Identification of key pathways and genes in psoriasis via gene microarray analysis

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Abstract. Psoriasis is a common chronic inflammatory, immune-mediated skin disease with a high incidence worldwide. It is a multifactorial disease and its exact pathogenesis has remained largely elusive. The purpose of the present study was to uncover the key pathways and genes associated with the incidence of psoriasis. Gene expression profiles (dataset no. GSE13355) were downloaded from Gene Expression Omnibus. Differentially expressed genes between skin samples from patients with lesional psoriasis or non-lesional psoriasis and those of normal healthy controls were identified using Bioconductor version 2.13 based in R. Kyoto Encyclopedia of Genes and genomes (KEGG) pathways significantly enriched in patients with lesional psoriasis were identified using gene set enrichment analysis (GSEA). Key KEGG pathways were then identified using leading-edge analysis of the results of GSEA. Differentially expressed genes involved in the significantly enriched KEGG pathways were considered as key genes. Several KEGG pathways which are known to be associated with lesional psoriasis, including autoimmune thyroid disease signaling, natural killer cell-mediated cytotoxicity signaling, as well as several novel pathways, including FCγR-mediated phagocytosis and neurotrophin signaling pathway, were identified. Several verified and novel genes were also got. The present study revealed key pathways and genes associated with psoriasis, which may serve as important biomarkers for the diagnosis and treatment of psoriasis.

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Introduction

Psoriasis is an autoimmune chronic inflammatory skin disease that is characterized by sharply demarcated, red, scaly lesions of varying extent. It is a long-lasting disease with a prevalence of 0-11.8% worldwide and high recurrence rate at any time (1,2). In addition, psoriasis may increase the risk of certain other diseases, including stroke and myocardial infarction (3). Psoriasis is a multifactorial disease which can be influenced by genetic as well as environmental factors. Two hypotheses have been posed regarding the pathogenesis of psoriasis: i) Immune system disorders and ii) excessive growth of skin cells (2). However, the exact etiology of psoriasis has remained to be elucidated.

Previous studies have used microarray or RNA sequencing for comparing the gene expression profiles between skin of patients with lesional psoriasis or non-lesional psoriasis as well as that of healthy controls, and certain key genes or pathways have been identified. Through gene expression profiling of lesional/non-lesional skin from psoriasis patients and normal skin from healthy controls based on the Affymetrix HG-U133 plus 2 platform, Nair *et al* (4) found that the interleukin (IL)-23 and nuclear factor-kappaB pathways were closely associated with psoriasis. Krueger *et al* (5) identified IL-17A as an important molecule in the process of cell activation and inflammatory gene circuits in psoriasis patients through comparing the gene expression profiles between skin samples of patients with lesional psoriasis treated with LY2439821- or placebo at different time-points. In addition, the expression patterns of certain psoriasis-associated genes exhibited marked differences between lesional skin and non-lesional skin in patients with psoriasis (2). Genes associated with immune response or epidermal cell proliferation are usually upregulated in skin affected by lesional psoriasis. DOUX2 was found to be upregulated in lesional skin compared with non-lesional skin in patients with psoriasis and atopic dermatitis (6). As an autoimmune disease, psoriasis is largely mediated by the disorder of T-cells. Certain genes or proteins were found to be up- or down-regulated in numerous types of cell through the regulation of T-cells. Yin *et al* (7) reported that compared with those in normal controls the expression levels of mRNA and protein of Notch 1 and Hes-1 in CD34+ cells, which are

Table I. Significantly enriched KEGG pathways in lesional psoriasis obtained by gene set enrichment analysis of lesional skin samples from patients with psoriasis and skin samples from healthy individuals.

KEGG pathway	NES	FDR
Phenylalanine metabolism	-2.160	<0.001
Cytosolic DNA sensing pathway	-2.132	0.001
RIG I-like receptor signaling pathway	-2.045	0.004
Drug metabolism, other enzymes	-2.054	0.005
Cell cycle	-2.027	0.005
NOD-like receptor signaling pathway	-2.001	0.006
Purine metabolism	-1.989	0.006
Fructose and mannose metabolism	-2.001	0.007
Pyrimidine metabolism	-1.969	0.008
Oxidative phosphorylation	-1.921	0.012
Oocyte meiosis	-1.943	0.012
p53 signaling pathway	-1.933	0.012
Alzheimer's disease	-1.906	0.012
Toll-like receptor signaling pathway	-1.923	0.012
Primary immunodeficiency	-1.908	0.012
Linoleic acid metabolism	-1.845	0.018
Porphyrin and chlorophyll metabolism	-1.850	0.018
Base excision repair	-1.840	0.018
Natural killer cell-mediated cytotoxicity	-1.858	0.018
Huntington's disease	-1.816	0.018
Proteasome	-1.851	0.018
Antigen processing and presentation	-1.834	0.018
Homologous recombination	-1.808	0.018
Aminoacyl tRNA biosynthesis	-1.810	0.019
Cysteine and methionine metabolism	-1.861	0.019
Parkinson's disease	-1.826	0.019
Epithelial-cell signaling in *Helicobacter pylori* infection	-1.818	0.019
DNA Replication	-1.864	0.019
Apoptosis	-1.819	0.019
Chemokine signaling pathway	-1.794	0.021
RNA polymerase	-1.752	0.031
Arginine and proline metabolism	-1.742	0.031
Leishmania infection	-1.746	0.031
Autoimmune thyroid disease	-1.737	0.032
Graft-versus-host disease	-1.730	0.033
Progesterone-mediated oocyte maturation	-1.721	0.033
Riboflavin metabolism	-1.722	0.034
Alanine aspartate and glutamate metabolism	-1.722	0.035
Arachidonic acid metabolism	-1.708	0.037
Allograft rejection	-1.695	0.039
Vibrio cholerae infection	-1.697	0.039
Tyrosine metabolism	-1.690	0.040
Amyotrophic lateral sclerosis	-1.679	0.042
Cytokine-cytokine receptor interaction	-1.675	0.043
Type I diabetes mellitus	-1.680	0.043
Systematic lupus erythematosus	-1.658	0.047
T-cell receptor signaling pathway	-1.661	0.047

The Affymetrix HG-U133 plus2 platform (GPL570) was used for the genome expression profiling of 58 skin samples of patients with lesional psoriasis and 64 skin samples from healthy controls. Negative values signify downregulation. NES, normalized enrichment score; KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate.

Table II. Significantly enriched KEGG pathways in lesional psoriasis obtained by gene set enrichment analysis of lesional and non-lesional skin samples from patients with psoriasis.

KEGG pathway	NES	FDR
NOD-like receptor signaling pathway	-1.982	0.005
Phenylalanine metabolism	-1.971	0.005
Pyrimidine metabolism	-1.967	0.005
Oocyte meiosis	-2.043	0.005
Oxidative phosphorylation	-1.983	0.005
Vibrio cholerae infection	-1.992	0.005
Parkinson's disease	-1.953	0.005
Huntington's disease	-1.960	0.005
Cell cycle	-1.984	0.005
Purine metabolism	-1.998	0.006
Leishmania infection	-1.927	0.006
Primary immunodeficiency	-1.935	0.006
Alzheimer's disease	-1.942	0.006
RIG I-like receptor signaling pathway	-2.055	0.006
Toll-like receptor signaling pathway	-2.014	0.006
Natural killer cell-mediated cytotoxicity	-1.928	0.006
Fructose and mannose metabolism	-1.999	0.006
Chemokine signaling pathway	-1.904	0.007
Cytosolic DNA sensing pathway	-2.055	0.008
DNA replication	-1.875	0.010
Drug metabolism, other enzymes	-2.061	0.011
Cysteine and methionine metabolism	-1.865	0.011
Antigen processing and presentation	-1.858	0.011
Base excision repair	-1.846	0.011
Arginine and proline metabolism	-1.839	0.011
Apoptosis	-1.848	0.012
p53 signaling pathway	-1.840	0.012
Proteasome	-1.828	0.012
Riboflavin metabolism	-1.828	0.013
Epithelial-cell signaling in *Helicobacter pylori* infection	-2.069	0.015
Porphyrin and chlorophyll metabolism	-1.790	0.018
Progesterone-mediated oocyte maturation	-1.782	0.019
Cytokine-cytokine receptor interaction	-1.779	0.019
Type I diabetes mellitus	-1.752	0.019
N-glycan biosynthesis	-1.773	0.019
T-cell receptor signaling pathway	-1.774	0.019
Systematic lupus erythematosus	-1.753	0.019
FCγR-mediated phagocytosis	-1.754	0.020
Aminoacyl tRNA biosynthesis	-1.758	0.020
FCεRI signaling pathway	-1.755	0.020
Homologous recombination	-1.765	0.020
α-linoleic acid metabolism	-1.759	0.020
Autoimmune thyroid disease	-1.760	0.021
Allograft rejection	-1.732	0.022
JAK/STAT signaling pathway	-1.735	0.022
Amyotrophic lateral sclerosis	-1.727	0.023
Gysosome	-1.717	0.025
Alanine aspartate and glutamate metabolism	-1.707	0.026
Mismatch repair	-1.707	0.026
Glycolysis, gluconeogenesis	-1.694	0.028
Intestinal immune network for IgA production	-1.687	0.030

Table II. Continued.

KEGG pathway	NES	FDR
Pentose phosphate pathway	-1.675	0.032
Neurotrophin signaling pathway	-1.662	0.034
Ether lipid metabolism	-1.666	0.034
Tyrosine metabolism	-1.663	0.034
Amino sugar and nucleotide sugar metabolism	-1.658	0.035
Bladder cancer	-1.651	0.036
Citrate cycle, tricarboxylic acid cycle	-1.626	0.041
VEGF signaling pathway	-1.623	0.041
Glutathione metabolism	-1.626	0.041
RNA polymerase	-1.628	0.041
Linoleic acid metabolism	-1.632	0.042
B-cell receptor signaling pathway	-1.629	0.042
Starch and sucrose metabolism	-1.607	0.046
Galactose metabolism	-1.605	0.046
Pathogenic *Escherichia coli* infection	-1.595	0.049

Negative values signify downregulation. NES, normalized enrichment score; KEGG, Kyoto Encyclopedia of Genes and Genomes; FDR, false discovery rate; IgA, immunoglobulin A.

largely mediated by T cells, were upregulated in patients with psoriasis. However, previous studies on psoriasis have reported hundreds of differentially expressed genes (DEGs) and pathways or biological processes they were involved in, and their data require further processing for discarding of genes and pathways with only minor changes in psoriasis.

Gene set enrichment analysis (GSEA) is the most well-known enrichment analysis method and is contained in numerous freely available platforms, such as R, Java and GenePattern. It can be used to analyze gene expression microarray data based on the gene expression levels between different statuses (such as tumor vs. normal samples) and the pre-defined gene sets in the Molecular Signatures Database (http://www.broadinstitute.org/gsea/msigdb/index.jsp) (8). Compared with the traditional DEG analysis (DEGA) method, GSEA can detect subtle changes in individual genes in diseases, which can be helpful in the detection of biomarkers that can be missed by other methods. The leading-edge analysis of GSEA results is useful for identifying gene sub-sets from the pool of DEGs (8).

The present study applied the traditional DEGA method and GSEA on a microarray dataset from Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/). Gene expression levels in lesional skin from patients with psoria were compared with those in non-lesional skin from patients with psoriasis as well as skin samples from healthy controls to identify the DEGs. GSEA was performed to identify overrepresented KEGG pathways in lesional skin from patients with psoriasis and leading-edge analysis was then performed for identifying key KEGG pathways. DEGs that were contained in the overrepresented KEGG pathways were considered as key genes. The present study identified several previously known as well as novel biomarkers associated with psoriasis.

Materials and methods

Microarray data and pre-processing. Gene expression profiles were extracted from the study by Nair *et al* (4), whose data were deposited in the GEO database with the accession number GSE13355. A total of 180 samples were contained in the dataset, which included 58 lesional (PP) and non-lesional (PN) skin samples from patients with psoriasis and 64 normal skin (NN) samples from healthy controls. The Affymetrix HG-U133 plus2 platform (GPL570; Affymetrix, Inc., Santa Clara, CA, USA) was used for the genome expression profiling, which contains 54,675 probes.

The raw CEL files were imported into R. Background correction, normalization and log2 transformation were performed based on the robust multiarray average method embedded in the Affy package (9) in Bioconductor version 2.13 (https://bioconductor.org/). The annotation packages hgu133plus2.db, hgu133plus2cdf and hgu133plus2probe were used to transform the probe-level data into the gene-level data. The mean expression value was calculated for genes corresponding to multiple probes.

Identification of DEGs. DEGs of PP compared with PN and NN were obtained through Student's *t*-test and Benjamini-Hochberg correction based on the R limma package (10). Screening thresholds for DEGs were adjusted to $P<0.05$ and fold change >2.

GSEA. GSEA is supported by the Broad Institute website (http://www.broadinstitute.org/gsea/index.jsp) and mainly embedded in three platforms: R, Java and GenePattern. It can be used to determine whether the members of a gene set are primarily distributed in the top or the bottom of the ranked gene list or randomly distributed in the list. In the present

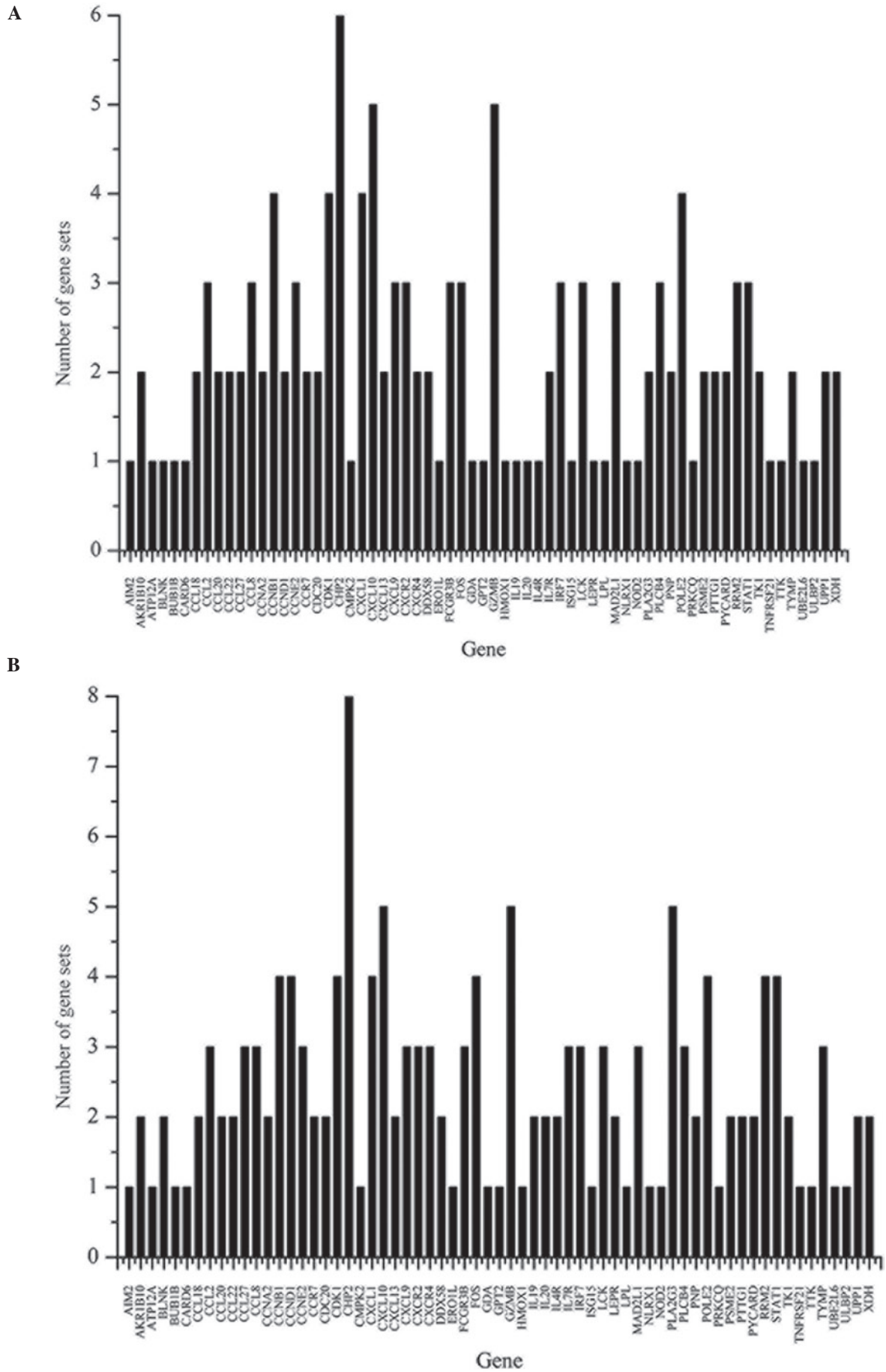

Figure 1. Number of KEGG pathways that key genes are involved in. (A) KEGG pathways identified by GSEA of lesional skin samples from patients with psoriasis and skin of healthy controls. (B) KEGG pathways identified by GSEA of lesional and non-lesional skin samples from patients with psoriasis. GSEA, gene set enrichment analysis; Kyoto Encyclopedia of Genes and Genomes.

Table III. Significantly enriched gene ontology terms of key genes in PP obtained through GSEA of PP vs NN and PP vs PN.

Category	Gene ontology name	FDR	Gene
BP	Immune response	$1.421x10^{-10}$	AIM2, BLNK, CCL18, CCL2, CCL20, CCL22, CCL27, CCL8, CCR7, CXCL1, CXCL10, CXCL13, CXCL9, CXCR4, DDX58, FCGR3B, IL19, IL4R, IL7R, NLRX1, NOD2, PNP, ULBP2
BP	Chemotaxis	$3.453x10^{-10}$	CCL18, CCL2, CCL20, CCL22, CCL27, CCL8, CCR7, CXCL1, CXCL10, CXCL13, CXCL9, CXCR2, CXCR4, TYMP
BP	Taxis	$3.455x10^{-10}$	CCL18, CCL2, CCL20, CCL22, CCL27, CCL8, CCR7, CXCL1, CXCL10, CXCL13, CXCL9, CXCR2, CXCR4, TYMP
MF	Chemokine activity	$5.374x10^{-10}$	CCL18, CCL2, CCL20, CCL22, CCL27, CCL8, CXCL1, CXCL10, CXCL13, CXCL9
MF	Chemokine receptor binding	$9.911x10^{-10}$	CCL18, CCL2, CCL20, CCL22, CCL27, CCL8, CXCL1, CXCL10, CXCL13, CXCL9
BP	Inflammatory response	$1.534x10^{-8}$	BLNK, CCL18, CCL2, CCL20, CCL22, CCL8, CCR7, CXCL1, CXCL10, CXCL13, CXCL9, CXCR2, CXCR4, FOS, HMOX1, IRF7
BP	Response to wounding	$1.615x10^{-7}$	BLNK, CCL18, CCL2, CCL20, CCL22, CCL8, CCNB1, CCR7, CXCL1, CXCL10, CXCL13, CXCL9, CXCR2, CXCR4, FOS, HMOX1, IRF7, PRKCQ
BP	Defense response	$1.797x10^{-7}$	BLNK, CCL18, CCL2, CCL20, CCL22, CCL8, CCR7, CXCL1, CXCL10, CXCL13, CXCL9, CXCR2, CXCR4, DDX58, FOS, HMOX1, IRF7, NLRX1, NOD2
BP	Locomotory behavior	$3.182x10^{-7}$	CCL18, CCL2, CCL20, CCL22, CCL27, CCL8, CCR7, CXCL1, CXCL10, CXCL13, CXCL9, CXCR2, CXCR4, TYMP
MF	Cytokine activity	$1.261x10^{-6}$	CCL18, CCL2, CCL20, CCL22, CCL27, CCL8, CXCL1, CXCL10, CXCL13, CXCL9, IL19, IL20
BP	Behavior	$1.261x10^{-6}$	CCL18, CCL2, CCL20, CCL22, CCL27, CCL8, CCR7, CXCL10, CXCL13, CXCL9, CXCR2, CXCR4, FOS, LEPR, TYMP, CXCL1
CC	Extracellular space	$1.261x10^{-6}$	CCL18, CCL2, CCL20, CCL22, CCL27, CCL8, CXCL1, CXCL10, CXCL13, CXCL9, HMOX1, IL19, IL20, ISG15, LEPR, LPL, PLA2G3, ULBP2
CC	Cytosol	$1.261x10^{-4}$	BUB1B, CCNB1, CCND1, CCNE2, CDC20, CDK1, GDA, GZMB, HMOX1, LCK, MAD2L1, NOD2, PLCB4, PNP, PTTG1, PYCARD, RRM2, TK1, TYMP, UPP1, XDH
CC	Extracellular region part	$1.261x10^{-4}$	CCL18, CCL2, CCL20, CCL22, CCL27, CCL8, CXCL1, CXCL10, CXCL13, CXCL9, HMOX1, IL19, IL20, ISG15, LEPR, LPL, PLA2G3, ULBP2
BP	Mitotic cell cycle checkpoint	0.002	BUB1B, CCNA2, CCND1, MAD2L1, TTK,CDK1
BP	Cell cycle checkpoint	0.005	BUB1B, CCNA2, CCND1, CDK1, MAD2L1, TTK,CCNE2
BP	Regulation of protein modification process	0.010	BUB1B, CCND1, CDC20, CDK1, IL20, MAD2L1, NOD2, PSME2, TTK, CCNB1
BP	Response to virus	0.014	CCL22, CCL8, CXCR4, IRF7, ISG15, STAT1, DDX58
BP	Anaphase-promoting complex -dependent proteasomal ubiquitin-dependent protein catabolic process	0.017	BUB1B, CDC20, CDK1, MAD2L1, PSME2, CCNB1
BP	Regulation of ubiquitin-protein ligase activity during mitotic cell cycle	0.026	BUB1B, CDC20, CDK1, MAD2L1, PSME2, CCNB1
BP	Positive regulation of protein modification process	0.032	CCND1, CDC20, CDK1, IL20, NOD2, PSME2, TTK, CCNB1
BP	Regulation of ubiquitin-protein ligase activity	0.041	BUB1B, CDC20, CDK1, MAD2L1, PSME2, CCNB1
BP	Regulation of ligase activity	0.049	BUB1B, CDC20, CDK1, MAD2L1, PSME2, CCNB1

BP, biological process; CC, cellular component; MF, molecular function; FDR, false discovery rate.

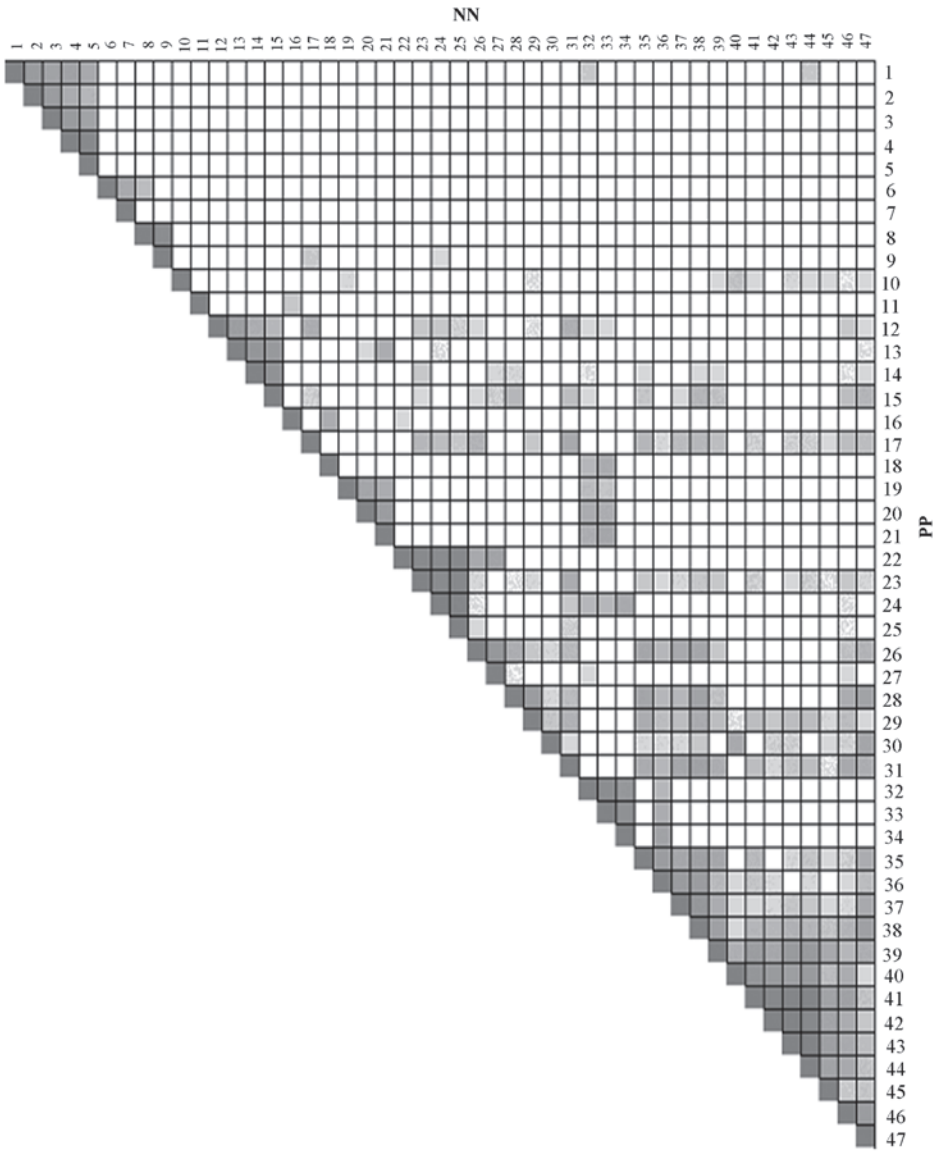

Figure 2. Overlap between the leading-edge sub-sets of every two Kyoto Encyclopedia of Genes and Genomes pathways identified by gene set enrichment analysis of lesional skin samples from patients with psoriasis and skin samples of healthy controls. The darker the color, the greater the overlap between the leading-edge subsets. 1, Alanine aspartate and glutamate metabolism; 2, arginine and proline metabolism; 3, cysteine and methionine metabolism; 4, phenylalanine metabolism; 5, tyrosine metabolism; 6, fructose and mannose metabolism; 7, riboflavin metabolism, 8, linoleic acid metabolism; 9, arachidonic acid metabolism; 10, proteasome; 11, aminoacyl tRNA biosynthesis; 12, p53 signaling pathway; 13, cell cycle; 14, oocyte meiosis; 15, progesterone-mediated oocyte maturation; 16, porphyrin and chlorophyll metabolism; 17, amyotrophic lateral sclerosis; 18, drug metabolism, other enzymes; 19, homologous recombination; 20, base excision repair; 21, DNA replication; 22, oxidative phosphorylation; 23, Alzheimer's disease; 24, Huntington's disease; 25, Parkinson's disease; 26, epithelial cell signaling in *Helicobacter pylori* infection; 27, *Vibrio cholerae* infection; 28, chemokine signaling pathway; 29, cytokine-cytokine receptor interaction; 30, primary immunodeficiency; 31, apoptosis; 32, purine metabolism; 33, pyrimidine metabolism; 34, RNA polymerase; 35, NOD-like receptor signaling pathway; 36, cytosolic DNA sensing pathway; 37, RIG I-like receptor signaling pathway; 38, Toll-like receptor signaling pathway; 39, Leishmania infection; 40, antigen processing and presentation; 41, graft-versus-host disease; 42, autoimmune thyroid disease; 43, allograft rejection; 44, type I diabetes mellitus; 45, systematic lupus erythematosus; 46, natural killer cell-mediated cytotoxicity; 47, T-cell receptor signaling pathway.

study, GSEA and traditional DEGA were combined to identify the potential biomarkers of psoriasis. GSEA was conducted based on the Java implementation and was performed using the KEGG pathway gene sets in the Molecular Signatures Database against two probe-level expression matrices: One was comprised of the PP and PN samples and another was comprised of PP and NN samples. GSEA was performed using default parameters, with a number of genes in the gene sets of 15-500 and a permutation test time of 1,000. Cut-off of the false-discovery rate (FDR) was set to 0.05 for the significant KEGG pathways. Furthermore, DEGs contained in significant KEGG pathways were considered as key genes involved in the incidence of psoriasis. The Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 (11) (http://david.abcc.ncifcrf.gov/) was used to annotate the key genes, and gene ontology (GO) terms with FDR<0.05 were selected.

Leading-edge analysis. Leading-edge analysis can be used to extract the core members in the gene sets, i.e. leading-edge

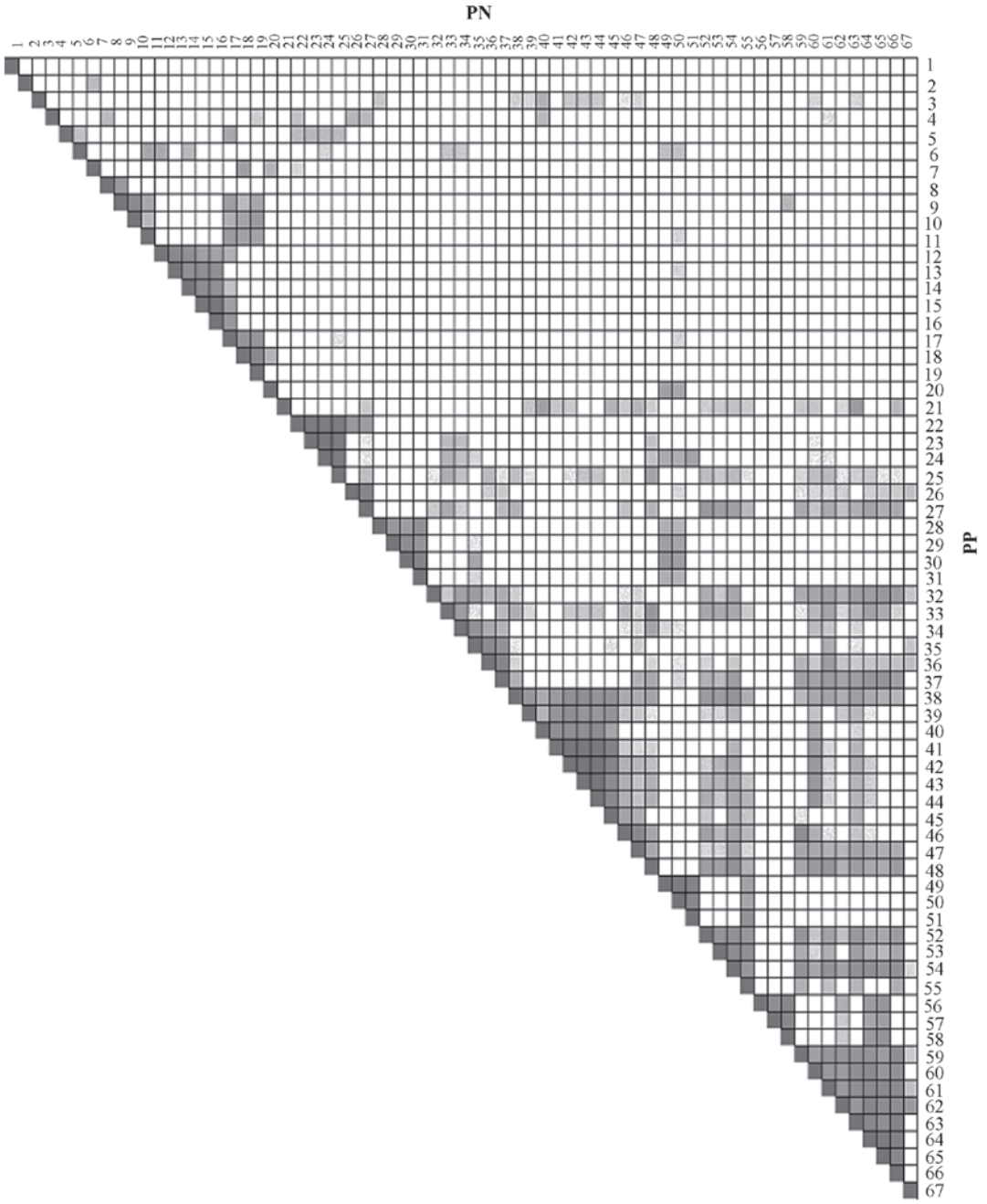

Figure 3. Overlap between the leading-edge sub-sets of every two Kyoto Encyclopedia of Genes and Genomes pathways identified by gene set enrichment analysis of lesional and non-lesional skin samples from patients with psoriasis. The darker the color, the greater the overlap between the leading-edge subsets. 1, *N*-glycan biosynthesis; 2, aminoacyl tRNA biosynthesis; 3, proteasome; 4, lysosome; 5, citrate cycle, tricarboxylic acid cycle; 6, glutathione metabolism; 7, porphyrin and chlorophyll metabolism; 8, riboflavin metabolism; 9, fructose and mannose metabolism; 10, amino sugar and nucleotid sugar metabolism; 11, pentose phosphate pathway; 12, arginine and proline metabolism; 13, alanine aspartate and glutamate metabolism; 14, cysteine and methionine metabolism; 15, phenylalanine metabolism; 16, tyrosine metabolism; 17, glycolysis, gluconeogenesis; 18, starch and sucrose metabolism; 19, galactose metabolism; 20, drug metabolism, other enzymes; 21, primary immunodeficiency; 22, oxidative phosphorylation; 23, Parkinson's disease; 24, Huntington's disease; 25, Alzheimer's disease; 26, *Vibrio cholerae* infection; 27, epithelial cell signaling in *Helicobacter pylori* infection; 28, homologous recombination; 29, base excision repair; 30, DNA replication, 31, mismatch repair; 32, bladder cancer; 33, amyotrophic lateral sclerosis; 34, p53 signaling pathway; 35, cell cycle; 36, oocyte meiosis; 37, progesterone-mediated oocyte maturation; 38, leishmania infection; 39, systematic lupus erythematosus; 40, antigen processing and presentation; 41, autoimmune thyroid disease; 42, allograft rejection; 43, type I diabetes mellitus; 44, graft-versus-host disease; 45, intestinal immune network for immunoglobulin A production; 46, cytokine-cytokine receptor interaction; 47, JAK/STAT signaling pathway; 48, apoptosis; 49, pyrimidine metabolism; 50, purine metabolism; 51, RNA polymerase; 52, NOD-like receptor signaling pathway; 53, RIG I-like receptor signaling pathway, 54, Toll-like receptor signaling pathway; 55, cytosolic DNA-sensing pathway; 56, ether lipid metabolism; 57, alpha linoleic acid metabolism; 58, linoleic acid metabolism; 59, chemokine signaling pathway; 60, natural killer cell-mediated cytotoxicity; 61, neurotrophin signaling pathway; 62, FC gamma R-mediated phagocytosis; 63, T-cell receptor signaling pathway; 64; FC epsilon RI signaling pathway; 65, VEGF signaling pathway; 66, B-cell receptor signaling pathway; 67, pathogenic *Escherichia coli* infection.

sub-sets. The significant gene sets can be grouped based on the common genes in their leading-edge sub-sets, which can reveal gene sets highly associated with the disease. In the present study, the GSEA results for PP and PN, and PP and NN were subjected to leading-edge analysis to identify key KEGG pathways in psoriasis.

Results

DEGs. A total of 540 genes were found to be differentially expressed in PP compared with NN, of which, 167 were downregulated and 374 were upregulated. In addition, 452 DEGs between PP and PN were identified, which contained 121 downregulated and 331 upregulated ones. A total of 422 genes were shared between the two lists of DEGs.

GSEA and key genes. GSEA of PP and NN samples revealed that 47 KEGG pathways were significantly enriched in PP (Table I). Furthermore, GSEA of PP and PN samples resulted in 67 significantly enriched KEGG pathways in PP (Table II). All of the KEGG pathways identified were downregulated in PP and 46 of these pathways overlapped between PP and PN. 65 of the 422 overlapping DEGs were involved in the 46 overlapping KEGG pathways and those genes were considered as key genes associated with the incidence of psoriasis. The number of KEGG pathways that every key gene was involved in is shown in Fig. 1. Biological processes, including immune response, chemokine activity and inflammatory response were found to be significantly enriched in those genes. The full list of enriched GO terms is shown in Table III.

Key KEGG pathways identified by leading-edge analysis. Through leading-edge analysis of the GSEA results for PP and PN, and PP and NN, the numbers of overlapping genes in leading-edge sub-sets of their significant KEGG pathways were obtained, which are illustrated in Figs. 2 and 3. According to the number of overlapping genes, every KEGG pathway was assigned a score and the ones with score >2 were selected as the key KEGG pathways in psoriasis. A total of 9 (Table IV) and 19 (Table V) key KEGG pathways were obtained, respectively, by analysis of the GSEA results of the PP vs. NN and PP vs. PN samples, among which seven overlapping pathways were found.

Table IV. Key KEGG pathways obtained by leading-edge analysis of the results of the gene set enrichment analysis of lesional and skin samples from patients with psoriasis and skin of normal controls.

KEGG pathway	Score
Chemokine signaling pathway	2.064
Allograft rejection	2.094
Antigen processing and presentation	2.111
Epithelial cell signaling in *Helicobacter pylori* infection	2.125
Leishmania infection	2.202
Alzheimer's disease	2.491
Autoimmune thyroid disease	2.617
Oxidative phosphorylation	2.793
Graft-versus-host disease	3.330

KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table V. Key KEGG pathways identified by leading-edge analysis of the gene set enrichment analysis of lesional and non-lesional skin samples from patients with psoriasis.

KEGG pathway	Score
Antigen processing and presentation	2.026
FCγR-mediated phagocytosis	2.062
Natural killer cell-mediated cytotoxicity	2.115
Systematic lupus erythematosus	2.132
Chemokine signaling pathway	2.138
Epithelial cell signaling in *Helicobacter pylori* infection	2.142
Amyotrophic lateral sclerosis	2.153
Neurotrophin signaling pathway	2.164
NOD-like receptor signaling pathway	2.261
Apoptosis	2.271
Parkinson's disease	2.322
Progesterone-mediated oocyte maturation	2.350
Bladder cancer	2.357
Toll-like receptor signaling pathway	2.488
Type I diabetes mellitus	2.604
Oxidative phosphorylation	2.850
Allograft rejection	3.359
Leishmania infection	3.402
Autoimmune thyroid disease	3.739

KEGG, Kyoto Encyclopedia of Genes and Genomes.

Discussion

Psoriasis is a common skin disease which is associated with inflammation and immune disorders and may be accompanied by numerous other diseases. In spite of the large number of studies performed, the precise etiologies of psoriasis have largely remained elusive. The present study performed a combination of traditional DEGA and GSEA to identify previously known as well as novel key KEGG pathways, such as NOD-like receptor signaling pathway, and genes associated with psoriasis, and therefore provided valuable targets for the treatment or diagnosis of psoriasis.

The DEGA method identified 540 DEGs between PP and NN and 452 DEGs between PP and PN. In addition, 422 overlapping DEGs were identified. These results indicated that the gene expression patterns in psoriasis patients are markedly different from those in the skin of healthy individuals, while gene expression is nearly identical among lesional and non-lesional skin samples of patients with psoriasis. Furthermore, by using fibre-optic confocal imaging technology, Suihko and Serup (12) found that there was no significant difference in dermal papillae and cell size between non-lesional psoriasis skin and healthy skin, while significant differences in dermal papillae, cell size and the number of cells existed between lesional psoriasis skin and healthy skin (13). The clinical symptoms of non-lesional psoriasis skin may be sustained by inflammation and immune-associated genes or pathways. In a study by Seifert *et al* (14), DDK-1, an inhibitor of the Wnt signaling pathway with important role in inflam-

mation and immune mechanisms, was found to be increased at the mRNA and protein level in non-lesional psoriasis skin compared with that in lesional psoriasis skin and healthy skin. Therefore inflammation- or immune-associated pathways or genes may be potential targets for preventing the occurrence of lesions in patients with non-lesional psoriasis.

Through the combination of GSEA and DEGA, 65 key genes were identified in the present study, which contained numerous genes known to be associated with psoriasis, including IL19 and IL20, as well as novel genes, including CHP2 and GZMB. Among the 65 key genes, CHP2 was found to be involved in the most significant KEGG pathways in the GSEA results for PP as well as for PN. CHP2 encodes a small calcium-binding protein, which regulates the cell pH by controlling the activity of plasma membrane-type Na^+/H^+ exchange (15). In a study of Li *et al* (16), CHP2 was reported to have important roles in the activation of the calcineurin/nuclear factor of activated T cells signaling pathway, which has been reported to be linked with the incidence of psoriasis. In the present study, enrichment analysis using DAVID identified the involvement of a number of well-studied biological processes in psoriasis, including immune response, inflammatory response and chemokine activity. Certain members of the chemokine family, including CXCL1, CCL2, CCL22, CXLC9 and CCL8, were found to be clustered in numerous GO terms. Chemokines are the largest family of cytokines in human immunophysiology (17). They can be divided into two major families and two sub-families: CC chemokine ligands, CC chemokine receptors, CXC chemokine ligands and CXC chemokine receptors. Several of them have been identified to be associated with the incidence and development of psoriasis. Kono *et al* (18) reported that the expression of CCR5 and CCL5 was associated with the development of psoriasiform hyperplasia and microabscess. Also, through managing multiple chemokines, keratinocytes actively participate in the inflammatory response in psoriasis patients (19). Therefore, chemokines may serve as potential biomarkers for the diagnosis and treatment of psoriasis. Furthermore, certain members of the IL family, including IL19, IL20, IL4R and IL7R, were also enriched in the KEGG pathways identified in the present study. IL19, IL20 and IL24 have critical roles regarding the symptoms of psoriasis (20). Through the comparison of gene expression profiles between lesional psoriasis skin and non-lesional psoriasis skin, Xie *et al* (2) identified IL7R as an important indictor for distinguishing non-lesional from lesional skin in patients with psoriasis.

The autoimmune thyroid disease signaling pathway had a high score in the key KEGG pathways for PP and PN in the present study. Autoimmune thyroid diseases include Hashimoto's thyroiditis, chronic autoimmune thyroiditis, Graves' disease and autoimmune atrophic thyroiditis and primary myxedema, which are mainly mediated by T cells and have, to a certain extent, a similar pathogenesis to that of psoriasis. Natural killer (NK) cell-mediated cytotoxicity signaling was also identified as a key KEGG pathways using leading-edge analysis of the GSEA results for PP and PN, and PP and NN samples. NK cells are large granular lymphocytes which have important roles in the formation of the innate immune system. The cytotoxic NK cells can kill cells expressing stress-induced molecules and have been validated to have important roles in psoriatic arthritis (21). Furthermore, the distribution of NK cells in psoriasis patients was shown to be different from that in healthy controls (22). Certain well-studied KEGG pathways in psoriasis, including chemokine signaling pathways, epithelial cell signaling in *Helicobacter pylori* infection as well as antigen processing and presentation, were also obtained in the present study.

In conclusion, the present study used a combination of the traditional DEGA method and GSEA of microarray data from GEO to identify key genes and KEGG pathways which may represent potential biomarkers for the incidence and development of psoriasis. GO enrichment analysis of key genes illustrated the reliability of the results. However, further molecular biological experiments are required to confirm the implication of the identified genes in psoriasis as well as their utilization as biomarkers and molecular targets for the treatment of psoriasis.

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