosphatase, A	U45975	22q11.2-q13.2	20.0 ± 2.6	7.2 ± 0.4	67%	2.8 ± 0.4	6.6E-02	2.7E-04	—
CNP	2',3'-cyclic nucleotide 3' phosphodiesterase	D13146	17q21	49.9 ± 6.4	17.8 ± 1.4	78%	2.8 ± 0.4	1.3E-01	4.9E-05	4.8E-03
UPP1	Uridine phosphorylase 1	X90858	7p12.3	21.4 ± 1.3	7.6 ± 0.6	89%	2.8 ± 0.2	5.1E-02	2.4E-07	6.1E-07
AMPD2	Adenosine monophosphate deaminase 2 (isoform L)	M91029	1p13.3	30.3 ± 4.0	11.2 ± 0.8	78%	2.7 ± 0.4	6.6E-02	8.6E-05	9.8E-03
BCAT2	Branched chain aminotransferase 2, mitochondrial	U62739	19q13	18.8 ± 2.0	7.1 ± 0.3	78%	2.7 ± 0.3	3.1E-02	1.0E-04	1.2E-02
HSD17B3	Hydroxysteroid (17-beta) dehydrogenase 3	U05659	9q22	21.4 ± 2.6	8.1 ± 0.5	78%	2.7 ± 0.3	8.0E-02	1.6E-04	2.5E-02
GARS	Glycyl-tRNA synthetase	U09587	7p15	29.7 ± 2.5	11.2 ± 0.7	78%	2.7 ± 0.2	7.6E-02	3.7E-06	7.1E-05
PTGS1	Prostaglandin-endoperoxide synthase 1	M59979	9q32-q33.3	14.2 ± 2.3	5.5 ± 0.4	67%	2.6 ± 0.4	7.9E-02	1.6E-03	—
TGM1	Transglutaminase 1	L34840	14q11.2, 3p22-p21.33	18.4 ± 2.3	7.2 ± 0.7	67%	2.6 ± 0.3	1.9E-01	9.2E-05	1.1E-02
AOAH	Acylxyacyl hydrolase	M62840	7p14-p12	25.2 ± 5.4	9.2 ± 0.8	67%	2.5 ± 0.7	2.3E-01	4.8E-03	—
GNPDA1	Glucosamine-6-phosphate deaminase 1	D31766	5q21	13.8 ± 2.0	5.5 ± 0.3	56%	2.5 ± 0.4	6.7E-02	6.4E-04	—
AARS	Alanyl-tRNA synthetase	D32050	16q22	18.1 ± 2.5	7.4 ± 0.3	67%	2.5 ± 0.3	1.6E-01	9.7E-04	—
SETDB1	SET domain, bifurcated 1	D31891	1q21	18.6 ± 2.5	7.4 ± 0.7	67%	2.5 ± 0.3	8.9E-02	1.5E-04	2.3E-02
DCTD	Deoxycytidylate deaminase gene	L39874	4q35.1	25.0 ± 3.0	9.8 ± 0.8	67%	2.5 ± 0.3	8.0E-02	5.4E-05	5.5E-03
HUMNOSB	Inducible nitric oxide synthase	D29675		14.1 ± 2.6	5.3 ± 0.2	67%	2.4 ± 0.6*	2.6E-01	4.1E-03	—
MGLL	Monoglyceride lipase	U67963	3q21.3	18.9 ± 2.0	7.2 ± 0.5	89%	2.4 ± 0.5	9.8E-02	2.3E-04	4.4E-02
DAO	D-amino-acid oxidase	D11370	12q24	14.9 ± 2.2	6.2 ± 0.2	56%	2.4 ± 0.4	9.7E-02	1.1E-03	—
DDT	D-dopachrome tautomerase	U49785	22q11.23	24.9 ± 2.9	10.5 ± 0.6	56%	2.4 ± 0.3	4.9E-02	1.1E-04	1.5E-02
TPI1	Triosephosphate isomerase 1	M10036	12p13	73.3 ± 8.5	30.0 ± 2.6	78%	2.4 ± 0.3	2.7E-02	1.0E-04	1.3E-02
SULT1A3	Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 3	U20499	16p11.2	20.3 ± 2.3	8.3 ± 0.6	67%	2.4 ± 0.3	1.7E-01	7.6E-05	8.5E-03
GLA	Galactosidase, alpha	X14448	Xq22	20.6 ± 2.2	8.6 ± 0.9	78%	2.4 ± 0.3	2.8E-01	1.0E-04	1.3E-02
PPGB	Protective protein for beta-galactosidase	M22960	20q13.1	83.4 ± 8.6	35.2 ± 2.4	78%	2.4 ± 0.2	2.6E-02	4.9E-05	4.8E-03
FAH	Fumarylacetoacetate hydratase	M55150	15q23-q25	18.7 ± 1.1	7.9 ± 0.5	89%	2.4 ± 0.1	4.4E-02	6.8E-07	3.4E-06
CDA	Cytidine deaminase	L27943	1p36.2-p35	26.9 ± 5.8	10.5 ± 1.0	56%	2.3 ± 0.7	6.4E-02	4.6E-03	—
HYAL2	Hyaluronoglucosaminidase 2	AJ000099	3p21.3	24.9 ± 3.7	10.9 ± 0.8	67%	2.3 ± 0.3	6.4E-02	1.7E-03	—
INPP5D	Inositol polyphosphate-5-phosphatase	U57650	2q36-q37	42.8 ± 5.6	18.8 ± 1.3	67%	2.3 ± 0.3	8.9E-02	4.6E-04	—
CEST	Carboxylesterase 1	L07765	16q13-q22.1	14.0 ± 2.0	6.2 ± 0.2	56%	2.3 ± 0.3	1.0E-01	1.2E-03	—
ACADVL	Acyl-Coenzyme A dehydrogenase	D43682	17p13-p11	41.4 ± 3.8	18.2 ± 1.6	67%	2.3 ± 0.2	2.0E-02	3.2E-05	2.4E-03

Continued

Table 2. Continued

<i>SULT1A1</i>	Sulfotransferase family, cytosolic, 1A, phenol-preferring, member 1	L19999	16p12.1	21.0 ± 2.3	9.2 ± 1.1	56%	2.3 ± 0.2	2.9E-01	9.3E-05	1.1E-02
<i>PRDX6</i>	Peroxidoxin 6	D14662	1q24.1	36.4 ± 2.7	16.1 ± 2.1	67%	2.3 ± 0.2	1.0E-01	4.4E-05	4.0E-03
<i>UGCR1</i>	Ubiquinol-cytochrome c reductase core protein 1	L16842	3p21.3	24.1 ± 3.3	10.1 ± 0.8	78%	2.2 ± 0.5	7.5E-02	5.4E-04	—
<i>GUSB</i>	Glucuronidase, beta	M15182	7q21.11	18.9 ± 2.2	8.5 ± 0.5	56%	2.2 ± 0.3	4.9E-02	1.2E-04	1.6E-02
<i>HK3</i>	Hexokinase 3	U51333	5q35.2	47.1 ± 7.3	20.0 ± 2.0	56%	2.1 ± 0.5	3.4E-01	1.6E-03	—
<i>CHKL</i>	Choline kinase-like	U62317	22q13.33	60.8 ± 7.0	26.6 ± 1.5	67%	2.1 ± 0.4	1.1E-01	5.0E-04	—
<i>PGM1</i>	Phosphoglucomutase 1	M83088	1p31	16.7 ± 1.5	7.8 ± 0.5	56%	2.1 ± 0.2	2.4E-01	4.6E-05	4.2E-03
<i>PCSK6</i>	Proprotein convertase subtilisin/kexin type 6	M80482	15q26	10.7 ± 1.1	5.1 ± 0.1	44%	2.1 ± 0.2	3.7E-02	1.7E-04	2.6E-02
<i>PMM1</i>	Phosphomannomutase 1	U86070	22q13.2	13.8 ± 1.3	6.6 ± 0.2	56%	2.1 ± 0.2	3.6E-02	1.7E-04	2.7E-02
<i>TALDO1</i>	Transaldolase 1	L19437	11p15.5-p15.4	85.3 ± 9.9	37.7 ± 3.0	78%	2.0 ± 0.5	1.2E-01	6.4E-04	—
Extracellular matrix										
<i>EPB49</i>	Erythrocyte membrane protein band 4.9 (demaftin)	L19713	8p21.1	38.7 ± 4.3	9.9 ± 0.8	100%	3.9 ± 0.4	2.8E-02	3.1E-06	5.2E-05
<i>SPARC</i>	Secreted protein, acidic, cysteine-rich (osteonectin)	J03040	5q31.3-q32	57.8 ± 8.2	24.4 ± 3.5	56%	2.4 ± 0.3	1.1E-01	4.0E-04	—
Integral intracellular membrane										
<i>CPT1B</i>	Carnitine palmitoyltransferase 1B (muscle)	Y08682	22q13.33	13.6 ± 1.9	4.9 ± 0.2	67%	2.8 ± 0.4	8.0E-02	3.3E-04	—
<i>STX5A</i>	Syntaxin 5A	U26648	11q12.3	19.9 ± 2.2	7.9 ± 0.6	89%	2.5 ± 0.3	1.8E-02	1.9E-05	9.0E-04
<i>HAX1</i>	HS1 binding protein	U68566	1q22	31.0 ± 2.9	13.9 ± 1.2	67%	2.2 ± 0.2	7.7E-02	4.9E-05	4.6E-03
<i>BAP1</i>	BRCA1 associated protein-1	D87462	3p21.31-p21.2	12.9 ± 1.0	6.2 ± 0.3	56%	2.1 ± 0.2	1.4E-02	5.4E-05	5.6E-03
<i>BZRP</i>	Benzodiazepine receptor (peripheral)	L21954	22q13.31	127.9 ± 26.1	50.5 ± 4.3	56%	2.3 ± 0.6	1.9E-01	4.8E-03	—
<i>BCL2</i>	B-cell CLL/lymphoma 2	M14745	18q21.33	16.2 ± 2.1	7.5 ± 0.6	44%	1.9 ± 0.5	1.4E-01	8.0E-04	—
<i>HERPUD1</i>	Homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1	D14695	16q12.2-q13	16.6 ± 3.1	7.0 ± 0.6	56%	1.9 ± 0.7	4.2E-01	7.8E-03	—
Integral plasma membrane										
<i>STAB1</i>	Stabilin 1	D87433	3p21.31	30.2 ± 6.7	4.7 ± 0.2	100%	6.4 ± 1.4*	1.8E-01	6.3E-05	6.8E-03
<i>IGHG3</i>	Immunoglobulin heavy constant γ 3	M87789	14q32.33	105.6 ± 38.7	15.8 ± 2.5	67%	6.3 ± 2.6	1.2E-01	1.4E-02	—
<i>ZYX</i>	Zyxin	X95735	7q32	38.0 ± 6.5	10.5 ± 1.0	78%	3.6 ± 0.6	4.1E-02	4.7E-04	—
<i>SELP1G</i>	Selectin P ligand	U25956	12q24	75.9 ± 10.0	21.1 ± 1.8	78%	3.6 ± 0.5	4.4E-02	2.2E-05	1.3E-03
<i>PRF1</i>	Perforin	M31951	10q22	16.9 ± 6.6	25.0 ± 3.8	67%	-3.4 ± 1.2	1.0E + 00	—	—
<i>CD151</i>	CD151 antigen	D29963	11p15.5	29.2 ± 4.9	8.7 ± 0.8	78%	3.4 ± 0.6	2.9E-01	1.3E-04	1.8E-02
<i>CD63</i>	CD63 antigen	X62654	12q12-q13	41.8 ± 6.7	12.4 ± 1.3	89%	3.4 ± 0.5	1.4E-01	1.0E-04	1.2E-02
<i>IFNGR2</i>	Interferon γ receptor 2	U05875	21q22.11	37.9 ± 6.9	11.5 ± 1.1	56%	3.3 ± 0.6	1.3E-01	3.4E-04	—
<i>TRIP12</i>	Thyroid hormone receptor interactor 12	D28476	2q37.1	27.4 ± 3.7	8.9 ± 1.0	78%	3.1 ± 0.4	2.4E-01	1.4E-04	2.1E-02
<i>LENG4</i>	Leukocyte receptor cluster member 4	S82470	19q13.4	26.8 ± 3.9	8.7 ± 0.6	78%	3.1 ± 0.4	8.6E-02	1.6E-04	2.5E-02
<i>CSF3R</i>	Colony stimulating factor 3 receptor	M59820	1p35-p34.3	33.3 ± 6.9	10.2 ± 0.8	67%	3.0 ± 0.8	5.9E-02	4.2E-03	—
<i>GP1BB</i>	Glycoprotein Ib (platelet), β polypeptide	U59632	22q11.21	97.2 ± 16.2	30.5 ± 3.3	67%	3.0 ± 0.7	6.9E-02	7.1E-04	—
<i>FLOT2</i>	Flotillin 2	M60922	17q11-q12	50.4 ± 7.9	16.1 ± 1.2	78%	2.8 ± 0.7	2.8E-02	3.9E-03	—
<i>VAT1</i>	Vesicle amine transport protein 1 homolog	U18009	17q21	12.3 ± 1.2	4.5 ± 0.1	78%	2.8 ± 0.3*	1.1E-01	2.5E-05	1.6E-03
										2.327

Continued

Table 2. Continued

MCL1	Myeloid cell leukemia sequence 1	L08246	1q21	88.7 ± 9.7	32.2 ± 3.9	78%	2.8 ± 0.3	6.6E-02	5.7E-05	6.0E-03
HLA-DQA1	Major histocompatibility complex, class II, DQ α 1	M34996	6p21.3	80.6 ± 18.7	25.1 ± 3.7	56%	2.7 ± 1.0	2.2E-01	6.8E-03	—
MICB	MHC class I chain-related gene B	U65416	6p21.3	17.3 ± 2.1	6.5 ± 0.2	67%	2.7 ± 0.3	1.2E-01	2.0E-04	3.5E-02
CD7	CD7 antigen	D00749	17q25.2-q25.3	69.9 ± 8.8	25.8 ± 2.1	67%	2.7 ± 0.3	4.3E-03	1.7E-04	2.9E-02
LILRA2	Leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 2	U82275	19q13.4	25.4 ± 4.7	8.8 ± 0.8	67%	2.6 ± 0.7	1.4E-01	2.1E-03	—
HEM1	Hematopoietic protein 1	M58285	12q13.1	38.4 ± 5.1	14.6 ± 2.0	67%	2.6 ± 0.3	1.2E-01	1.7E-04	2.7E-02
FCGR1	Fc fragment of IgG, receptor, transporter, alpha	U12255	19q13.3	78.6 ± 13.7	29.2 ± 2.5	67%	2.5 ± 0.6	1.1E-01	2.8E-03	—
SPG7	Spastic paraplegia 7 homolog	X65784	16q24.3	21.6 ± 2.2	8.5 ± 0.5	78%	2.5 ± 0.3	6.4E-02	3.2E-05	2.4E-03
IL10RB	Interleukin 10 receptor, beta	Z17227	21q22.1-q22.2	15.8 ± 1.6	6.2 ± 0.4	78%	2.5 ± 0.3	3.7E-02	3.6E-05	2.9E-03
SELP	Selectin P	M25322	1q22-q25	15.0 ± 1.2	5.9 ± 0.4	78%	2.5 ± 0.2	1.0E-01	1.5E-06	1.4E-05
CEACAM4	Carcinoembryonic antigen-related cell adhesion molecule 4	D90276	19q13.2	13.3 ± 2.1	5.5 ± 0.2	56%	2.4 ± 0.4*	5.8E-02	1.7E-03	2.018
CD3E	CD3E antigen, epsilon polypeptide	M23323	11q23	45.8 ± 5.8	18.9 ± 1.0	67%	2.4 ± 0.3	4.0E-02	4.0E-04	—
AAMP	Angio-associated, migratory cell protein	M95627	2q35	22.1 ± 3.2	9.4 ± 0.7	56%	2.4 ± 0.3	5.1E-02	1.1E-03	—
LAMP1	Lysosomal-associated membrane protein 1	J04182	13q34	47.8 ± 4.5	20.2 ± 2.0	67%	2.4 ± 0.2	7.4E-02	3.3E-05	2.6E-03
BST2	Bone marrow stromal cell antigen 2	D28137	19p13.2	46.3 ± 9.5	16.4 ± 1.6	78%	2.3 ± 0.8	2.5E-01	9.3E-03	—
CD33	CD33 antigen	M23197	19q13.3	19.4 ± 3.1	7.6 ± 0.7	56%	2.3 ± 0.5	2.3E-01	1.3E-03	—
ACRV1	Intra-acrosomal protein	S65583	11p12-q13	12.8 ± 2.3	5.5 ± 0.2	56%	2.3 ± 0.4*	7.3E-02	2.9E-03	—
PITG1IP	Pituitary tumor-transforming 1 interacting protein	Z50022	21q22.3	30.0 ± 3.3	13.0 ± 0.9	56%	2.3 ± 0.3	6.0E-02	1.7E-04	2.7E-02
HDLBP	High density lipoprotein binding protein (vigilin)	M64098	2q37	18.8 ± 2.3	8.3 ± 0.6	67%	2.3 ± 0.3	5.3E-02	2.9E-04	—
ICAM3	Intercellular adhesion molecule 3	X69819	19p13.3-p13.2	52.2 ± 4.4	22.6 ± 1.7	67%	2.3 ± 0.2	8.2E-03	2.0E-05	1.1E-03
OS-9	Amplified in osteosarcoma	U41635	12q13	58.6 ± 7.7	24.2 ± 1.8	67%	2.2 ± 0.5	8.3E-02	9.9E-04	—
EMP3	Epithelial membrane protein 3	U62101	19q13.3	159.3 ± 21.9	65.2 ± 4.9	67%	2.2 ± 0.5	1.5E-03	1.1E-03	—
HA-1	Minor histocompatibility antigen HA-1	D86976	19p13.3	103.1 ± 11.3	43.5 ± 2.0	78%	2.2 ± 0.4	3.5E-02	1.7E-04	2.8E-02
EBI3	Epstein-Barr virus induced gene 3	L08187	19p13.3	12.8 ± 1.7	5.8 ± 0.2	56%	2.2 ± 0.3	3.1E-02	5.4E-04	—
SPN	Sialophorin	M61827	16p11.2	33.2 ± 4.5	15.0 ± 1.3	67%	2.2 ± 0.3	8.3E-02	6.9E-04	—
CD19	CD19 antigen	M84371	16p11.2	14.6 ± 1.3	6.7 ± 0.3	67%	2.2 ± 0.2	1.0E-02	1.1E-04	1.5E-02
ITGAX	Integrin, α X	M81695	16p11.2	32.2 ± 4.8	13.8 ± 1.0	44%	2.1 ± 0.5	2.1E-01	1.1E-03	—
P2RX5	Purinergic receptor P2X, ligand-gated ion channel, 5	U49395	17p13.3	17.4 ± 1.8	8.2 ± 0.7	56%	2.1 ± 0.2	3.1E-02	1.7E-04	2.8E-02
HLA-DOA	Major histocompatibility complex, class II, DO alpha	M31525	6p21.3	13.6 ± 1.6	6.4 ± 0.4	56%	2.1 ± 0.2	7.8E-02	3.5E-04	—
KLRK1	Killer cell lectin-like receptor subfamily K, member 1	X54870	12p13.2-p12.3	31.3 ± 3.8	13.4 ± 1.5	67%	2.0 ± 0.5	1.2E-03	6.8E-04	—
ITGB2	Integrin, β 2 (antigen CD18 (p95), lymphocyte function-associated antigen 1)	M15395	21q22.3	86.1 ± 7.9	37.8 ± 3.9	78%	2.0 ± 0.4	1.2E-01	1.8E-04	3.1E-02
LAPTM5	Lysosomal-associated multispinning membrane protein-5	U51240	1p34	146.9 ± 21.8	61.8 ± 5.4	67%	1.9 ± 0.6	1.2E-01	2.8E-03	—

Continued

Table 2. Continued

UCP2	Uncoupling protein 2	U94592	11	Mitochondrial 50.2 ± 6.6	22.5 ± 2.0	56%	2.0 ± 0.5	4.8E-03	7.9E-04	—
IER2	Immediate early response 2	M62831	19p13.13	Other 105.7 ± 17.3	28.7 ± 1.7	78%	3.5 ± 0.7	6.4E-02	3.7E-04	—
PTMA	Prothymosin, alpha	M14483	2q35-q36	65.9 ± 17.1	109.3 ± 8.0	44%	-3.4 ± 1.5	9.7E-01	3.9E-02	—
CRIP2	Cysteine-rich protein 2	D42123	14q32.3	25.6 ± 3.2	8.1 ± 0.6	78%	3.2 ± 0.4	1.5E-01	1.1E-04	1.4E-02
PHC2	Polyhomeotic-like 2	U89278	1p34.3	23.4 ± 3.0	7.8 ± 0.3	89%	3.0 ± 0.4	6.3E-02	1.7E-04	2.7E-02
PTH1	Ferritin, heavy polypeptide 1	L20941	11q13	279.0 ± 16.1	122.8 ± 5.7	67%	2.3 ± 0.1	2.9E-02	2.4E-07	7.2E-07
PFC	Properdin P factor, complement	M83652	Xp11.3-p11.23	46.6 ± 8.4	19.1 ± 1.8	56%	2.2 ± 0.6	1.8E-01	1.2E-02	—
IFI44	Interferon-induced protein 44	D28915	1p31.1	13.6 ± 2.5	5.5 ± 0.3	56%	2.0 ± 0.7	7.2E-01	5.5E-03	—
				Proteases or inhibitors						
PCOLN3	Procollagen (type III) N-endopeptidase	U58048	16q24.3	17.3 ± 2.3	5.4 ± 0.2	78%	3.2 ± 0.4	8.0E-02	1.4E-04	2.0E-02
MME	Membrane metallo-endopeptidase	J03779	3q25.1-q25.2	24.6 ± 3.4	7.8 ± 0.7	67%	3.1 ± 0.4	2.2E-01	1.9E-04	3.1E-02
ADAM8	A disintegrin and metalloproteinase domain 8	D26579	10q26.3	31.6 ± 4.1	10.6 ± 0.9	67%	3.0 ± 0.4	6.7E-02	4.9E-05	4.5E-03
SERPINB6	Serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 6	S69272	6p25	24.7 ± 2.8	8.7 ± 0.5	89%	2.8 ± 0.3	3.0E-02	1.9E-05	9.5E-04
TIMP2	Tissue inhibitor of metalloproteinase 2	M32304	17q25	25.7 ± 3.3	8.8 ± 0.8	78%	2.7 ± 0.5	6.2E-02	2.0E-04	3.3E-02
CTSD	Cathepsin D	M63138	11p15.5	82.9 ± 16.7	30.3 ± 2.0	56%	2.7 ± 0.6	6.4E-02	1.0E-03	—
SPINT2	Serine protease inhibitor, Kunitz type, 2	U78095	19q13.1	23.9 ± 1.9	10.5 ± 0.9	89%	2.3 ± 0.2	1.0E-01	7.2E-06	2.1E-04
NOMO1	PM5 protein, centromeric copy	X57398	16p13.11	27.3 ± 2.9	11.5 ± 0.9	78%	2.4 ± 0.2	5.6E-02	1.2E-04	1.5E-02
PSME1	Proteasome (prosome, macropain) activator subunit 1	L07633	14q11.2	84.3 ± 11.3	34.9 ± 3.2	56%	2.2 ± 0.5	1.1E-01	5.5E-04	—
PSMD2	Proteasome 26S subunit, non-ATPase, 2	D78151	3q27.3	36.2 ± 4.5	16.0 ± 1.2	56%	2.3 ± 0.3	1.3E-01	2.1E-04	3.7E-02
CTSB	Cathepsin B	M14221	8p22	71.3 ± 11.1	29.8 ± 2.8	56%	2.1 ± 0.6	2.2E-01	6.6E-03	—
PSMA4	Proteasome subunit, α type, 4	D00763	15q24.1	42.8 ± 5.9	17.8 ± 2.6	67%	2.2 ± 0.5	2.9E-01	5.7E-04	—
				Ribosomal						
MIRPL28	Mitochondrial ribosomal protein L28	U19796	16p13.3	22.0 ± 2.7	6.2 ± 0.3	100%	3.5 ± 0.4	1.2E-01	6.2E-06	1.7E-04
RPL39	Ribosomal protein L39	D79205	Xq22-q24	465.1 ± 21.5	226.3 ± 24.3	22%	2.1 ± 0.1	1.3E-01	1.9E-05	8.7E-04
RPS4Y1	Ribosomal protein S4, Y-linked 1	M58459	Yp11.3	15.1 ± 7.7	32.2 ± 6.4	78%	-5.5 ± 1.4	9.7E-01	—	—
				Signal transduction						
CSRP1	Cysteine and glycine-rich protein 1	M76378	1q32	21.0 ± 2.7	5.7 ± 0.4	89%	3.7 ± 0.5	1.3E-01	2.9E-05	2.0E-03
GNAZ	Guanine nucleotide binding protein ((G protein), α z polypeptide)	J03260	22q11.22	24.9 ± 3.9	7.6 ± 0.5	67%	3.3 ± 0.5	1.0E-01	2.1E-04	3.6E-02
CDC25B	Cell division cycle 25B	S78187	20p13	63.9 ± 8.5	19.1 ± 1.5	89%	3.3 ± 0.4	8.5E-02	5.6E-05	5.9E-03
ILK	Integrin-linked kinase	U40282	11p15.5-p15.4	29.2 ± 4.1	8.3 ± 0.7	78%	3.3 ± 0.7	5.6E-02	2.8E-04	—
PTRN	Protein tyrosine phosphatase, receptor type, N	L18983	2q35-q36.1	20.8 ± 2.9	6.4 ± 0.4	78%	3.3 ± 0.5	9.5E-02	1.1E-04	1.4E-02
TSC2	Tuberous sclerosis 2	L48546	16p13.3	30.4 ± 3.3	9.2 ± 0.6	78%	3.3 ± 0.4	3.8E-02	1.2E-05	4.3E-04
BRD2	Bromodomain containing 2	X62083	6p21.3	70.9 ± 8.0	21.2 ± 2.8	78%	3.4 ± 0.4	1.2E-01	1.9E-05	9.8E-04
CLU	Clusterin	M63379	8p21-p12	222.8 ± 26.1	72.8 ± 6.8	78%	3.1 ± 0.4	3.6E-02	1.6E-05	7.2E-04
INPP5E	Inositol polyphosphate-5-phosphatase	U45974	9q34.3	15.1 ± 1.3	4.7 ± 0.2	89%	3.2 ± 0.3*	1.1E-01	3.9E-06	8.9E-05
LTK	Leukocyte tyrosine kinase	D16105	15q15.1-q21.1	23.8 ± 3.2	7.2 ± 0.8	89%	3.3 ± 0.4	4.1E-01	2.1E-05	1.2E-03
NRGN	Neurogranin	X99076	11q24	230.1 ± 27.2	76.5 ± 7.2	78%	3.0 ± 0.4	3.4E-02	2.5E-05	1.6E-03

Continued

Table 2. Continued

PLCB2	Phospholipase C, β 2	M95678	15q15	84.0 \pm 7.8	25.6 \pm 1.9	100%	3.3 \pm 0.3	1.9E-02	6.4E-07	2.6E-06	2.064
PKM2	Pyruvate kinase, muscle	X56494	15q22	60.0 \pm 6.5	18.8 \pm 1.5	78%	3.2 \pm 0.3	1.3E-02	7.2E-06	2.2E-04	
PSD	Pleckstrin and Sec7 domain containing	X99688	10q24	23.4 \pm 3.7	7.8 \pm 0.6	67%	3.0 \pm 0.5	2.0E-01	2.8E-04	—	
MAP2K3	Mitogen-activated protein kinase kinase 3	D87116	17q11.2	32.9 \pm 4.1	11.2 \pm 1.1	78%	2.9 \pm 0.4	8.5E-02	4.9E-05	4.6E-03	
IKBKE	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon	D63485	1q32.1	18.9 \pm 2.1	6.7 \pm 0.4	78%	2.8 \pm 0.3	1.2E-01	1.4E-04	2.0E-02	
FASTK	FAST kinase	X86779	7q35	20.3 \pm 2.4	7.0 \pm 0.5	78%	2.9 \pm 0.3	2.8E-02	8.5E-05	9.7E-03	
LSP1	Lymphocyte-specific protein 1	M33552	11p15.5	48.1 \pm 7.2	15.9 \pm 1.8	78%	3.0 \pm 0.5	4.5E-04	6.9E-05	7.5E-03	
RABGGTA	Rob geranylgeranyltransferase, α subunit	Y08200	14q11.2	23.6 \pm 3.1	8.6 \pm 0.5	67%	2.7 \pm 0.4	5.4E-02	1.4E-04	2.1E-02	
MADD	MAP-kinase activating death domain	AB002356	11p11.2	31.7 \pm 4.1	11.2 \pm 0.7	67%	2.8 \pm 0.4	1.5E-01	1.0E-04	1.3E-02	
CSNK2A2	Casein kinase 2, α prime polypeptide	M55268	16p13.3-p13.2	17.3 \pm 1.8	6.1 \pm 0.2	78%	2.9 \pm 0.3	1.7E-01	4.3E-05	3.8E-03	2.056
CCND3	Cyclin D3	M92287	6p21	68.3 \pm 6.2	23.6 \pm 2.3	78%	2.9 \pm 0.3	5.0E-02	3.9E-06	8.5E-05	
CENTB1	Centaurin, β 1	D30758	17p13.2	61.9 \pm 6.3	22.6 \pm 1.7	67%	2.7 \pm 0.3	1.2E-02	2.5E-05	1.6E-03	
PRKACG	Protein kinase, cAMP-dependent, catalytic, γ	M34182	9q13	41.6 \pm 3.1	15.1 \pm 1.5	89%	2.8 \pm 0.2	1.2E-02	1.7E-06	2.2E-05	
YWHAH	Tyrosine 3-monooxygenase/typtophan 5-monooxygenase activation protein, eta polypeptide	D78577	22q12.3	85.7 \pm 5.7	31.1 \pm 4.3	89%	2.8 \pm 0.2	8.9E-02	2.8E-05	1.9E-03	
NCF1	Neutrophil cytosolic factor 1	M55067	7q11.23	72.3 \pm 13.7	22.1 \pm 1.7	78%	3.1 \pm 0.8	1.3E-02	1.2E-03	—	
ARHGFE2	Rho/rac guanine nucleotide exchange factor 2	U72206	1q21-q22	25.6 \pm 5.7	8.7 \pm 0.7	56%	2.7 \pm 0.8	2.9E-02	8.8E-03	—	
TRAF1	TNF receptor-associated factor 1	U19261	9q33-q34	20.4 \pm 2.6	7.3 \pm 0.3	78%	2.6 \pm 0.5	1.0E-01	3.4E-04	—	
TRAF4	TNF receptor-associated factor 4	X80200	17q11-q12	15.1 \pm 2.2	6.0 \pm 0.3	56%	2.5 \pm 0.4	6.0E-02	4.6E-04	—	
ARAF1	V-raf murine sarcoma 3611 viral oncogene homolog	U01337	Xp11.4-p11.2	32.7 \pm 2.8	11.8 \pm 0.9	89%	2.8 \pm 0.2	1.6E-02	1.6E-06	1.7E-05	
Csk	C-src tyrosine kinase	X59932	15q23-q25	62.4 \pm 8.0	22.5 \pm 1.6	78%	2.8 \pm 0.4	9.4E-03	1.2E-04	1.6E-02	
FKBP4	FK506 binding protein 4, 59kDa	M88279	12p13.33	23.1 \pm 3.2	8.8 \pm 0.4	67%	2.6 \pm 0.4	4.8E-02	4.8E-04	—	
GNG10	Guanine nucleotide binding protein (G protein), γ 10	U31383	9q32	18.3 \pm 3.0	6.7 \pm 0.7	56%	2.7 \pm 0.4	2.8E-01	3.7E-04	—	
TNFRSF14	Tumor necrosis factor receptor superfamily, member 14	U70321	1p36.3-p36.2	28.1 \pm 2.6	10.5 \pm 0.7	78%	2.7 \pm 0.2	7.5E-03	5.4E-06	1.4E-04	
ARHGGEF16	Rho guanine exchange factor 16	D89016	1p36.3	18.8 \pm 3.0	6.6 \pm 0.4	67%	2.6 \pm 0.6	3.4E-01	2.9E-03	—	
TNFRSF1B	Tumor necrosis factor receptor superfamily, member 1B	M32315	1p36.3-p36.2	67.4 \pm 11.4	24.6 \pm 2.6	67%	2.7 \pm 0.5	4.9E-02	2.6E-04	4.9E-02	
PIM1	Pim-1 oncogene	M16750	6p21.2	34.9 \pm 4.5	13.9 \pm 1.1	67%	2.3 \pm 0.5	1.0E-01	6.0E-04	—	
STK19	Serine/threonine kinase 19	BC016916	6p21.3	12.8 \pm 1.5	5.4 \pm 0.3	78%	2.4 \pm 0.3*	4.3E-02	1.7E-04	2.8E-02	
NDRG1	N-myc downstream regulated gene 1	D87953	8q24.3	39.6 \pm 5.4	15.5 \pm 1.1	67%	2.6 \pm 0.3	1.3E-01	4.7E-04	—	
PXN	Paxillin	U14588	12q24	39.1 \pm 3.1	15.3 \pm 1.8	89%	2.6 \pm 0.2	4.2E-02	1.5E-05	6.7E-04	
IHPK1	Inositol hexaphosphate kinase 1	D87452	3p21.31	14.1 \pm 1.4	5.6 \pm 0.2	67%	2.5 \pm 0.2	2.4E-02	1.0E-04	1.3E-02	
STAT5A	Signal transducer and activator of transcription 5A	U43185	17q11.2	27.6 \pm 4.3	10.4 \pm 0.8	78%	2.7 \pm 0.4	5.4E-02	2.8E-04	—	
SQSTM1	Sequestosome 1	U46751	5q35	98.8 \pm 10.3	40.8 \pm 2.5	67%	2.4 \pm 0.3	1.6E-02	4.6E-05	4.1E-03	
HDFG	Hepatoma-derived growth factor	BC018991	X	41.6 \pm 5.0	17.3 \pm 1.6	67%	2.4 \pm 0.3	8.6E-02	2.1E-04	3.7E-02	

Continued

Table 2. Continued

MPP1	Membrane protein, palmitoylated 1	M64925	Xq28	34.9 ± 4.9	15.5 ± 2.1	67%	2.2 ± 0.3	7.4E-01	7.1E-04	—
RGL2	Ral guanine nucleotide dissociation stimulator-like 2	U68142	6p21.3	15.7 ± 1.2	6.6 ± 0.3	78%	2.4 ± 0.2	4.5E-02	9.1E-06	3.1E-04
MX1	Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78	M33882	21q22.3	30.1 ± 8.8	8.7 ± 1.0	67%	3.0 ± 1.2	9.3E-01	1.4E-02	—
RPS6KA1	Ribosomal protein S6 kinase, 90kDa, polypeptide 1	L07597	3	28.8 ± 4.3	11.3 ± 1.1	67%	2.5 ± 0.4	1.2E-01	1.3E-04	1.8E-02
ITPK1	Inositol 1,3,4-triphosphate 5/6 kinase	U51336	14q31	49.0 ± 6.3	20.2 ± 1.4	56%	2.4 ± 0.3	5.7E-02	1.5E-04	2.2E-02
ARF3	ADP-ribosylation factor 3	M74491	12q13	54.1 ± 5.9	23.2 ± 2.7	56%	2.3 ± 0.3	5.7E-02	1.6E-04	2.4E-02
FKBP1A	FK506 binding protein 1A	M34539	20p13	42.8 ± 5.0	17.5 ± 1.3	67%	2.4 ± 0.3	1.9E-02	1.1E-04	1.4E-02
PRKAG1	Protein kinase, AMP-activated, γ 1 non-catalytic subunit	U42412	12q12-q14	15.4 ± 2.0	6.7 ± 0.3	67%	2.3 ± 0.3	5.4E-02	1.4E-03	—
BIRC1	Baculoviral IAP repeat-containing 1	U80017	.5q12.2-q13.3	12.5 ± 1.5	5.8 ± 0.3	44%	2.1 ± 0.3	7.5E-02	2.3E-04	4.1E-02
ARHGEF1	Rho guanine nucleotide exchange factor 1	U64105	19q13.13	43.6 ± 3.7	19.2 ± 1.4	67%	2.3 ± 0.2	1.0E-02	1.5E-05	6.0E-04
AVPR1B	Arginine vasopressin receptor 1B	L37112	1q32	14.3 ± 1.4	6.4 ± 0.4	56%	2.2 ± 0.2	1.1E-01	5.8E-05	6.2E-03
DGKZ	Diacylglycerol kinase, zeta	U51477	11p11.2	32.6 ± 2.9	13.8 ± 0.8	67%	2.4 ± 0.2	2.8E-02	9.1E-06	3.2E-04
PARK7	Parkinson disease (autosomal recessive, early onset) 7	D61380	1p36.33-p36.12	58.4 ± 5.9	26.2 ± 3.0	56%	2.2 ± 0.2	1.3E-01	1.8E-04	2.9E-02
RGS2	G0/G1 switch regulatory gene # 8	L13391	1q31	60.3 ± 12.5	21.4 ± 3.2	67%	2.3 ± 0.8	2.7E-01	8.1E-03	—
PPM1F	Protein phosphatase 1F (PP2C domain containing)	D13640	22q11.22	29.0 ± 4.3	11.2 ± 1.0	67%	2.4 ± 0.6	1.7E-01	1.3E-03	—
ARF5	ADP-ribosylation factor 5	M57567	7q31.3	42.4 ± 6.2	16.2 ± 1.4	78%	2.4 ± 0.5	5.1E-02	4.0E-04	—
PTK2B	PTK2B protein tyrosine kinase 2 beta	U43522	8p21.1	13.2 ± 2.2	5.8 ± 0.3	56%	2.3 ± 0.4	5.0E-03	1.5E-03	—
PIM1	Pim-1 oncogene	M54915	6p21.2	54.7 ± 6.4	23.5 ± 1.8	78%	2.1 ± 0.4	1.1E-01	3.4E-04	—
INPPL1	Inositol polyphosphate phosphatase-like 1	L36818	11q23	19.4 ± 2.8	8.2 ± 0.9	56%	2.4 ± 0.3	1.3E-01	2.1E-04	3.8E-02
PCMT1	Protein-L-isoaspartate (D-aspartate) O-methyltransferase	D25547	6q24-q25	13.2 ± 2.3	5.8 ± 0.2	44%	2.3 ± 0.4	1.3E-01	1.4E-03	—
ARHGAP1	Rho GTPase activating protein 1	U02570	11p12-q12	25.6 ± 3.3	11.8 ± 0.8	56%	2.2 ± 0.3	3.4E-02	6.9E-04	—
FKBP2	FK506 binding protein 2, 13kDa	M75099	11q13.1-q13.3	23.6 ± 3.1	10.8 ± 0.9	56%	2.2 ± 0.3	7.0E-02	9.7E-04	—
TNIP1	TNFAIP3 interacting protein 1	D30755	5q32-q33.1	29.1 ± 4.0	12.8 ± 0.9	56%	2.3 ± 0.3	5.0E-02	2.2E-04	4.0E-02
IRAK1	Interleukin-1 receptor-associated kinase 1	L76191	Xq28	32.7 ± 2.8	15.5 ± 1.1	56%	2.1 ± 0.2	8.1E-02	2.0E-05	1.0E-03
RHOG	Ras homolog gene family, member G (rho G)	X61587	11p15.5-p15.4	51.9 ± 8.5	19.7 ± 1.6	78%	2.2 ± 0.7	8.0E-03	4.4E-03	—
RASSF2	Ras association (RalGDS/AF-6) domain family 2	D79990	20pter-p12.1	29.3 ± 4.5	12.3 ± 1.7	56%	2.1 ± 0.6	4.3E-01	3.7E-03	—
NEDD8	Neural precursor cell expressed, developmentally down-regulated 8	D23662	14q11.2	53.1 ± 6.8	23.8 ± 2.6	67%	2.0 ± 0.5	1.9E-01	8.6E-04	—
CAP1	CAP, adenylate cyclase-associated protein 1	L12168	1p34.2	134.7 ± 18.9	58.4 ± 5.4	67%	2.1 ± 0.5	4.2E-02	3.1E-03	—
ZAP70	Zeta-chain (TCR) associated protein kinase	L05148	2q12	36.6 ± 4.3	16.3 ± 1.4	78%	2.0 ± 0.4	4.4E-02	5.1E-04	—
FKBP8	FK506 binding protein 8	L37033	19p12	27.4 ± 3.7	12.3 ± 1.1	56%	2.0 ± 0.5	1.4E-02	7.6E-04	—

Continued

Table 2. Continued

GRK6	G protein-coupled receptor kinase 6	L16862	5q35	23.4 ± 3.6	10.5 ± 1.0	56%	2.0 ± 0.5	3.0E-02	1.3E-03	—
MAP2K2	Mitogen-activated protein kinase kinase 2	L11285	7q32	29.1 ± 4.0	12.9 ± 0.7	67%	2.0 ± 0.5	1.5E-02	1.3E-03	—
PTP4A2	Protein tyrosine phosphatase type IVA, member 2	U14603	1p35	76.6 ± 9.4	35.3 ± 2.6	56%	1.9 ± 0.4	2.0E-02	5.7E-04	—
RAC1	Ras-related C3 botulinum toxin substrate 1	NM_005908	7p22	20.1 ± 3.4	8.9 ± 0.6	56%	2.0 ± 0.5	3.4E-02	3.2E-03	—
MX2	Myxovirus (influenza virus) resistance 2	M30818	21q22.3	20.6 ± 3.9	8.7 ± 0.9	56%	1.9 ± 0.7	7.0E-01	8.6E-03	—
FYB	FYN binding protein (FYB-120/130)	U93049	5p13.1	5.2 ± 0.7	10.8 ± 1.9	67%	-2.3 ± 0.2	4.3E-01	7.1E-03	—
Structural										
MYL9	Myosin, light polypeptide 9, regulatory	J02854	20q11.23	17.9 ± 4.7	4.4 ± 0.2	67%	4.1 ± 1.1*	1.4E-01	1.1E-03	—
PLEC1	Plectin 1, intermediate filament binding protein	U53204	8q24	37.8 ± 6.0	9.6 ± 0.9	78%	3.9 ± 0.6	3.0E-02	3.4E-05	2.8E-03
GFAP	Glial fibrillary acidic protein	S40719	17q21	19.9 ± 3.2	7.2 ± 0.5	67%	2.8 ± 0.4	1.9E-01	3.5E-04	—
BECN1	Beclin 1	L38932	17q21	40.3 ± 4.0	14.8 ± 1.2	78%	2.7 ± 0.3	5.7E-02	1.6E-05	7.4E-04
MYH9	Myosin, heavy polypeptide 9, non-muscle	M31013	22q13.1	149.8 ± 13.7	55.4 ± 4.4	78%	2.7 ± 0.2	5.8E-02	6.7E-06	1.9E-04
KRT1	Keratin 1	M98776	12q12-q13	16.7 ± 2.9	6.2 ± 0.3	67%	2.5 ± 0.6	4.9E-01	3.0E-03	—
NUMA1	Nuclear mitotic apparatus protein 1	Z14227	11q13	25.7 ± 3.4	9.5 ± 0.6	78%	2.7 ± 0.4	5.5E-02	3.4E-04	—
PDLIM1	PDZ and LIM domain 1 (elfin)	U90878	10q22-q26.3	26.6 ± 2.9	11.0 ± 0.8	56%	2.4 ± 0.3	3.6E-02	2.5E-05	1.6E-03
MYL6	Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	M31212	12	360.2 ± 18.0	138.0 ± 13.6	89%	2.6 ± 0.1	2.4E-02	3.9E-06	8.1E-05
SAFB	Scaffold attachment factor B	L43631	19p13.3-p13.2	25.4 ± 2.6	10.3 ± 0.7	67%	2.5 ± 0.3	9.9E-02	2.8E-05	1.9E-03
MAPT	Microtubule-associated protein tau	AH005895	17q21.1	26.3 ± 4.6	10.1 ± 0.8	56%	2.4 ± 0.6	9.5E-02	2.2E-03	—
TPM3	Tropomyosin 3	BC000771	149.2 ± 16.9	64.5 ± 5.5	67%	2.3 ± 0.3	7.7E-02	1.7E-04	2.9E-02	—
HSU34301	Nonmuscle myosin heavy chain IIB	U34301	17	17.0 ± 2.1	7.4 ± 0.9	56%	2.3 ± 0.3	1.0E-01	1.2E-04	1.7E-02
KNS2	Kinesin 2 60/70kDa	L04733	14q32.3	16.1 ± 2.2	6.5 ± 0.4	56%	2.5 ± 0.3	6.5E-02	1.6E-04	2.4E-02
TUBB2C	Tubulin, β 2C	AK026167	9q34	53.2 ± 4.8	23.9 ± 2.5	67%	2.2 ± 0.2	1.7E-01	3.9E-05	3.2E-03
TUBA3	Tubulin, α 3	X01703	12q12-12q14.3	37.7 ± 5.5	14.5 ± 1.8	67%	2.4 ± 0.5	3.2E-01	9.7E-04	—
TNMC1	Troponin C, slow	M37984	3p21.3-p14.3	11.2 ± 1.2	5.1 ± 0.1	56%	2.2 ± 0.2*	6.7E-02	1.7E-04	2.7E-02
MSN	Moesin	M69066	Xq11.2-q12	178.8 ± 27.9	72.8 ± 6.9	67%	2.2 ± 0.6	5.4E-02	4.1E-03	—
Transcription										
RELA	V-rel reticuloendotheliosis viral oncogene homolog A	L19067	11q13	39.8 ± 4.3	11.1 ± 1.1	100%	3.6 ± 0.4	4.1E-03	7.9E-07	4.8E-06
FOS	V-fos FBJ murine osteosarcoma viral oncogene homolog	V01512	14q24.3	51.4 ± 14.0	13.9 ± 3.4	67%	3.4 ± 1.1	2.8E-02	4.5E-03	—
NFE2	Nuclear factor (erythroid-derived 2)	S77763	12q13	32.3 ± 4.1	9.5 ± 1.4	78%	3.4 ± 0.4	3.5E-01	1.5E-05	6.1E-04
IRF5	Interferon regulatory factor 5	U51127	7q32	29.0 ± 4.6	10.2 ± 1.0	78%	2.9 ± 0.5	8.4E-02	2.0E-04	3.6E-02
ZNFpT1	Zinc-finger protein	X65230	15.2 ± 2.2	5.3 ± 0.1	78%	2.9 ± 0.4	6.4E-02	5.9E-04	—	—
SF1	Splicing factor 1	L49380	11q13	46.4 ± 4.3	16.2 ± 0.6	78%	2.9 ± 0.3	3.7E-02	1.5E-05	6.4E-04
HCFC1	Host cell factor C1	L20010	Xq28	26.0 ± 2.9	8.9 ± 0.5	78%	2.9 ± 0.3	2.7E-02	2.5E-05	1.6E-03
SREBF1	Sterol regulatory element binding transcription factor 1	U00968	17p11.2	25.6 ± 2.1	8.9 ± 0.6	89%	2.9 ± 0.2	6.3E-02	1.6E-06	1.9E-05
POLR2A	Polymerase (RNA) II (DNA directed) polypeptide A	X74874	17p13.1	18.9 ± 2.7	6.8 ± 0.5	67%	2.8 ± 0.4	7.3E-02	7.7E-05	8.7E-03

Continued

Table 2. Continued

MAZ	MYC-associated zinc finger protein	M94046	16p11.2	29.7 ± 3.1	10.7 ± 0.6	78%	2.8 ± 0.3	7.9E-03	3.1E-05	2.3E-03
TCFL1	Transcription factor-like 1	D43642	1q21	38.2 ± 5.3	14.3 ± 0.7	67%	2.7 ± 0.4	9.9E-02	4.7E-04	—
IRF3	Interferon regulatory factor 3	Z56281	19q13.3-q13.4	25.1 ± 1.7	9.5 ± 0.5	78%	2.6 ± 0.2	2.2E-02	9.3E-07	2.303
BTG2	BTG family, member 2	U72649	1q32	40.3 ± 8.1	14.8 ± 1.1	67%	2.5 ± 0.7	8.1E-02	3.0E-03	—
VGLL4	Vestigial like 4	D50911	3p25.2	16.4 ± 2.1	6.5 ± 0.3	67%	2.5 ± 0.3	1.3E-01	3.1E-04	—
RNPC2	RNA-binding region (RNP1, RRM) containing 2	L10910	20q11.23	16.1 ± 2.1	6.4 ± 0.6	67%	2.5 ± 0.3	2.4E-01	4.7E-04	—
NBL1	Neuroblastoma, suppression of tumorigenicity 1	D28124	1p36.13-p36.11	16.6 ± 1.9	6.8 ± 0.6	67%	2.4 ± 0.3	2.4E-01	1.2E-04	1.6E-02
NCOR2	Nuclear receptor co-repressor 2	U37146	12q24	26.6 ± 2.9	11.2 ± 0.8	67%	2.4 ± 0.3	4.2E-02	5.1E-05	5.0E-03
JUND	Jun D proto-oncogene	X56681	19p13.2	114.4 ± 9.6	47.5 ± 3.9	89%	2.4 ± 0.2	1.4E-02	3.0E-06	4.8E-05
TRIM28	Tripartite motif-containing 28	U95040	19q13.4	44.0 ± 5.2	17.3 ± 1.2	67%	2.3 ± 0.5	1.4E-02	2.2E-04	4.0E-02
POLR2E	Polymerase (RNA) II (DNA directed) polypeptide E	D38251	19p13.3	28.4 ± 3.5	11.2 ± 1.1	78%	2.3 ± 0.5	7.7E-02	6.5E-04	—
BCL6	B-cell CLL/lymphoma 6 (zinc finger protein 51)	U00115	3q27	12.0 ± 2.1	5.2 ± 0.2	56%	2.3 ± 0.4	5.3E-02	2.6E-03	—
PML	Promyelocytic leukemia	M79462	15q22	10.7 ± 1.3	4.7 ± 0.2	56%	2.3 ± 0.3*	1.5E-02	5.1E-04	—
CEBPB	CCAAT/enhancer binding protein (C/EBP), β	X52560	20	80.6 ± 14.7	32.2 ± 2.9	67%	2.2 ± 0.7	3.7E-01	1.3E-02	—
SFRS17	Splicing factor, arginine/serine-rich 11	M74002	1p31	20.0 ± 2.3	8.4 ± 0.8	78%	2.2 ± 0.5	1.4E-01	3.4E-04	—
SRF	Serum response factor	J03161	6p21.1	18.6 ± 2.5	8.3 ± 0.6	56%	2.2 ± 0.3	1.7E-01	3.9E-04	—
YY1	YY1 transcription factor	M77698	14q	13.4 ± 1.5	6.3 ± 0.6	56%	2.1 ± 0.2	2.2E-01	2.8E-04	—
LYL1	Lymphoblastic leukemia derived sequence 1	M22638	19p13.2	14.2 ± 1.2	6.8 ± 0.3	56%	2.1 ± 0.2	4.2E-02	4.3E-05	3.7E-03
SUPT4H1	Suppressor of Ty 4 homolog 1	U43923	17q21-q23	20.1 ± 3.2	8.9 ± 0.8	56%	2.0 ± 0.5	2.1E-01	2.9E-03	—
TAF15	TAF15 RNA polymerase II	U51334	17q11.1-q11.2	23.9 ± 2.8	11.1 ± 1.0	67%	1.9 ± 0.4	3.9E-02	6.5E-04	—
EIF3S9	Eukaryotic translation initiation factor 3, subunit 9 eta	U78525	7p22.3	19.6 ± 2.2	9.0 ± 0.7	67%	2.2 ± 0.2	2.3E-02	2.6E-04	4.8E-02
TCIRG1	T-cell, immune regulator 1, ATPase, H + transporting, lysosomal V0 protein alpha isoform 3	U45285	11q13.4-q13.5	31.4 ± 3.3	6.7 ± 0.5	100%	4.7 ± 0.5	1.2E-01	1.6E-07	1.6E-07
TETRAN	Tetracycline transporter-like protein	L11669	4p16.3	26.8 ± 2.7	6.8 ± 0.5	100%	3.9 ± 0.4	1.2E-01	1.6E-06	1.8E-05
HD	Huntingtin (Huntington disease)	L12392	4p16.3	22.3 ± 2.7	6.5 ± 0.3	78%	3.5 ± 0.4	9.1E-02	4.9E-05	2.082
ATP6AP1	Human mRNA for ORF, Xq terminal portion.	D16469	Xq28	31.7 ± 4.2	9.0 ± 0.8	89%	3.5 ± 0.5	6.5E-02	8.8E-06	2.9E-04
GGA3	Golgi associated, γ adaptin ear containing, ARF binding protein 3	D63876	17q25.2	33.9 ± 3.3	10.1 ± 0.6	89%	3.4 ± 0.3	7.1E-02	2.3E-06	2.236
ATP6V0C	ATPase, H + transporting, lysosomal, V0 subunit c	M62762	16p13.3	69.7 ± 8.8	21.2 ± 1.9	89%	3.3 ± 0.4	9.8E-02	4.3E-05	3.7E-03
AP2M1	Adaptor-related protein complex 2, mu 1 subunit	D63475	3q28	42.6 ± 5.5	14.2 ± 1.3	67%	3.0 ± 0.4	4.1E-02	1.3E-04	1.8E-02
SLC2A3	Solute carrier family 2 (facilitated glucose transporter), member 3	M20681	12p13.3	26.0 ± 2.3	9.3 ± 1.0	89%	2.8 ± 0.2	1.3E-01	9.2E-06	3.3E-04

Continued

Table 2. Continued

SLC9A1	Solute carrier family 9 (sodium/hydrogen exchanger), isoform 1 (antipporter, Na ⁺ /H ⁺ , amiloride sensitive)	S68616	1p36.1-p35	19.0 ± 2.4	6.8 ± 0.2	67%	2.8 ± 0.4	4.2E-02	2.3E-04	4.2E-02
SLC11A1	Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1	D50402	2q35	19.1 ± 3.0	6.8 ± 0.6	78%	2.8 ± 0.4	1.9E-01	4.7E-04	—
MILC1	Megalencephalic leukoencephalopathy with subcortical cysts 1	D25217	22q13.33	16.1 ± 2.6	5.8 ± 0.5	56%	2.8 ± 0.4*	2.2E-02	5.7E-04	—
SEC24C	FLJ44715 gene product	D38555	10q22.3	21.3 ± 2.9	8.0 ± 0.6	67%	2.7 ± 0.4	8.3E-02	3.3E-04	—
CLTA	Clathrin, light polypeptide (Lca)	M20471	9p13	73.6 ± 9.1	26.7 ± 1.9	89%	2.8 ± 0.3	4.6E-02	3.8E-05	3.2E-03
AP2B1	Adaptor-related protein complex 2, β 1 subunit	M34175	17q11.2-q12	28.2 ± 4.0	10.3 ± 1.2	67%	2.5 ± 0.5	1.7E-01	2.3E-04	4.2E-02
AP1B1	Adaptor-related protein complex 1, β 1 subunit	L13939	22q12.2	23.2 ± 3.0	9.5 ± 0.8	67%	2.4 ± 0.3	4.3E-02	6.1E-04	—
TXN2	Thioredoxin 2	U78678	22q13.1	18.9 ± 2.5	7.5 ± 0.5	67%	2.5 ± 0.3	3.8E-02	2.8E-04	—
CRIP1	Cysteine-rich protein 1 (intestinal)	U09770	7q11.23	35.3 ± 3.9	15.9 ± 1.1	56%	2.2 ± 0.2	9.2E-02	1.4E-04	2.0E-02
NAPA	N-ethylmaleimide-sensitive factor attachment protein, alpha	U39412	19q13.33	15.0 ± 2.0	6.4 ± 0.4	67%	2.1 ± 0.5	5.5E-02	3.2E-03	—
Ubiquitin										
UBE1	Ubiquitin-activating enzyme E1	M58028	Xp11.23	46.4 ± 6.9	11.3 ± 1.0	89%	4.1 ± 0.6	8.6E-03	3.3E-05	2.5E-03
USP11	Ubiquitin specific protease 11	U44839	Xp11.23	60.2 ± 7.4	18.9 ± 1.1	78%	3.2 ± 0.4	1.2E-02	5.0E-05	4.9E-03
UBC	Ubiquitin	M26880	12q24.3	198.0 ± 14.3	66.3 ± 10.5	89%	3.0 ± 0.2	2.4E-01	7.8E-06	2.4E-04
UBE1L	Ubiquitin-activating enzyme E1-like	L13852	3p21	54.8 ± 4.6	18.6 ± 1.3	89%	2.9 ± 0.2	4.7E-02	9.4E-07	7.5E-06
CUL7	Cullin 7	D38548	6p21.1	14.7 ± 2.0	5.8 ± 0.3	56%	2.5 ± 0.3	6.6E-02	2.2E-04	4.0E-02
RAD23A	RAD23 homolog A (S. cerevisiae)	D21235	19p13.2	15.6 ± 1.6	6.6 ± 0.3	78%	2.4 ± 0.2	8.8E-02	2.0E-04	3.4E-02
UBE2V1	Homo sapiens UBEV-1	BC000468	20q13.2	25.9 ± 3.6	11.2 ± 1.3	67%	2.3 ± 0.3	5.3E-01	4.4E-04	—
UFD1L	Ubiquitin like protein	U64444	22q11.21	22.8 ± 2.7	10.1 ± 0.9	67%	2.0 ± 0.4	1.3E-01	5.7E-04	—
USP4	Ubiquitin specific protease 4 (proto-oncogene)	U20657	3p21.3	14.8 ± 1.7	6.6 ± 0.3	67%	2.0 ± 0.4	4.0E-02	5.7E-04	—
Unknown										
LRRC14	Leucine rich repeat containing 14	D25216	8q24.3	31.7 ± 4.1	9.2 ± 1.1	89%	3.4 ± 0.4	5.3E-02	8.8E-06	2.8E-04
WDR42A	WD repeat domain 42A	U06631	1q22-q23	23.6 ± 2.2	7.4 ± 0.5	89%	3.2 ± 0.3	8.1E-02	3.1E-06	5.6E-05
C1orf16	Chromosome 1 open reading frame 16	D87437	1q35	19.3 ± 2.2	6.5 ± 0.3	78%	3.0 ± 0.3	4.8E-02	2.8E-05	2.1
C1orf19	Chromosome 6 open reading frame 9	U89336	6p21.3	41.6 ± 4.1	13.8 ± 1.3	78%	2.8 ± 0.3	7.6E-05	3.9E-06	2,116
KIAA0056	KIAA0056 protein	D29954	11q25	17.4 ± 2.8	6.2 ± 0.4	67%	2.6 ± 0.6	2.7E-01	1.5E-03	—
C21orf2	Chromosome 21 open reading frame 2	U84569	21q22.3	25.2 ± 3.3	9.8 ± 0.9	67%	2.6 ± 0.3	8.5E-02	1.0E-04	1.2E-02
PRCC	Papillary renal cell carcinoma	X99720	1q21.1	20.1 ± 2.1	7.7 ± 0.6	78%	2.6 ± 0.3	1.4E-01	2.7E-05	1.8E-03
KIAA0226	KIAA0226 gene product	D86979	3q29	20.6 ± 2.3	7.8 ± 0.5	78%	2.6 ± 0.3	6.4E-02	5.4E-05	5.5E-03
ARMCX6	Hypothetical protein FLJ20811	L20773	Xq21.33-q22.3	25.6 ± 2.7	10.1 ± 0.9	56%	2.5 ± 0.3	4.6E-02	3.0E-05	2.1E-03
UBAP2L	Ubiquitin associated protein 2-like	D63478	1q22	13.2 ± 1.9	5.4 ± 0.2	67%	2.5 ± 0.3	5.5E-02	7.3E-04	—
ARMET	Arginine-rich, mutated in early stage tumors	M83751	3p21.1	21.2 ± 2.5	8.8 ± 0.5	56%	2.4 ± 0.3	4.9E-02	1.1E-04	1.5E-02
KIAA0174	KIAA0174 gene product	D79996	16q22.2	28.6 ± 3.2	12.0 ± 1.3	67%	2.4 ± 0.3	2.1E-01	1.1E-04	1.4E-02

Continued

Table 2. Continued

TATDN2	TatD DNase domain containing 2	D86972	3p25.3	13.8 ± 1.5	5.8 ± 0.5	67%	2.4 ± 0.3	1.4E-01	1.0E-04	1.3E-02
PFAAP5	Phosphonoformate immuno-associated protein 5	U50535	13	15.7 ± 1.5	6.8 ± 0.6	67%	2.3 ± 0.2	1.7E-01	7.2E-05	8.0E-03
HSHRTPSN	Retrotransposon	Z48633		10.6 ± 2.1	4.4 ± 0.2	44%	2.2 ± 0.6*	7.9E-02	6.6E-03	—
TAGLN2	Transgelin 2	D21261	1q21-q25	278.8 ± 30.1	111.3 ± 10.9	89%	2.2 ± 0.5	1.6E-02	1.3E-03	—
TRIM26	Tripartite motif-containing 26	U09825	6p21.3	21.4 ± 2.8	9.6 ± 0.6	56%	2.2 ± 0.3	1.4E-01	1.0E-03	—
NUP188	Nucleoporin 188kDa	D79991	9q34.13	13.7 ± 1.3	6.2 ± 0.2	67%	2.2 ± 0.2	4.2E-02	1.6E-04	2.3E-02
FAM53B	Family with sequence similarity 53, member B	D50930	10q26.2	16.8 ± 2.8	7.2 ± 0.4	56%	2.1 ± 0.5	9.7E-02	3.8E-03	—
C21orf33	Chromosome 21 open reading frame 33	U53003	21q22.3	12.2 ± 1.1	5.7 ± 0.2	44%	2.1 ± 0.2	6.7E-02	2.1E-05	1.2E-03
CYFIP2	Cytoplasmic FMR1 interacting protein 2	L47738	5q34	29.2 ± 3.9	13.4 ± 1.2	67%	2.0 ± 0.5	2.6E-02	1.4E-03	—
DXYS155E	DNA segment on chromosome X and Y (unique) 155 expressed sequence	L03426	Xp22.32, Ypter-p11.2	15.9 ± 2.1	6.7 ± 0.4	78%	2.0 ± 0.5	2.5E-03	1.3E-03	—
BRD3	Bromodomain containing 3	D26362	9q34	15.9 ± 1.8	7.2 ± 0.6	67%	2.0 ± 0.4	2.6E-01	5.1E-04	—
FAM50A	DNA segment on chromosome X (unique) 9928 expressed sequence	D83260	Xq28	16.6 ± 1.7	7.6 ± 0.4	33%	2.0 ± 0.2	6.3E-04	3.0E-05	2.2E-03
NK4	Natural killer cell transcript 4	M59807	16p13.3	116.7 ± 18.5	49.2 ± 6.4	56%	1.9 ± 0.6	6.7E-03	2.8E-03	—

Data are mean ± SE except * average ± SE. Genes were filtered based on the average frequency of 10 and average change in expression of at least 2-fold. Fold changes for genes with increased expression are represented as the ratio of RA average frequency/normal average frequency, whereas genes with reduced expression are represented as the negative reciprocal of that ratio. SE was also calculated for the fold change. Average frequency of expression and its SE were calculated for 9 RA and 13 control samples. The results of three separate statistical analyses performed on the data are shown. A Student *t* test was performed to identify statistically significant differences between samples with a threshold of $P < 0.5$. Two Welch ANOVA (26) analyses using different multiple testing corrections were performed. The first, performed according to the methods of Benjamini and Hochberg (28) to calculate the FDR, was used with a limit of 0.05. The second, based on Bonferroni (29,30) FWER, was calculated with P value cutoff of <0.05 . The rightmost column represents genes that comprise the class prediction analysis. Chromosomal map units shown are based on GenBank information. * Genes with an asterisk have control samples with frequencies <5 ppm and are called absent in $>50\%$ of samples. Therefore, the fold change calculation may not accurately reflect the actual difference in expression. Annotation was based on GO, Gene, and PubMed to categorize the genes. Genes with different Affymetrix identifiers were not removed from the table.

fold change of RA frequency compared with that of the controls (Table 2). This analysis clustered the genes into 19 functional classes and highlighted one chromosomal location. Ten genes with increased expression in the RA PBMCs compared with normal controls map to an RA susceptibility locus, 6p21.3 (27) (Table 3). The functional classes are diverse and include genes involved in calcium binding, chaperones, cytokines, transcription, translation, signal transduction, extracellular matrix, integral to plasma membrane, integral to intracellular membrane, mitochondrial, ribosomal, structural, enzymes, and proteases. Many of these 330 genes or gene products are known to be differentially regulated in RA. Twenty-five genes were classified as unknown because they either coded for a hypothetical protein or were identified as an open reading frame of unknown function.

The k -nearest neighbor analysis identified genes that may be preferentially regulated in the RA samples. Of the 29 genes identified by the class prediction analysis (Figure 2B) to be expressed in the RA PBMCs compared with the controls, only *RELA* (NF κ B *p65*) (28), *IGF2* (insulin-like growth factor 2) (29), *FTH1* (ferritin heavy chain) (30), and *SELP* (selectin P) (31) have previously been associated with RA. Furthermore, both NF- κ B and selectin P have been used as therapeutic targets in animal models (32,33). *INPP5E* (inositol polyphosphate-5-phosphatase E), *STAB1* (stabilin), *AGPAT1* (1-acylglycerol-3-phosphate *O*-acyltransferase 1), *TCIRG1* (T-cell, immune regulator 1, ATPase, H⁺ transporting, lysosomal V0 protein A isoform 3), *HD* (Huntingtin), *SREBF1* (sterol regulatory element binding factor 1), and *IRF3* (interferon regulatory factor 3) are examples of genes that have not previously been associated with RA.

DISCUSSION

In this study, the mRNA levels of 6800 genes were measured in PBMCs from RA patients with active disease and normal individuals. All patients were on

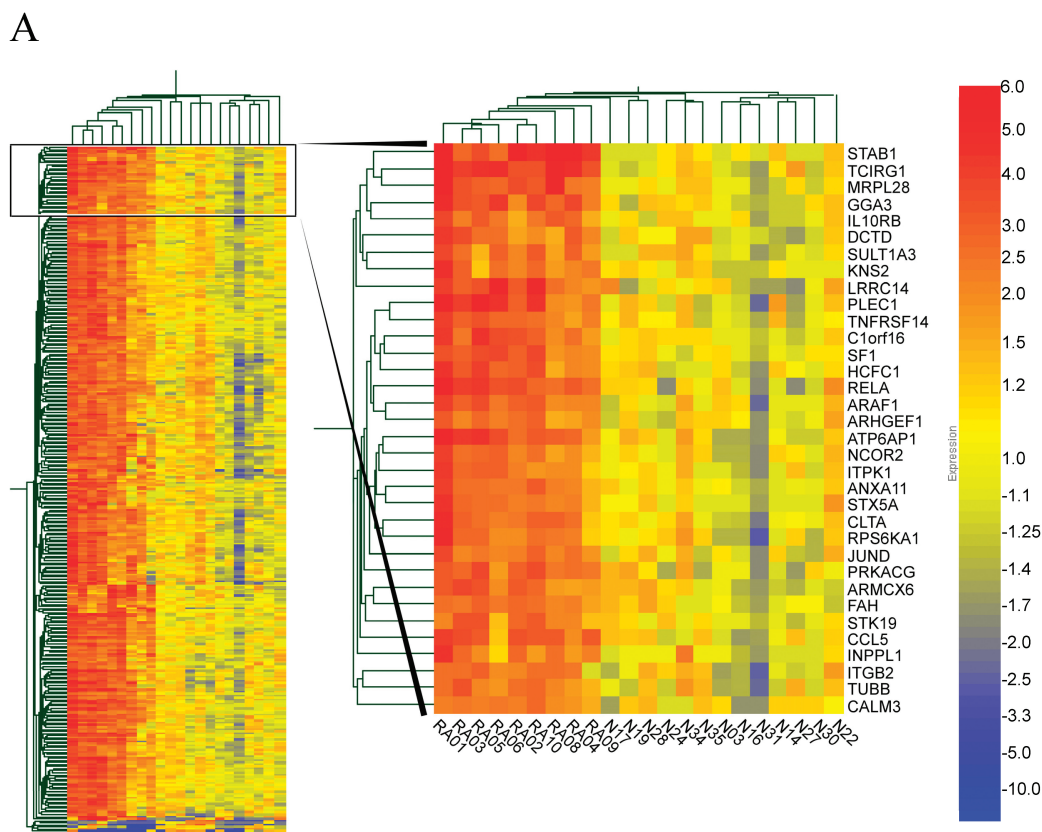


Figure 1. Unsupervised hierarchical cluster analysis of RNA from 9 RA and 13 control PBMC samples. Total RNA samples were analyzed on oligonucleotide arrays as described. In no case were samples pooled. Genes were selected for analysis if they had a present call, a frequency greater than 10 ppm, and two-fold change expression in five of nine RA samples. The expression patterns of 330 genes are displayed in a dendrogram where columns represent each sample and rows represent individual genes. Genes are colored on a gradient (from -10 -fold to 10 -fold), with those increase in expression relative to the average of the control in red. Those that decrease are in blue, and those with little or no change are in yellow. A, region where expression levels in the RA samples were increased compared with the normal samples.

DMARD therapy that included methotrexate. Three hundred thirty differentially expressed transcripts were detected in at least 50% of the patients and exhibited a minimum of a two-fold change in expression from normal individuals. A number of genes previously thought to be involved in RA pathogenesis were detected in this study. These include the transcripts for TNF receptor TNFRSF1B (*p75*) and CCL5 (RANTES). TNF α has a key role in RA, and the expression of mRNA and protein of TNF receptors is increased in RA synovial membranes and sera (34-36). In murine models, as well as TNF α transgenic and receptor knockout mice, the pathogenic activity of TNF has been well docu-

mented. Furthermore, both the soluble form of the TNF receptor and antibodies against TNF are efficacious in animal models and are effective therapies for RA (4,6-8,37,38). CCL5 is a chemokine expressed in the serum and synovial joints of patients with RA and is likely to play important roles in recruitment of inflammatory cells (39). A polyclonal antibody to RANTES improved symptoms in animals with adjuvant induced arthritis (40). RNA transcripts encoding proteins from a number of signaling pathways, including NF- κ B, were present in increased amounts in individuals with RA, and many of these are targets for therapeutic blockade (41). NF- κ B (*RELA*) has important roles in the production of in-

flammatory cytokines such as IL-1 and TNF (28). The presence of these known genes in the data set further validates the array data and analysis.

A *k*-nearest neighbor analysis was applied to the data set to identify genes preferentially expressed in the PBMCs from RA patients compared with controls. Twenty-nine genes were identified. Some of these genes have been previously identified as being differentially regulated in RA and include *IGF2* (29), *FTH1* (30), and *SELP* (31). *SELP* contributes to many inflammatory diseases and has been shown to mediate leukocyte interaction with endothelial cell wall (42). Levels of *SELP* are increased in the synovial fluid of RA patients (43). In the

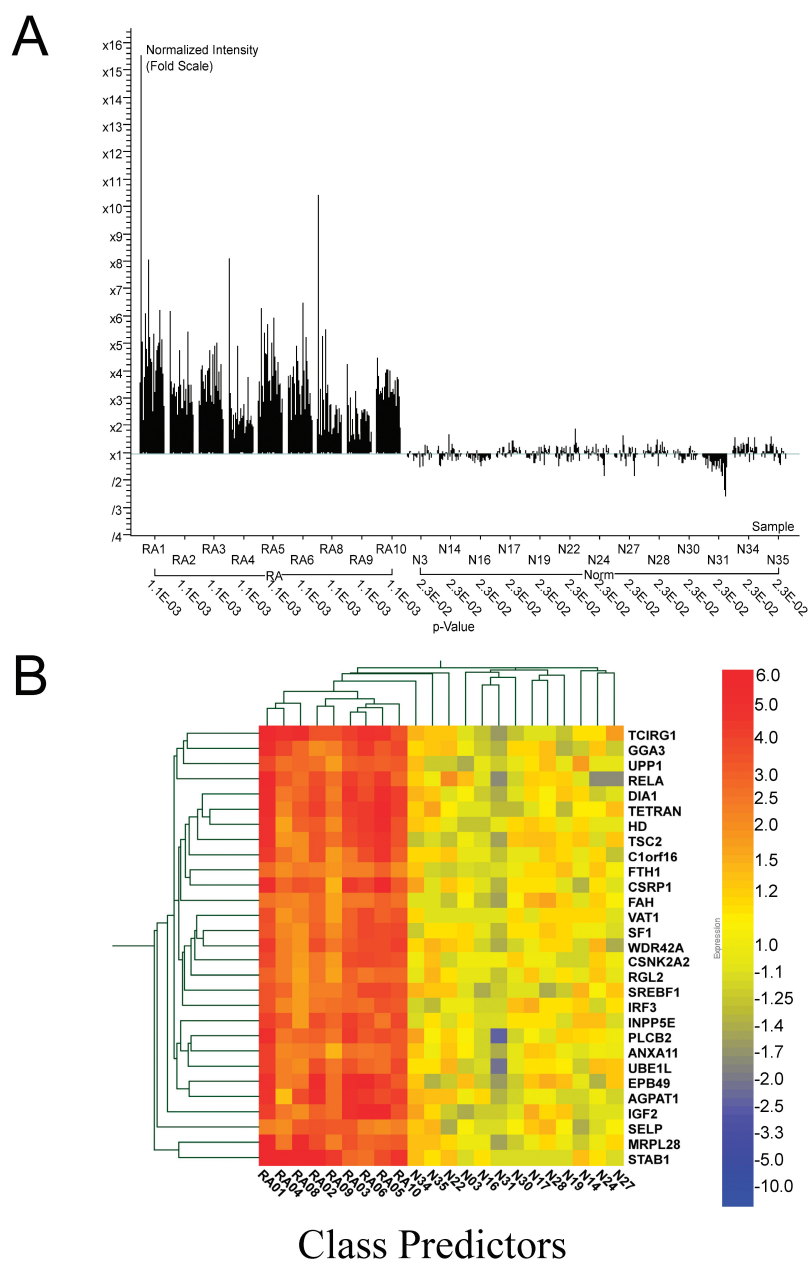


Figure 2. Class prediction. Using a class prediction algorithm, a list of genes that most consistently distinguished diseased vs. normal samples was generated. Classification was generated by the *k*-nearest neighbors algorithm (26). The number of neighbors selected was six, with a decision cutoff for *P* value ratio of 0.2. The final list was determined by an iterative cross-validation process in which the best combination of number of genes and neighbors was found to derive the most discriminating list. In the cross-validation mode, each sample in turn was set aside as the test article, and the remainder of the samples were used to generate the model, which was then evaluated on the test article. (A) Fold change and *P* values of the 29 prediction genes. (B) Unsupervised hierarchical cluster analysis of the 29 genes. The expression patterns of 29 genes are displayed in a dendrogram where columns represent each sample and rows represent individual genes. Genes are colored on a gradient (from -10-fold to 10-fold) with those increase in expression relative to the average of the control in red. Those that decrease are in blue, and those with little or no change are in yellow.

murine collagen-induced arthritis model, the deletion of *SELP* resulted in more severe disease compared with wild-type mice (44).

Many genes not previously known as being differently regulated in RA were also identified, for example, *TCIRG1* (T-cell, immune regulator 1), *INPP5E* (inositol polyphosphate-5-phosphatase E), and *STAB1* (stabilin). *TCIRG1* is a seven-transmembrane, novel T cell protein that plays a role in T cell activation (45). Antibodies to *TCIRG1* (*TIRC7*) prevent human T cell proliferation in vitro, inhibit type I subset-specific $\text{IFN}\gamma$ and IL-2, but not the type II subset cytokine IL-4. A *TIRC7* antibody prolonged survival in a rat model of acute kidney allograft rejection (45). *TIRC7*-null mice have disrupted T and B cell responses in vitro and in vivo, suggesting that *TIRC7* may play a role in T and B lymphocyte balance (46).

INPP5E, a member of the inositol polyphosphate 5-phosphatase family, similar to *INPP5D* (Table 2), regulates PI-3 kinase signal transduction (47). *AGPAT1* (1-acylglycerol-3-phosphate *O*-acyltransferase 1) catalyzes the conversion of lysophosphatidic acid (LPA) to phosphatidic acid (PA). LPA and PA are two phospholipids involved in signal transduction and phospholipid synthesis (48). Overexpression of *AGPAT-1* in cell lines leads to the expression of both $\text{TNF}\alpha$ and IL-6 in cells stimulated with IL-1 β , suggesting that *AGPAT-1* overexpression may amplify cellular signaling responses from cytokines (49).

Interestingly, 10 transcripts, including *AGPAT1*, differentially regulated in the RA PBMC from this study map to chromosome region 6p21.3, the major histocompatibility (MHC) locus III (27) (Table 3). Many of the genes in the MHCIII region have fundamental roles in a variety of cellular functions and include the inflammatory cytokines $\text{TNF}\alpha$, LTA, LTB, and the advanced glycation end product receptor, RAGE (*AGER*) (27). Multifactor interactions contribute to the disease process at several levels. One hypothesis is that dys-

Table 3. Genes with increased expression in RA compared with normal PBMCs at the RA susceptibility locus 6p21.3

Gene symbol no.	Gene name	GenBank acc.
<i>MLN</i>	Motilin	X15393
<i>AGPAT1</i>	1-acylglycerol-3-phosphate O-acyltransferase 1	U56417
<i>HLA-DQA1</i>	Major histocompatibility complex, class II, DQ α 1	M34996
<i>MICB</i>	MHC class I chain-related gene B	U65416
<i>HLA-DOA</i>	Major histocompatibility complex, class II, DO alpha	M31525
<i>BRD2</i>	Bromodomain containing 2	X62083
<i>STK19</i>	Serine/threonine kinase 19	BC016916
<i>RGL2</i>	Ral guanine nucleotide dissociation stimulator-like 2	U68142
<i>C1orf19</i>	Chromosome 6 open reading frame 9	U89336
<i>TRIM26</i>	Tripartite motif-containing 26	U09825

regulation of genes in a locus could contribute to the etiology of the disease, perhaps through coordinated transcription of regions of a chromosome in response to stress or inflammation. RA is a complex autoimmune disorder, and expression analysis of a larger number of patients may validate this hypothesis.

STAB1 [also known as common lymphatic endothelial and vascular receptor (CLEVER-1 or FEEL-1)] was overexpressed in the RA PBMCs. This gene, identified by the *k*-nearest neighbor analysis, was expressed in 100% of RA PBMC samples and exhibited the highest fold change in this study (64-fold). Stabilin 1 is a large glycoprotein, multifunction scavenger receptor. Characterized as FEEL-1, this protein demonstrated a role as a scavenger receptor that binds to both advanced glycation end products as well as gram-positive and gram-negative bacteria (50,51). The receptor was shown to be expressed on mononuclear cells, tissue macrophages, and endothelial cells (50-52). An antibody to FEEL-1 demonstrated a marked reduction in cell-to-cell interaction in a Matrigel tube formation assay, suggesting a role for the receptor in angiogenesis (50). CLEVER-1 has been demonstrated to be involved in the PMBC transmigration through vascular and lymphatic endothelium (52). The CLEVER-1 gene is encoded by 69 exons, and multiple isoforms are expressed in the endothelium (52). The potential function of CLEVER-1 in RA remains to be elucidated.

Several studies of gene expression in RA have been reported. Devauchelle et al. (53) focused on differences in expression in synovia isolated from RA patients compared with that of synovia from osteoarthritis patients. Watanabe et al. (54) reported on differences in expression between RA and normal synovial fibroblasts, and van der Pouw Kraan et al. (55) identified differences in gene expression in RA synovia, allowing the classification of different disease subtypes. A recent study by Bovin et al. (56), using a 12,000-gene oligonucleotide microarray, examined changes in gene expression between PBMCs from 14 RA patients vs. 7 sex- and age-matched controls, and they identified 25 genes that were discriminative. Although different filter criteria were applied to the data sets present here and the report from Bovin et al. (56), there were nine genes that overlapped between the two studies, including *S100A12*, *NCF4*, and *GNG10*. Of the genes that did not overlap, four were not present on the microarray used in this study, three showed changes but did not meet the strict data filtration criteria, and four were not called present in any of the samples. Another study by Olsen et al. (57), using a 4300-gene cDNA microarray, identified a gene expression signature for early-onset rheumatoid arthritis in PBMCs. In that study, the authors segregated the data based on those with longstanding and early-onset disease. There is some overlap between the Olsen et al. (57) study and the results presented

here. Of the 44 genes identified, eight from Olsen, et al. also appeared in the present study. Of the 30 that do not, four were not on the human FL6800 array, 15 were not called present in any of the samples, and the others were not included due to the filtration criteria. In the results presented here, patients were selected from the high disease activity cohort, and during analysis, several filtration criteria were applied to the data set with several statistical analyses and a minimum expression criteria of at least 50% of the patients. These measures ensured that the resulting defined gene signature was as robust as possible.

It must be noted that RA patients possess a broad spectrum of disease severity and time of onset, and the comparisons above serve to highlight the multiple differences in patient selection criteria, study materials, protocols, and data analysis that exist in studies so far. Combining the data from our study with that of others, however, does point to several consistent changes in gene expression that would be useful to investigate further. For example, the increased expression of the RAGE ligand *S100A12* has been observed in more than 1 study and, as a result, has highlighted the RAGE pathway as potentially important in RA; it is now subject to further study by our group.

The information from this study can be used in two major ways. First, it allows genes important in the pathogenesis of RA to be identified. These genes can then be investigated in detail to determine their potential roles in disease. Second, the power of DNA microarray profiling, with its ability to monitor the expression of multiple genes simultaneously, may allow the identification of patterns of gene expression associated with RA. This may enable rapid diagnosis of RA and predictions of prognosis, as well as response to, and side effects of, DMARDs. The use of these techniques is most advanced in oncology, where predictions of prognosis can be made for certain cancers (17). This provides clinically useful information that guides decisions about

how aggressive a treatment regimen should be for a given patient. There is a marked difference in the clinical features of RA between individuals, and molecular phenotyping (or patient profiling) may identify or characterize different disease subgroups and courses of disease progression.

A weakness of global gene expression analysis techniques lies in identifying the relationship of changes in gene expression to the disease process. Changes in gene expression may either cause a disease process or occur as a consequence of it. The presence of gene expression changes in genes that have been associated with RA validates the data set. However, not all genes are primarily regulated by changes in mRNA levels, with many being subject to posttranscriptional regulation. TNF, the best-validated molecular therapeutic target in RA, does not emerge from this type of analysis. This study examined expression in nine RA patients and identified a set of genes that is preferentially expressed in RA patients compared with controls. Although the data are intriguing, samples from a larger number of patients would aid in a class prediction to determine which genes are most associated with disease state and type of prognosis. It is interesting to note that a recent study of PBMC expression profiles in several autoimmune diseases showed that, whereas all diseases displayed profiles that differed from a normal immune response, not all diseases could be clearly distinguished from each other (58).

Gene expression studies on PBMCs may not exactly represent the situation within the inflamed synovial membranes of RA. RA is a systemic disease, however, and differences in cytokine production and phenotype of PBMCs in RA have been demonstrated (59-61). This approach has the advantage of being a rapid and minimally invasive way of obtaining cells from patients. The usefulness of assaying tissue samples in RA is limited by availability and sampling bias due to regional differences in disease activity in synovia. However, if

the diagnostic/predictive results of a gene expression profile can be demonstrated, PBMCs are a readily accessible source of cells.

The use of oligonucleotide microarrays enables a broader view of complex inflammatory diseases, such as RA. The simultaneous measurement of multiple mRNA transcripts allows an increased understanding of the complexity of proteins that may be interacting in a disease state rather than focusing on one or two at a time. This study identified 330 mRNA transcripts that were differentially regulated in the PBMCs from RA patients compared with normal volunteers. Having demonstrated that these techniques can be used with PBMCs, the next step involves looking at patterns of gene expression in individuals over time and detailed phenotypic examination of these individuals to determine patterns of gene expression associated with different features of RA.

ACKNOWLEDGMENTS

The authors thank Dr. James C. Keith, Jr., for reviewing the manuscript.

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