MARMARA UNIVERSITY
INSTITUTE FOR GRADUATE STUDIES
IN PURE AND APPLIED SCIENCES

USING SYSTEMS BASED MODELS TO UNCOVER THE DISEASE NETWORK OF PSORIASIS AND ITS ASSOCIATIONS WITH OTHER AUTOIMMUNE-RELATED DISEASES

TUBA SEVİMOĞLU

Ph.D. THESIS

Department of Bioengineering

Thesis Supervisor

Assoc. Prof. Kazım Yalçın ARĞA

ISTANBUL, 2015

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Tuba SEVİMOĞLU, a Doctor of Philosophy student of Marmara University Institute for Graduate Studies in Pure and Applied Sciences, defended her thesis entitled "Using Systems Based Models to Uncover the Disease Network of Psoriasis and its Associations with other Autoimmune-related Diseases", on October 14, 2015 and has been found to be satisfactory by the jury members.

Jury Members

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APPROVAL

Marmara University Institute for Graduate Studies in Pure and Applied Sciences Executive Committee approves that Tuba SEVİMOĞLU be granted the degree of Doctor of Philosophy in Department of Bioengineering, Bioengineering Program on (Resolution no:).

Director of the Institute
Prof. Dr. Uğur YAHŞİ

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Tez Başlığı: Sistem esaslı modeller kullanarak sedef hastalığı ağının ve diğer bağışıklık sistemi ile ilintili hastalık ağlarının oluşturulması

Sedef hastalığı kompleks, zarar veren ve günlük yaşam kalitesini olumsuz yönde etkileyen otoimmün bir deri hastalığıdır. Bu hastalık konusunda birçok mikrodizi analizi yapılmış ve hastalık patojenezi ile ilintili bazı genler belirlenmiş olsa da sedef mekanizmasını anlamaktan halen çok uzağız. Bu çalışma insan biyolojik ağları ile üç farklı mikrodizi platformundan omiks veri setlerini bütünleşik bir şekilde kullanarak ve bunları yeni bir korelasyon metodu ile birleştirerek sedef hastalığının mekanizmasını ortaya çıkarmayı amaçlamıştır. Ana gen ekspresyonu farklılaşmış genler, protein etkileşim ağları ve onların topolojik ve modüler analizi, transkripsiyonel regülasyon mekanizmalarının aydınlatılması ve sedef ilintili genlerin korelasyon analizini birleştiren sistem bazlı yaklaşım sonucunda büyük oranda korele ve birbiri ile ilişkili bir sedef hastalık ağı ortaya çıkarılmıştır. ELISA ve RT-PCR kullanılarak yapılan deneysel çalışmalar bulgularımızı destekler niteliktedir. Sedef hastalığı ile diğer otoimmün ilintili hastalıkların (Romatoid artrit, atopik dermatit ve sistemik lupus eritematosus.) bağlantısı aynı sistem bazlı yaklaşım ile araştırılmıştır. Romatoid artrit ile sedef hastalığı arasındaki ilişki psoriatik artrit ve bozuk immün rejenerasyon sistemi ile olduğu düşünülmektedir. Atopik dermatit ve sedef hastalığı benzer cilt sorunları ile karşımıza çıkar. Aynı zamanda kemokin sinyal yolizi gibi ortak sinyal iletim yolları da vardır. Sistemik lupus ile sedef hastalığının birlikte görülmesi olasılığı düşüktür ancak ortak immün tepki genleri vardır. Sonuç itibariyle bu çalışma sedef hastalığının moleküler mekanizmasını daha iyi anlayabilmek için ve SUB1 ve IFI44 gibi birkaç biyobelirteç adayını ileride yapılacak olan deneysel çalışmalar için önermek ve aynı zamanda diğer kompleks insan hastalıklarına uyarlanabilecek bir yapı kurmak konusunda önemli bir çabayı ortaya koymaktadır. Bu çalışma aynı zamanda sedef hastalığının diğer otoimmün ilintili hastalıklarla olan bağlantısını anlamak için kullanılabilecek veriler sunmaktadır. Dahası bu araştırma diğer kompleks genetik hastalıklar ile sedef hastalığı arasında ortak genetik bir sebep ortaya koymaktadır.

ABSTRACT

Thesis Title: Using Systems Based Models to Uncover the Disease Network of Psoriasis and its Associations with other Autoimmune-related Diseases

Psoriasis is a complex and debilitating autoimmune disease of skin that greatly impacts the quality of life. Though various microarray studies have already revealed genes that could be implicated in the disease pathogenesis, we are far from understanding the mechanism of psoriasis. This study employs integrated analysis of human biological networks with omics datasets from three different microarray platforms in conjunction with a novel correlation approach to reveal the disease mechanism of psoriasis. A systems-based approach incorporating identification of core differentially expressed genes, reconstruction of protein-protein interaction network and its topological and modular analysis, elucidation of the transcriptional regulatory mechanisms and correlation analysis of psoriasis-associated genes resulted with a highly correlated and interconnected disease network of psoriasis. Experimental studies using ELISA and RT-PCR was done to confirm our findings. The association of psoriasis and three other autoimmune diseases (Rheumatoid arthritis, atopic dermatitis and syslemic lupus erythematosus) have been confirmed by using the same system based approach employed in psoriasis. The association of rheumatoid arthritis and psoriasis is thru psoriatic arthritis as well as defective immune regeneration. Atopic dermatitis and psoriasis is associated with similar skin related symptoms as well as signaling pathways such as chemokine signaling. The coexistence of psoriasis and systemic lupus erythematosus is rare but they do share common genes implicated in immune response.

Overall these results implement a comprehensive approach to figure out the molecular framework of psoriasis, and propose several biomarker candidates, such as SUB1 and IFI44 for further experimental studies as well as establishing a new framework that can be applied to other complex human diseases. Furthermore it demonstrates the common genetic causes between psoriasis and the selected autoimmune diseases.

CLAIM FOR ORIGINALITY

Psoriasis is a complex autoimmune disease with no known cure to date. Though numerous studies have been dedicated to the elucidation of the genetic mechanism of the disease there are still gaps regarding the cause and the origin of disease progression. This thesis aimed at elucidating the disease mechanism of psoriasis and its association with three other autoimmune diseases (rheumatoid arthritis, atopic dermatitis and systemic lupus ertyhtematosus) using a systems based approach. Integrative analysis employing transcriptomics and interactome datasets together with biological networks enabled us to understand the disease mechanism in detail. Statistical analysis of psoriasis disease datasets also led to biomarker candidates which were confirmed in experimental studies. Furthermore an updated JAK/STAT signaling pathway for psoriasis was proposed.

In addition, the association between psoriasis and other autoimmune-related diseases (rheumatoid arthritis, atopic dermatitis and systemic lupus erythematosus) was investigated.

This study is a significant endeavor in providing a better understanding of molecular mechanism of psoriasis as well as suggesting biomarker candidates. By understanding the molecular mechanism of psoriasis potential therapeutic targets in the treatment of human psoriasis can be established. This study is also beneficial in understanding the relationship between psoriasis and other autoimmune-related diseases. Moreover, this research provides a common standard operating procedure in the investigation of other complex genetic diseases.

October 2015

Tuba Sevimoğlu Assoc. Prof. K. Yalçın Arğa

ABBREVIATIONS

AD : Atopic Dermatitis

AMP : Antimicrobial Peptide

C_T Threshold cycle

DEG : Differentially Expressed Gene

FC : Fold change

FCC : Fold Change Correlation

GO Gene Ontology

IFN : Interferon

IRF Interferon Regulatory Factor

ISG : Interferon Stimulated Gene

PPI : Protein Interaction Network

PsA : Psoriatic Arthritis

RA : Rheumatoid Arthritis

SLE : Systemic Lupus Erythematosus

TF : Transcription Factor

TRN : Transcriptional Regulatory Network

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1. INTRODUCTION

Psoriasis is a complex, autoimmune skin disease that is still under investigation. The complexity of diseases like psoriasis has led the research efforts towards systems biology and more specifically to the area of functional genomics. In this thesis psoriasis and its association with three other autoimmune diseases: rheumatoid arthritis, systemic lupus erythematosus and atopic dermatitis has been investigated within a systems biology approach.

The rest of this chapter provides a literature review of the selected autoimmune diseases as well as insights into the nomenclature used in the application of system biology approach such as functional genomics, genomics, proteomics and interactomics. The efforts that have contributed to the elucidation of disease mechanisms of psoriasis and the other autoimmune diseases have been discussed. The aim of this thesis has also been given in Chapter 1. Chapter 2 describes the materials and methodology used in this thesis. Chapter 3 gives the results of the analyses and their discussions. Finally Chapter 4 summarizes the results of this thesis and gives suggestions for further research.

1.1. Autoimmune Diseases

Autoimmune diseases (ADs) are chronic conditions that originate from the loss of immunological tolerance to self-antigens. They represent a heterogeneous set of disorders that distress individual organs or organ systems (Anaya, 2012). Autoimmunity occurs when central and peripheral tolerance system fails to develop leading to an elevated number of B cells (Lleo et al., 2010).

The relationships between genetic and environmental factors that contribute to the development of autoimmune diseases are still uncertain. Furthermore the understanding of contributions by T cells, B cells, myeloid cells, and dendritic cells, to disease pathogenesis needs improvement (Smilek and St. Clair, 2015).

1.2. Psoriasis

Psoriasis is a common, complex, multigenic, inflammatory autoimmune disease that presents itself as skin lesions and joint pain. It affects 2-3% of the world's population. It can appear after trauma or surgery, as a result of emotional distress, or as a consequence

of environmental, immunological or genetic triggers (Perera et al., 2012). *Plaque psoriasis* which accounts for 90% of psoriasis incidents (Griffiths and Barker, 2007) usually present itself as patches of inflamed skin with silvery scales. Other phenotypes include *Inverse psoriasis* marked by red and inflamed lesions in the axillae and groin, *Seborrheic psoriasis* in the areas of scalp or eyebrows, *guttate psoriasis* observed after Streptococci, *Guttate psoriasis*, *Pustular psoriasis*, *non-pustular palmar-plantar psoriasis* and *Nail psoriasis* (Roberson et al. 2010). Psoriasis patients are accustomed to outbreaks followed by brief alleviation.

Terminal differentiation is incomplete in the epidermis of psoriatic skin due to rapid reproduction and maturation of the keratinocytes. The scaling and breaks in the protective barrier of the skin is caused by the failure of psoriatic corneocytes to stack normally and to secrete extracellular lipids. The detectable rosy skin lesions of psoriasis are caused by expansion of blood vessels in the dermis (Balato et al., 2010).

Psoriatic Arthritis (PsA) is seen in around 20% of psoriasis sufferers. PsA is characterized by chronic inflammatory arthritis in the presence of psoriasis. The symptoms of PsA are similar to rheumatoid arthritis, besides some other autoimmune diseases such as Type 1 Diabetes have been associated with PsA previously (Castelino and Barton, 2010).

There is still no cure for psoriasis. Currently the therapies related to psoriasis are for the control of the symptoms of the disease so that the patient can live a more normalized life. There are several treatment types for the patients of psoriasis including topical, for mild psoriasis, phototherapy, oral therapies and even biologics for more severe types of the disease.

1.3. Other Autoimmune Diseases in this Study

This section gives a literature review of the autoimmune diseases selected for this study: Rheumatoid Arthritis, Atopic Dermatitis and Systemic Lupus Erythamatosus.

1.3.1. Rheumatoid arthritis

Rheumatoid arthritis (RA) is a complex autoimmune disease which presents itself by chronic inflammation and destruction of synovial joints leading to joint and structural bone damage. Genetic and environmental factors are involved in the progression of the

disease (Orozco et al., 2006). The progress of the disease causes pain, stiffness and disability which affect a patient's quality of life.

RA is a symmetric polyarticular arthritis affecting the small diarthrodial joints of the hands and feet. It presents itself as inflammation in the joint lining (synovium). Furthermore pannus which is the aggressive front of tissue invades and destroys local articular structures. The synovium has a delicate intimal lining which is infiltrated by CD4+ T cells, B cells and macrophages during the onset of RA resulting in hyperplasia. Enzymes such as metalloproteinases and serine proteases digest the extracellular matrix and destroy the articular structures (Firestein, 2003). Female patients have an increased tendency to develop RA with implications of exogenous hormonal influences (Silman and Pearson, 2002). Though RA has been associated with genes of the human leucocyte antigen (HLA) complex, the molecular mechanism of the disease in not quite fully understood hence proper treatment of patients is a work in progress (John, 2001).

1.3.2. Atopic dermatitis

Atopic dermatitis (AD) (eczema) is a chronic, inflammatory skin disease. There are several factors involved in the pathogenesis of AD such as genetics, immunological factors, epidermal barrier abnormalities and the environment; however the cellular and molecular mechanisms governing the disease have still not been fully understood. The reduced cell-mediated immunity as well as the deficiency of antimicrobial peptides in AD cause bacterial and viral infections generating adverse affects on a patients quality of life (Bieber, 2010). Scratching extensively to relieve the itching induced by AD causes excoriation and lichenification, triggering flares, producing papular eruptions, therefore causing the disease to spread to other regions (Abramovitz, 2005). Patients with atopic dermatitis display IgE autoreactivity to proteins demonstrating that autoimmune mechanisms are implicated in the development of the disease (Mittermana et al, 2004).

1.3.3. Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune disease affecting multiple organ systems. Genetics, age, hormonal factors and environmental triggers are factors that are involved in the manifestation of the disease (Neill and Cervera, 2010).

A patient might have SLE if they have renal, neurological, haematological and immunological disorders or malar or discoid rashes (Petri, 2002). Remission and relapses of the disease at any part of the body is a well known aspect of the disease. The most characteristic feature of the disease is fatigue accompanied by fever, lymphadenopathy and weight loss which can also be mistaken for other diseases such as malignancy. Various autoantibodies are linked to SLE which are helpful in identifying the disease (Smith and Gordon, 2010). Type I interferon (IFN) is a central player in the occurance of SLE by inducing the activation of peripheral dendritic cells. IFN also directly affects T cells and B cells. Furthermore activation of genes such as IRF5 which control IFN production may take part in the disease progression (Pascual et al., 2006). Although a large number of studies have been conducted regarding SLE, the pathogenesis of this disease still bemuses researchers.

1.4. Functional Genomics

Functional genomics has arisen from the need to understand the biochemical and physiological function of gene products, and the complex interplay between them using a holistic approach. Comprehensive analyses of diverse molecular organizations have been enabled by rapid progress in the area of high throughput technologies (Colebatch et al., 2001). Functional genomics makes the transition from gene to genome, transcript to transcriptome, protein to proteome, metabolite to metabolome possible using high throughput technologies.

Functional genomics tools enable us to examine biological processes. The data generated by the help of high throughput technologies such as microarray analysis for gene expression profiling or mapping of physical interactions of proteins may guide us in exploring possible gene or gene sets (protein or protein sets) involved in the formation of a disease. We can also interpret the logic behind the dysfunctioning of a biological pathway through the aid of these technologies. Integrating diverse datasets to create biological networks, cell signatures and developmental markers are effective ways of using functional genomics tools (Fraser and Marcotte, 2004).

Genomic profiling can be used in diagnosis, prognosis, classification and monitoring of diseases based on expression patterns of as many as several thousand of RNAs (Tang et al., 2012).

1.4.1 Genomics

The term “Genomics” was first proposed by Dr. Thomas H. Roderick, a geneticist at the Jackson Laboratory in 1986 (Yadav, 2007). Genomics integrates computational analysis methods such as bioinformatics with experimental methods such as DNA sequencing or gene expression analysis to investigate the characteristics of a given genome. Genomics is instrumental in analyzing the fundamental molecular mechanisms of disease development and its relevance to healthy state. Advances in this discipline in the area of identification of novel biomarkers and therapeutic targets have caused it to become an indispensable tool that clarifies complex situations that might not have been possible by conventional approaches (Tong et al., 2015).

1.4.2. Transcriptomics

The transcriptome is the total RNA sequences transcribed in a cell. Transcriptome analysis using microarrays helps identify the differential expression of genes which allows us to seize information about genes that are active and indicative of disease (Horgan and Kenny, 2011).

In complex biological systems such as humans the proportion of the non-protein-coding transcribed sequences might be greater. As a result of alternative splicing or substitute transcription initiation or termination sites more than one variant of mRNA can exist. Hence the information we acquire from the transcriptome of an organism is more complex than of a genome sequence (Adams, 2008).

1.4.3. Interactomics

Interactomics is a discipline that focuses on interactions between proteins of a biological system. The proteins depicted by nodes and their relationships depicted by edges elucidate the mechanisms of cell functions. The approximately 30.000 genes identified by the Human Genome Project are in a continuous interaction with each other and together they function in such a way that results in disease or healthy states. Gene and protein interactions need to be mapped in order to have a complete understanding of a genome (Cesarani et al., 2005). Different experimental techniques are established to measure physical protein interactions, with Y2H (Yeast 2 Hybrid) (Fields and Song, 1989) and AP-MS (Affinity purification mass spectrometry) (Bauer and Kuster 2003)

being the most widely used. There are publicly available PPI databases that collect and store interaction data from these experimental studies. Some of the most notable PPI databases are: BioGRID (the Biological General Repository for Interaction Datasets) (Stark et al., 2006), HPRD (the Human Protein Reference Database) (Mathivanan et al, 2008), DIP (the Database of Interacting Proteins) (Salwinski et al., 2004), IntAct (the IntAct molecular interaction database) (Hermjakob et al., 2004) and BIND (Biomolecular Interaction Network Database) (Bader et al., 2001).

There are also databases that collect and store data for protein – DNA interactions. These databases are useful in understanding the regulatory relationship between a gene and its transcription factor (TF). A TF is a protein that binds to a molecule of DNA to regulate its function and to increase or decrease gene expression. There are various techniques used to capture TF-DNA interactions such as Electrophoretic mobility shift assay (Garner and Revzin, 1981) and Chromatin immunoprecipitation (Hebbes et al., 1988). Some of the TF-gene databases are: TRANSFAC (Transcription Factor Database) (Wingender et al., 2000), TRED (Transcriptional Regulatory Element Database) (Jiang et al., 2007) and HTRIdb (The Human Transcriptional Regulation Interactions database) (Bovolenta et al., 2012).

1.5. Functional Genomics Studies in Psoriasis

The complexity of psoriasis has led to many studies in the area of functional genomics. More often these studies are individual transcriptome analysis. There are very few integrative analysis that can be found whilst literature mining.

Gudjonsson and coworkers (2010) carried out a gene expression study of lesional and nonlesional psoriatic skin and healthy controls. Their analysis yielded a catalog of previously unreported differentially expressed genes. The results of their enrichment analysis indicated upregulation of immune and defense response as well as keratinocyte differentiation and downregulation of processes that regulate fatty acids and lipid.

Ohara and coworkers (2010) used DNA microarray to identify gene expression in ATP stimulated human keratinocytes. Their results indicated that IL-6, IL-20, CXCL1-3, and ATF3 were overexpressed in these keratinocytes. Furthermore two-phased activation of STAT3 was pointed out. Coda et al (2010) explored gene expression of lesional and non lesional psoriatic skin as well as blood samples using Affymetrix microarrays. Their

study identified novel “hot spots” at specific genomic locations, which might present targets for susceptibility *loci* in future studies.

Suarez-Farinas and coworkers (2010) employed Gene Set Enrichment Analysis (GSEA) in order to evaluate transcriptomes of four different psoriasis microarray studies. Tian and coworkers (2012) carried out meta-analysis on microarray data sets. The results of their analysis indicated a set of reliable psoriasis differentially expressed genes (DEGs) as well as the activation of Atherosclerosis Signaling and Fatty Acid Metabolism pathways. They also identified novel genes which were involved in cardiovascular development and lipid metabolism. Madonna and coworkers (2012) found out that the inhibitors of PI3K/AKT axis in epidermal keratinocytes may be used for treatment of psoriasis, as well as other skin diseases.

Williamson and coworkers (2013) employed keratome skin biopsy enriched in extracellular proteins to identify proteins consistently overexpressed in lesional versus non-lesional skin of psoriasis including S100A7 and FABP5. They have also suggested Profilin 1 as a candidate plasma biomarker of psoriasis. Swindell and coworkers (2013) analyzed existing psoriasis datasets to identify differentially expressed psoriasis genes. Their results indicated that epidermal differentially expressed genes might be induced by AP-1 in response to IL-1, IL-17A and IL-20 cytokine families. Furthermore various inflammatory and cytokine related patterns were evident in psoriatic skin.

Lu et al (2013) attempted to illustrate psoriasis disease mechanism via transcriptional regulatory network construction using microarray data. Their results indicated E2F1, JUN, NF-kB1, STAT1, STAT3 and SP3 were pivotal in the network. These transcription factors may regulate major processes and pathways such as cell proliferation process, cell adhesion molecule pathway; cell cycle pathway, Toll-like receptor signaling pathway and steroid hormone biosynthesis pathway in the progression of the disease.

Guo and coworkers (2014) used three feature selection algorithms to identify candidate biomarkers using gene expression profiles. These biomarker genes are associated with uncontrolled skin cell proliferation.

1.6. Functional Genomics Studies in Other Autoimmune Diseases

This section summarizes previous functional genomics efforts for rheumatoid arthritis, atopic dermatitis and systemic lupus erythamatosus.

1.6.1. Functional genomics studies in rheumatoid arthritis

Gene expression profiling is most commonly used in the area of patient classification and prognosis improvement to illuminate the molecular mechanism of rheumatoid arthritis (Jarvis and Frank, 2010).

Zanders and coworkers (2000) used subtractive hybridization of cDNAs in conjunction with high-density array hybridization to identify rheumatoid arthritis genes. Their results indicated abundance of immunoglobulins and HLA-DR. Macrophage, B cell and plasma cell infiltration were also present in gene expression profiling with suggestion of interferon induction. Watanabea and coworkers (2002) compared the gene expression profile of rheumatoid synovial fibroblasts (RSFs) to normal synovial fibroblasts in an attempt to analyze the aberrant growth properties of rheumatoid synoviocytes. Their results indicated that RSFs showed sensitivity to the cell proliferative effect of PDGF (platelet derived growth factor). Van der Pouw Kraan and coworkers (2003) aimed to elucidate the molecular mechanism of RA by applying DNA microarray analysis to samples taken from the affected joint tissues from RA patients. The gene expression profiles of synovial tissues from RA patients showed that STAT1 played a central role in these tissues. Jeonga and coworkers (2004) used a cDNA microarray to explore IL1B effect on rheumatoid arthritis associated genes. Inflammatory mediators, matrix-modifying enzymes, and apoptosis-associated molecules were the categories of genes that were significantly different in expression. . They proposed that IL1B enhances inflammatory cytokines, causes abnormal MMPs and TIMP production, and dysregulation of apoptosis in rheumatoid synovial fibroblasts.

Bovin and coworkers (2004) used oligonucleotide-based DNA chip microarrays to identify differentially expressed genes of RA patients. Their results indicated that several genes were overexpressed such as DEFA, RNASE2, S100A8 and S100A12 known to be involved in immunoinflammatory responses.

van der Pouw Kraan and coworkers (2007) identified peripheral blood (PB) gene expression profiles by cDNA microarrays to illuminate RA subtypes. Their results indicated that IFN type I is a characteristic of a sub-group of RA patients. Furthermore the activation of the innate defence system, coagulation and complement cascades, and fatty acid metabolism is evident in RA. Lee et al (2011) investigated the expression of major chemokines and receptors in samples of synovial tissue and peripheral blood from patients with different forms of arthritis including RA. They concluded that various chemokines and receptors might play key roles in inflammatory joint disorders such as RA.

Heruth and coworkers (2012) used Illumina RNA sequencing to identify differential expression in rheumatoid arthritis synvovial fibroblasts transcriptomes. They proposed several new genes associated with RA ond offered novel dysregulated pathways such as Cellular Growth and Proliferation, and Cell Morphology.

Yoshida and coworkers (2012) aimed to identify the expression patterns of microdissected synovial lining cells of patients with RA. They performed laser microdissection (LMD) for subsequent cDNA microarray analysis, and confirmed significant gene expression through immunohistochemical methods. They proposed that synovial lining cells have major roles in the inflammatory and proliferative mechanisms of this disease.

1.6.2. Functional genomics studies in atopic dermatitis

In order to illuminate the disease progress and identify potential biomarkers in AD a considerable amount of functional genomics studies were carried out. A microarray study performed by Nomura and coworkers (2003) showed distinctive gene expression patterns related to the infiltration of TH2 cells, eosinophils, and mast cells in AD and the infiltration of TH1 cells and neutrophils in psoriasis providing potential signature markers for the respective diseases.

Sugiara and coworkers (2005) performed microarray analysis of atopic skin lesions. They reported upregulation of S100A8 and S100A7 and downregulation of loricrin and filaggrin. Their results suggested that abberant epidermal differentiation and insufficient defense mechanism are major dysfunctions in AD. Ogawa and coworkers (2005) implemented DNA microarray analysis in skin samples of AD patients. They concluded

that upregulation of the tenascin-C expression is specific to AD lesions, and it plays a regulatory role in inflammatory processes. Microarray studies of de Jongh and coworkers (2005) indicated that atopic dermatitis epidermis expresses lower levels of host defense proteins compared with psoriasis epidermis. They also observed that antimicrobial peptides were overexpressed in psoriasis keratinocytes rather than AD.

Hijnen et al (2005) performed transcriptomic profiling of peripheral blood, unstimulated CD4+ T cells in AD patients. The results of their study indicated differentially expressed genes involved in tissue homing, proliferation, and apoptosis mechanisms. Their analysis did not result in significant expression of atopy genes.

Lü and coworkers (2009) identified hub and candidate genes (MMP1 and MMP10) that are significantly up-regulated in AD patients, both at the cellular level and in serum through cDNA microarray and interactomic analyses. mRNA expression analyses done by Rebane et al. (2012) revealed that apoptosis-related genes in keratinocytes and skin and immune system-related genes in lesional skin of AD patients were upregulated. Their results indicated increased IFNG responses in skin of patients with AD and proposed new apoptosis and inflammation associated factors in the progress of AD.

A comparative analysis study to identify dysregulated expression in AD and psoriatic lesions using gene expression data was performed by Choy et al (2012). Their results indicated a correlation between AD gene set score and measures of allergic inflammation. They also demonstrated that neutrophilic inflammation as well as allergic inflammation are characteristics of AD. Recently, Suarez-Farinas and coworkers (2015) provided additional insights into the lesional AD transcriptome using RNA-seq and microarrays performed on the same cohort. Results of this study indicated commonly identified AD genes which included S100A8/A9/A12, CXCL1, OASL, K16 and CLDN8. Additionally CCL2, CCL3, IL1R1 related and IL-36 isoform genes were also identified. They concluded that TREM-1 pathway and the IL-36 cytokine were activated in this disease.

1.6.3. Functional genomics studies in systemic lupus erythematosus

Genomic profiling studies of SLE exhibited dysregulated inflammatory cytokines, chemokines, and immune response-related genes, furthermore genes functioning in pathways such as apoptosis, signal transduction, and the cell cycle were also activated.

Additionally analysis of peripheral blood and kidney glomeruli samples also show overexpression of IFN regulated genes indicating that interferon stimulation is important in SLE (Qinga and Putterman, 2004). Rus et al. (2002) used PBMC from SLE patients in transcriptomic profiling analysis. They have proposed genes belonging to TNF/death receptor, IL-1 cytokine family, and IL-8 and its receptor familes which have formerly not been associated with SLE. Baechler et al. (2003) employed gene expression profiles of peripheral blood mononuclear cells (PBMC) to determine differentially expressed genes of SLE. Their research concluded that IFN pathway was dysregulated in SLE. Rus et al. (2004) also investigated transcriptomic profiles consequently applying the ‘‘nearest shrunken centroids’’ method to identify a set of genes in PBMC of SLE patients. They identified genes that were previously not associated with the disease. Their study may help interpret pathways associated with SLE.

Nakou et al. (2008) analyzed bone marrow mononuclear cells and PBMC from SLE patients using genome-scale DNA microarrays. They identified genes that are active in cell death, growth, signaling, and proliferation. They proposed that apoptosis and granulocytes play a major role in the disease progress. Nikpour et al. (2008) sought to determine the correlation of PBMC gene expression of SLE patients with disease activity. Disease activity in SLE is measured using the SLE Disease Activity Index 2000 (SLEDAI-2K), a scoring system to enumerate persistent activity in rash, mucous membranes, alopecia, and proteinuria. They used a custom microarray to profile differentially expressed genes of SLE patients. Their results indicated that SLEDAI-2K is correlated with overexpression of IFN genes. They proposed that transciptome profiling can be useful in detecting disease activity. Gene expression analysis done by Li et al. (2010) using blood samples resulted in the overexpression of IFN genes correlating with IgG autoantibodies expression. They also suggested that overexpression of IFNA may help autoantibody class shift from IgM to the IgG. Jeffries and coworkers (2011) implemented a genome-wide DNA methylation study in CD4 (+) T cells in SLE patients and healthy controls. Their results demonstrated hypomethylated genes such as CD9, MMP09, PDGFRA and BST2 in SLE patients. Overexpression of genes involved in folate biosynthesis was also apparent. Furthermore transcription

factors such as RUNX3 and HNF4a were found to affect differentially methylated genes. Activation of apoptosis pathway was also evident.

Becker et al. (2013) determined the transcriptomic profiles of isolated leukocyte subsets obtained from SLE patients. Their results indicated an overexpression of CD38, CD63, CD107a and CD169. Furthermore SLE lymphoid and myeloid subsets displayed elevated levels of transcripts for cytosolic RNA and DNA sensors and downstream effectors mediating IFN and cytokine production. Absher et al. (2013) analyzed DNA methylation, in SLE patients and healthy controls. They have identified reduction in methylation in close proximity of the genes that respond to interferon, and suggested sensitivity to interferon in patients with SLE. They hypothesized that this sensitivity may explain the recurrence of the disease.

1.7. Systems Biomedicine

Systems biomedicine is an emerging field that specializes in applying systems biology principles to understand the complex mechanism of humans and suggest therapeutics and drug targets that help to improve a patient's quality of life. Figure 1.1 gives a brief explanation of how this is done. High-throughput gene expression profiling (i.e., transcriptomics) technologies permit the identification of disease-related genes by exposing the difference between healthy and disease states. Integration of transcriptomics and protein interaction data can help us to gain knowledge of the disease mechanism (Pache et al., 2008) as well as suggesting therapeutic intervention (i.e. drug targets) (Bartfai et al., 2012). Moreover, disease genes may be highly expressed, have tissue-specific expression patterns and a higher mutation rate over evolutionary time (Oti and Brunner, 2007). With this information at hand different studies have been performed on omics platforms at various levels to identify, predict or prioritize disease genes, some of which are stated in Sevimoglu and Arga (2014).

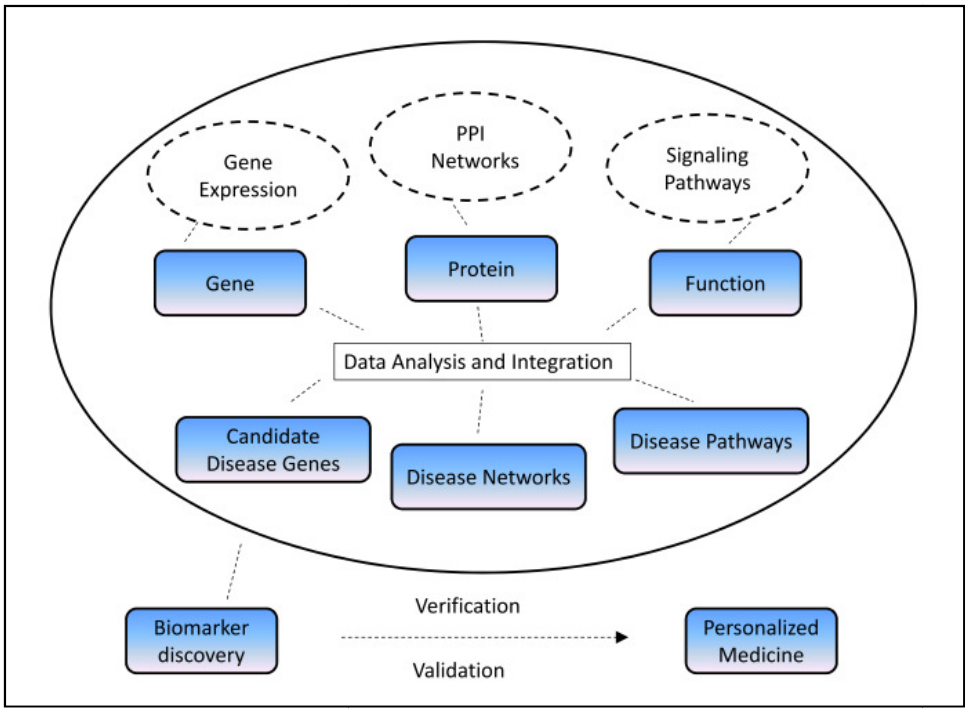

Figure 1.1 The systems biomedicine approach (Sevimoglu and Arga, 2013)

1.8. Aim of this Thesis

The aim of this thesis is to elucidate the molecular mechanism of psoriasis, identify potential biomarkers and therapeutic targets as well as shedding light on the genetic connections between psoriasis and three other autoimmune-related diseases: rheumatoid arthritis, systemic lupus erythematosus and atopic dermatitis. Moreover, this research introduces a novel integrative approach that will help investigate the molecular mechanisms of other complex genetic diseases as well.

2. MATERIALS AND METHODS

This chapter describes the materials and methods employed in the elucidation of the disease mechanism of psoriasis and its association with other autoimmune diseases using a systems biology approach.

2.1. Materials

In this section, the materials used to elucidate the disease mechanism of psoriasis as well as the other selected autoimmune diseases are given.

2.1.1. Transcriptome datasets

The raw data of high throughput gene expression datasets associated with psoriasis, RA, MS and SLE from a total of 21 studies have been obtained from Gene Expression Omnibus (GEO) (Barrett et al., 2013) and analyzed (Tables 2.1 and 2.2). These datasets were from three different microarray platforms: Affymetrix, Illumina and Agilent. For psoriasis; samples of the datasets were often taken from skin, but there were also samples from bone marrow mesenchymal stem cells (GSE40033) and from dermal mesenchymal stem cells (GSE42632). Lesional versus non-lesional samples were analyzed for ten of the psoriasis datasets and psoriasis versus normal samples were analyzed for the remaining two (GSE40033, GSE42632). For RA, samples were taken from synovial tissue of RA patients and healthy donors. Samples were taken from the skin for AD datasets and for SLE datasets blood samples were used.

Table 2.1 Transcriptomics datasets of psoriasis employed in the present study

Dataset No	GEO ID	Sample Size	Platform	#of Probesets	Description	Reference
1	GSE14905	82	Affymetrix Human Genome U133 Plus 2.0 Array	54675	Analysis of lesional and non-lesional skins from patients with psoriasis.	Yao et al., 2008
2	GSE34248	28	Affymetrix Human Genome U133 Plus 2.0 Array	54675	Analysis of lesional and non-lesional skins from patients with psoriasis.	Bigler et al., 2013
3	GSE41662	48	Affymetrix Human Genome U133 Plus 2.0 Array	54675	Analysis of lesional and non-lesional skins from patients with psoriasis.	Bigler et al., 2013
4	GSE30999	170	Affymetrix Human Genome U133 Plus 2.0 Array	54675	Analysis of lesional and non-lesional skins from patients with moderate-to-severe psoriasis.	Suárez-Fariñas et al., 2012
5	GSE13355	180	Affymetrix Human Genome U133 Plus 2.0 Array	54675	Analysis of lesional and non-lesional skins from patients with psoriasis as well as normal skin from control individuals.	Nair et al., 2009
6	GSE26866	37	Affymetrix Human Genome U133A 2.0 Array	22277	Analysis of paired lesional and non-lesional skins from patients with psoriasis.	Mitsui et al., 2012
7	GSE6710	26	Affymetrix Human Genome U133A Array	22280	Analysis of lesional and non-lesional skins from patients with plaque-type psoriasis.	Reischl et al., 2007
8	GSE40263	10	Affymetrix Human Gene 1.0 ST Array	32321	Analysis of skins from patients with psoriasis as well as normal skin from healthy control individuals.	Unpublished data
9	GSE2737	11	Affymetrix Human Genome U95A Array	12626	Analysis of paired lesional and non-lesional skins from patients with psoriasis as well as normal skin of healthy control individuals.	Kulski et al., 2005
10	GSE41745	6	Illumina Genome Analyzer IIx (Homo Sapiens)	33655	Analysis of paired lesional and non-lesional skins from patients with psoriasis.	Jabbari et al., 2012
11	GSE42632	12	Agilent-026652 Whole Human Genome Microarray 4x44K v2	28908	Analysis of dermal mesenchymal stem cells between psoriatic patients and normal adults.	Unpublished data
12	GSE40033	14	Agilent-028004 SurePrint G3 Human GE 8x60K Microarray	42405	Analysis of bone marrow mesenchymal stem cells between psoriatic patients and normal adults.	Unpublished data

Table 2.1 Transcriptomics datasets of other autoimmune diseases employed in the present study

Disease	GEO ID	Sample Size	Platform	#of Probesets	Description	Reference
Rheumatoid Arthritis	GSE1919	10	Affymetrix Human Genome U95A Array	12626	Analysis of synovial tissues from rheumatoid arthritis patients in comparison to normal donors were investigated	Ungethuem et al., 2010
	GSE10500	8	Affymetrix Human Genome U95 Version 2 Array	12625	Macrophages from Rheumatoid Arthritis synovial fluids were compared to primary human blood-derived macrophages.	Yarilina et al., 2008
	GSE55457	23	Affymetrix Human Genome U133A Array	22283	Identification of rheumatoid arthritis in patients by transcriptome-based rule set generation	Woetzel et al., 2014
Atopic Dermatitis	GSE16161	18	Affymetrix Human Genome U133 Plus 2.0 Array	54675	Genomic analysis of atopic dermatitis to identify defects of epidermal cornification	Guttman-Yassky et al., 2009
	GSE27887	17	Affymetrix Human Genome U133 Plus 2.0 Array	54675	Genomic profiling of atopic dermatitis in both lesional and non-lesional skin	Tintle et al., 2011
	GSE32924	12	Affymetrix Human Genome U133 Plus 2.0 Array	54675	Genomic profile of paired samples of atopic non lesional and lesional skin from patients compared with normal human skin	Suárez-Fariñas et al., 2011
Systemic Lupus Erythematosus	GSE45923	9	Affymetrix Human Genome U133 Plus 2.0 Array	54675	Analysis of peripheral blood cells from lupus patients and healthydonors	Zhao et al., 2013
	GSE46907	10	Affymetrix Human Genome U133B Array	22645	Analysis of gene expression profile of Systemic Lupus Erythematosus monocytes.	Rodriguez-Pla et al., 2014
	GSE61635	129	Affymetrix Human Genome U133 Plus 2.0 Array	54675	Analysis of mRNA from the blood of a Systemic Lupus Erythematosus cohort and healthy controls.	Unpublished data

2.1.2. Protein-protein interaction datasets

The physical *Homo sapiens* protein-protein interaction dataset was retrieved from iRefIndex Database (Razick et al., 2008). The visualization and topological analysis of the PPI network was performed via Cytoscape (Shannon et. al., 2003).

2.1.3. Transcription factor-gene regulation data

The regulatory associations between transcription factors (TFs) and their downstream effectors in the regulatory network were obtained from different resources: Transcriptional Regulatory Element Database (TRED) (Jiang et al., 2007), GENOMATIX (Genomatix Software Inc, Ann Arbor, MI, USA), and The Human Transcriptional Regulation Interactions database (HTRIdb) (Bovolenta et al., 2012).

2.1.4. Gene – protein associations

To avoid possible ambiguity due to different identifiers employed in different microarray platforms and for ease of comparison between gene sets, all the identifiers of different platforms and series were converted to ENTREZ identifiers, accordingly. The gene–protein associations were obtained from UniProt ID Mapping tool (Uniprot Consortium, 2014) and conversions were done using bioDBnet platform (Mudunuri et al., 2009).

2.1.5. Gene Ontology annotations

The Gene Ontology annotations are obtained from Gene Ontology Consortium (GOC, http://www.geneontology.org) which is a bioinformatics resource that uses structured, controlled vocabularies to classify gene product function. They use molecular function, biological process and cellular component as a means of organization (The Gene Ontology Consortium, 2013).

2.1.6. Protein – Pathway/Process/Disease associations

The pathway, disease and Gene Ontology (GO) enrichment analyses were carried out through DAVID bioinformatics tool (Huang et. al., 2007). Pathways associated with disease genes were collected from Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al., 2012).

2.1.7 Patient characterization

After patient consent, blood and skin samples were taken from 4 female and 3 male psoriasis patients and 3 female and 2 male healthy controls with ages ranging between 20 and 48. Table 2.3 gives the PASI scores of seven psoriasis patients involved in the experiments. A PASI (Psoriasis Area and Severity Index) score is a tool used to measure the severity and extent of psoriasis.

Table 2.2 Psoriasis Area and Severity Index (PASI) of psoriatic patients

Patient No	Gender	PASI Score
1	Female	13
2	Female	9
3	Female	4.4
4	Female	6
5	Male	5.2
6	Male	6.3
7	Male	9.2

2.1.8. Chemicals and kits used in experimental studies

The ELISA kits used for the experimental studies are given in Table 2.4.

Table 2.3 ELISA kits utilized

Protein Symbol	Kit Name-Catalog Number
IFI44	MyBiosource- MBS925861
IRF9	MyBiosource - MBS921012
IFIT1	MyBiosource - MBS925861
RSAD2	MyBiosource - MBS931736
OAS2	MyBiosource - MBS2022768
PI3	Abnova - KA1771
SUB1	Blugene – E01A2002
WIF1	Bluegene – E01W0018

Prior to the PCR studies, RNase contamination from the work place and equipments were removed by spraying with RNaseZap® RNase Decontamination Solution from Ambion™ and rinsing off with RNase-free water.

2.2. Methods

In this section, the methods employed to analyze the selected disease datasets for this thesis are explained, including the identification of DEGs, construction and analysis of disease PPI networks, construction of a TF-gene network and Fold Change Correlation Analysis of psoriasis DEGs.

2.2.1. Identification of differentially expressed genes

RMA normalization (Irizarry et al., 2003) and linear models for microarray data (LIMMA) method (Smyth, 2004) was followed in statistical analysis of each dataset in order to identify DEGs. DEGs were selected according to computed p-values <0.05 and the same p-value cut-off were used for comparison across datasets of all microarray platforms. Up/down-regulation of genes was identified according to fold changes. The DEGs with fold change greater than 1.5 were accepted as up-regulated and the DEGs with fold change of less than 0.5 were accepted as down-regulated.

2.2.2. Construction and analysis of protein- protein interaction networks

The interactions associated with proteins corresponding to DEGs were identified and PPI networks consisting of down and up-regulated genes were reconstructed and enriched with first interacting neighbours. The PPI networks were converted into undirected graphs. A graph is a pair of sets (V, E), where V is the set of vertices and E is the set of edges, structured by pairs of vertices. Here the vertices represent the proteins and edges the binary interactions between them.

The dual-metric approach (Karagoz et al., 2015) and Cytohubba plugin (Chin et al., 2014) was employed in determination of hub proteins. These topological metrics are; degree (a local metric), which is defined by the number of adjacent nodes of a node in the network, and betweenness centrality (a global metric), which characterizes nodes by how repeatedly they exist on the shortest path among two other nodes in the network. The intersection of the top ten proteins based on these two metrics was selected as hub proteins.

2.2.3. Reconstruction of transcriptional regulatory network

A directed graph of TF – Gene associations has been constructed, in which an edge between a TF and a gene represent the regulation of the gene by the specific TF. All connections in the network correspond to direct interactions of TFs with genes.

2.2.4. Fold change correlation analysis

Fold change (FC) values of individual DEGs in each dataset were calculated using their gene expression profiles in order to present a FC profile for each DEG among datasets. These FC profiles were used to check for correlations between selected DEGs utilizing Pearson correlation coefficients (PCC). DEGs with PCC values greater than 0.7 were accepted as positively correlated. A correlation network was formed between DEGs based on these cutoff values. Modules within the correlation network were identified using Cytoscape plugin Clust&See (Spinelli et al., 2013). Hubs of the correlation network as well as each module were identified using Cytohubba (Chin et al., 2014).

2.2.5. Functional enrichment analysis

The pathway, disease and Gene Ontology (GO) enrichment analyses were carried out through DAVID bioinformatics tool (Huang et. al., 2007). Enrichment results with p-value <0.05 were considered as statistically significant (Boyle et al., 2004). DAVID tool has options to enrich gene sets according to Genetic Association Database (GAD) disease and disease class as well as Gene Ontology terms: biological process, molecular function, cellular component and KEGG, PANTHER and REACTOME pathways.

2.2.6. Enzyme linked immunosorbent essay (ELISA) procedures

The plasma was collected using heparin as an anticoagulant and centrifuged for 15 minutes at 1000 ×g at 2-8°C within 30 minutes of collection. Samples were aliquoted and stored at -80°C. Commercial ELISA kits were used according to manufacturer's protocols (Table 2.4) to identify the pre-selected protein concentrations in plasma of 7 psoriasis patients and 5 healthy controls. The results of the ELISA tests were read with BIO-TEK ELx800. Test results were calculated and interpreted using www.elisaanalysis.com through a four parameter logistic regression analysis (4PL) then using a t-test to calculate the significance. 4PL is usually used for curve-fitting analysis in ELISA. The formula for 4PL is:

$$y = \mathrm{d} + \frac{\mathrm{a} - \mathrm{d}}{1 + (\mathrm{x}/\mathrm{c})^{\mathrm{b}}}$$

Where,

y = OD (Optical Density)

x = Concentration

and, a, b, c and d are constants.

The constants used in calculations are given in Appendix A.

2.2.7. Quantitative RT-PCR

Skin samples were taken using a 4 mm punch biopsy. The samples were promptly frozen in liquid nitrogen and stocked at -80˚C. Cell disruption was carried out using Cellcrusher®. Total RNA was extracted using the RNeasy® Mini Kit (Cat. No. 47104) (Qiagen, Milano, Italy) according to manufacturer's protocols. The RNA concentrations were measured using IMPLEN® Nanophotometer P-Class.

Primers were designed using Primer3Plus (Untergasser et al., 2007) and PrimerQuest (www.idtdna.com) except for OAS2 (Schmeisser et al., 2010) (Table 2.5). RT-PCR was performed on the Roche – Lightcycler 1.5. Lightcycler® RNA Master SYBR Green I kit was used for one step PCR application. RPLP0 was used as housekeeping genes. The data was evaluated using the Livak Method (Livak and Schmittgen, 2001).

The formulas used are:

$$\Delta C_{T(sample)} = C_{T(sample)} - C_{T(housekeeping)}$$

$$\Delta C_{T(control)} = C_{T(control)} - C_{T(housekeeping)}$$

$$\Delta\Delta C_T = \Delta C_{T(sample)} - \Delta C_{T(control)}$$

$$\text{Expression ratio} = 2^{-\Delta\Delta CT}$$

C_T is the threshold cycle, which demonstrates the fractional cycle number that the amplified target reaches a fixes threshold. ΔC_T is the cycle difference between target and reference. ΔC_T graphs are given in Appendix A.

Table 2.4 Primers designed and employed in Real-Time PCR analyses

Gene Symbol	Forward	Reverse
IFIT1	GATGAAGGACAGGAAGCTGA	TAGCAAAGCCCTATCTGGTG
RSAD2	GGATAGCATGAAGGAGCAGA	CCTTGCAACATGGTATGTGA
IRF9	GCCTGTAACACACTGCCTCT	CTTGTAGGGCTCAGCAACAT
IFI44	GTAATGAATGATGCCCTTCG	GGAACTAATCCCCTGCAAAT
OAS2	ACAGCTGAAAGCCTTTTGGA	GCATTAAAGGCAGGAAGCAC
PI3	CAGCTGAAGCAGAGGCTTAC	CAGGCTTAGTGGAGACTGGA
NMI	CGCAGTGTGGTCAGAAATA	GGCAGAGTGTTACCCAATAA
WIF1	TGAGTGGGAGACCAGAAG	GGGAGAAGAGGCAGAGAA
SUB1	GCTTACTTCCTGGTTCCT	GTTTCCTGCCTCAACTATTC
Housekeeping Gene		
RPLP0	TTTAGGTTTCACCGCGTTAG	CTAGAATAACAGCCCCAGCA

3. RESULTS AND DISCUSSIONS

This chapter is presented in five main sections: 1) Integrative Analysis of Psoriasis Datasets and Experimental verification of Psoriasis Biomarker Candidates, 2) Integrative Analysis of Rheumatoid Arthritis Datasets,3) Integrative Analysis of Atopic Dermatitis Datasets, 4) Integrative Analysis of Systemic Lupus Erythamatosus Datasets and 5) Comparative Analysis of the Inspected diseases.

3.1. Integrative Analysis of Psoriasis Datasets

In this section, the results and discussions of the psoriasis datasets are given, including the analysis of differentially expressed genes, the reconstruction of the PPI network, identifying central proteins of this PPI network, analysis of TF-gene regulation as well as reconstruction of a TF-gene network and identification of central TFs and lastly analysis of FCC and construction of a FCC network between DEGs of psoriasis.

3.1.1. Differentially expressed genes of psoriasis

In the present study, we utilized twelve publicly available gene expression datasets of psoriasis which included samples of lesional and non-lesional skin as well as bone marrow and dermal mesenchymal stem cells (Table 2.1). Statistical analyses of these datasets led to the identification of DEGs. The number of DEGs across datasets ranges between 572 (GSE40263) and 3184 (GSE41662). In all datasets (except GSE2737), the number of up-regulated genes were higher than that of down-regulated genes (Figure 3.1).

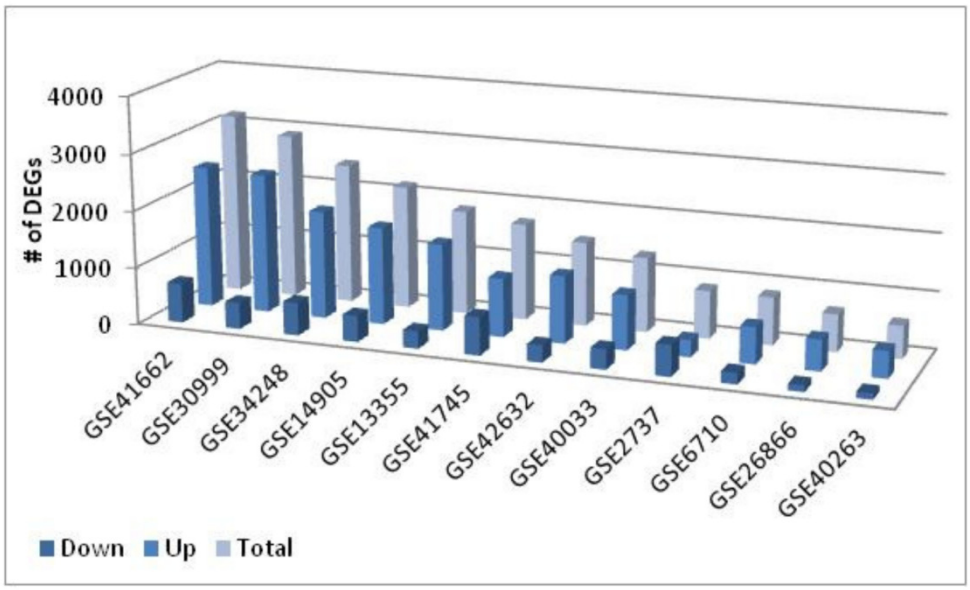

Figure 3.1 The numbers of differentially expressed genes in psoriasis datasets

The DEGs in psoriasis datasets have major associations with infectious, immune system and cardiovascular diseases as well as other diseases such as neurological disorders (Figure 3.2).

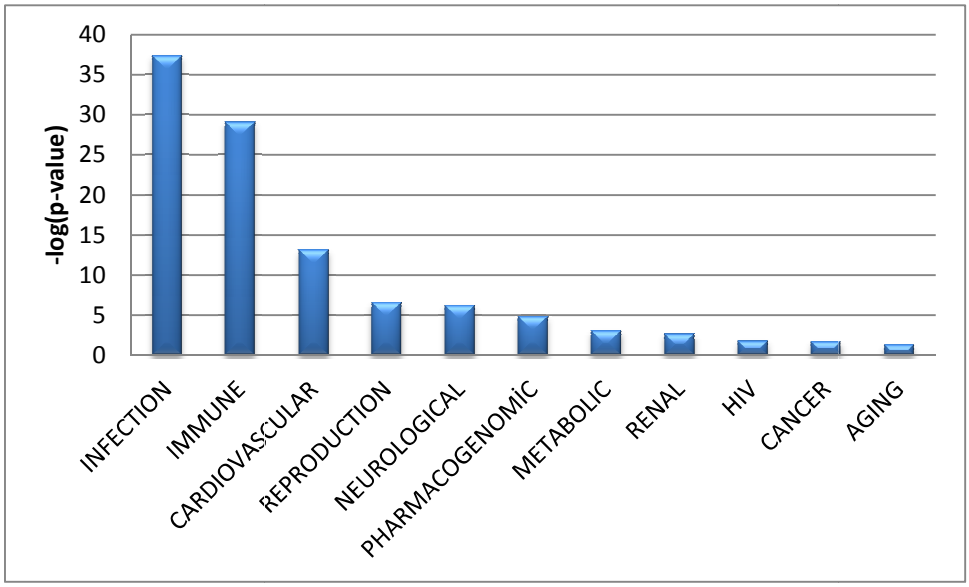

Figure 3.2 Disease classes associated with differentially expressed genes in psoriasis (Classification was based on GAD database)

IFI44, which is an interferon stimulated gene (ISG) encoding an interferon induced, cytoplasmic protein with antiviral activity, was the sole common DEG among 11 of the 12 datasets. Though its function is still unknown, it is believed to be involved in host defense. Recombinant expression of IFI44 alone is sufficient to inhibit cell proliferation indicating that it does not require the presence or activity of any additional ISGs (Hallen et al., 2007). It is upregulated with a FC as high as 4.8 in samples taken from various tissues. Ten DEGs (IFIT1, OAS2, PI3, STAT1, NMI, TRIM22, RSAD2, WIF1, SUB1 and MAD2L1) were found in ten of the twelve datasets. These DEGs along with IFI44 will be named as "core DEGs" for the rest of this thesis since they are commonly identified in at least ten of the twelve datasets. Eight of the core DEGs are cytoplasmic proteins (GO:0005737) except for WIF1 and PI3, which are in the extracellular region and SUB1 which is located inside the nucleus. Six of the DEGs (OAS2, NMI, IFI44, RSAD2, STAT1, and TRIM22) share a common GO Biological Process Term (response to stimulus, GO:0050896), while NMI, SUB1, STAT1 and TRIM22 share the same GO Molecular Function Terms (transcription factor binding, GO:0003712 and transcription cofactor activity, GO:0008134). Six of these core DEGs are ISG's (IFI44, IFIT1, OAS2, STAT1, TRIM22 and RSAD2).

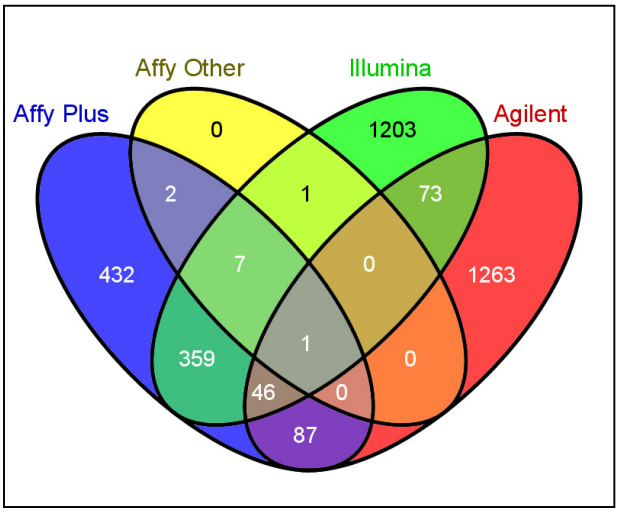

Figure 3.3 Comparative analysis of differentially expressed genes in psoriasis according to microarray platforms ("Affy plus": Affymetrix Human Genome U133 Plus 2.0 Array. "Affy other": Affymetrix Human Genome arrays other than U133 Plus 2.0.)

The common DEGs across datasets were also analyzed according to the microarray platform employed in the analyses (Figure 3.3). The highest number of common DEGs (934) was in the five datasets of Affymetrix Human Genome U133 Plus 2.0 Array. This is possibly due to the fact that it is the latest series of Affymetrix platform and contains the highest number of probesets (54675) which covers approximately twenty thousand genes.

3.1.2 Transcription factor-differentially expressed gene relationships in psoriasis

The regulatory relationship between some of the core DEGs and their TFs (which were also differentially expressed in psoriasis datasets) were depicted (Figure 3.4). Seven of the cores DEGs have TFs associated with them. TRIM22, RSAD2, IFIT1, STAT1 and OAS2 are all ISGs so it not a surprise that they are regulated by either IRFs (Interferon Regulatory Factors) or IFNG. STAT1 and NMI are both TF's and they also regulate each other. Three of the TFs involved in the regulatory relationships with the core DEGs (IRF1, IRF7 and IRF9) are members of the interferon regulatory transcription factor family. They are multifunctional transcription factors that play major roles in the regulation of immune cells along with cell cycle regulation and apoptosis in response to a variety of stimuli (Schwartz et al., 2011, Ning et al., 2011). STAT1 and STAT2 associate to form a heterodimer, which in turn recruits IRF9 and forms the IFN-stimulated gene factor 3 (ISGF3) complex which is a TF regulating the expression of IFIT1. These three TFs (STAT1, STAT2 and IRF9) can work together as a complex and also individually to regulate different processes (Fink and Grandvaux, 2013). STAT1 is a major TF by which cytokines induce transcription (Delgoffe and Vignali, 2013). MYC is a TF regulating the expression of STAT1 and NMI as well as expression of PI3 which activates the transcription of growth related genes.

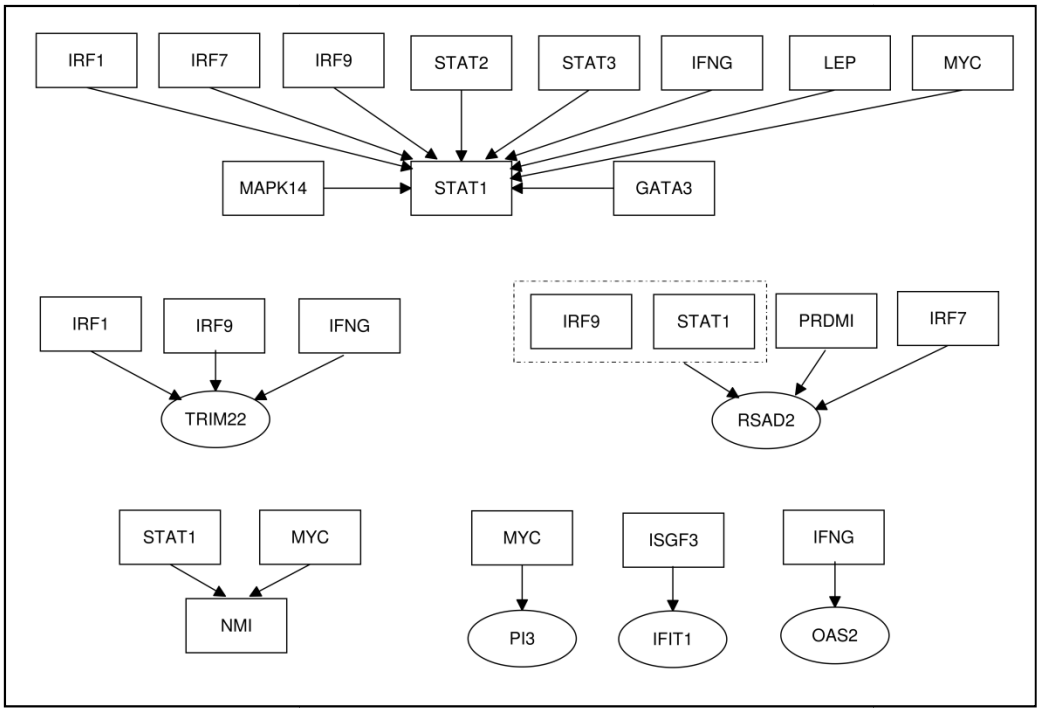

Figure 3.4 The transcriptional regulatory modules controlling the core DEGS in psoriasis (TF: ▭ , core DEG: ⬭).

3.1.3. Protein-protein interaction (PPI) networks associated with psoriasis

A PPI network associated with psoriasis has been reconstructed. The reconstructed PPI network of psoriasis consisted of 576 binary interactions between 534 proteins, which were physically interacting with the core DEGs (Figure 3.5).

Analyses of reconstructed psoriasis network identified five proteins as hubs (STAT1, MAD2L1, CYCS, NMI and SUB1), which exhibit high topological centrality in the network (Table 3.1). In order to consider both local and global features of nodes within the network, two metrics of graph theory were simultaneously employed in determination of hub proteins: a local-based metric (degree), and a global-based metric (betweenness centrality). These hub proteins deserve attention for further studies, since they indicate significant potential for being candidate biomarkers for psoriasis.

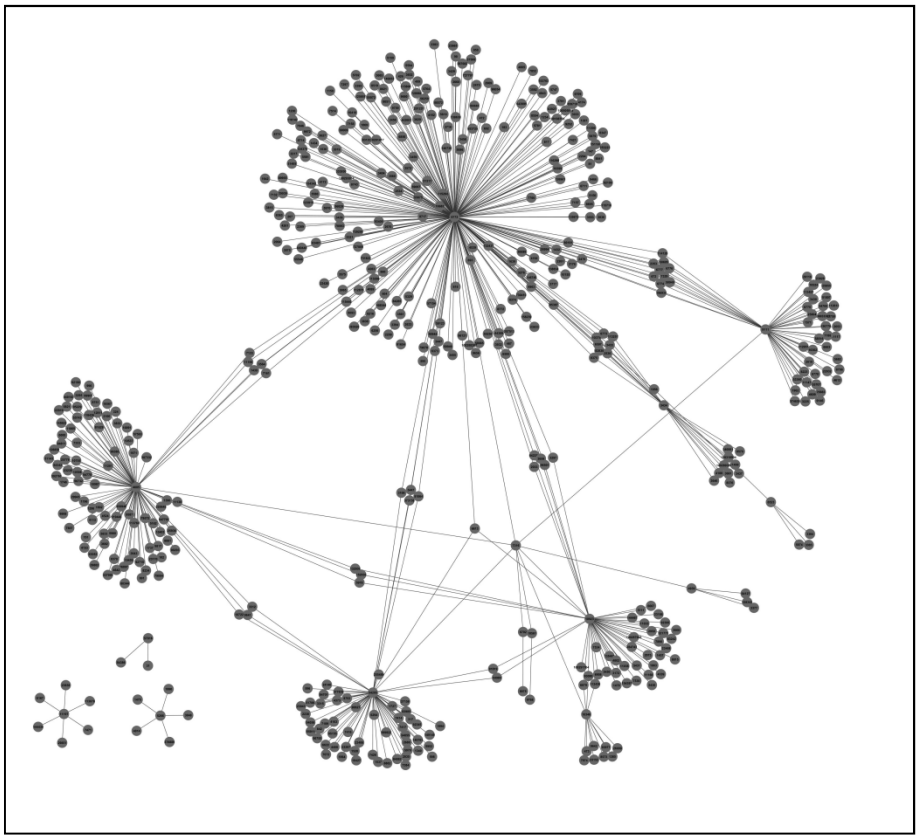

Figure 3.5 Psoriasis PPI Network of core DEGs (with Entrez ID's)

Table 3.1 Central proteins (hubs) of the reconstructed psoriasis network

Protein Symbol	Degree	Degree Rank	Betweenness	Betweenness Rank
STAT1	266	1	101661	1
MAD2L1	99	2	41626	2
CYCS	63	3	26730	3
NMI	52	4	19275	4
SUB1 (PC4)	50	5	19114	5

Four of the hub proteins of the reconstructed psoriasis network were also in our core DEG list. STAT1 is from the family of STAT (signal transducer and activator of transcription) proteins which play an important role in processes such as, cell growth and differentiation, cell survival and apoptosis and immune responses. They bind to receptors and function as transcription factors that trigger gene activation (Shuai, 2000). STAT1 is overexpressed with a FC ranging between 1.65 and 3.75. MAD2L1 (mitotic

arrest deficient-like 1) is needed for the execution of spindle assembly checkpoint during mitosis to ensure the proper segregation of chromosomes under normal growth conditions (Xu et al., 1997). It is overexpressed with a FC range of 1.87 and 5.5. NMI (N-myc (and STAT) interactor) is a TF that can enhance STAT1-mediated transcription, implicating a broader role for NMI in cytokine signaling (Zhu et al., 1999). The FC for NMI is between 1.65 and 2.37. SUB1 (also known as PC4: Positive Cofactor 4) plays a dual role in regulation of gene transcription as an activator or repressor and functions in distinct stages of the transcription process (Conesa and Acker, 2010). A possible role of SUB1 in the initial reaction to DNA damage was also proposed by noticing single-stranded DNA and facilitating the consequent steps of DNA repair (Mortusewicz et al., 2008). It is upregulated with a FC range of 1.54 – 3.26. CYCS (Cytochrome c), which was not one of the core DEGs but a DEG in nine of the analyzed datasets, is a component of the electron transport chain of mitochondria (Gonzales and Neupert, 1990). It is active in cell apoptosis (programmed cell death) (Liu et al., 1996) and in antioxidant defense system of mitochondria (Skulachev, 1998). It was up-regulated in psoriasis (FC ranging between 1.54 and 2.85).

3.1.4. Fold change correlation (FCC) analysis of psoriasis

A comprehensive literature survey on signaling pathways and biological processes associated with common DEGs of psoriasis datasets and central proteins of reconstructed psoriasis network yielded a set of 145 genes (182 probesets), which were differentially expressed in at least five datasets. The correlation analysis in the gene expression levels was based on fold changes instead of raw expression data.

A correlation network was reconstructed based on the results of the FCC Analysis, which consisted of 182 nodes (representing the probesets) and 4152 edges (representing significant correlation with Pearson>0.70). Topological analysis of the network indicated a highly dense, scale-free degree distribution with average connectivity of 45.6. SUB1, IL13RA1 and SOCS1 have the highest number of correlations (86 of the 182 probesets) (Table 3.2). DEFB4A has the lowest number of correlations with only four of the DEGs (PI3, CCNB2, UBA6 and LEPR).

Table 3.2 Central DEGs (hubs) of the FCC network of psoriasis and its modules

Protein symbol	Degree	Protein symbol	Betweenness
Overall network			
SUB1-1	86	CSF2RA-3	474.6
SOCS1	86	LIFR-1	394.4
IL13RA1-3	86	SHC1-2	288.1
IL12RB2	85	IL12RB1	266.7
TRIM14-2	84	LEPR-2	243.01
MAPKAPK3	82	CTSC	228.6
NMI	82	SUB1-1	223.9
OAS1	81	ITGA4	216.5
CDK1-2	80	IL13RA1-3	216.4
Module 1			
SUB1-1	86	CTSC	753.4
SOCS1	86	SUB1-1	642.9
IL12RB2	85	TRIM14-2	542.3
TRIM14-2	84	SOCS1	471.8
MAPKAPK3	82	ATP1A2	469.9
NMI	82	IL12RB2	461.3
Module 2			
CDK1-1	77	LIFR-1	2652.3
CDC6-1	75	SHC1-2	1225.1
CXCR2	73	CDK1-1	711.2
CDK1-3	72	SLPI	666.3
SHC1-2	70	CDK1-3	627.5
SLPI	69	CDC6-1	616.4
LIFR-1	69	CXCR2	545.9
Module 3			
CSF2RA-3	76	CSF2RA-3	2646.9
ITGA4	56	LEPR-2	2005.2
STAT3	55	STAT3	1471.8
LEPR-2	45	ITGA4	1463.9
Module 4			
IRF9	62	IRF9	1543.8
S100A9	56	CSF2RA-4	1190.2
LIFR-2	49	S100A9	1030.7
CSF2RA-4	45	LIFR-2	633.4

Four modules have been identified as a result of clustering analysis. Module 1 appears to be the central module of the FCC network of psoriasis with a high-level connection to Module 2 (Figure 3.6). The hubs of Module 1 shares proteins with the global hub analysis of the FCC Network as well as PPI network of psoriasis. Pathway enrichment analysis for the 145 DEGs and the modules has been given in Table 3.3.

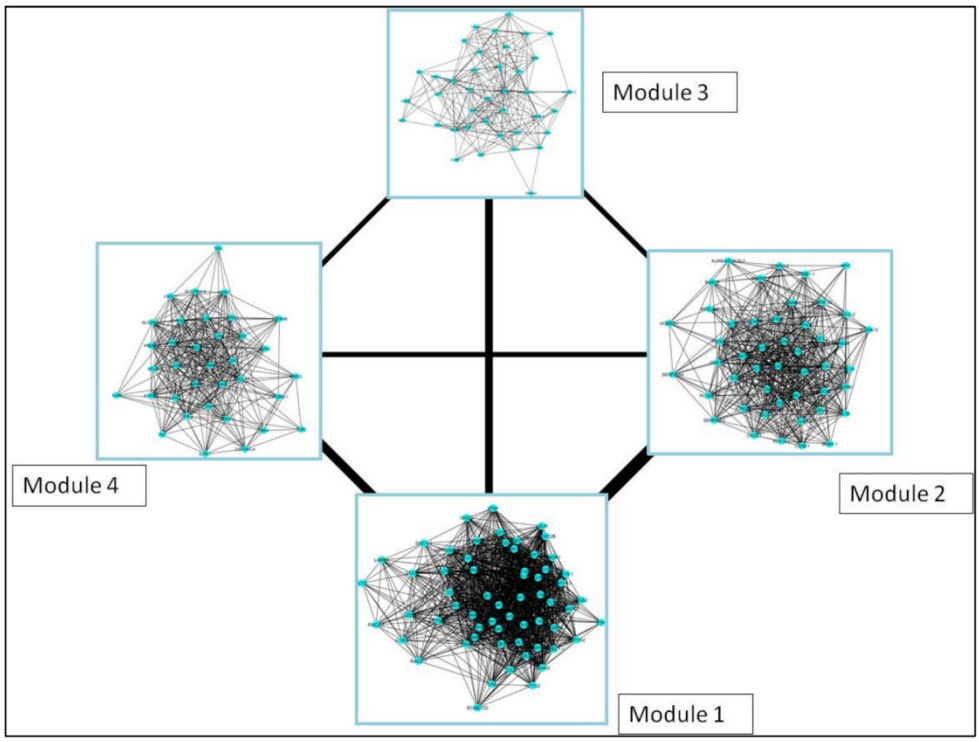

Figure 3.6 Modules of the FCC network of psoriasis (The line widths are proportional with the correlations between the modules)

Table 3.3 Pathway enrichment in psoriasis network

Pathway (KEGG ID)	P-value				
	Overall	***Module 1***	***Module 2***	***Module 3***	***Module 4***
Jak-STAT signaling pathway (hsa04630)	1.16×10^{-12}	8.43×10^{-3}	3.91×10^{-2}	4.68×10^{-3}	3.34×10^{-9}
Cytokine-cytokine receptor interaction (hsa04060)	1.67×10^{-8}	-	7.94×10^{-3}	-	4.01×10^{-6}
Cell cycle (hsa04110)	2.64×10^{-8}	-	1.34×10^{-10}	-	-
Chemokine signaling pathway (hsa04062)	2.03×10^{-5}	-	1.22×10^{-2}	-	-
NOD-like receptor signaling (hsa04621)	9.59×10^{-5}	-	-	-	-
Oocyte meiosis (hsa04114)	1.22×10^{-4}	-	1.13×10^{-5}	-	-
Progesterone-mediated oocyte maturation (hsa04914)	7.43×10^{-4}	-	7.36×10^{-4}	-	-
RIG-I-like receptor signaling (hsa04622)	8.08×10^{-3}	-	-	-	-
Toll-like receptor signaling (hsa04620)	8.58×10^{-3}	-	-	-	-
Pathways in cancer (hsa05200)	1.22×10^{-2}	-	-	-	-
Hematopoietic cell lineage (hsa04640)	1.76×10^{-2}	-	-	-	2.57×10^{-2}
p53 signaling pathway (hsa04115)	3.16×10^{-2}	-	4.34×10^{-2}	-	-
Adipocytokine signaling pathway (hsa04920)	-	-	-	3.21×10^{-3}	-

3.1.5. Experimental analysis of selected differentially expressed psoriasis genes

This section gives the results of ELISA and RT-PCR analysis performed for the identification of biomarkers of psoriasis.

3.1.5.1. ELISA assay results

ELISA was performed to identify pre-selected DEGs in psoriasis patients and healthy controls. The results indicated that PI3 levels were higher (as much as 11.2 times) in psoriasis patients than the healthy controls (pvalue = $4x10^{-4}$) (Figure 3.7).

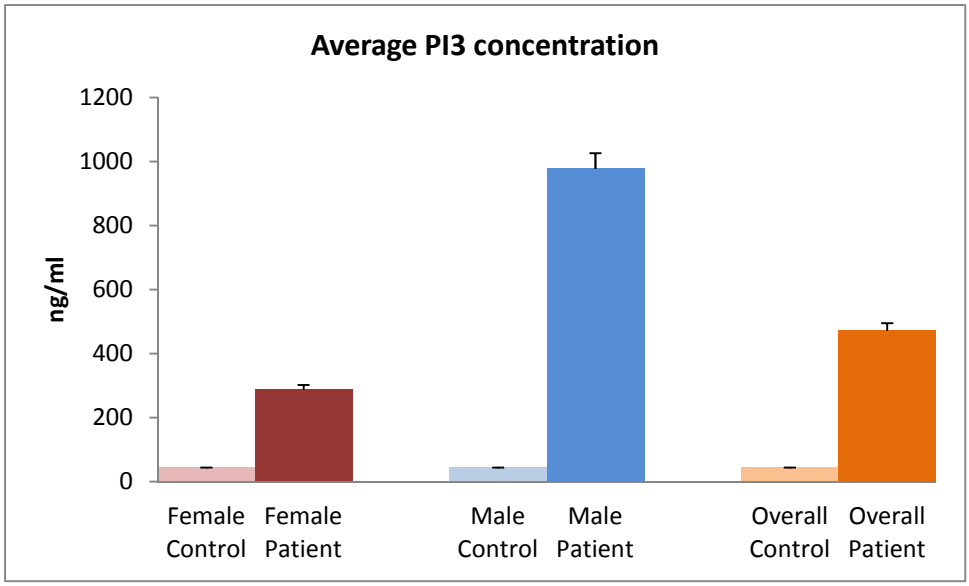

Figure 3.7 Average PI3 protein concentrations of female, male and overall psoriasis patients and healthy controls.

There was also a significant difference between female controls and female psoriasis patients (pvalue = $2.42x10^{-6}$) with a fold change of 6.8. The highest difference was among male controls and male psoriasis patients with (pvalue = $1.9x10^{-3}$) with 23.2 fold.

Analysis of the amount of SUB1 protein indicated that there was a difference only between male controls and male psoriasis patients (pvalue = $1.13x10^{-5}$) with a fold change of 1.6 (Figure 3.8). There was no significant difference in overall as well as female.

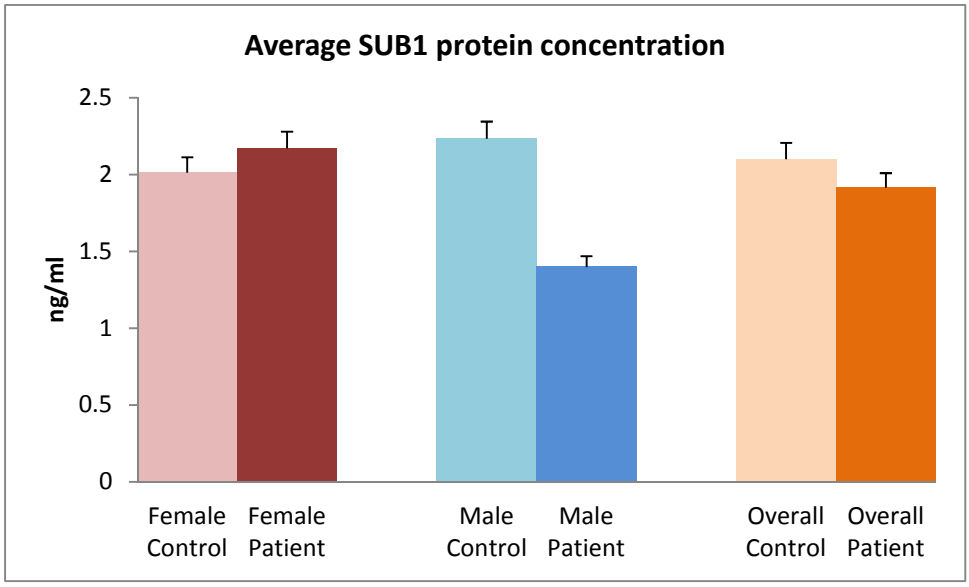

Figure 3.8 Average SUB1 protein concentrations of female, male and overall psoriasis patients and healthy controls.

The protein levels for WIF1 also showed significant difference when psoriasis patients and healthy controls are compared (pvalue = 3.8×10^{-2} by 1.10 fold) (Figure 3.9). There is also a significant difference between WIF1 protein levels of female control and female psoriasis patients (pvalue = 1.96×10^{-4} by 1.2 fold).

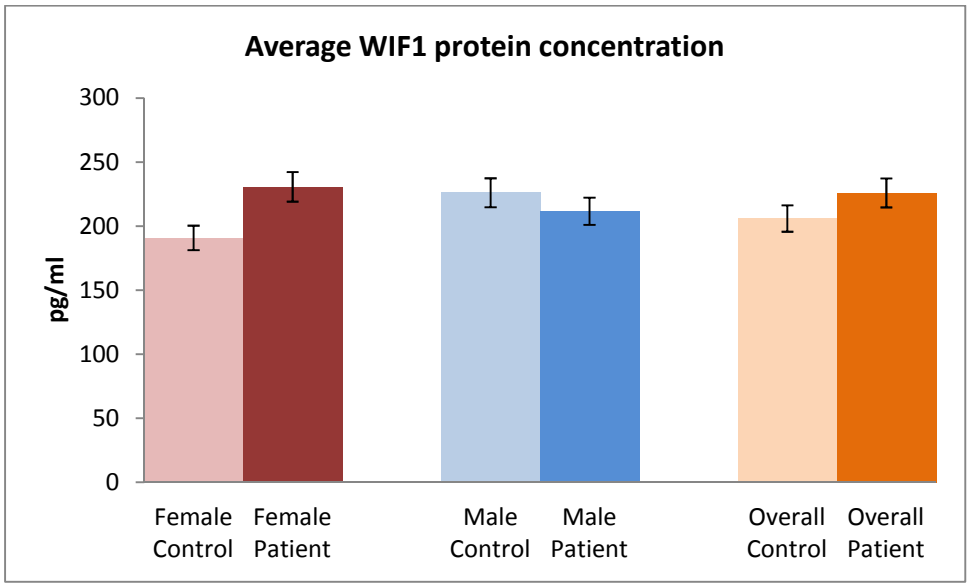

Figure 3.9 Average WIF1 protein concentrations of female, male and overall psoriasis patients and healthy controls.

The protein levels for IFIT1, IRF9, IFI44, RSAD2 and OAS2 did not show any significant difference (p value < 0.05) between psoriasis patients and healthy controls.

3.1.5.2. Quantitative RT-PCR Analysis

Quantitative RT-PCR experiments were performed to validate the results of microarray analysis of psoriasis datasets. The results of the RT-PCR analysis confirmed that there were significant differences between psoriatic skin and healthy controls.

Table 3.4 RNA concentrations in the skin samples taken from patients

Patient No	State	Gender	RNA concentration1 (ng/µl)	A260/A280	RNA concentration2 (ng/µl)	A260/A280
1	Disease	Female	114	2.036	118	1.967
2		Female	134	1.982	143	1.994
3		Female	530	1.949	514	1.932
4		Female	158	1.975	174	1.977
5		Male	248	1.908	252	1.909
6		Male	250	1.953	252	1.969
7		Male	344	2.024	362	2.011
8	Healthy	Female	29.6	2.000	38.8	2.021
9		Female	126	1.909	136	2.000
10		Female	50	1.923	50	1.923
11		Male	30	2.143	36	2.205
12		Male	76	2.000	78	2.053

The maximum absorbance of nucleic acids and proteins can be at 260 and 280 nm, respectively. The ratio of absorbances at these wavelengths has been used as a measure of purity in both nucleic acid and protein extractions. A pure RNA typically has a 260/280 ratio of ~2.0.

The overall results of PCR analysis using the Livak method is given in Table 3.5.

Table 3.5 Summary of RT-PCR analysis results

Gene Name	Patient Average (ΔCt)	Control Average (ΔCt)	ΔΔCt	FC (2^-ΔΔCt)
IFI44	-1.103	0.071	-1.174	**2.257**
IFIT1	-6.534	-5.726	-0.808	**1.751**
IRF9	-4.397	-4.138	-0.259	1.197
NMI	5.353	3.573	1.780	0.291
OAS2	-2.800	-2.025	-0.775	**1.711**
PI3	-4.290	-3.933	-0.356	1.280
RSAD2	-2.633	-3.949	1.316	**0.402**
SUB1	1.621	2.251	-0.630	1.548
WIF1	6.107	7.547	-1.410	2.713

These results confirm that there is a significant difference between psoriasis patients and healthy controls for the selected genes in transcriptomic level with WIF1 having the highest FC = 2.713 and NMI having the lowest FC = 0.291.

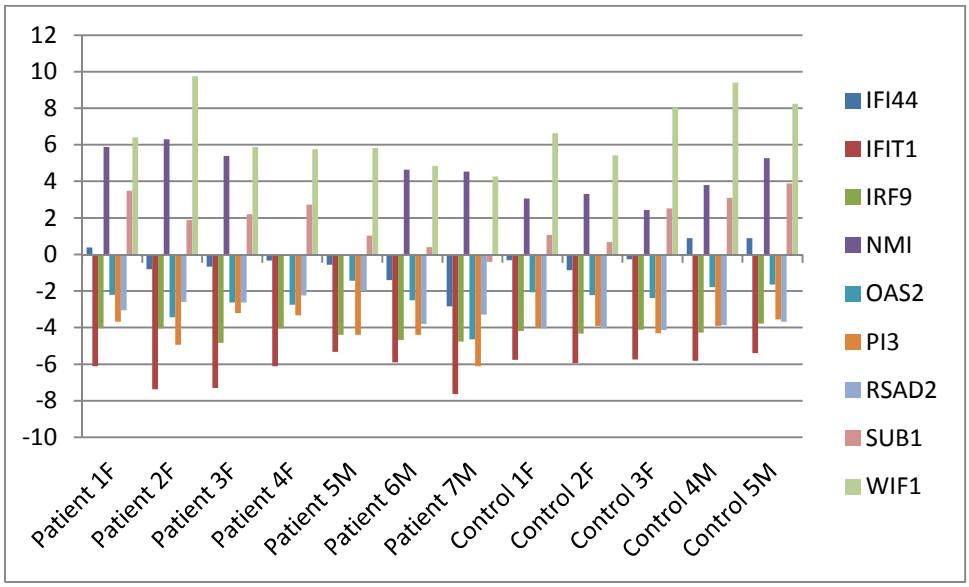

Figure 3.10 Comparison of ΔC_T values of genes employed in RT-PCR analyses (F: Female, M: Male)

A closer look at Figure 3.10 shows that there might be correlation among some of the results. To further investigate these, Pearson correlations of the ΔC_T results have been calculated and those above 0.60 are accepted as correlated (Table 3.6). The results

indicate that IFI44 has been correlated with almost all the other selected genes (except for NMI and RSAD2). IFIT1 is highly correlated with OAS2, IRF9 is correlated with SUB1, OAS2 is correlated with PI3 which is also correlated with SUB1.

Table 3.6 Pairwise comparison of expression correlations of studied genes based on ΔC_T values (Pearson correlation coefficients were employed)

	IFI44	**IFIT1**	**IRF9**	**NMI**	**OAS2**	**PI3**	**RSAD2**	**SUB1**	**WIF1**
IFI44	1.00								
IFIT1	0.62	1.00							
IRF9	0.70	0.48	1.00						
NMI	-0.01	-0.49	-0.06	1.00					
OAS2	0.79	0.87	0.40	-0.30	1.00				
PI3	0.74	0.46	0.33	-0.13	0.70	1.00			
RSAD2	-0.12	-0.29	-0.08	-0.26	-0.13	0.05	1.00		
SUB1	0.89	0.31	0.69	0.14	0.49	0.67	0.06	1.00	
WIF1	0.66	0.13	0.62	0.25	0.28	0.16	-0.13	0.63	1.00

The visualization of the correlation results can be seen in Figure 3.11. It appears that IFI44 is the central gene. This might mean that IFI44 may be a major participant in the progression of psoriasis. There is not much research done on IFI44 hence there is still very little known about this ISG. Further experiments are needed to understand the role it plays during the onset of the disease. SUB1 is correlated with four other genes which is in line with our findings that it is a hub in psoriasis PPI network as well as FCC analysis.

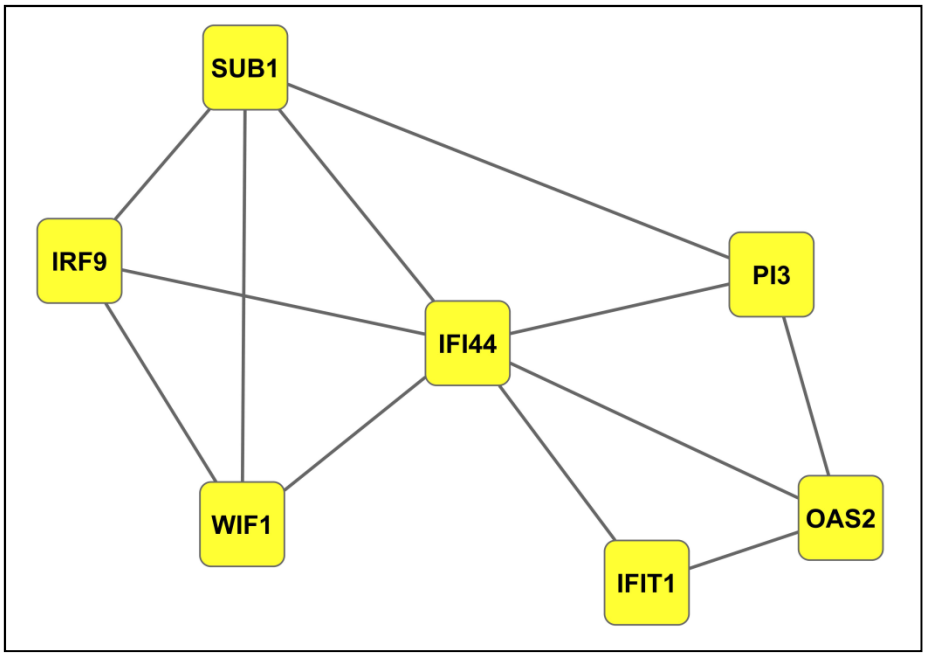

Figure 3.11 Highly-correlated gene cluster in psoriasis

To understand gender effect on the results, female and male results were also examined individually (Table 3.7). The results indicated that indeed, there is a difference in the disease presentation between male and female psoriasis patients (Figure 3.12).

Table 3.7 Analysis of RT-PCR results upon gender dependency

	Female				**Male**			
	Patient Av.	Control Av.	ΔΔCt	FC(2^-ΔΔCt)	Patient Av.	Control Av.	ΔΔCt	FC (2^-ΔΔCt)
IFI44	-0.600	-0.475	-0.125	**1.091**	-1.607	0.890	-2.497	**5.644**
IFIT1	-6.725	-5.810	-0.915	**1.886**	-6.280	-5.600	-0.680	**1.602**
IRF9	-4.236	-4.212	-0.025	**1.017**	-4.612	-4.028	-0.584	**1.499**
NMI	5.863	2.938	2.925	**0.132**	4.588	4.525	0.063	**0.958**
OAS2	-2.756	-2.227	-0.530	**1.444**	-2.858	-1.723	-1.136	**2.197**
PI3	-3.783	-4.067	0.284	**0.821**	-4.965	-3.733	-1.233	**2.350**
RSAD2	-2.629	-4.067	1.438	**0.369**	-2.643	-3.773	1.130	**0.457**
SUB1	2.579	1.425	1.154	**0.449**	0.343	3.490	-3.147	**8.856**
WIF1	6.017	6.698	-0.682	**1.604**	4.985	8.820	-3.835	**14.271**

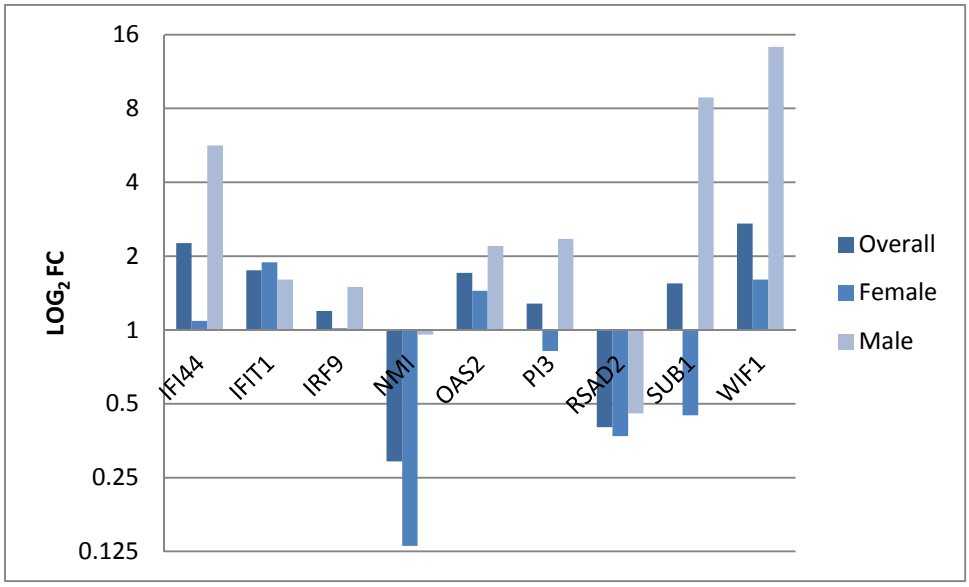

Figure 3.12 The expression comparison of selected genes based on gender dependency

The relationship between PASI index and individual FC values of each psoriasis patient has also been examined. These relationships are depicted in Figure 3.13 thru Figure 3.21. These graphs indicate that there is a linear relationship between the PASI Score and FC of all the genes except for NMI.

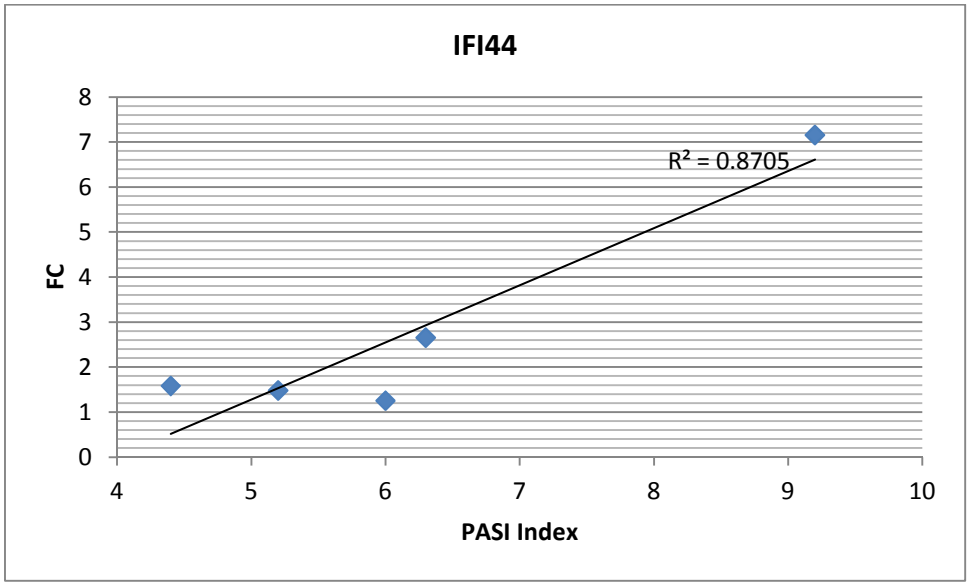

Figure 3.13 Plot of PASI Score versus Fold Change (FC) for the linear regression model corresponding to IFI44 (Goodness of fit is represented by R^2 values)

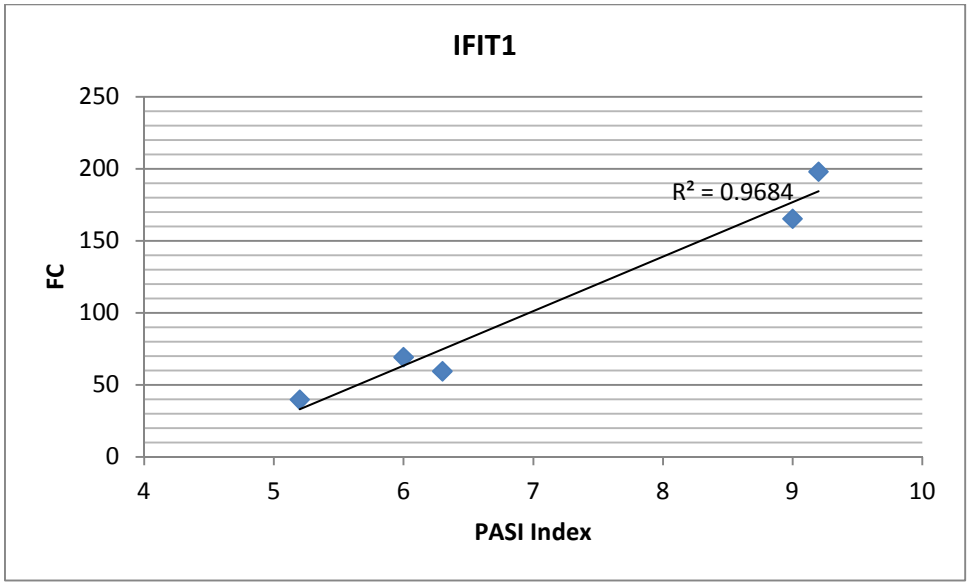

Figure 3.14 Plot of PASI Score versus Fold Change (FC) for the linear regression model corresponding to IFIT1 (Goodness of fit is represented by R^2 values)

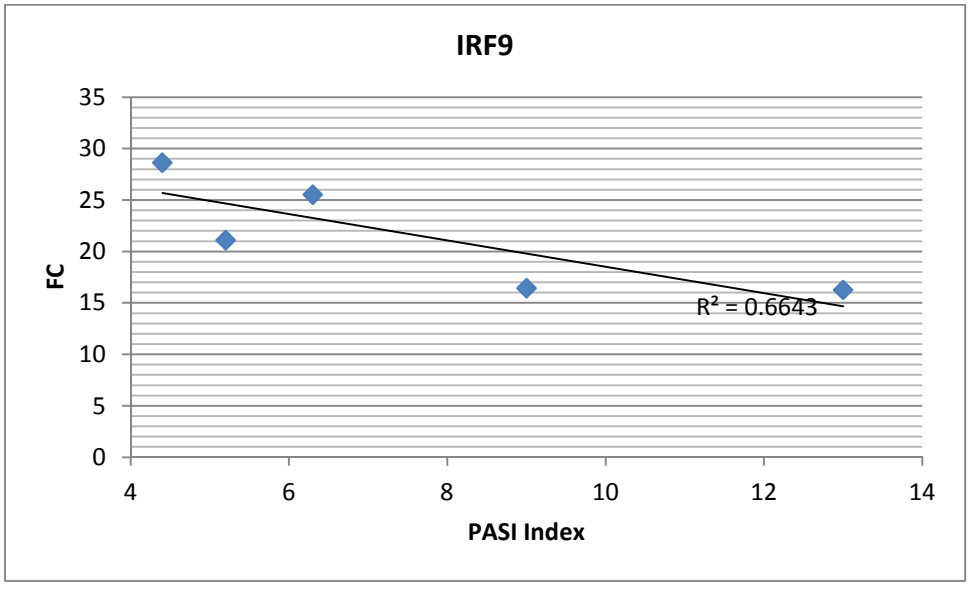

Figure 3.15 Plot of PASI Score versus Fold Change (FC) for the linear regression model corresponding to IRF9 (Goodness of fit is represented by R^2 values)

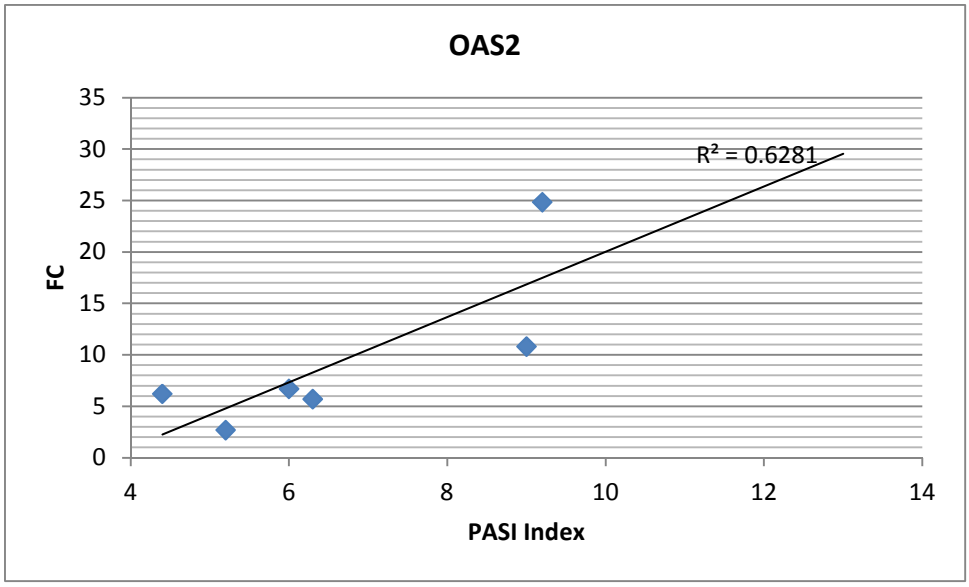

Figure 3.16 Plot of PASI Score versus Fold Change (FC) for the linear regression model corresponding to OAS2 (Goodness of fit is represented by R^2 values)

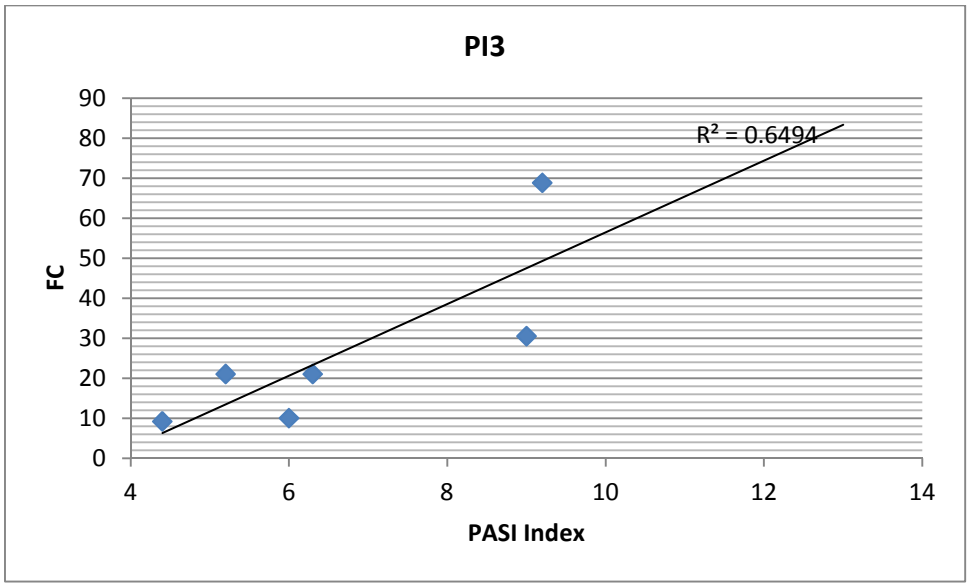

Figure 3.17 Plot of PASI Score versus Fold Change (FC) for the linear regression model corresponding to PI3 (Goodness of fit is represented by R^2 values)

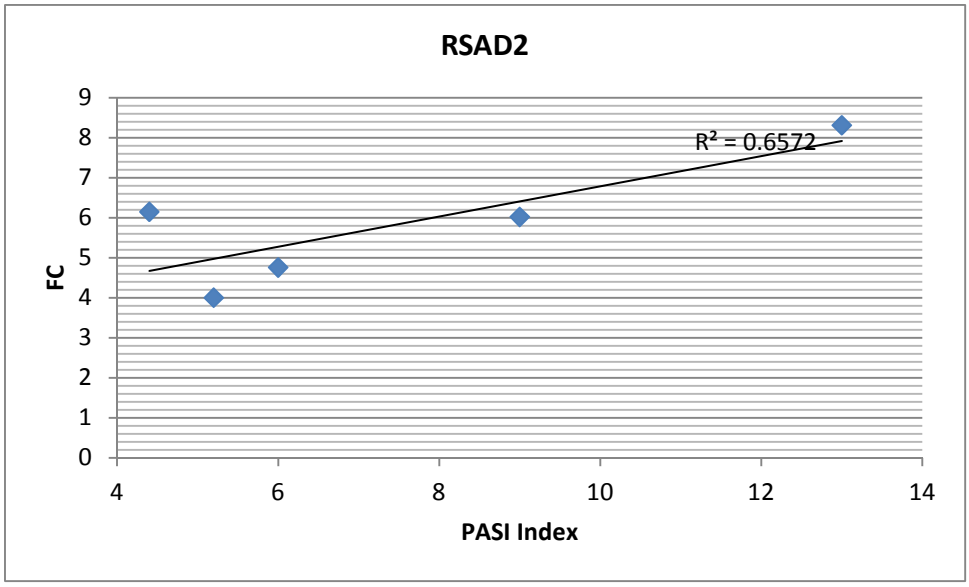

Figure 3.18 Plot of PASI Score versus Fold Change (FC) for the linear regression model corresponding to RSAD2 (Goodness of fit is represented by R^2 values)

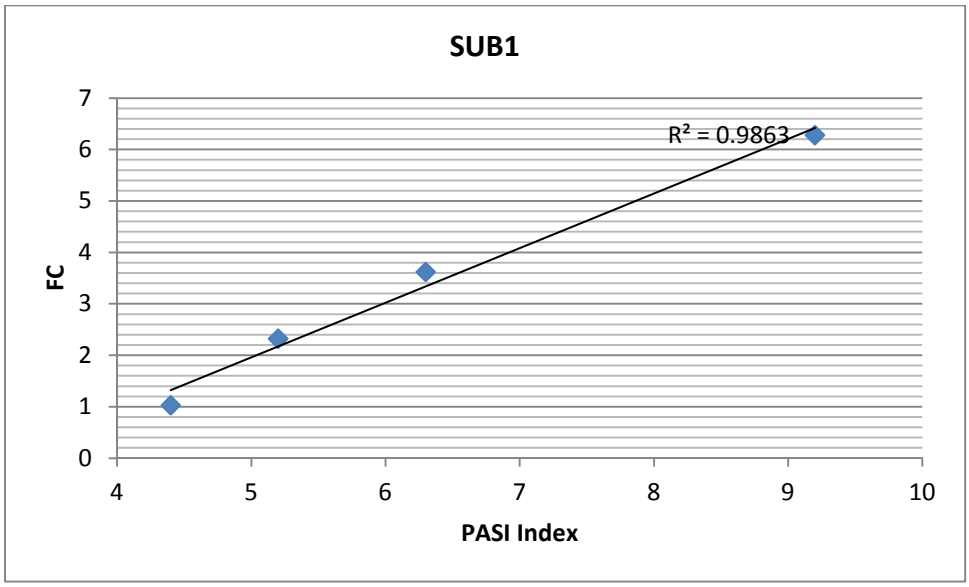

Figure 3.19 Plot of PASI Score versus Fold Change (FC) for the linear regression model corresponding to SUB1 (Goodness of fit is represented by R^2 values)

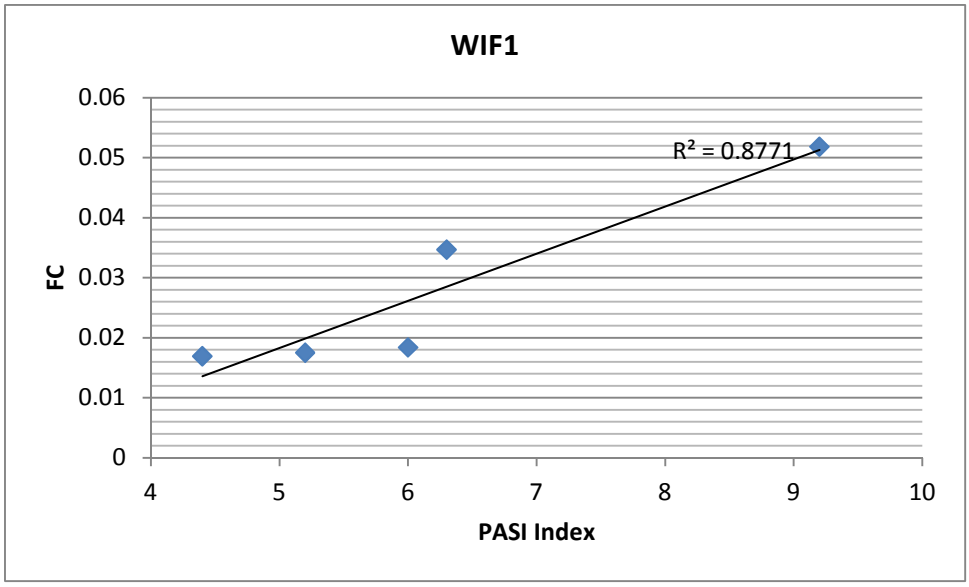

Figure 3.20 Plot of PASI Score versus Fold Change (FC) for the linear regression model corresponding to WIF1 (Goodness of fit is represented by R^2 values)

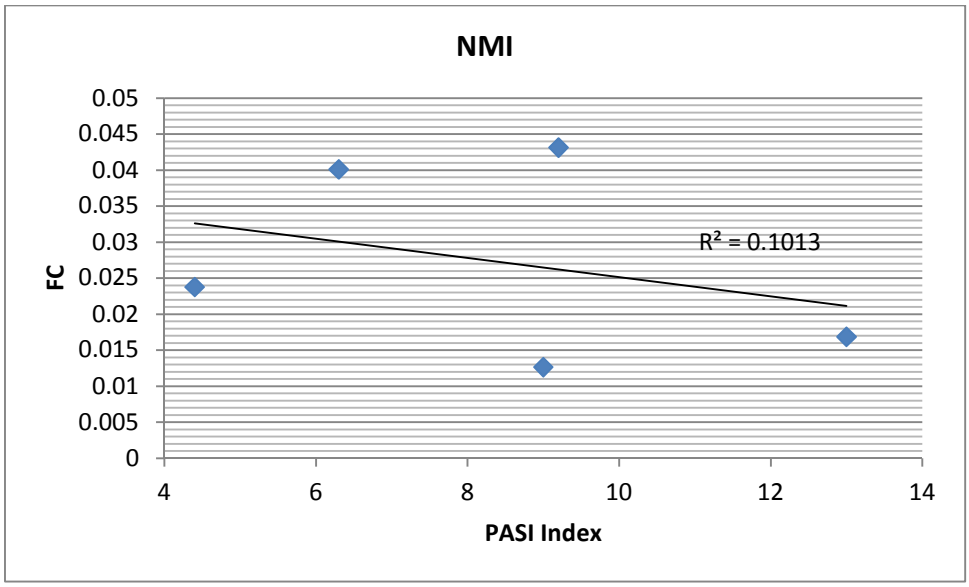

Figure 3.21 Plot of PASI Score versus Fold Change (FC) for the linear regression model corresponding to NMI (Goodness of fit is represented by R^2 values)

3.1.6. Discussion of computational and experimental analysis for psoriasis

In this study, the largest set of microarray datasets to date has been explored to investigate a comprehensive pool of DEGs in psoriasis. In addition to statistical analysis

of the datasets, the holistic approach comprising the reconstruction and topological analysis of biological networks around DEGs was enriched with a correlation analysis based on fold changes to provide deep insight into the disease mechanism of psoriasis. Furthermore experimental studies based on the integrative analysis provided additional understanding of this mechanism.

Some of the well-recognized genes involved in psoriasis such as IL17, IL22 and INOS were not detected as DEGs by most of the twelve studies. This might be due to the fact that expressions of these genes are usually low on microarray platforms; hence fold changes are not accurately measured. This is a major limitation of microarrays for the study of these genes (Suarez-Farinas et al., 2010).

The studied datasets each have hundreds of DEGs when individually analyzed, however they lack a common gene when all datasets were compared. This might be due to platform differences, naming issues, and heterogeneity of patient selection criteria. The numbers of DEGs that are common fall sharply to a small number when a comparison between platforms was performed (Figure 3.3). Even within the Affymetrix platform the number of common DEGs shows a great decline between the newest and early generations. The main difference between the three different platforms is the number of probe sets. Arrays with the highest number of probe sets happen to be in the Affymetrix platform: Human Genome U133 Plus 2.0 array, which include 54675 probe sets that collectively target 20026 human genes. The comparative analysis of the five datasets (GSE 14905, GSE34248, GSE41662, GSE30999, and GSE13355) employing this array resulted with 934 common DEGs, pointing out a great agreement between these datasets. On the other hand, the lowest number of probe sets is also in an Affymetrix platform, Human Genome U95A Array, which is an early generation array with only 12626 probe sets.

Though individual transcriptomics studies on psoriasis represented significant findings, conclusions were not sufficient to uncover molecular mechanisms behind the disease. Several research groups have portrayed the psoriasis transcriptome by comparing lists of differentially expressed genes (Gudjonsson et al., 2010; Suarez-Farinas et al., 2010; Bigler et al., 2013). The absence of agreement between these studies might be due to threshold affects (e.g. p-value, fold change, false discovery rate) on selection of over

and underexpressed genes and also parameter differences in analyzing microarrays (Pan et al., 2005).

An alternate to utilizing microarray data is employing expression level correlation to identify new functional modules and gene sets (Ye and Eskin, 2006). Reynier et al. (2011) suggest that a correlation between gene expression levels can allow us to identify the activated mechanisms at the cellular level. A different approach was taken for this study. Instead of using gene expression levels to calculate correlation, Fold Change (FC) values of the DEGs have been used. This new approach is called Fold Change Correlation (FCC) Analysis.

The holistic approach coupled with FCC analysis has been employed in the present study to overcome these inconsistencies. In the first step, we started with the statistical analysis of the individual gene expression datasets and comparatively analyzed the overlapping results. This analysis resulted in 11 core DEGs. Then, to uncover the biological mechanism of the disease, PPI network around these core DEGs was reconstructed and central proteins (called hubs) were identified based on a local (i.e., degree) and a global topological metric (i.e., betweenness centrality).

The hub proteins (STAT1, MAD2L1, CYCS, NMI and SUB1) require special attention since they can be considered as candidates for biomarker studies and potential drug targets. Three of these hub proteins were transcription factors (STAT1, NMI and SUB1), which means they control the flow (transcription) of genetic information from DNA to messenger RNA. SUB1, which is upregulated in our datasets, appears to play a dual role (as an activator or repressor) in gene expression and has multiple effects in distinct steps of the transcription cycle, consisting of initiation, elongation, termination and reinitiation (Conesa and Acker, 2010). NMI is a transcription cofactor that augments IFNG induced transcription activity. It can potentiate STAT-dependent transcription and also augment coactivator protein recruitment (Zhu et al., 1999). Another hub protein, STAT1 is also one of our upregulated core DEGs and is a member of STAT proteins which play a central role in Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway and cytokine signaling (Bromberg and Darnell, 2000).

In addition, the literature were further surveyed for signaling pathways associated with the proteins encoded by the core DEGs to extract other protein encoding DEGs functionally associated with our core DEGs. As a result, a comprehensive pool of 145 DEGs consisting of transcription factors, cytokines, receptors, enzymes and interferon-stimulated genes (ISGs), was constructed (Table 3.8).

Among the 145 DEGs, 32 of them were involved in chemokine signaling pathways, 21 of which are particularly in JAK/STAT pathway. JAK/STAT pathway presents a direct mechanism to transduce an extracellular signal into a transcriptional response. The activation of this pathway stimulates cell proliferation, differentiation, cell migration and apoptosis which are critical to processes such as immune development, and adipogenesis (Rawlings et al., 2004). The JAKs and STATs are essential intracellular mediators of immune cytokine action and lack of these proteins causes immunological defects (Ivashkiv, 2000).

Table 3.8 Genes associated with the proposed psoriasis pathway (The core DEGs are marked with *)

Protein Symbol	Protein Name	Molecular Function	Direction of Regulation	Comments
Module 1				
ID1	Inhibitor of DNA binding 1	Transcription Regulator	↑	
IFI16	Interferon, gamma-inducible protein 16	Transcription Regulator	↑	Interferon Stimulated Gene
NMI*	N-myc (and STAT) interactor	Transcription Regulator	↑	
STAT1*	signal transducer and activator of transcription 1	Transcription Regulator	↑	JAK/STAT pathway and chemokine signaling pathway
SUB1*	SUB1 homolog	Transcription Regulator	↑	
TRIM22	tripartite motif containing 22	Transcription Regulator	↑	Interferon Stimulated Gene
CYCS	cytochrome c, somatic	Transporter	↑	Sulfur metabolism
SLC5A1	solute carrier family 5 (sodium/glucose cotransporter), member 1 (SGLT1)	Transporter	↑	Carbohydrate digestion and absorption, and mineral absorption and bile secretion
CCL2	chemokine (C-C motif) ligand 2	Cytokine	↑	Chemokine signaling pathway
IL1RN	interleukin 1 receptor antagonist	Cytokine	↑	
IL12RB2	interleukin 12 receptor, beta 2	Receptor	↑	JAK/STAT pathway
IL13RA1	interleukin 13 receptor, alpha 1	Receptor	↑	JAK/STAT pathway
FZD5	frizzled class receptor 5	Receptor	↑	WNT signaling pathway
LDLR	low density lipoprotein receptor	Receptor	↑	Bile secretion
IFIH1	interferon induced with helicase C domain 1	Hydrolase (EC:3.6.4.13)	↑	RIGI like receptor signaling pathway; Interferon Stimulated Gene
ATP1A2	ATPase, Na+/K+ transporting, alpha 2 polypeptide	Hydrolase (EC:3.6.3.9)	↓	Mineral absorption, carbohydrate digestion and absorption
CTSC	cathepsin C	Hydrolase (EC:3.4.14.1)	↑	Lysosome
LYZ	lysozyme	Hydrolase (EC:3.2.1.17)	↑	Salivary secretion
ISG20	interferon stimulated exonuclease gene	Hydrolase (EC:3.1.13.1)	↑	Interferon Stimulated Gene

Table 3.8 Genes associated with the proposed psoriasis pathway (The core DEGs are marked with *) continued.

Protein Symbol	Protein Name	Molecular Function	Direction of Regulation	Comments
CA6	carbonic anhydrase VI	Lyase (EC:4.2.1.1)	↓	Nitrogen metabolism
HMOX1	heme oxygenase (decycling) 1	Oxidoreductase (EC:1.14.99.3)	↑	Mineral absorption
ALOX12B	arachidonate 12-lipoxygenase, 12R type	Oxidoreductase (EC:1.13.11.-)	↑	Epidermal barrier function
OAS1	2'-5'-oligoadenylate synthetase 1	Transferase (EC:2.7.7.84)	↑	Interferon Stimulated Gene
MAPK13	mitogen-activated protein kinase 13	Transferase (EC:2.7.11.24)	↑	Cell cycle, RIGI like receptor signaling pathway
CDK1	cyclin-dependent kinase 1	Transferase (EC:2.7.11.22)	↑	Cell cycle and oocyte meiosis
MAPKAPK3	mitogen-activated protein kinase-activated protein kinase 3	Transferase (EC:2.7.11.1)	↑	
HK2	hexokinase 2	Transferase (EC:2.7.1.1)	↑	Carbohydrate digestion and absorption
ACTG2	actin, gamma 2, smooth muscle, enteric	Other	↓	
CKS2	CDC28 protein kinase regulatory subunit 2	Other	↑	Cell cycle
CLDN8	claudin 8	Other	↓	
HOMER1	homer homolog 1	Other	↑	
IFI44*	interferon-induced protein 44	Other	↑	Interferon Stimulated Gene
IFIT1*	interferon-induced protein with tetratricopeptide repeats 1	Other	↑	Interferon Stimulated Gene
ISG15	ISG15 ubiquitin-like modifier	Other	↑	RIGI like receptor signaling pathway
KIAA0101	KIAA0101	Other	↑	Cell cycle
LAMB4	laminin, beta 4	Signaling protein	↓	
LAMP3	lysosomal-associated membrane protein 3	Signaling protein	↑	Autophagy

Table 3.8 Genes associated with the proposed psoriasis pathway (The core DEGs are marked with *) continued.

Protein Symbol	Protein Name	Molecular Function	Direction of Regulation	Comments
MAD2L1*	MAD2 mitotic arrest deficient-like 1	Other	↑	Cell cycle and oocyte meiosis
MX1	MX dynamin-like GTPase 1	Other	↑	Interferon Stimulated Gene
PLSCR1	phospholipid scramblase 1	Other	↑	Interferon Stimulated Gene
RAC2	ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)	Other	↑	Chemokine signaling pathway
RSAD2*	radical S-adenosyl methionine domain containing 2	Other	↑	Interferon Stimulated Gene
SCGB1D2	secretoglobin, family 1D, member 2	Signaling protein	↓	
SERPINB1	serpin peptidase inhibitor, clade B (ovalbumin), member 1	Other	↑	
SOCS1	suppressor of cytokine signaling 1	Other	↑	JAK/STAT pathway; Interferon Stimulated Gene
TRIM14	tripartite motif containing 14	Other	↑	Interferon Stimulated Gene
SHC1	SHC (Src homology 2 domain containing) transforming protein 1	Other	↑	Chemokine signaling pathway; also in module 2
IL7R	interleukin 7 receptor	Receptor	↑	JAK/STAT pathway; also in module 4
Module 2				
CCNE1	Cyclin E1	Transcription Regulator	↑	Cell Cycle and oocyte meiosis
MYC	v-myc avian myelocytomatosis viral oncogene homolog	Transcription Regulator	↑	JAK/STAT pathway
OVOL1	ovo-like zinc finger 1	Transcription Regulator	↑	Cell cycle
ABCA12	ATP-binding cassette, sub-family A (ABC1), member 12	Transporter	↑	Epidermal barrier function
CXCL2	chemokine (C-X-C motif) ligand 2	Cytokine	↑	Chemokine signaling pathway

Table 3.8 Genes associated with the proposed psoriasis pathway (The core DEGs are marked with *) continued.

Protein Symbol	Protein Name	Molecular Function	Direction of Regulation	Comments
CXCL8	chemokine (C-X-C motif) ligand 8	Cytokine	↑	JAK/STAT pathway and Chemokine signaling pathway
IL19	interleukin 19	Cytokine	↑	JAK/STAT pathway and RIGI like receptor signaling pathway
IL1B	interleukin 1, beta	Cytokine	↑	
IL12RB1	interleukin 12 receptor, beta 1	Receptor	↑	JAK/STAT pathway
CXCR2	chemokine (C-X-C motif) receptor 2	Receptor	↑	JAK/STAT pathway and chemokine signaling pathway
KLK13	kallikrein-related peptidase 13	Hydrolase (EC:3.4.21.-)	↑	
PLCB4	phospholipase C, beta 4	Hydrolase (EC:3.1.4.11)	↓	Chemokine signaling pathway and WNT signaling pathway
CDKN3	cyclin-dependent kinase inhibitor 3	Hydrolase (EC:3.1.3.16)	↑	Cell cycle
ADCY2	adenylate cyclase 2	Lyase (EC 4.6.1.1)	↓	Oocyte meiosis and chemokine signaling pathway
TTK	TTK protein kinase	Transferase (EC:2.7.12.1)	↑	Cell cycle
AURKA	aurora kinase A	Transferase (EC:2.7.11.1)	↑	Cell cycle and oocyte meiosis
BUB1	BUB1 mitotic checkpoint serine/threonine kinase	Transferase (EC:2.7.11.1)	↑	Cell cycle and oocyte meiosis
BUB1B	BUB1 mitotic checkpoint serine/threonine kinase B	Transferase (EC:2.7.11.1)	↑	Cell cycle
TGM1	transglutaminase 1	Transferase (EC:2.3.2.13)	↑	Epidermal barrier function
CCNB2	cyclin B2	Other	↑	Cell cycle and oocyte meiosis
CDC45	cell division cycle 45	Other	↑	Cell cycle
CDC6	cell division cycle 6	Other	↑	Cell cycle
CYP4F22	cytochrome P450, family 4, subfamily F, polypeptide 22	Other	↑	Epidermal barrier function
FBXO5	F-box protein 5	Other	↑	Cell cycle and oocyte meiosis

Table 3.8 Genes associated with the proposed psoriasis pathway (The core DEGs are marked with *) continued.

Protein Symbol	Protein Name	Molecular Function	Direction of Regulation	Comments
GNA15	guanine nucleotide binding protein (G protein), alpha 15 (Gq class)	Other	↑	
MALL	mal, T-cell differentiation protein-like	Other	↑	
PCNA	proliferating cell nuclear antigen	Other	↑	Cell cycle
S100A12	S100 calcium binding protein A12	Other	↑	
SERPINB3	serpin peptidase inhibitor, clade B (ovalbumin), member 3	Other	↑	
SERPINB4	serpin peptidase inhibitor, clade B (ovalbumin), member 4	Other	↑	
SLPI	secretory leukocyte peptidase inhibitor	Signaling protein	↑	
TPX2	TPX2, microtubule-associated	Other	↑	
GZMA	granzyme A (granzyme 1, cytotoxic T-lymphocyte-associated serine esterase 3)	Hydrolase (EC:3.4.21.78)	↑	
KYNU	Kynureninase	Hydrolase (EC:3.7.1.3)	↑	Tryptophan metabolism; also in module 3
CCNA2	cyclin A2	Other	↑	Cell cycle; also in module 3
CCNB1	cyclin B1	Other	↑	Cell cycle and oocyte meiosis; also in module 3
MPZL2	myelin protein zero-like 2	Signaling protein	↑	Also in module 4
LIFR	leukemia inhibitory factor receptor alpha	Receptor	↓	JAK/STAT pathway; also in module 4
Module 3				
ID4	Inhibitor of DNA binding 4	Transcription Regulator	↓	
IRF1	Interferon regulatory factor 1	Transcription Regulator	↑	Interferon Stimulated Gene
IRF7	Interferon regulatory factor 7	Transcription Regulator	↑	RIGI like receptor signaling pathway, Interferon Stimulated Gene

Table 3.8 Genes associated with the proposed psoriasis pathway (The core DEGs are marked with *) continued.

Protein Symbol	Protein Name	Molecular Function	Direction of Regulation	Comments
STAT3	signal transducer and activator of transcription 3	Transcription Regulator	↑	JAK/STAT pathway and chemokine signaling pathway
TCN1	transcobalamin I (vitamin B12 binding protein, R binder family)	Transporter	↑	
CCL20	chemokine (C-C motif) ligand 20	Cytokine	↑	Chemokine signaling pathway
WNT5A	wingless-type MMTV integration site family, member 5A	Cytokine	↑	WNT signaling pathway
GPC4	glypican 4	Receptor	↓	WNT signaling pathway
DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58 (RIGI)	Hydrolase (EC 3.6.3.14)	↑	RIGI like receptor signaling pathway
ENO1	enolase 1, (alpha)	Lyase (EC:4.2.1.11)	↑	
SOD2	superoxide dismutase 2, mitochondrial	Oxidoreductase (EC:1.15.1.1)	↑	
AKR1B10	aldo-keto reductase family 1, member B10 (aldose reductase)	Oxidoreductase (EC 1.1.1.21)	↑	
OAS2*	2'-5'-oligoadenylate synthetase 2	Transferase (EC:2.7.7.84)	↑	Interferon Stimulated Gene
CDC20	cell division cycle 20	Other	↑	Cell cycle and oocyte meiosis
DEFB4A	defensin, beta 4A	Signaling protein	↑	
KRT16	keratin 16	Other	↑	
MGB2	secretoglobin, family 2A, member 1 (SCGB2A1)	Signaling protein	↓	
MPHOSPH6	M-phase phosphoprotein 6	Other	↑	Cell cycle
NOD2	nucleotide-binding oligomerization domain containing 2	Other	↑	
RGS1	regulator of G-protein signaling 1	Other	↑	
RGS20	regulator of G-protein signaling 20	Other	↑	

Table 3.8 Genes associated with the proposed psoriasis pathway (The core DEGs are marked with *) continued.

Protein Symbol	Protein Name	Molecular Function	Direction of Regulation	Comments
SOCS3	suppressor of cytokine signaling 3	Other	↑	JAK/STAT pathway
WIF1*	WNT inhibitory factor 1	Signaling protein	↓	WNT signaling pathway
ITGA4	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)	Receptor	↑	
LEPR	leptin receptor	Receptor	↓	JAK/STAT pathway
LEP	Leptin	Growth Factor	↓	JAK/STAT pathway
MMP12	matrix metallopeptidase 12 (macrophage elastase)	Hydrolase (EC:3.4.24.65)	↑	
MMP9	matrix metallopeptidase 9	Hydrolase (EC:3.4.24.35)	↑	
CFB	complement factor B	Hydrolase (EC:3.4.21.47)	↑	
PNP	purine nucleoside phosphorylase	Hydrolase (EC:2.4.2.1)	↑	
CSF2RA	colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage)	Receptor	↑	JAK/STAT pathway; also in module 4
Module 4				
BCL3	B-cell CLL/lymphoma 3	Transcription Regulator	↑	
IRF9	Interferon regulatory factor 9	Transcription Regulator	↑	JAK/STAT pathway
PRDM1	PR domain containing 1, with ZNF domain	Transcription Regulator	↑	
SLC23A2	solute carrier family 23 (ascorbic acid transporter), member 2	Transporter	↑	
CXCL1	chemokine (C-X-C motif) ligand 1	Cytokine	↑	Chemokine signaling pathway
CXCL9	chemokine (C-X-C motif) ligand 9	Cytokine	↑	Chemokine signaling pathway
IL20	interleukin 20	Cytokine	↑	JAK/STAT pathway
L2RA	interleukin 2 receptor, alpha	Receptor	↑	JAK/STAT pathway
IL2RG	interleukin 2 receptor, gamma	Receptor	↑	JAK/STAT pathway

Table 3.8 Genes associated with the proposed psoriasis pathway (The core DEGs are marked with *) continued.

Protein Symbol	Protein Name	Molecular Function	Direction of Regulation	Comments
IL4R	interleukin 4 receptor	Receptor	↑	JAK/STAT pathway
TLR2	toll-like receptor 2	Receptor	↑	
GZMB	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)	Hydrolase (EC:3.4.21.79)	↑	
PRSS8	protease, serine, 8	Hydrolase (EC:3.4.21.-)	↑	Epidermal barrier function
UBA6	ubiquitin-like modifier activating enzyme 6	Ligase (EC 6.3.2.19)	↑	
IDO	indoleamine 2,3-dioxygenase 1	Oxidoreductase (EC:1.13.11.52)	↑	Tryptophan metabolism
TDO	tryptophan 2,3-dioxygenase	Oxidoreductase (EC:1.13.11.11)	↑	Tryptophan metabolism
OAS3	2'-5'-oligoadenylate synthetase 3	Transferase (EC:2.7.7.84)	↑	Interferon Stimulated Gene
PIM1	Pim-1 proto-oncogene, serine/threonine kinase	Transferase (EC:2.7.11.1)	↑	JAK/STAT pathway and Cell cycle
JAK3	Janus kinase 3	Transferase (EC:2.7.10.2)	↑	JAK/STAT pathway and chemokine signaling pathway
BIRC3	baculoviral IAP repeat containing 3	Other	↑	
DBF4	DBF4 zinc finger	Other	↑	Cell cycle
PI3*	peptidase inhibitor 3, skin-derived (SKALP)	Signaling protein	↑	
S100A7	S100 calcium binding protein A7 (psoriasin)	Other	↑	
S100A8	S100 calcium binding protein A8	Cytokine	↑	
S100A9	S100 calcium binding protein A9	Other	↑	
SOST	sclerostin	Signaling protein	↑	WNT signaling pathway
SPRR2C	small proline-rich protein 2C	Other	↑	Epidermal barrier function
TSPAN8	tetraspanin 8	Other	↓	

The chemotactic cytokines (chemokines) found in the psoriasis DEG pool were CXCL1, CXCL2, CXCL8 (also known as IL8), CXCL9, CCL2, and CCL20 along with their G-protein coupled receptor (GPCR) CXCR2. They were all upregulated with CXCL8 having the highest FC value of 27.1. CXCL8 induces a time- and concentration-dependent activation of JAK3 activity in neutrophils (Henkels et al., 2011), and the phosphorylation of JAK3 leads to the recruitment and activation of STAT1 (Wang et al., 1999; Walker et al., 2006). Chemokines coordinate immune cell trafficking both during the development of the immune system and during responses to exogenous or infectious agents by signaling through their receptors (Moratz et al., 2004). Upregulation of chemokines and their receptors enables cell-to-cell communication, whereas negative regulators of signaling help resolve the IFN-induced state and facilitate the return to cellular homeostasis (Schneider et al., 2014). The GPCRs are regulated by RGS (regulator of G protein signaling) proteins, which stimulate G-protein inactivation by downregulating the intracellular response to repeated ligand stimulation. The two RGS proteins in our DEG pool were RGS1 and RGS20, which were both upregulated in psoriasis. RGS1 is a key regulator of leukocyte trafficking and is critical in downregulating the response to sustained chemokine signaling (Patel et al., 2013).

IL19 and IL20, both of which were upregulated in psoriasis, are the cytokines involved in the JAK/STAT pathway. Among skin cells, keratinocytes were found to be important targets of IL19. They also increase the production of three S100 family proteins S100A7, S100A8, S100A9, and to a moderate extent IL1B, IL20, CXCL8 and MMP1. The protein encoded by the S100A7 gene is also known as psoriasin, which is overexpressed in hyperproliferative skin diseases and exhibits antimicrobial activities against bacteria and induces immunomodulatory activities (Celis et al., 1990). IL19 is also known to activate the transcription factor STAT3 (Witte et al., 2014). IL-20 has a distinct role in promoting hyperproliferation of keratinocytes hence modulating inflammation in the skin. The ability of keratinocytes to release pro-inflammatory factors when stimulated by cytokines or physical distress allows them to recruit inflammatory cells and regulate their behavior (Rich and Kupper, 2001). While there were 10 cytokine receptors (IL4R, IL7R, IL2RA, IL12RB1 IL12RB2, IL13RA1, IL2RG, LIFR, LEPR and CSF2RA) involved in JAK/STAT pathway, none of the

receptors for IL19 and IL20 were differentially expressed in the transcriptomics datasets examined here, surprisingly. The cytokine receptors in the DEG pool were all upregulated except for LIFR and LEPR, which were downregulated.

As part of a feedback loop, cytokines up-regulate the suppressors of cytokine signaling (SOCS) that inhibit the activity of JAKs and STATs (Slattery et al., 2013). SOCS3 which has the great sequence homology to SOCS1, seems to inhibit JAK catalytic activity in a manner that is analogous to SOCS1, but SOCS3 relies on receptor binding, rather than a direct interaction with JAKs, to gain access to the JAK activation loop (Alexander, 2002). SOCS1 and SOCS3 were upregulated in psoriasis proving that the JAK/STAT pathway is activated with its key elements CXCL1, CXCL2, CXCL8, CXCR2, JAK3, STAT1, SOCS1 and SOCS3, in psoriasis (Figure 3.22).

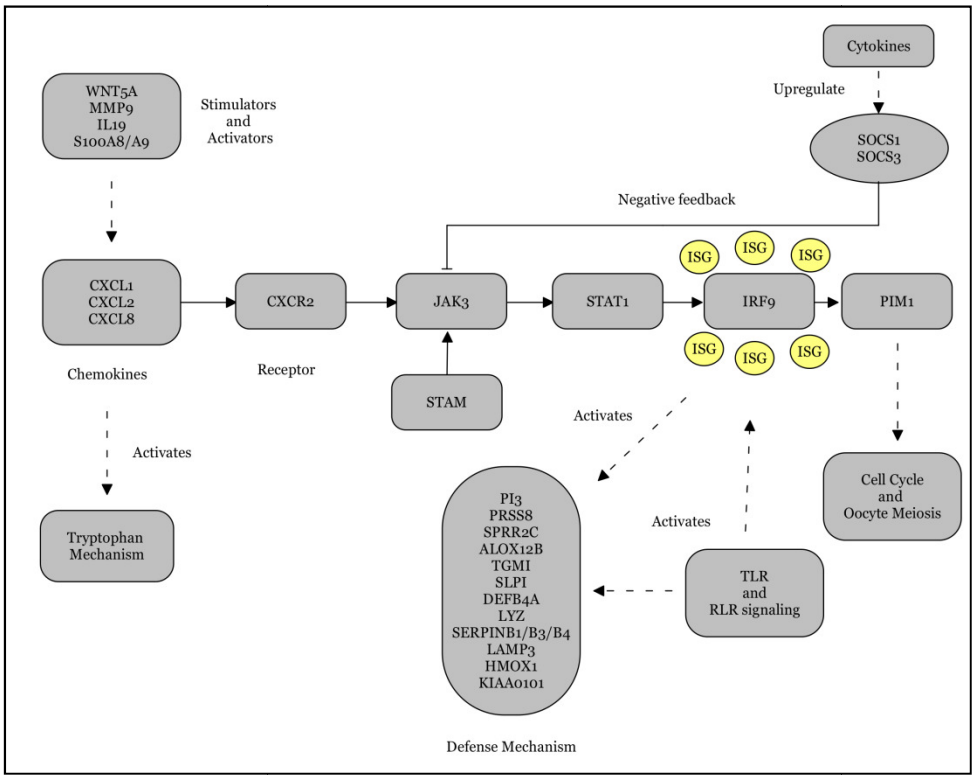

Figure 3.22: The proposed psoriasis pathway

Neutrophils are critical in the regulation of the innate immune response. They recruit chemoattractant (chemokine) gradients to the area of injury or infection which is

important for host defense mechanism (Kobayashi and DeLeo, 2009). The activation of neutrophils can be achieved through various extracellular stimuli, resulting in the production of inflammatory cytokines (Sadik and Luster, 2012). The enzymatic cleavage of chemokines by matrix metalloproteinases (MMPs) increases their potency to attract neutrophils. MMP9 cleaves and increases the chemotactic activity of CXCL1 and CXCL8. CXCL8 is the main chemokine produced by neutrophils, activating them through CXCR2 in an autocrine loop (Soehnlein and Lindbom, 2010). Dean and coworkers (2008) reported that macrophage-specific MMP12 might terminate neutrophil recruitment. The MMPs also function in the degradation and removal of Extra Cellular Matrix (ECM) molecules from tissues and have major roles in wound healing and tissue repair. The activities of MMPs are regulated by tissue inhibitors of metalloproteinases (TIMPs) (Nagase et al., 2006). MMP9 and MMP12 were overexpressed in the psoriatic skin with MMP12 with a high overexpression of 9.8 fold, and the MMP12 inhibitor TIMP3 was downregulated in psoriatic skin.

WNT5A, another DEG, is reported to increase the production of IL6, CCL2, CCL, CXCL1 and CXCL8. The WNT5A mediated WNT non-canonical pathway functions in the inflammatory response. WNT5A also binds to several members of the Frizzled receptor family, including FZD2, FZD5 and FZD8 (Jung et al., 2013). WIF1(WNT Inhibitory Factor 1) is an inhibitor of WNT proteins such as WNT3A, WNT4 and WNT5A, which are extracellular signaling molecules that play a role in embryonic development (Surmann-Schmitt et al., 2009). FZD5, which is also present in our DEG pool and is a receptor for WNT5A was upregulated and WIF1, which is a core DEG and an inhibitor for WNT5A, was down regulated in the datasets. It should also be noted that WNT5A, FZD5 and WIF1 along with GPC4, SOST and PLCB4 are the members of the WNT signaling pathway, which is an ancient and evolutionarily conserved pathway that regulates crucial aspects of cell fate determination, cell migration, cell polarity, neural patterning and organogenesis during embryonic development (Komiya and Habas, 2008). Of the three branches of this pathway, our results may indicate that the non-canonical Wnt/Ca2+ branch of the pathway was activated in psoriasis.

Studies of ISGs have increased our knowledge in areas of translational control, regulation of RNA stability and editing, protein transport and turnover. Some of the most studied ISGs are the OAS family GTPases, RSAD2 and ISG15, which are

associated with antiviral response (Borden and Williams, 2011). For instance, RSAD2, also known as Viperin, is an antiviral enzyme induced by at least two different innate immune pathways: via JAK/STAT signaling (Zhou et al., 2007) or via direct activation by IRF1 (Stimweiss et al., 2010). RSAD2 was upregulated in our datasets with FC as high as 16.23. STATs and the IFN regulatory factors (IRF) family members are important in cellular differentiation of hematopoietic cells, the regulation of gene expression and also amplifying the effects of ISGs (Tamura et al., 2008). IFIT proteins, another group of ISGs, may be induced through toll-like receptor (TLR) signaling as well as IRF and STAT activity (Fensterl and Sen, 2010). PLSCR1 (Phospholipid scramblase 1) may amplify and enhance other ISGs as well as altering the plasma membrane or binding DNA in the nucleus (Dong et al., 2004). The ISGs in the DEG pool included IRF1, IRF7, IRF9, IFIT1, IFI16, STAT1, OAS1, OAS2, OAS3, MX1, ISG15, ISG20, IFIH1, IFI44, PLSCR1 and RSAD2, some of which were also defined as core DEGs. In a study by Schoggins and Rice (2011) numerous ISG's were found to be activated in the absence of IFN signaling. Examples of these ISGs include STAT1, IRF and IRF7. Therefore, the transcriptional regulatory cascades that are mediated by IRFs might be spontaneous antiviral mechanisms that permit ISG expression before IFN itself can be produced. This might explain the lack of IFN's in our DEG pool while IRF1 and IRF7 along with IRF9 were overexpressed and a number of ISGs were present in the DEG pool. The silencing of ISGs may cause increased infection (Li et al, 2013). Our results may indicate that the ISGs are needed in psoriasis to activate the antimicrobial peptides (AMP's).

Of the 145 DEGs, 25 take a role in cell cycle and oocyte meiosis, one of which is MAD2L1, which is also among core DEGs. This group of DEGs included two TFs (CCNE1 and OVOL1) and nine enzymes, mostly transferases. All the DEGs in this group were upregulated proposing that cell cycle and oocyte meiosis processes are activated during the disease progress. Among them, AURKA is an essential molecule involved in regulating the functions of centrosomes, spindles and kinetochores and is therefore required for proper mitosis of cells (Marumoto et al., 2005). Its overexpression may induce checkpoint disruption, possibly leading to aneuploidy (Katayama et al., 2004).

Progression of the cell cycle requires the combination of cyclins and cyclin-dependent kinases (CDKs). CDK1 is a key regulator of resumption of meiosis and meiotic maturation of oocytes. Meiotic maturation is regulated by the CDK1-cyclin B complex (Dekel, 2005) and the checkpoints are mediated by cyclin-dependent kinase inhibitors (CKIs) in interphase. CDKN3 (cyclin-dependent kinase inhibitor 3), which is a CKI was upregulated in psoriasis datasets. Cyclin A (CCNA2) is a also a major regulator in cell cycle, related to cyclin-dependent kinases (CDK1 and CDK2) as well as S-phase progression and entry into mitosis. Upregulation of Cyclin A causes premature S-phase entry as well as inducing the extension of the S phase. Abnormal Cyclin A - CDK2 activation causes chromosomal double-strand breaks (Tane and Chibazakura, 2009). CKS (cyclin dependent-kinase subunit) proteins are essential for cell proliferation. They bind to CDK complexes during cell cycle phases when these are active (Egan and Solomon, 1998). Though Cyclin B (CCNB1 and/or CCNB2) is necessary for cells to enter mitosis and therefore necessary for cell division, inappropriate overexpression of CCNB1 causes non-specific cell death independent of mitotic arrest (Eichhorn et al, 2014). MAD2L1 is a mitotic spindle assembly checkpoint protein that also regulates CCNB1 (Manning and Dyson, 2012).

In addition to MAD2L1, BUB1B and BUB1 genes are also involved in the mitotic checkpoint, which serves as a surveillance mechanism. Over expression of these genes might demonstrate that alterations in mitotic arrest genes may play a role in psoriasis by disrupting control mechanism for the normal mitotic checkpoint. Mammary epithelial cells will divide even when chromosomes are not correctly attached to the spindle in loss of normal control, giving rise to aneuploidy and chromosomal instability (Percy et al., 2000). KIAA0101 is a PCNA (proliferating cell nuclear antigen) associated cell cycle-regulated phosphoprotein which localizes to sites of DNA damage. It is active in both DNA replication and the response to DNA damage (Emanuelea et al., 2011). In addition to these DEGs, leptin (LEP) and its receptor (LEPR) were also associated with the regulation of oocyte maturation and embryo development (Ryan et al., 2002). These two proteins, which also take a role in adipocytokine signaling and JAK/STAT pathways, were downregulated in psoriasis.

Kynurenine (KYNU), indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) of the tryptophan metabolism were also represented in the DEG

pool, all of which were overexpressed in all datasets. Previously, Nomura and coworkers (2003) reported KYNU among genes that were overexpressed in psoriasis when compared with atopic dermatitis. The present study incorporating all the published datasets verified the upregulation of KYNU in psoriasis, but its expression level was significantly elevated (i.e., up to 16 fold) in psoriatic skin when compared to healthy skin. Tryptophan metabolism is known to mediate both genetic and environmental mechanisms of depression which is well documented as a serious impairment in psoriasis patients (Krueger et al., 2001). Simultaneous presence of high producer alleles of pro-inflammatory cytokine genes determines the genetic predisposition to depression via up-regulation of IDO, while impact of environmental stresses is mediated via hormonal activation of TDO (Oxenkrug, 2010).

Abberant RIGI like receptor (RLR) signaling or abnornal RLR expression has been implicated in the development of autoimmune diseases (Loo and Gale, 2011). In the present study, 6 proteins that play central roles in RLR signaling were differentially overexpressed: RIGI (DDX58), ISG15, IFIH1, IRF7, CXCL8 and MAPK13. In addition, 7 proteins (TLR2, CXCL8, CXCL9, IRF7, IL1B, MAPK13 and STAT1) were differentially overexpressed in psoriasis, which play central roles in Toll-like receptor (TLR) signaling. A mechanism by which the innate immune system can sense the invasion of pathogenic microorganisms is Toll-like receptor (TLR) signaling mechanism, which recognizes specific molecular patterns that are present in microbial components (Akira and Takeda, 2004). It was reported that stimulation of different TLRs activates signals that are active in the induction of adaptive immune responses (Iwasaki and Medzhitov, 2004). The overexpressions of TLR2 as well as CXCL8 and STAT1 have previously been reported in psoriatic skin (Baker et al., 2003). The primary lesions in inflammatory diseases might affect proteins that modulate activation through TLRs (Beutler, 2004).

The epithelium serves as an interface between invading pathogens and the immune system of the host during inflammation. Under physiological conditions, polarized epithelia form a protective barrier, however, during inflammation; this protective mechanism becomes compromised by various stimuli that originate on both sides of the epithelial barrier. Consequently it is believed that decreasing epithelial permeability may have beneficial effects by limiting inflammatory responses. Thus, understanding

mechanisms that control the epithelial barrier disruption is important in identifying novel molecular targets for pharmacological modulation of mucosal inflammation (Ivanov et al., 2010). Previous studies show that PRSS8 (a serine protease) expression is essential for the epidermal permeability barrier therefore, fundamental for postnatal survival (Leyvraz et al., 2005). PRSS8 was upregulated in our datasets. SPRR2C (small proline rich protein 2C), which is also related to increased barrier function in epithelia, was highly overexpressed in our datasets. Furthermore, SPRR2C is found to be a marker for abnormal keratinocyte differentiation characteristic for psoriasis (Quekenborn-Trinquet et al., 2005). In addition to these proteins, ALOX12B plays a role in skin barrier formation and terminal differentiation (Heidt et al., 2000). TGM1 (transglutaminase-1) enzyme is active in the development of the cornified cell envelope (Kim et al., 1994). TGM1 and ALOX12B along with ABCA12, and CYP4F22 are also associated with autosomal recessive congenital ichthyosis (ARCI), a rare skin condition (Espositoa et al., 2009). These ARCI related DEGs were also upregulated in our datasets. ABCA12, a lipid transporter, from the large superfamily of the ATP-binding cassette (ABC) transporter genes, which bind ATP for the transport of numerous molecules across the cell membrane (Allikmets et al., 1996) and function in cellular lipid trafficking in keratinocytes (Lefevre et al., 2003). Overexpression of ABCA12 could cause barrier abnormality. CYP4F22 is from the cytochrome P450 family 4, subfamily F enzymes, with unknown epidermal functions. Sasaki and colleagues (2012) revealed that CYP4F22 is highly expressed at the site and the onset of keratinization during skin development and is involved in the metabolism of lipid substrates that are important to differentiation/keratinization of epidermal keratinocytes, at least during the fetal period.

Disruption of the tight junctions is believed to be one of the processes that lead to loss of cellular cohesion and aggressive growth. Claudins are essential in the formation and function of tight junctions. CLDN8, which is one of the cell adhesion molecules in endolethelial cells and a member of the claudin family, was downregulated in our datasets. Loss of expression for CLDN8 has also been reported in cancer studies (Escudero-Esparza et al., 2011).

The outermost layer of the skin, the stratum corneum, functions as the body's main protective barrier against physical and chemical damage, dehydration, and microbial

pathogens. During normal stratum corneum desquamation, the most superficial corneocytes are shed from the skin surface. Stratum corneum desquamation which is premature in psoriasis is a tightly regulated process, orchestrated by the combined function of serine proteases and their inhibitors within the intercorneal matrix. KLK13 of the KLK family is a serine protease that has a role in stratum corneum desquamation and was overexpressed in our datasets as high as 7.76 fold. SLPI and PI3 are also implicated in the regulation of desquamation (Borgono et al., 2007). PI3, one of our core DEGs, encodes the PI3 protein (peptidase inhibitor 3) also known as Elafin or skin-derived antileukoprotease (SKALP). It is an antimicrobial peptide which is cross-linked into the cornified cell envelopes from the inside of psoriatic keratinocytes (Nakane et al., 2002). PI3 was highly upregulated with a wide range of FC between 109.93 and 1.823. In addition to the regulation of desquamation, SLPI is also important in wound healing as well as limiting protease-mediated tissue injury associated with inflammation, especially at mucosal/epithelial surfaces (Zhu et al., 2002). It also exhibits antimicrobial properties and immunomodulatory activity (Doumas et al., 2005).

Patients with psoriasis have fewer skin infections than expected leading to the hypothesis that lesional psoriatic skin has a chemical shield against infections in the form of antimicrobial peptides (Harder and Schröder, 2005). Defensins are antimicrobial peptides secreted by various cells as a component of the innate host defence (Wehkamp et al., 2007). DEFB4A (defensin, beta 4A) was highly overexpressed in our datasets (as high 134.98 fold). The cytokines IL1B and IL1RN are regulators of DEFB4A (Liu et al., 2002). Another DEG that is an antimicrobial peptide was LYZ which encodes human lysozyme. It is the first antimicrobial protein found in human skin (Schröder and Harder, 2006) and is upregulated in psoriatic skin. S100A7 (psoriasin) of the S100 family of proteins is an antimicrobial peptide which is discovered and associated with psoriasis in 1990s (Celis et al., 1990). Besides S100A7, calprotectin (S100A8/S100A9 protein complex) is also an antimicrobial peptide induced in psoriatic skin. Nukui et al. (2008) proposed that S100A8/A9 can induce cytokine production in psoriatic epidermis. In addition, Lee et al. (2012) suggested that S100A8 and/or S100A9 function to generate a psoriatic milieu in human skin and proposed three possible mechanisms: 1) signaling via positive feedback to adjacent keratinocytes to produce pro-inflammatory and pro-angiogenic cytokines, thus exacerbating psoriatic

skin lesions; 2) attracting immune cells, thus facilitating the complex interaction with keratinocytes; and 3) promoting endothelial cell proliferation, survival, and angiogenesis both directly and by inducing keratinocytes to produce pro-angiogenic cytokines. S100A12, also known as Calgranulin C, MRP6, or EN-RAGE, is a calcium-binding pro-inflammatory protein predominantly secreted by granulocytes. Plasma S100A12 is increased in inflammatory disorders and is proposed as a marker of inflammation (Pietzsch and Hoppmann, 2009). S100A12 and S100A9 were highly overexpressed in our datasets with 47 fold and 36 fold respectively along with S100A7 and S100A8. Our analyses indicated that the antimicrobial peptides (DEFB4A, PI3, S100A8, S100A9 and S100A12) seemed to have higher overexpression in psoriatic skin than any other genes and we proposed that these peptides play significant roles as downstream effectors in the defense mechanism of the biological system in response to psoriasis. SERPINB1, SERPINB3 and SERPINB4 are members of the serpin family of proteinase inhibitors that are all overexpressed in psoriatic skin. This group of proteins protects tissues from damage at inflammatory sites. SERPINB3/B4 expression is found to be increased in the skin and serum of individuals with psoriasis (Suarez-Farinas et al., 2010). Among them SERPINB4 had the highest overexpression with 126.7 fold difference from non-lesional skin.

Of the DEGs, LAMP3 is a major lysosomal membrane protein and a member of the LAMP-family of proteins, active in the process of autophagy. Autophagy is a process that is crucial for discarding misfolded or aggregated proteins, clearing flawed organelles, as well as terminating intracellular pathogens. It is important for prevention of diseases such as cancer, autoimmune diseases and infections and its disregulation is being related to non-apoptotic cell death (Glick et al., 2010). Taking into consideration the suggestion of Higaki and coworkers (2009) that LAMP3-positive keratinocytes may act as antigen-presenting cells in psoriatic skin, and the upregulation of LAMP3 in our analyses, we proposed autophagy as a part of survival mechanism of the skin in response to proteomic changes in psoriasis.

Other DEGs such as ID1, ID4, KRT16 and HMOX1 which are previously indicated in psoriasis disease were also identified in the present study. Hanselmann et al. (2001) proposed a role for HMOX1(heme oxygenase (decycling) 1) in wound healing and psoriasis where it might be involved in heme degradation and in the protection of cells

from the toxic effects of reactive oxygen species also involved in the hyperproliferation of keratinocytes during wound healing and in psoriasis. ID1, from the inhibitor of DNA binding (Id) gene family, is a transcription factor which may be involved in a regulatory pathway in the epidermis in vivo (Ronpirin et al., 2010). Id family is also known to control cell proliferation and the progression of cell cycle with the exception of ID4 (Zebedee and Hara, 2001). Unlike ID1, another transcription factor ID4 is downregulated in psoriatic skin (Ruchusatsawat et al., 2011). KRT16, a basal-like cytokeratin which is overexpressed in psoriatic skin has been named a biomarker of the disease (Leigh et al., 1995).

The DEG pool work together to form a network and consist of proteins that are; 1) ISGs, 2) members of signaling and/or biological pathways (mostly JAK/STAT, cell cycle and oocyte meiosis) and 3) involved in the defense mechanisms (desquamation, autophagy, antimicrobial skin peptides, epidermal barrier function). The results of the FCC analysis revealed that the hubs of the FCC network of psoriasis show similarity to the hubs of the PPI network of psoriasis. SUB1 and NMI were present in both networks as hubs, which were also in our core DEG list. SUB1 was also a hub of Module 1.

The modular topology of the FCC network was further investigated to better assess the psoriasis disease network. In the modular view of the FCC network, Module 1 is the central module with 48 members including proteins that are interferon induced such as IFI44, IFIT1, RSAD2, MX1, IFIH1, OAS1, NMI, STAT1, SOCS1 and TRIM22, five of which are also in our core DEG list. There are 6 TFs and 13 enzymes in this module. The enrichment analysis results indicated that JAK/STAT signaling pathway is significant in all of the modules including Module 1. It is the only significant pathway of this module. Also GO-BP results show that most of the DEGs in Module 1 are involved in biological processes such as response to stress, chemical stimulus, biotic stimulus, in general response to stimulus. Module 2 has 39 members, 15 of which have molecular activities in cell cycle and oocyte meiosis. The most significant pathways of this module are Cell Cycle (p-value = 1.34×10^{-10}) and Oocyte Meiosis (p-value = 1.13×10^{-5}). Module 3 has 35 members which are mostly involved in immune system processes and positive regulation of biological processes. Module 4 has 32 members which are involved in biological processes such as immune, defense and inflammatory response. These 145 DEGs, that have been separated into four different modules,

function in an integrated manner as a defense mechanism of the cell in response to the biological processes that have been affected by psoriasis.

The results of experimental studies were threefold: 1) The selected psoriasis DEGs are correlated with each other in transcriptomic level. 2) There is significant gender difference in transcriptomic as well as proteomic level. 3) PASI scores correlate with fold change values in transcriptomic level.

Four of the DEGs selected for experimental studies are IFI44, IFIT1, RSAD2 and OAS2, which are ISG's. In PCR analysis three of them are correlated (IFI44, IFIT1 and OAS2). Interestingly only IFI44 is correlated with IRF9 which may mean that it is carrying the signals from other ISGs to IRF9. Further experimentation may be needed to check for correlation between IFI44 and other ISGs thus proving IFI44 to be a biomarker DEG for psoriasis. SUB1 is the other DEG that is a possible biomarker. Interestingly in proteomic level only male psoriasis patients have a significant difference.

Sex hormones, ethnic background, anatomy, physiology, immunity, genetics, epigenetics, as well as geographical, sociocultural, and environmental factors may be effective in the occurrence of gender difference in complex nature of psoriasis (Colomba et al., 2014). Sakai and coworkers (2005) confirm that psoriasis is more severe in male patients. In a study done by Cemil and coworkers (2015), sex hormones (testosterone and estradiol) were significantly different in psoriatic patients then the healthy controls. This study provided confirmation on the findings that psoriasis may present itself differently in different genders.

PASI scores are most frequently used to asses the severity of psoriasis in clinical trials (Jacobson and Kimball, 2004). This study confirms that there is a relationship between the PASI scores of psoriatic patients and the fold change values of the selected DEGs in transcriptomic level.

3.2. Integrative Analysis of Rheumatoid Arthritis Datasets

Three RA datasets have been examined for this study (Table 2.2). The comparison of probe sets is given in Figure 3.23. There were five common probesets which correspond to four DEGs.

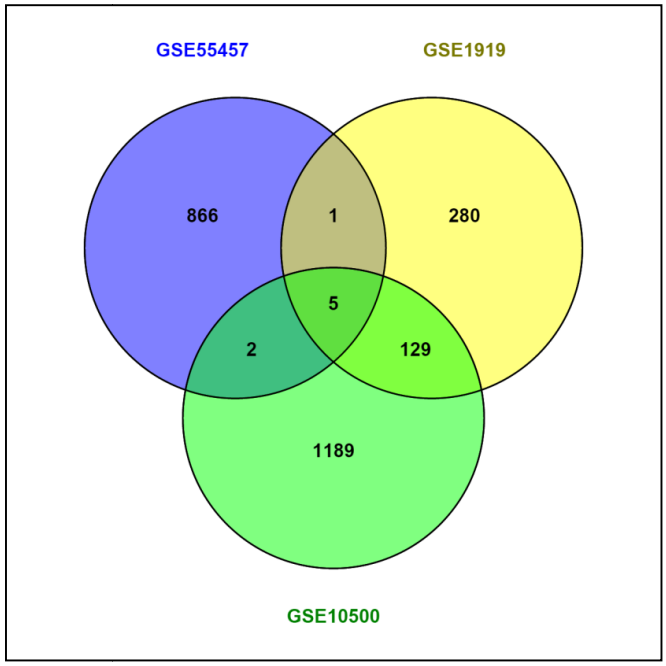

Figure 3.23 Comparison of the numbers of differentially expressed genes in rheumatoid arthritis datasets.

The remainder of this section gives details of DEGs of RA, PPI network of RA, transcriptional regulatory network of RA DEGs and enrichment analysis for this disease.

3.2.1. Differentially expressed genes of rheumatoid arthritis

The four common DEGs between the three analyzed RA datasets were: STAT1, BTN3A3, CD52 and MAFF, which were named as "core DEGs of RA" for the remainder of the thesis. To explore a more comprehensive set of genes, the mutual mutual of GSE1919 and GSE10500 were also taken into consideration, resulting in a list of 121 DEGs (134 probesets). These DEGs are listed in Appendix B.

STAT1 (Signal transducer and activator of transcription 1) is a major transcription factor that has been previously discussed in Sections 3.1.3 and 3.1.6.

BTN3A3 (butyrophilin, subfamily 3, member A3) of the butyrophilin (BTN) family of Ig superfamily receptors, which modulate the function of T cells in the adaptive immune response (Rhodes et al., 2015). The protein encoded by MAFF (v-maf avian

musculoaponeurotic fibrosarcoma oncogene homolog F) gene is from the family of MAF transcription factors that plays major roles in the control of mammalian gene expression and development. The MAFF transcript levels are regulated by pro-inflammatory cytokines such as IL1B and TNF suggesting a role for this gene in inflammatory response (Massrieh et al., 2006).

CD52 (cluster of differentiation 52) is a very small glycopeptide which is a lymphocyte differentiation antigen. It is believed to have anti-adhesion properties. Furthermore an association between the epididymal CD52 and sperm maturation was stated (Domagala and Kurpisz, 2001). High expression of CD52 has been also associated with certain types of lymphoma such as T-prolymphocytic leukemia and cutaneous T-cell lymphomas (Piccaluga et al., 2007).

3.2.2. Transcriptional regulation of differentially expressed genes in rheumatoid arthritis

The regulatory relationship between DEGs of RA and their TFs has been examined in this section. The transcriptional regulatory network (TRN) for RA has been constructed. The TRN of RA consists of 104 nodes and 342 edges (Figure 3.24). The RA DEGs are being regulated by 16 TFs.

The hubs of the TRN of RA are listed in Table 3.9. The protein encoded by CEBPD (CCAAT/enhancer-binding protein delta) is a transcription factor that is involved in the regulation of adipose tissue development, apoptosis and cell proliferation. It also functions as a tumor suppressor (Duitman et al., 2014).

PPARG (Peroxisome proliferator-activated receptor gamma) is a nuclear receptor involved in the regulation of genes associated with growth and differentiation. Like CEBPD, PPARG also regulates the development of adipose tissue as well as fatty acid storage and glucose metabolism (Farmer, 2005). There are also implications for its activity in the regulation of immune response and inflammation (Clark, 2002). PPARG is mainly present in adipose tissue, colon and macrophages and was implicated in the pathology of numerous diseases such as cancer and diabetes.

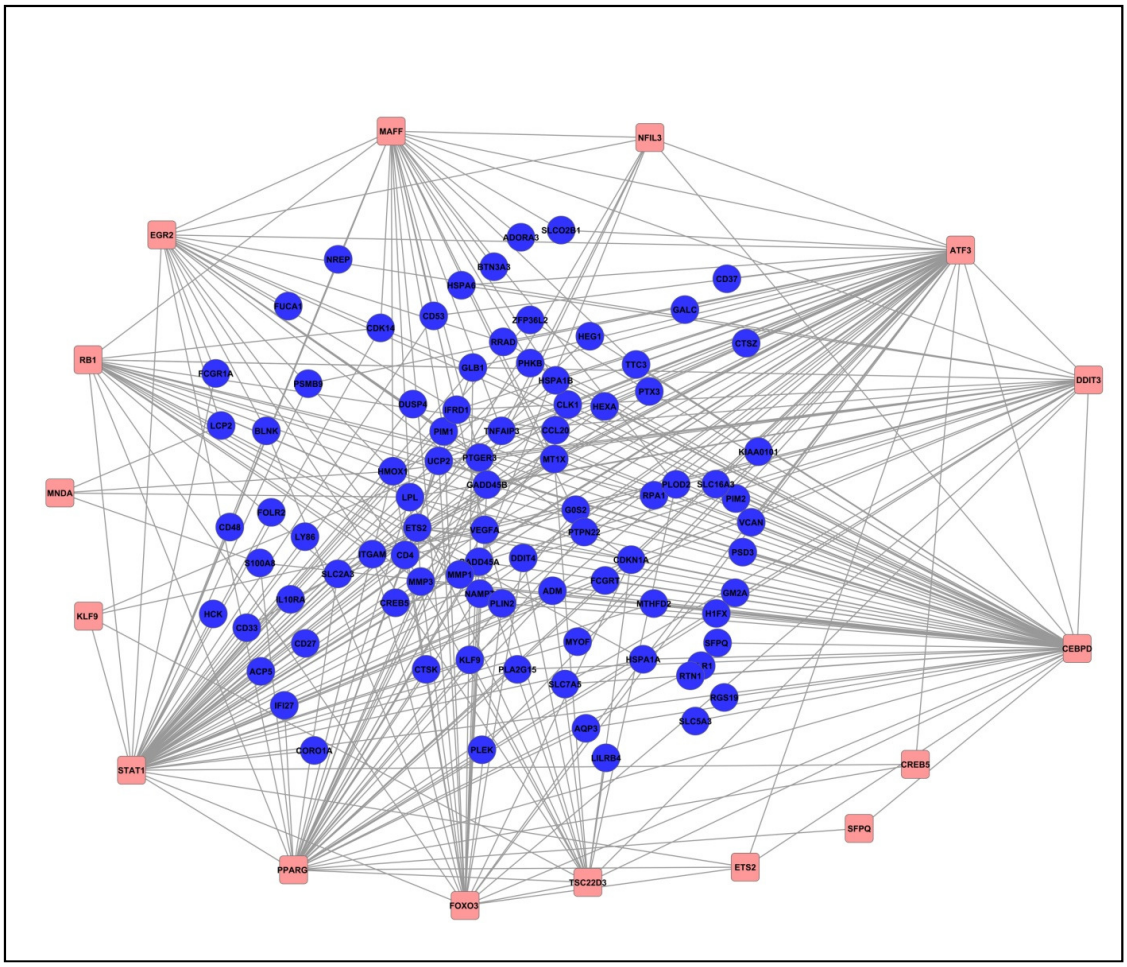

Figure 3.24 Transcriptional regulatory network of rheumatoid arthritis (Transcription Factor: pink rectangle, Target Gene: blue circle).

Table 3.9 Central proteins (hubs) of the transcriptional regulatory network of rheumatoid arthritis

Protein Symbol	Degree	Degree Rank	Betweenness	Betweenness Rank
STAT1	64	1	1737	1
CEBPD	60	2	1476	2
ATF3	57	3	1207	3
PPARG	42	4	678	4

ATF3 (activating transcription factor 3) is a transcription factor that is overexpressed as response to stress in various physiological conditions and in several cancer cells such as hepatocellular carcinoma (Xiaoyan et al., 2014) and colorectal cancer (Lee et al., 2014).

3.2.3. Protein-protein interaction networks associated with rheumatoid arthritis

A PPI network was constructed between RA DEGs and their interacting partners (Figure 3.25). The reconstructed RA PPI network has 5216 binary interactions between 3331 proteins. The central proteins of the PPI network of RA are listed in Table 3.10. The highest ranking hub of the PPI network is the protein encoded by KIAA0101 gene which is a PCNA-associated factor and is also in the curated list of psoriasis DEGs which was discussed in Section 3.1.6. STAT1, PPARG and CEBPD are transcription factors that are also central in TRN of RA.

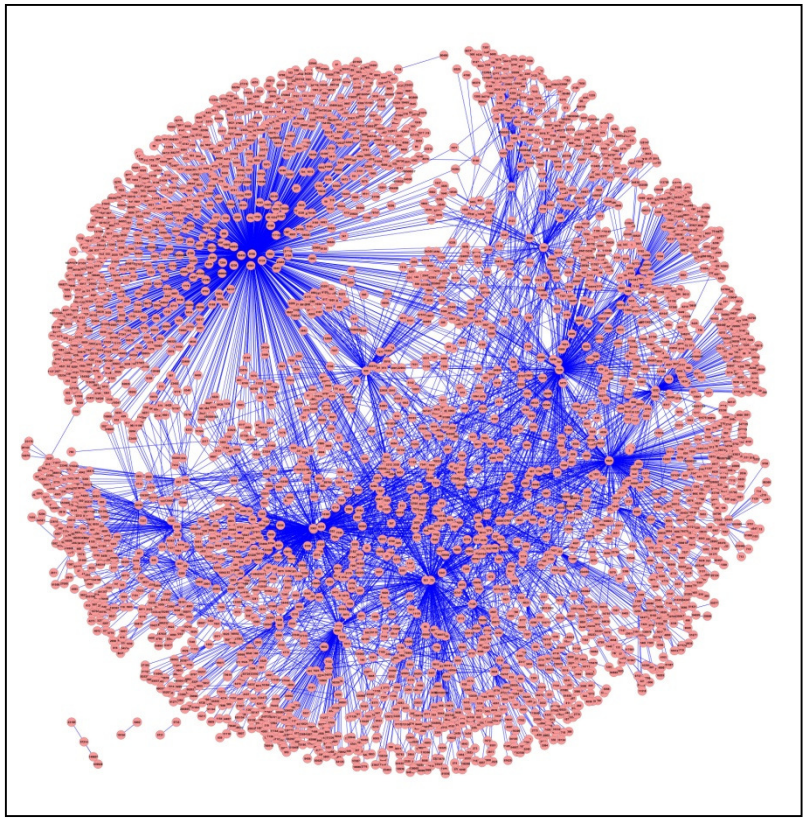

Figure 3.25 Protein-protein interaction network of rheumatoid arthritis

Table 3.10 The central proteins of the protein-protein interaction network of rheumatoid arthritis

Protein Symbol	Degree	Degree Rank	Betweenness	Betweenness Rank
KIAA0101	1122	1	2890757	1
CDKN1A	313	2	664209	4
RB1	294	3	688243	3
STAT1	264	4	612355	5
HSPA1B	214	5	268113	9
HSPA1A	214	6	268113	9
RPA1	173	7	401694	6
PPARG	154	8	358806	7
CEBPD	140	9	302833	8

3.2.4 Enrichment analysis of rheumatoid arthritis

The RA DEGs are associated with several disease classes such as immune, infection and cardiovascular diseases. Among these DEGs 25 of them are associated with immune related diseases and 21 of them are associated with cardiovascular diseases. Table 3.11 gives the disease class associations of RA DEGs.

Table 3.11 Disease class enrichment of differentially expressed genes of rheumatoid arthritis

Term	PValue
INFECTION	1.33×10^{-4}
IMMUNE	3.22×10^{-4}
CARDIOVASCULAR	0.001966
AGING	0.006565
OTHER	0.035624

The RA DEGs are associated with cardiovascular diseases such as coronary atherosclerosis (p-value = 4.87×10^{-4}) and autoimmune diseases such as rheumatoid arthritis (p-value = 2.05×10^{-3}) and psoriasis (p-value = 3.4×10^{-3}). The diseases associated with RA DEGs are given in Table 3.12.

Table 3.12 Disease associations of differentially expressed genes of rheumatoid arthritis (p–value < 0.05)

Term	PValue	Term	PValue
atherosclerosis, coronary	$4.87x10^{-4}$	kidney cancer	0.009654
ankylosing spondylitis	0.001039	atherosclerosis, coronary	0.009654
arthritis, rheumatoid	0.002047	gastric ulcer	0.009804
oral cancer	0.002816	Restenosis	0.017277
Psoriasis	0.003409	nasopharyngeal cancer	0.018829
heart disease, ischemic	0.004038	Cirrhosis	0.019059
Stroke	0.006961	cardiovascular disease	0.019059
abdominal aortic aneurysm	0.007096	Longevity	0.023176
rheumatoid arthritis	0.00726	Preeclampsia	0.024266
ovarian cancer	0.008953	subarachnoid hemorrhage	0.024643
diabetes, type 2	0.030669	myocardial infarct	0.038807
ulcerative colitis	0.030808	carotid atherosclerosis	0.041063
kawasaki disease	0.030809	myasthenia gravis	0.044731
Crohn's disease ulcerative colitis	0.031552	Leptin	0.045861
Lymphoma	0.031552	Alzheimer's disease dementia, vascular	0.045861
Periodontitis	0.032762	head and neck cancer	0.047814
diabetes, type 2; obesity	0.034098	lung function	0.048515

Approximately half of the RA DEGs were associated with the “response to stimulus” (p-value = 4.76×10^{-11}) term. The “Immune System Process” term was also highly significant (p-value = 1.33×10^{-9}). The list of enriched biological processes of RA is given in Appendix B.

42 of the RA DEGs encode plasma membrane proteins (p-value = $5.2x10^{-4}$). The GO Cellular component terms enriched in RA DEGs are listed in Table 3.13.

The enriched GO molecular function terms include “transcription factor activity” (p-value = $8.6x10^{-3}$) and “immunoglobin binding” (p-value = $1.47x10^{-4}$). The rest of the enriched MF terms are listed in Table 3.14.

Table 3.13 Gene Ontology cellular component terms associated with differentially expressed genes of rheumatoid arthritis (p–value < 0.05)

Term	PValue
GO:0005773~vacuole	7.61×10^{-6}
GO:0005764~lysosome	1.25×10^{-5}
GO:0000323~lytic vacuole	1.25×10^{-5}
GO:0005886~plasma membrane	5.20×10^{-5}
GO:0000267~cell fraction	0.013653
GO:0031225~anchored to membrane	0.016473
GO:0044459~plasma membrane part	0.035260
GO:0005624~membrane fraction	0.045637

Table 3.14 The enriched Gene Ontology molecular function terms of differentially expressed genes of rheumatoid arthritis (p–value < 0.05)

Term	PValue
GO:0019864~IgG binding	1.87×10^{-5}
GO:0019865~immunoglobulin binding	1.47×10^{-4}
GO:0046983~protein dimerization activity	0.001551
GO:0030246~carbohydrate binding	0.003607
GO:0004553~hydrolase activity, hydrolyzing O-glycosyl compounds	0.004414
GO:0003700~transcription factor activity	0.008634
GO:0016798~hydrolase activity, acting on glycosyl bonds	0.009489
GO:0060089~molecular transducer activity	0.014164
GO:0004871~signal transducer activity	0.014164
GO:0001871~pattern binding	0.023689
GO:0030247~polysaccharide binding	0.023689
GO:0043565~sequence-specific DNA binding	0.027878
GO:0004563~beta-N-acetylhexosaminidase activity	0.027968
GO:0004872~receptor activity	0.031856
GO:0019900~kinase binding	0.038103
GO:0015665~alcohol transmembrane transporter activity	0.041661
GO:0015166~polyol transmembrane transporter activity	0.041661
GO:0005529~sugar binding	0.049475

The results of the pathway enrichment analysis of RA are listed in Table 3.15. Lysosomes are the cell's main digestive compartment to which intra and extracellular

molecules are delivered for degradation. Mutations in the lysosome associated genes prevent the breakdown of these molecules; causing the undegraded materials to accumulate within the lysosomes and forming severe clinical symptoms. Elevated lysosomal cysteine protease activities, along with aspartate protease cathepsin D and lysosomal glycosidases, are associated with RA disease progression (Sohar et al., 2002).

Table 3.15 Pathway enrichment analysis of rheumatoid arthritis (p–value < 0.05)

Term	PValue
hsa04142:Lysosome	5.82×10^{-5}
hsa05219:Bladder cancer	0.012937
hsa00511:Other glycan degradation	0.014774
hsa04612:Antigen processing and presentation	0.0161
hsa04640:Hematopoietic cell lineage	0.018127
hsa03320:PPAR signaling pathway	0.047254
REACT_6900:Signaling in Immune system	1.10×10^{-4}
REACT_604:Hemostasis	0.010225

Antigen processing and presentation pathway is important for the body's ability to detect signs of infection or abnormal cell growth. First, the antigen-presenting cells digest proteins from inside or outside the cell, then they display the resulting antigenic peptide fragments on cell surface major histocompatibility complex molecules so that the T cells can recognize them. Cellular mechanisms such as autophagy are believed to participate in antigen processing and presentation. Autophagy can target pathogens that reside in the cytosol or within phagosomes for lysosomal degradation. (Vyas et al., 2008). PPAR signaling pathway is another significant pathway of RA disease progression and is involved in inflammation and immune response.

3.3. Integrative Analysis of Atopic Dermatitis

This section describes the results of the analysis of atopic dermatitis disease datasets. Three datasets of AD was analyzed for this study (Figure 3.26).

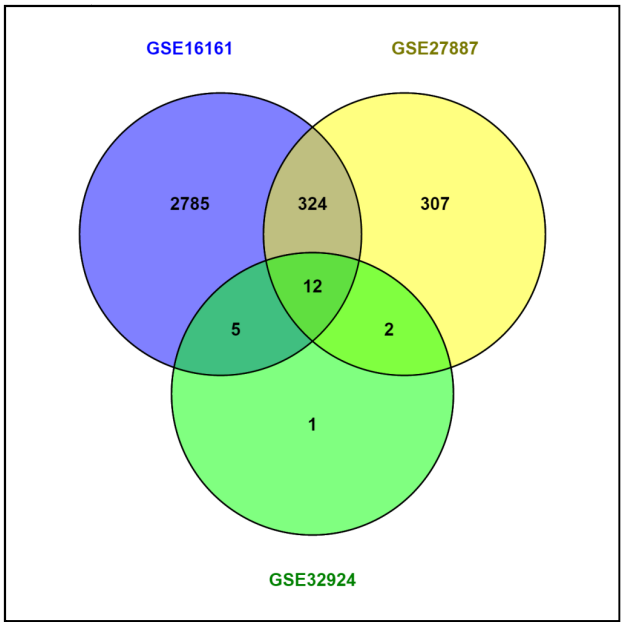

Figure 3.26 The comparison of the number of differentially expressed genes of atopic dermatitis datasets employed in this study.

3.3.1. Differentially expressed genes of atopic dermatitis

Even though three datasets of AD were analysed, the FC threshold selected did not produce enough DEGs for GSE32924 dataset, therefore it was excluded for the remainder of this study. Despite the big difference in number of DEGs in the two datasets (for GSE16161 total number of DEGS is 2487 while for GSE27887 it is 508), there were a total of 280 DEGs (corresponding to 336 probes) mutual between them. These DEGs are listed in Appendix B.

3.3.2. Transcription factor – differentially expressed gene relationship of atopic dermatitis

The transcriptional regulatory network of AD DEGs is depicted in Figure 3.27. There are 230 nodes and 563 edges in the network including 14 TFs. The hub TFs of the TRN of AD are listed in Table 3.16. IRF1 (Interferon Regulatory Factor 1) is from the TF family of interferon regulatory factors. The function of IRF1 has been previously discussed in Section 3.1.6. TEAD4 (TEA domain family member 4) gene encodes the TEF3 (Transcriptional enhancer factor 3) from the TEF family of transcription factors. It is usually expressed in skeletal muscle and might be active in the embryonic

development of skeletal muscle as well as regulating the expression of the unfolded protein response genes (Benhaddau et al., 2012).

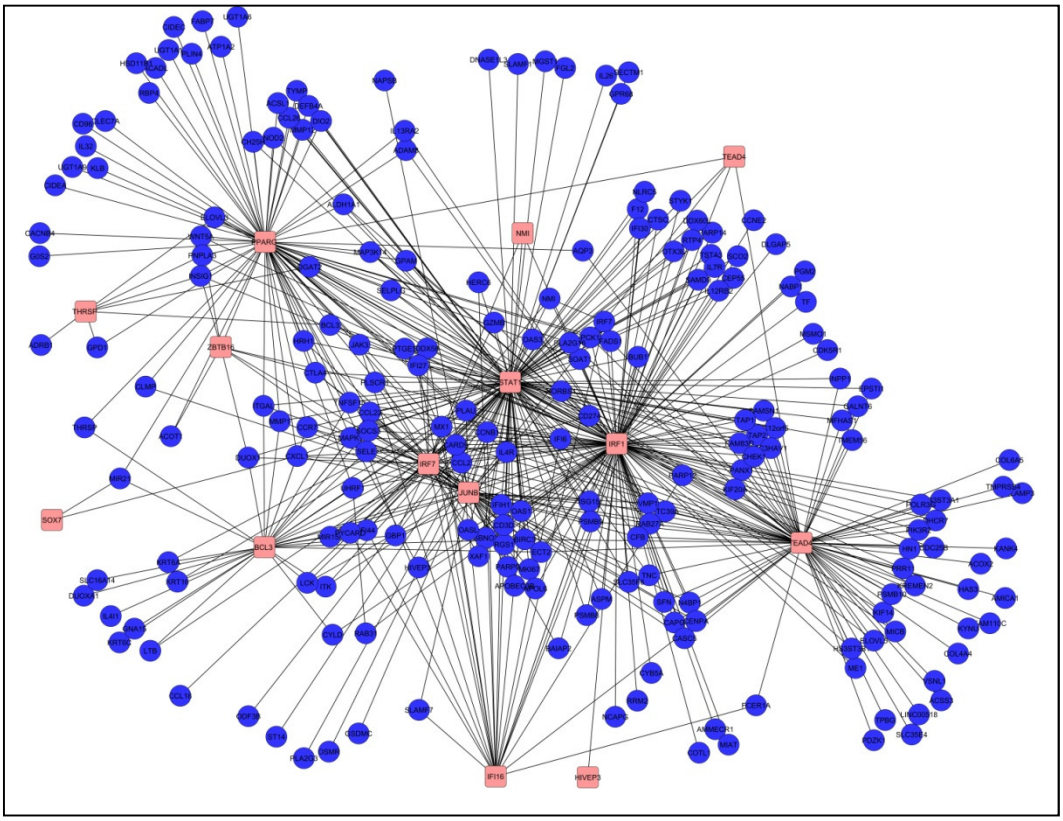

Figure 3.27 The transcriptional regulatory network of differentially expressed genes of atopic dermatitis (Transcription Factor: pink rectangle, Target Gene: blue circle)

Table 3.16 Central Proteins (Hubs) of the Transcriptional Regulatory Networks of atopic dermatitis

Protein Symbol	Degree	Degree Rank	Betweenness	Betweenness Rank
IRF1	131	1	9654	2
STAT1	129	2	9956	1
TEAD4	76	3	4831	4
PPARG	74	4	5554	3

3.3.3. Protein-protein interaction networks associated with atopic dermatitis

A PPI Network was constructed for AD DEGs. The reconstructed AD PPI network is very dense with 5899 binary interactions between 3796 proteins (Figure 3.28).

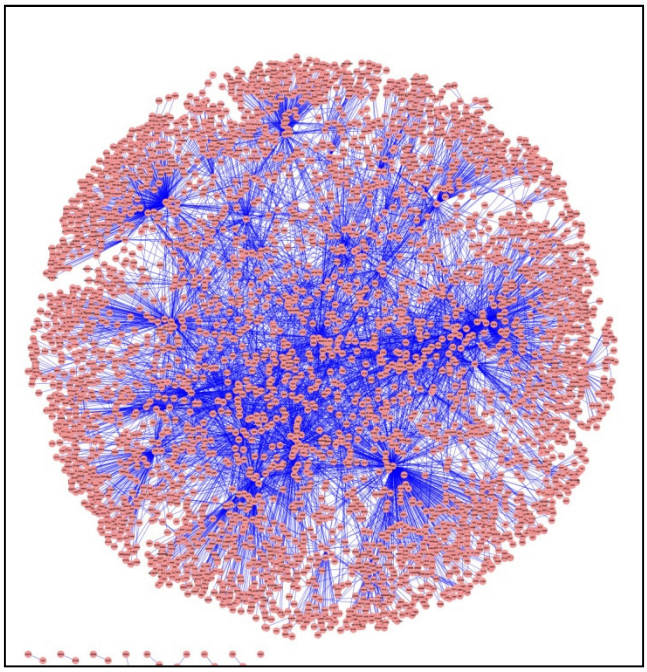

Figure 3.28 Protein-protein interaction network associated with atopic dermatitis

The central proteins (hubs) of the AD PPI network are depicted in Table 3.17. MAPK1 (mitogen-activated protein kinase 1) is from the MAP kinase family also known as the ERKs (extracellular signal-regulated kinases) which is crucial for a variety of signalling processes that are activated in inflammation and effect various mechanisms that are important in inflammation (Newton and Holden, 2003).

STAT1, IRF1 and PPARG are TFs previously mentioned in Section 3.1.6. Additionally PLSCR1 and CCNB1 are of the psoriasis DEGs described in the same section.

Table 3.17 The central proteins of protein-protein interaction network of atopic dermatitis (p–value < 0.05)

Protein Symbol	Degree	Degree Rank	Betweenness	Betweenness Rank
MAPK1	396	1	1206693	2
STAT1	264	2	837547	3
SFN	253	3	745692	4
PLSCR1	225	4	742010	5
MAP3K14	206	5	508558	8
LCK	203	6	539889	7
IL7R	183	7	549093	6
RRM2	163	8	419840	9
PPARG	154	9	406493	10
ZBTB16	153	10	405523	11
CCNB1	148	11	381147	12
IRF1	147	12	315242	13
UBC	136	13	2692142	1
MYO5A	113	14	310898	14

3.3.4. Enrichment analysis of atopic dermatitis

The disease classes associated with AD DEGs are immune (p value = 2.63×10^{-5}) and infection (p value = 2.21×10^{-4}). There are 17 DEGs associated with Type 1 Diabetes, 16 of the DEGs are associated with RA and 9 with psoriasis disease. The disease associations are given in Table 3.18.

Major biological processes enriched in AD DEGs are generalized terms such as, response to stimulus (p value = 9.12×10^{-13}) with 106 DEGs and immune system process (p value = 7.2×10^{-18}) with 58 DEGs. The whole list of enriched biological processes for AD is in Appendix B.

The proteins encoded by 142 of the AD DEGs are cytoplasmic, while 79 of them are plasma membrane proteins. There are also proteins found in endoplasmic reticulum and extracellular space. Table 3.19 gives the GO cellular component terms and their p-values.

Table 3.18 Diseases associated with differentially expressed genes of atopic dermatitis (p–value < 0.05)

Term	PValue
hepatitis B	$3.93x10^{-6}$
rheumatoid arthritis	$3.33x10^{-5}$
interferon response	$1.10x10^{-4}$
dermatitis, atopic	$1.52\ x10^{-4}$
juvenile arthritis	$1.92\ x10^{-4}$
diabetes, type 1	$2.30\ x10^{-4}$
Psoriasis	$8.60\ x10^{-4}$
multiple sclerosis	0.003451
hepatitis C	0.005498
Arthritis	0.006151
hepatitis C, chronic	0.006896
multiple sclerosis; IgA nephropathy	0.008786
ankylosing spondylitis	0.012395
acute coronary syndrome	0.01638
Behcet's disease	0.023172
sclerosis, systemic	0.025385
myocardial infarct	0.031944
Obesity	0.033326
celiac disease	0.035443
Spondyloarthropathies	0.035771
systemic lupus erythematosus	0.037065
ulcerative colitis	0.04439

Table 3.19 Cellular Component Terms Associated with differentially expressed genes of atopic dermatitis (p–value < 0.05)

Term	PValue
GO:0005737~cytoplasm	$1.01\ x10^{-4}$
GO:0005829~cytosol	$2.63\ x10^{-4}$
GO:0005792~microsome	$2.95\ x10^{-4}$
GO:0042598~vesicular fraction	$3.81\ x10^{-4}$
GO:0044459~plasma membrane part	$4.38\ x10^{-4}$
GO:0044444~cytoplasmic part	$5.74\ x10^{-4}$
GO:0005886~plasma membrane	0.001743
GO:0000267~cell fraction	0.002266

Table 3.19 Cellular Component Terms Associated with differentially expressed genes of atopic dermatitis (p–value < 0.05) continued.

Term	PValue
GO:0042825~TAP complex	0.004639
GO:0044421~extracellular region part	0.006428
GO:0042824~MHC class I peptide loading complex	0.007794
GO:0005625~soluble fraction	0.00887
GO:0005615~extracellular space	0.008999
GO:0005783~endoplasmic reticulum	0.01207
GO:0005887~integral to plasma membrane	0.024687
GO:0031226~intrinsic to plasma membrane	0.031602
GO:0005839~proteasome core complex	0.036851
GO:0005815~microtubule organizing center	0.040863
GO:0009897~external side of plasma membrane	0.046523
GO:0000793~condensed chromosome	0.047948

The results of the pathway enrichment analysis are given in Table 3.20. The most significant pathway in the enrichment results was the PPAR signaling pathway. PPARs regulate lipid, glucose, and amino acid metabolism as well as important cellular functions, such as cell proliferation, differentiation, and inflammatory responses. Sertznig and coworkers (2008) suggested that PPAR signaling pathway may represent therapeutic targets for various skin diseases such as psoriasis and atopic dermatitis.

Jak-STAT and chemokine signaling pathways are major signaling pathways for a variety of cytokines and growth factors and are previously described in Section 3.1.6.

NOD-like receptor families appear as crucial sensors of infection and stress in intracellular compartments. Mutations in several of the NOD-like receptor genes are associated with auto-immune and auto-inflammatory syndromes, drawing attention to the central roles of NOD-like receptors in the immune system (Shaw et al., 2010).

Table 3.20 Pathway enrichment analysis of atopic dermatitis (p–value < 0.05)

Term	PValue
hsa03320:PPAR signaling pathway	1.80×10^{-4}
hsa05340:Primary immunodeficiency	0.001194
hsa01040:Biosynthesis of unsaturated fatty acids	0.001505
hsa04621:NOD-like receptor signaling pathway	0.003056
hsa04630:Jak-STAT signaling pathway	0.00998
hsa04062:Chemokine signaling pathway	0.011685
hsa04660:T cell receptor signaling pathway	0.012795
hsa04960:Aldosterone-regulated sodium reabsorption	0.014816
hsa04060:Cytokine-cytokine receptor interaction	0.018794
P00031:Inflammation mediated by chemokine and cytokine signaling pathway	2.80×10^{-4}
REACT_602:Metabolism of lipids and lipoproteins	0.003236
REACT_6900:Signaling in Immune system	0.00478
REACT_16888:Signaling by PDGF	0.009553

3.4. Integrative Analysis of Systemic Lupus Erythematosus

This section describes the results of the analysis of systemic lupus erythematosus disease datasets.

3.4.1. Differentially Expressed Genes of Systemic Lupus Erythematosus

Three datasets of SLE were examined for this study (Figure 3.29). There were a total of 56 DEGs (corresponding to 73 probes) mutual in these datasets. The list of SLE DEGs is given in Appendix B. All the SLE DEGs were upregulated.

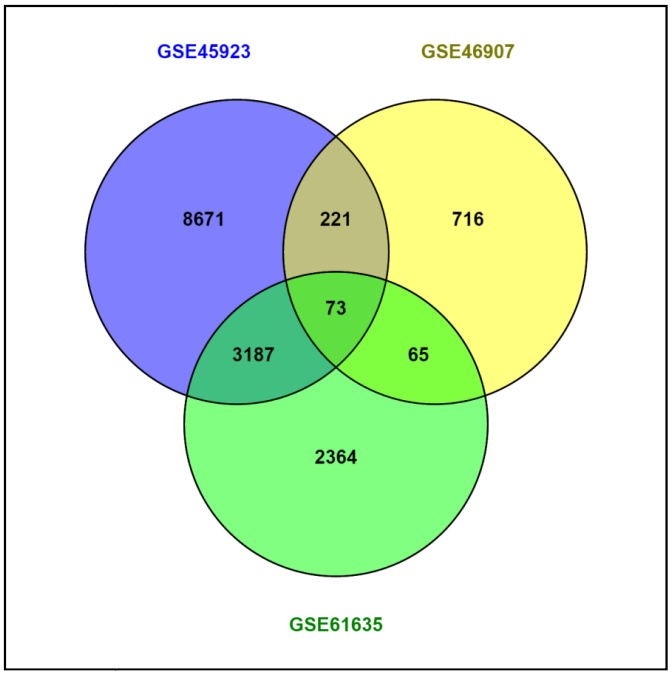

Figure 3.29 Comparison of number of differentially expressed genes across systemic lupus erythematosus datasets

3.4.2. Transcription factor – differentially expressed gene relationship of systemic lupus erythematosus

The transcriptional regulatory network of SLE consists of 79 nodes and 180 edges (Figure 3.30). There are five TFs with STAT1 being the major TF that regulates the other DEGs.

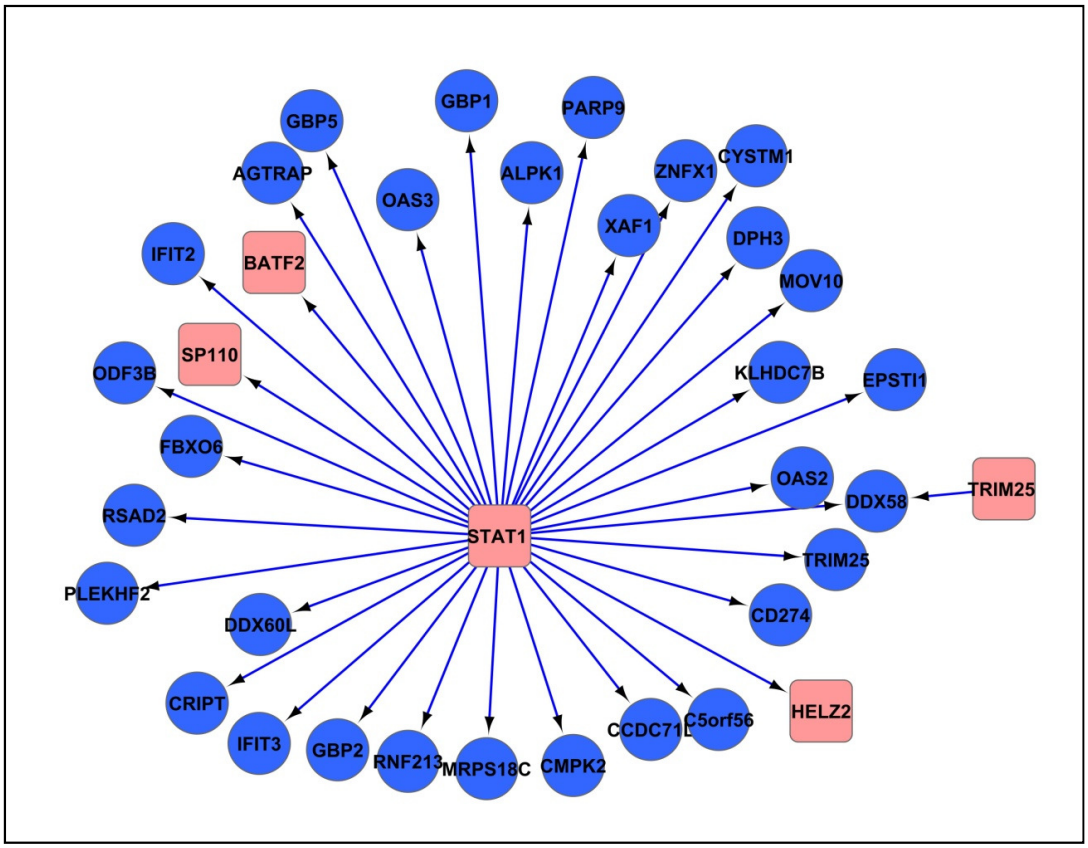

Figure 3.30 The transcriptional regulatory network for differentially expressed genes of systemic lupus erythematosus (Transcription Factor: pink rectangle, Target Gene: blue circle)

3.4.3 Protein-protein interaction networks associated with systemic lupus erythematosus

A PPI subnetwork was constructed between SLE DEGs and their interacting partners (Figure 3.31). The reconstructed SLE PPI network has 769 binary interactions between 865 interacting proteins.

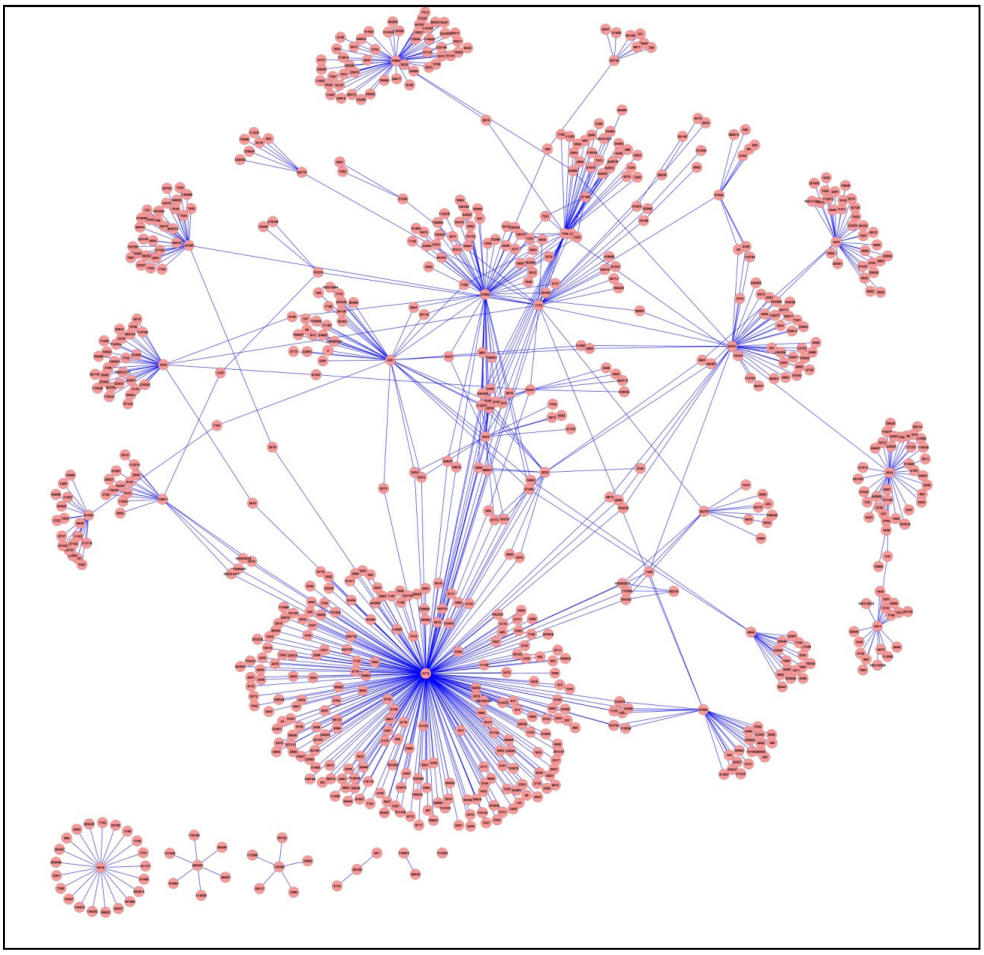

Figure 3.31 Protein-protein interaction network of systemic lupus erythematosus

The central proteins (hubs) of SLE are listed in Table 3.21. STAT1 is the major hub of SLE. Another hub, DDX58 (DEAD (Asp-Glu-Ala-Asp) Box Polypeptide 58) encodes the RIG-I (retinoic acid-inducible gene 1) which is a RIG-I-like receptor dsRNA helicase enzyme. It is involved in RNA binding, alteration of RNA secondary structure as well as immune response (Cui et al., 2005).

Table 3.21 Central proteins of the protein-protein interaction network of systemic lupus erythematosus

Protein Symbol	Degree	Degree Rank	Betweenness	Betweenness Rank
STAT1	264	1	166331	1
DDX58	63	2	43762	4
PLEKHF2	52	3	35134	7
USP25	41	4	27942	10
TRIM25	40	5	81161	2
IFIT3	37	6	29185	8
GBP2	37	7	38273	5

3.4.4. Enrichment analysis of systemic lupus erythematosus

Surprisingly the pathway enrichment analysis did not result in any significant functional pathways for SLE DEGs. The individual datasets produced significant pathways but there were no mutual pathways among the three datasets. The mutual pathways in two of the disease datasets were: Ubiquitin mediated proteolysis (hsa0412) and Signaling by Rho GTPases (REACT_11044).

Ubiquitin mediated proteolysis is important for many basic cellular processes such as, regulation of cell cycle, DNA repair, immune and inflammatory responses and biogenesis of organelles. This pathway has been activated in many diseases due to its association with the above mentioned biological processes. Proteolysis is the breakdown of proteins into smaller polypeptides or amino acids. The degradation process of proteins via the ubiquitin mediated proteolysis pathway occurs by the attachment of ubiquitin molecules to the substrate and then degradation of the tagged protein and recycling of ubiquitin (Ciechanover, 2000).

Rho GTPases are a subfamily of the Ras superfamily proteins which are effective in processes such as the regulation of cell shape change, cytokinesis, cell adhesion, and cell migration as well as gene expression. Rho signaling has appeared as an important regulator of actin cytoskeleton in many of these processes (Lu et al., 2009). Furthermore activation of Rho GTPases is crucial in the regulation of immune responses and in the activation of T cells. The deregulation of Rho GTPase-mediated pathways may be critical in the pathogenesis of SLE (Pernis, 2008).

The disease enrichment results indicate that SLE DEGs are also associated with infectious diseases ($2.6x10^{-3}$) and hepatitis (p value = $0.9x10^{-5}$).

Table 3.22 Gene Ontology enrichment results of differentially expressed genes of systemic lupus erythematosus (p–value < 0.05)

	Term	PValue
BP	GO:0006955~immune response	7.46×10^{-4}
	GO:0002376~immune system process	0.006095
	GO:0009615~response to virus	0.025205
CC	GO:0009898~internal side of plasma membrane	0.036696
MF	GO:0017076~purine nucleotide binding	0.013304
	GO:0003723~RNA binding	0.013601
	GO:0000166~nucleotide binding	0.016177
	GO:0005524~ATP binding	0.047462

Table 3.22 lists the enriched GO terms of the SLE DEGs. The GO BP enrichment results give us three general processes associated with SLE. The SLE DEGs that are involved in the immune response are GBP1, RSAD2, GBP2, OAS2, GBP5, CD274, DDX58 and OAS3. Four of these DEGs are also in the curated psoriasis DEG list: RSAD2, OAS2 and OAS3 which are ISGs and DDX58 which is involved in the regulation of immune response. GBP1, GBP2 and GBP5 are from the dynamin superfamily of large GTPases, which are also ISGs. These guanylate-binding proteins (GBPs) are involved in interferon-induced gene transcription and in the identification of interferon response (Vestal and Jeyaratnam, 2011). These biological processes are indicative of autoimmune properties of SLE.

3.5. Comparative Analysis of Psoriasis and Other Disease Datasets

In this section, the DEG lists, TFs and their hubs, PPI networks and their hubs and enrichment analysis results of RA, AD and SLE were compared with psoriasis datasets analysis results.

A total of nine disease datasets of the selected three autoimmune diseases (RA, AD and SLE) were examined for this study. The total number as well as up and downregulated genes for each disease dataset was given in Figure 3.32.

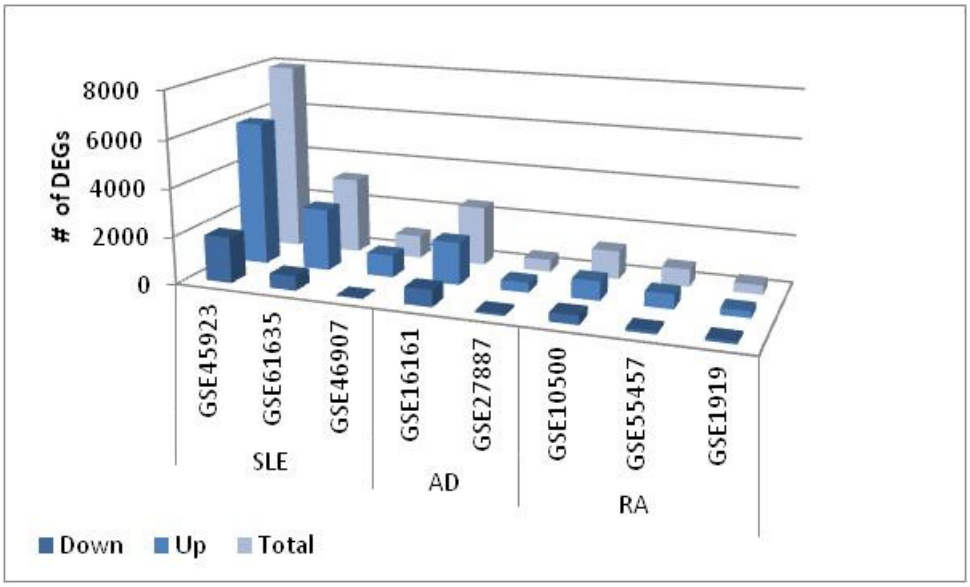

Figure 3.32 The numbers of differentially expressed genes employed in other autoimmune disease datasets.

The highest number of DEGs was in GSE45923 (SLE) with 6027 upregulated and 1927 downregulated DEGs. The lowest number of DEGs was in GSE1919 (RA) with only 278 upregulated and 116 downregulated DEGs. For all the disease datasets the upregulated DEGs were greater in number than of the downregulated DEGs.

3.5.1. Comparison of differentially expressed genes in autoimmune diseases

A comparison between the curated psoriasis DEG list (Table 3.8) and the DEG list of other autoimmune diseases has led to the identification of mutual DEGs between psoriasis and these diseases (Figure 3.33). The sole mutual DEG between all the studied diseases is STAT1.

STAT1 is a major, complex transcriptional factor with various conflicting functions. At the time of activation it causes the overexpression of some genes while having an adverse effect on the transcription of others. It promotes the crosstalk between signal

transduction pathways. STAT1 can also function in the absence of inducer-mediated activation (Ramana et al., 2000). The roles of STAT1 in Jak-STAT pathway and its functions have been discussed in Section 3.1.6.

Among the seven mutual DEGs between RA and psoriasis, five of them (STAT1, S100A8, CCL20, HMOX1 and RAC2) are response to stimulus genes. Three of these DEGs (S100A8, CCL20 and HMOX1) are active in the defense mechanism (such as response to wounding). HMOX1 and PIM1 regulate transcription factor activity and DNA binding. Five of these DEGs are in the nucleus except for CCL20 which is in the extracellular region and S100A8 which is located in the cytoplasm. These DEGs were overexpressed in RA disease progression as well as in psoriatic skin.

All of the mutual DEGs between psoriasis and SLE are response to stimulus genes. OAS2, OAS3, DDX58 are in cytoplasm, STAT1 is in nucleus and RSAD2 is an endoplasmic reticulum membrane protein. OAS2, OAS3 and STAT1 were previously linked to hepatitis B (p-value = $2x10^{-4}$). These DEGs are also overexpressed in SLE as well as psoriatic skin.

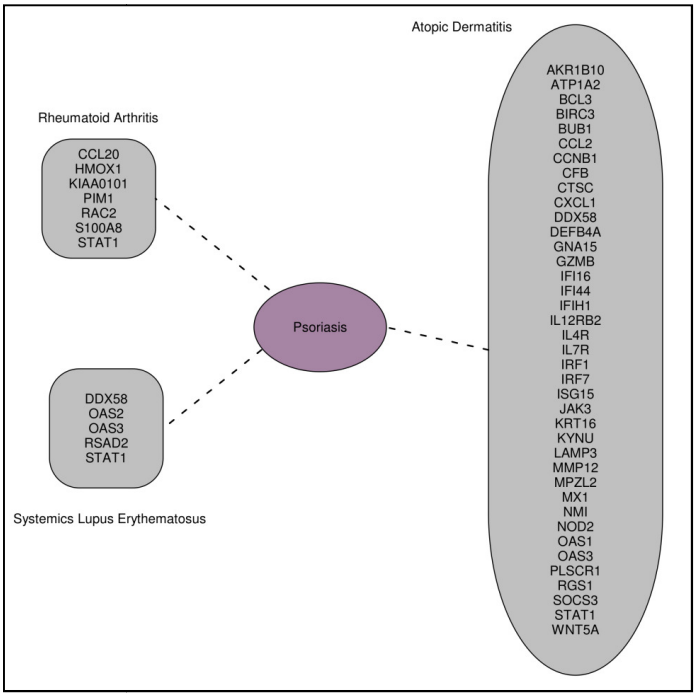

Figure 3.33 Mutual DEGs between investigated diseases and psoriasis

There are 39 mutual DEGs between psoriasis and AD. 25 of them are in the cytoplasm and nine are in the extracellular region. 29 of these DEGs are response to stimulus genes of which twelve (BCL3, DDX58, NMI, CCL2, CXCL1, CFB, DEFB4A, IFIH1, IRF7, KYNU, MX1 and NOD2) are in the defense mechanism. Nine of these mutual DEGs (OAS1, OAS3, CCL2, IRF1, IL12RB2, IL4R and SOCS3) have also been linked to diabetes (p value = $1.3x10^{-5}$) in other studies and six of them (STAT1, OAS1, OAS3, IRF1, IL4R and MX1) were also linked to hepatitis B (p-value = $1.3x10^{-5}$). 38 of these genes were upregulated. Only ATP1A2 was downregulated. It was also downregulated in psoriatic skin.

Detailed information on the DEGs mutual between psoriasis and the other autoimmune diseases can be found in Section 3.1.6.

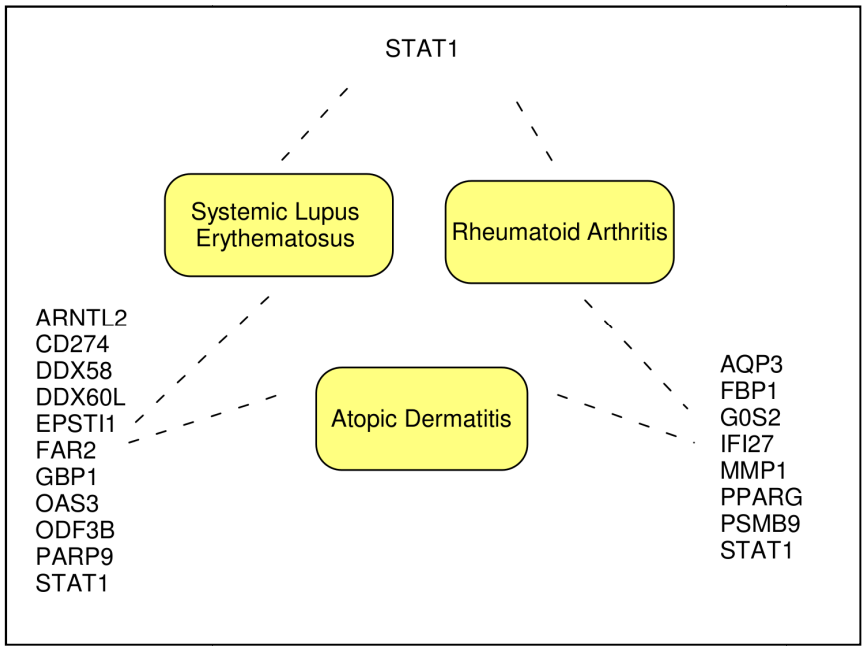

Figure 3.34 Mutual DEGs of atopic dermatitis, rheumatoid arthritis and systemic lupus erythematosus

Figure 3.34 gives mutual DEGs among the selected autoimmune diseases. There are twelve mutual DEGs between SLE and AD. Among these DEGs GBP1, DDX58, DDX60L, CD274 and OAS3, ARNTL2 and STAT1 are response to stimulus genes. Seven of them are enzymes involved in catalytic activity (OAS3, DDX58, DDX60L,

FAR2, GBP1, ODF3B and PARP9). EPSTI1 (Epithelial-Stromal Interaction Protein 1 (breast)) is a protein coding gene that is activated in response to interferon and may be involved in tissue organization (Villadsen et al., 2008).

STAT1 is the only mutual DEG between RA and SLE. The reason for this might be that SLE patients do not have arthritis related symptoms.

There are eight mutual DEGs between AD and RA five of which are response to stimulus genes (AQP3, FBP1, PPARG, PSMB9 and STAT1). IFI27 is an ISG. PSMB9 and FBP1 are enzymes. PPARG, FBP1 and PSMB9 are cytosol proteins. G0S2 (G0/G1switch 2) is a protein coding gene which promotes apoptosis. G0S2 is known for its direct inhibitory capacity on the rate-limiting lipolytic enzyme adipose triglyceride lipase. Other key features include sub-cellular localization, expression profiles and regulation, and possible functions in cellular proliferation and differentiation (Heckmann et al., 2013).

STAT1 is the major hub of the disease PPI networks as well as the TF in all the studied diseases. In addition PPARG is a mutual TF and a PPI hub between AD and RA datasets. PPARG was previously discussed in Section 3.2.2.

The PPI network of AD is the densest network since it is showing the interactions of the highest number of proteins compared with the other diseases.

3.5.2. Comparison of enrichment analysis of the investigated diseases

The biological process comparison of psoriasis, AD, RA and SLE resulted in two mutual processes: immune system process and immune response which prove that these diseases are immune mediated. The triple comparison of Psoriasis, RA and AD yielded in 36 mutual biological processes including cell activation, response to nutrient levels, cell proliferation, response to wounding and inflammatory response. The highest number of mutual BP (99) is between Psoriasis and AD with processes such as JAK-STAT cascade, regulation of apoptosis, and regulation of adaptive and innate response standing out.

The biological process comparisons of psoriasis versus other autoimmune diseases are listed in Appendix B.

The mutual BP between psoriasis and, AD and RA are significantly higher than of SLE. This is due to the small number of enriched biological processes of SLE. The biological process bi-comparison of RA, AD and SLE are given in Appendix B.

The enriched signaling pathway comparisons of psoriasis versus other autoimmune diseases resulted in Table 3.23. Impaired immune regeneration is a defect of both psoriasis and RA. In RA, bone marrow hematopoietic stem cells (HSCs) are functionally defective which compromises the immune system. Proper HSC function is necessary to restore immune health in RA patients (Colmegna et al., 2008). Hematopoietic stem cells (HSCs) are at the foundation of the adult blood differentiation hierarchy, and provide continuous hematopoietic cell production throughout life. Hematopoietic development occurs in several mesodermal lineages (Dzierzak and Speck, 2008).

The RA DEGs CD4, CD33 and CD37 are cluster of differentiation (CD) molecules involved in the Hematopoietic cell lineage pathway. CD4 is a glycoprotein and a member of the immunoglobin superfamily that is crucial for the development and function of the immune system. They are also called T helper (Th) cells or T4 cells (Hanna et al., 1994).

The synovial membrane in patients with rheumatoid arthritis has hyperplasia, increased vascularity, and inflammatory cells. These cells are primarily CD4+ T cells, which are the orchestrator of cell-mediated immune responses. Activated CD4+ T cells stimulate B cells through cell-surface contact and through the binding of $\alpha_L\beta_2$ integrin, CD154 (CD40 ligand), and CD28, to produce immunoglobulins, including rheumatoid factor. They also express osteoprotegerin ligands that stimulate osteoclastogenesis. (Choy and Panayi, 2001). Osteoclast is derived from the pluripotent hematopoietic stem cell and any intervention in osteoclastogenesis stages could result in serious adverse effects from the hematopoietic system (Yavropoulou and Yovos, 2008). CD33 also from the immunoglobin superfamily, is a cell surface glycoprotein receptor specific for the myeloid lineage (Andrews et al., 1983). CD37 is another cell surface glycoprotein expressed on mature human B cells which might be crucial in T-cell–B-cell interactions (Knobeloch et al., 2000).

IL2RA, IL4R, IL1B and IL7R are the psoriasis DEGs involved in the Hematopoietic cell lineage pathway. IL2RA (interleukin 2 (IL2) receptor alpha) is an integral membrane protein. IL4R (interleukin 4 receptor) is a receptor for both IL4 and IL13 and is involved in Th2 differentiation. IL7R (interleukin 7 receptor) is crucial in the development of immune cells (Gregory et al., 2007).

Table 3.23 Disease linkages based on cellular pathways

Psoriasis – RA	hsa04640:Hematopoietic cell lineage
Psoriasis – AD	hsa04630:Jak-STAT signaling pathway hsa04060:Cytokine-cytokine receptor interaction hsa04062:Chemokine signaling pathway
Psoriasis – SLE	-
RA - AD	hsa03320:PPAR signaling pathway REACT_6900:Signaling in Immune system

Jak-STAT and chemokine signaling pathways, which have been discussed in detail in Section 3.1.6., are the mutual signaling pathways of AD and psoriasis. PPAR signaling pathway is a mutual pathway for RA and AD has also been previously discussed.

3.5.3 Discussions of computational analysis of comparative investigation of the disease datasets

To understand the association between psoriasis and the selected autoimmune diseases we have to explore the individual disease mechanisms primarily. For this each disease has been analyzed independently to explore the genes that are differentially expressed and the resulting DEGs have been enriched to determine the relevant biological processes and significant functional pathways. The transcription factors regulating these DEGs have been identified and the interaction network involving the disease proteins and their interacting partners have been constructed. This analysis aids us to perceive the biological mechanisms of each disease independently therefore guides us to identify the association between the selected autoimmune diseases and psoriasis.

The associations of psoriasis and other autoimmune diseases have been long documented (Ali and Warren, 2013). In a study performed by Wu and coworkers (2012)

patients with psoriasis were more likely to have at least 1 other autoimmune disease and the strongest association was with rheumatoid arthritis.

The strong connection between rheumatoid arthritis and psoriasis is due to psoriatic arthritis as well as defective immune regeneration. Psoriatic arthritis (PsA) can sometimes be difficult to differentiate from rheumatoid arthritis. Patients with PsA are subject to the same quality of life issues as RA patients because of joint damage. Furthermore the addition of skin related problems cause the worsening of physical functions. While the major mutual features of rheumatoid arthritis and psoriasis (and PsA) are joint inflammation the distinctive difference between the two diseases is that PsA affects fewer joints than rheumatoid arthritis (RA), and it often has an asymmetrical distribution of the affected joints rather than the symmetrical pattern seen in RA (Lee et al., 2010).

Conforming to this study, individuals with inflammatory conditions such as rheumatoid arthritis and psoriasis also experience higher rates of cardiovascular disease. The risk can be associated with the disease severity or inflammatory markers. (Gabriel, 2008).

The highest number of mutual DEGs were between psoriasis and AD which are mostly immune and dermal inflammation related. This evidently leads to mutual disease features such as dry, scaly skin and disturbed epidermal differentiation (Bowcock and Cookson, 2004). The mutual DEGs between AD and psoriasis are to a great extent overexpressed which implies that signaling pathways are activated in both diseases rather than being silenced. The difference in the progression of both diseases can be explained by the difference of the expression level of these genes.

As has been expressed previously genetics and environmental factors are effective in the progression of both psoriasis and AD. The environmental triggers of psoriasis include infections of group A streptococcal antigens (Baker et al., 1997). Though infections exacerbate psoriasis symptoms it does not cause infections on the psoriatic skin as a result of AMPs. Patients with AD may suffer from colonization and infection from Staphylococcus aureus. The lack of AMPs, skin barrier dysfunction and skin inflammation promote the increase of Staphylococcus aureus colonization in AD skin lesions (Lin et al., 2007).

The spectrum of human lupus ranges from only skin related symptoms to systemic disease, all with a characteristic tissue damage. Systemic disease affects a number of organs and tissues with relapses and remitting courses and high morbidity (Banchereau and Pascual, 2006).

The coexistence of psoriasis and SLE is rare (Berthelot et al., 2007). Yet there is a genetic cause common between these two diseases. One of the mutual DEGs of psoriasis and SLE is DDX58 (also known as RIG-I), which stimulates dendritic cell maturation. Dendritic cells induce resistance to infection (Steinman and Banchereau, 2007). Infections are very common in the induction or exacerbation of systemic lupus erythematosus (SLE) and they account for 30-50% of morbidity and mortality (Doria et al., 2008). Infections do not cause mortality in psoriasis which is a major difference between the two studied diseases.

4. CONCLUSIONS

The disease mechanism of psoriasis is giving a hard time to its researchers due to its complex nature. In this study, to identify DEGs associated with psoriasis, we have analyzed expression patterns from twelve microarray studies with the largest cohort of patients to date (a total of 534 patients). Eleven core DEGs (IFI44, IFIT1, MAD2L1, STAT1, RSAD2, NMI, OAS2, TRIM22, WIF1, SUB1 and PI3) were identified and TF – core DEG relationships were displayed. Seven of the core DEGs have TFs associated with them. The core DEGs that are ISG's (TRIM22, RSAD2, IFIT1, STAT1 and OAS2) are regulated by either IRFs or IFNG. The PPI network of these core DEGs was also reconstructed. Five proteins (STAT1, MAD2L1, CYCS, NMI and SUB1) were identified as hubs of this reconstructed PPI network. A DEG pool which are thought to have associations with psoriasis disease was formed from 145 DEGs present in at least five of the twelve analyzed datasets to elucidate the psoriasis mechanism as a whole, including our core DEGs and DEGs which are found in related signaling pathways as a result of literature survey. Instead of utilization of a gene co-expression network analysis to describe the correlation patterns among gene expression levels across microarray samples, FC values were recruited to analyze the correlation between DEGs. The FCC analysis led to a highly correlated network. The central DEGs of the overall FCC Network included SUB1, SOCS1, OAS1 and NMI. To understand the modular topology of the 145 DEGs, a clustering analysis was done which resulted in the identification of four modules. Module 1 is the central module with a high-level connection to Module 2. The hub DEGs (SUB1, SOCS1, NMI) of Module 1 show similarity to the hubs of the overall FCC Network. Identification of the central molecules and highly interconnected modules of the reconstructed FCC network resulted with a summary of the gene profiles located centrally in the modules, which illuminate the disease mechanism of psoriasis. The hubs of the overall FCC network show similarity to PPI network of psoriasis constructed using core DEGs and their interactions. The enrichment analysis indicated that JAK/STAT signaling pathway is significant in all the modules as well as the overall network. The FCC analysis method appears to be uncomplicated and requires fewer amounts of data compared to computing the correlations of gene expression levels. In addition possible uncertainties

that may arise from high variance are eliminated, and the effect of false positives is possibly reduced.

ELISA tests and quantitative RT-PCR was done to verify the findings of the computational analysis which proved that psoriasis DEGs function in correlation with each other and IFI44 and SUB1 appear to be central DEGs. The gender difference in the onset of psoriasis disease has also been proven in protein (SUB1, WIF1 and PI3) and transcriptomic levels (e.g., WIF1, SUB1, PI3 and IFI44). In transcriptomic level the selected DEGs have a higher fold change in male patients than the female counterparts. Also in protein level, e.g. in SUB1 there is only an observed difference in plasma of male patients than the healthy controls. PASI scores of psoriasis patients are also correlated with fold change values of the DEGs in transcriptomic level. SUB1 and IFI44 need further experimentation to check for biomarker characteristics since they are central DEGs and their expression levels show significant difference in transcriptomic and/or protein levels.

A disease pathway for psoriasis was proposed which is a modified version of JAK/STAT signaling pathway. This pathway includes the stimulators and the activators such as WNT5A, S100A8/A9 and IL19, the chemokines (CXCL1, CXCL2, CXCL8) and their receptor (CXCR2), JAK3, STAT1, IRF9, the ISGs such as IFI44, IFIT1, RSAD2, the inhibitors SOCS1 and SOCS3, the defense mechanism (eg, PI3, DEFB4A, HMOX1 and KIAA0101, which eventually activates the biological pathways cell cycle and oocyte meiosis.

The associations of psoriasis with other autoimmune diseases have also been examined. The same methodology was employed in the analysis of three selected autoimmune diseases: rheumatoid arthritis, atopic dermatitis and systemic lupus erythematosus. Three microarray datasets of each of the diseases were examined. PPI networks and TF regulatory networks were constructed and hubs of these networks were identified. The disease DEGs were analyzed to identify the signaling networks, and gene ontology terms associated with these diseases. For RA, there were four mutual DEGs (STAT1, BTN3A3, CD52 and MAFF) between the three datasets investigated. The DEG list was extended to better analyze the disease. These DEGs were regulated by 16 TFs. The hubs of the TRN were STAT1, CEBPD, ATF3 and PPARG. A dense PPI network was also

reconstructed with STAT1, PPARG and CEBPD being the hub proteins along with KIAA0101. Pathway enrichment analysis pointed out that Antigen processing and presentation, Hematopoietic cell lineage as well as PPAR signaling pathways were important in RA.

The analysis of the Atopic dermatitis disease datasets yielded a total of 280 DEGs. There were 14 TFs regulating these DEGs. The hubs of the TRN network of AD included IRF1, STAT1 and PPARG. A very dense PPI network was reconstructed and hub proteins such as IRF1, STAT1, MAPK1 and PPARG were identified. Some of the signaling pathways associated with AD are JAK/STAT, PPAR signaling and NOD-like receptor signaling.

The integrative analysis of systemic lupus erythematosus resulted in a total of 56 DEGs and five TFs (STAT1, TRIM25, HELZ2, SP110 and BATF2). A sparse PPI network was constructed and hub proteins of this network such as STAT1, DDX58 and IFIT3 were identified. Ubiquitin mediated proteolysis and signaling by Rho GTPases were significant pathways of this disease.

Comparative analysis of the three diseases and psoriasis was also done. STAT1 is the sole mutual DEG of all the studied diseases. There were seven mutual DEGs (CCL20, HMOX1, KIAA0101, PIM1, RAC2, S100A8 and STAT1) between RA and psoriasis, five mutual DEGs (DDX58, OAS2, OAS3, RSAD2 and STAT1) between SLE and psoriasis and 39 mutual DEGs between AD and psoriasis. The disease linkages based on cellular pathways yielded Hematopoietic cell lineage pathway for Psoriasis and RA, JAK/STAT signaling and Chemokine signaling for Psoriasis and AD, and PPAR signaling for RA and AD.

Overall these results implement a comprehensive approach to figure out the molecular framework for better therapeutic studies in the treatment of psoriasis, propose several hypotheses and point out target molecules for further experimental studies, and establish a new framework that can be applied to other complex human diseases. Furthermore it demonstrates common genetic causes between psoriasis and the selected autoimmune diseases.

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APPENDICES

Appendix A – Supplementary tables and figures associated with experiments

Table A.1 Constants/Parameters used in ELISA analysis

	a	B	c	D	R-squared
PI3	28.4618	-1.17771	109990	0.0651885	0.9986794
SUB1	3.0127736	0.4746029	0.6030064	-0.655537	0.9925264
WIF1	2.09924609	1.47749332	206.6615645	-0.08406871	0.996374

Table A.2 Raw C_T data for RT-PCR

	IFI44	IFIT1	IRF9	NMI	OAS2	PI3	RPLP0	RSAD2	SUB1	WIF1
Negative Control	36.84	28	30.85		38.04	31.79	>40.00	35.88	>40.00	37.49
Negative Control	36.46	28.9	29.54		>40.00	32.69	>40.00	>40.00	37.77	36.6
Patient 1	31.14	24.53	26.57	36.49	28.32	27.23	30.81	27.56	33.28	37.88
Patient 1	30.87	24.5	26.63	36.54	28.51	26.67	30.44	27.58	34.94	36.17
Patient 2	28.02	21.56	25.21	35.58	25.81	24.04	29.06	26.55	31.17	39.3
Patient 2	28.56	21.89	24.9	35.22	25.51	24.29	29.13	26.46	30.8	38.38
Patient 3	32.07	25.17	27.59	>40.00	29.79	29.29	32.83	29.8	34.64	38.43
Patient 3	31.55	25.17	27.68	37.87	29.89	29.26	32.12	29.91	34.73	38.29
Patient 4	32.05	25.59	27.54	>40.00	29.09	28.15	31.73	29.57	34.82	[>40.00]
Patient 4	30.72	25.61	27.81	>40.00	28.85	28.63	31.7	29.36	34.07	37.48
Patient 5	30.94	25.69	26.65	>40.00	29.76	26.7	31.04	29.33	32.06	37.25

Patient 5	29.97	25.73	26.6	>40.00	29.43	26.56	31.01	28.72	32.06	36.47
Patient 6	29.93	25.21	26.43	35.87	28.73	26.82	31.03	27.27	31.72	36.08
Patient 6	29.59	25.34	26.56	35.75	28.59	26.73	31.31	27.46	31.41	35.96
Patient 7	28.76	24.01	27.02	36.21	27.06	25.58	31.6	28.34	31.5	36.09
Patient 7	28.97	24.14	26.87	36.27	27.08	25.62	31.81	28.5	31.11	35.86
Control 1	30	24.63	26.2	33.33	28.47	26.23	30.22	26.34	31.32	36.84
Control 1	30.15	24.63	26.19	33.58	28.15	26.56	30.55	26.34	31.59	37.2
Control 2	29.91	25	30.16	34.23	28.61	27.11	30.99	26.91	31.32	36.22
Control 2	30.21	24.98	26.59	34.23	28.77	26.91	30.85	26.87	31.9	36.46
Control 3	29.49	23.93	25.46	31.91	27.22	25.29	29.55	25.46	32.32	37.55
Control 3	29.14	23.72	25.45	32.1	27.17	25.25	29.59	25.43	31.85	37.67
Control 4	30.9	24.74	26.19	33.99	28.82	26.81	29.76	26.69	33.27	39.93
Control 4	31.85	24.6	26.21	34.55	28.55	26.33	31.2	26.52	33.89	39.82
Control 5	31.58	25.53	27.07	35.7	29.03	27.19	30.15	26.94	34.16	38.11
Control 5	31.47	24.97	26.66	36.1	28.95	26.98	31.13	27	34.88	39.66

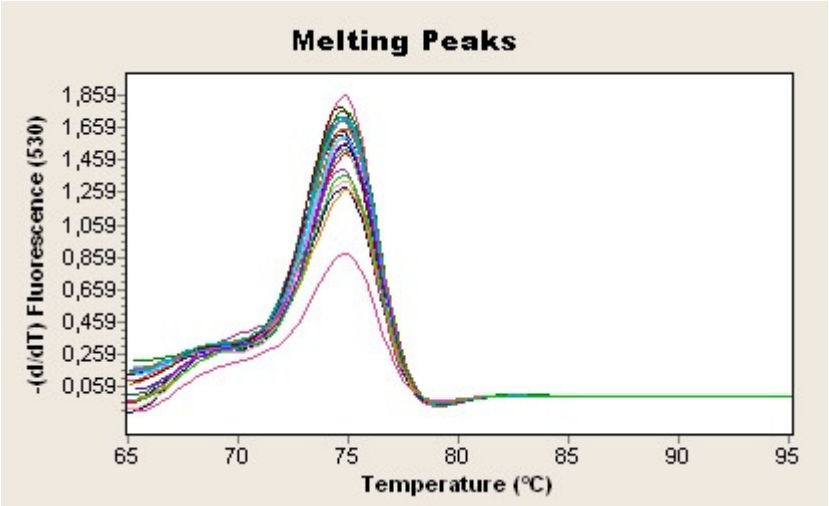

Figure A.1 Melting Peaks of IFI44

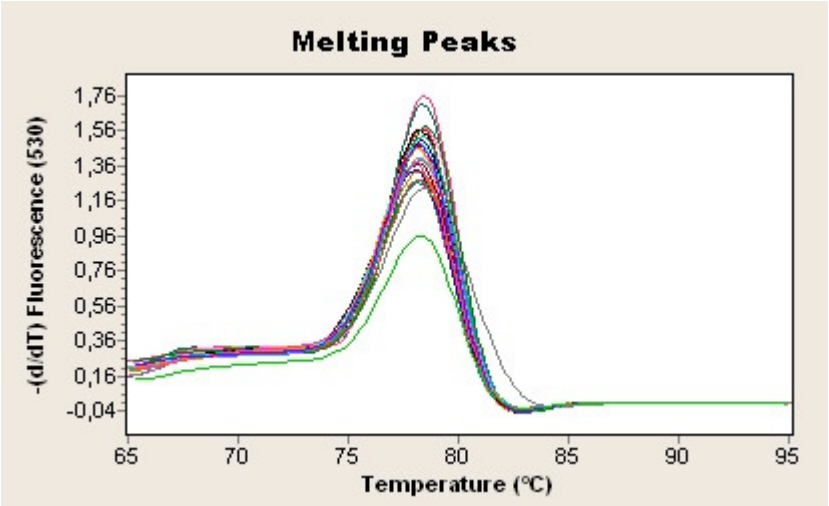

Figure A.2 Melting Peaks of IFIT1

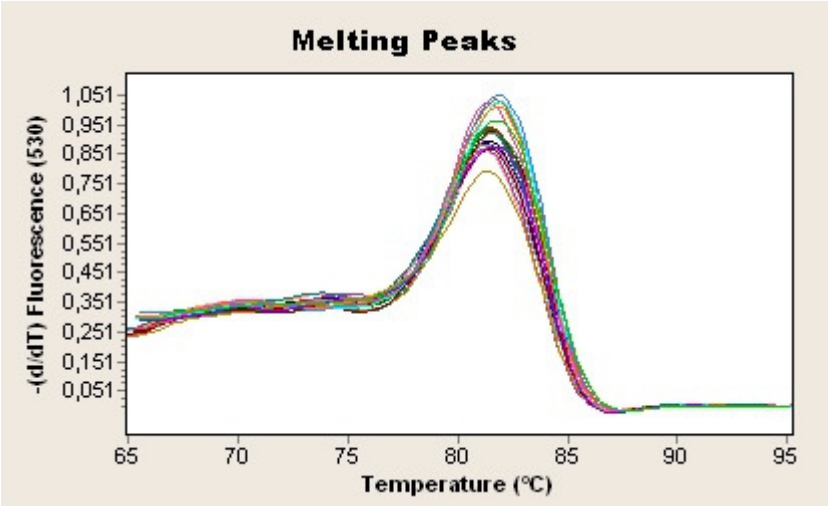

Figure A.3 Melting Peaks of IRF9

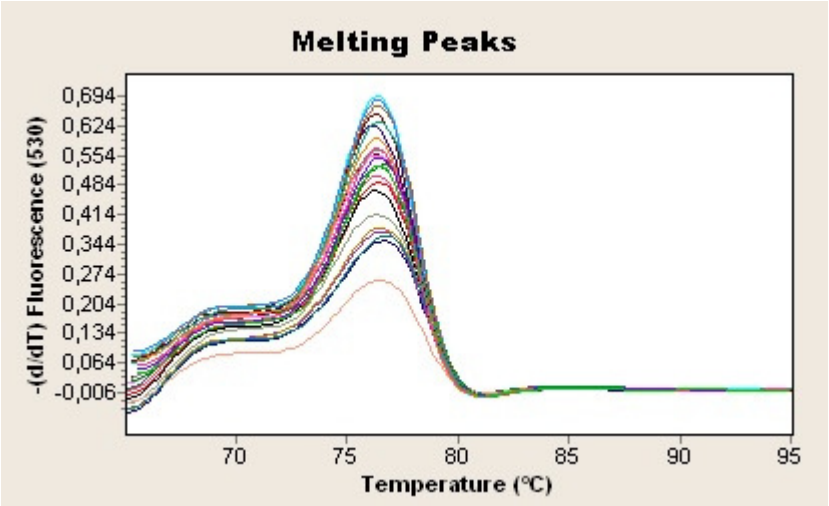

Figure A.4 Melting Peaks of NMI

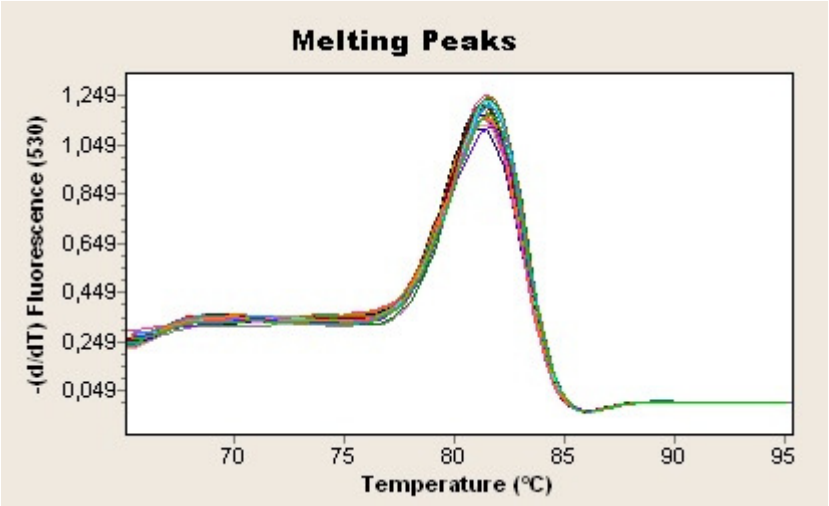

Figure A.5 Melting Peaks of OAS2

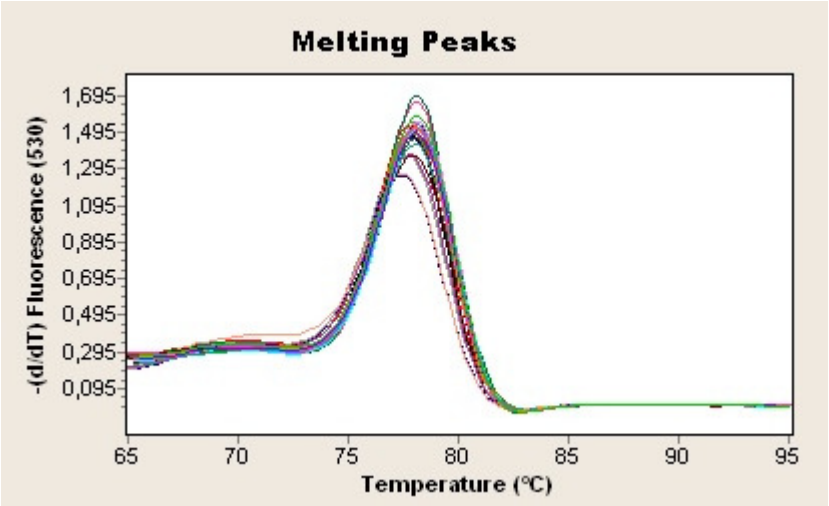

Figure A.6 Melting Peaks of PI3

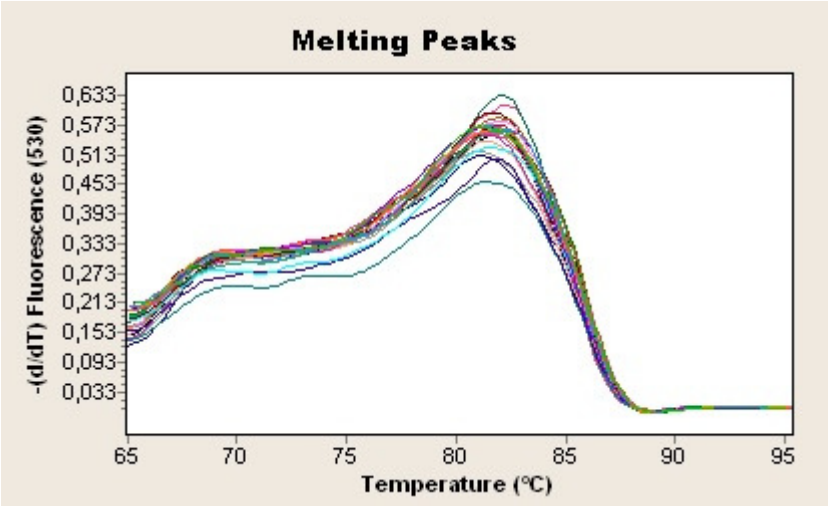

Figure A.7 Melting Peaks of RPLP0

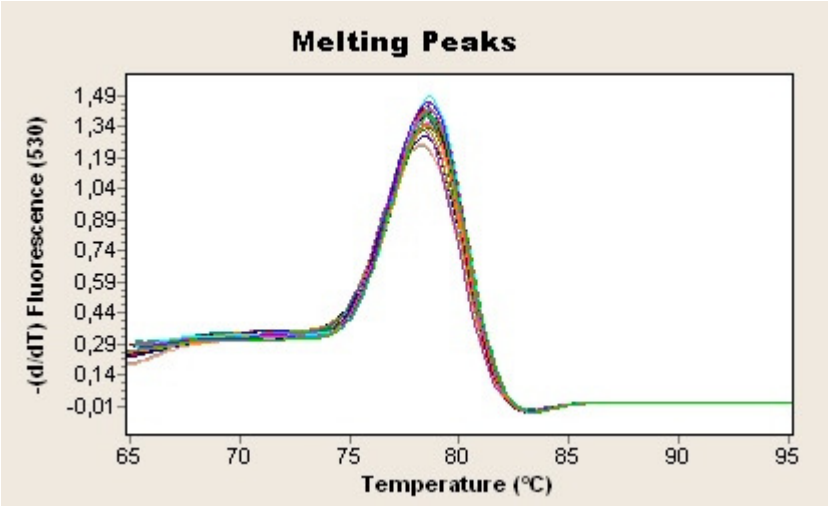

Figure A.8 Melting Peaks of RSAD2

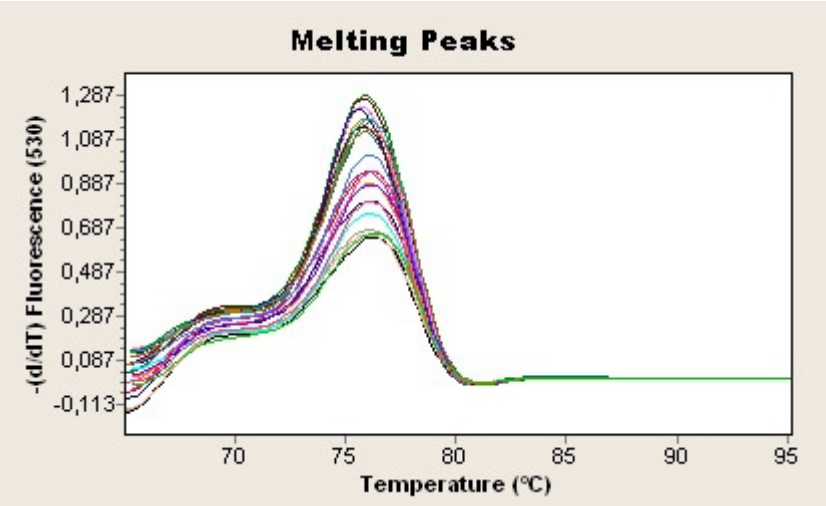

Figure A.9 Melting Peaks of SUB1

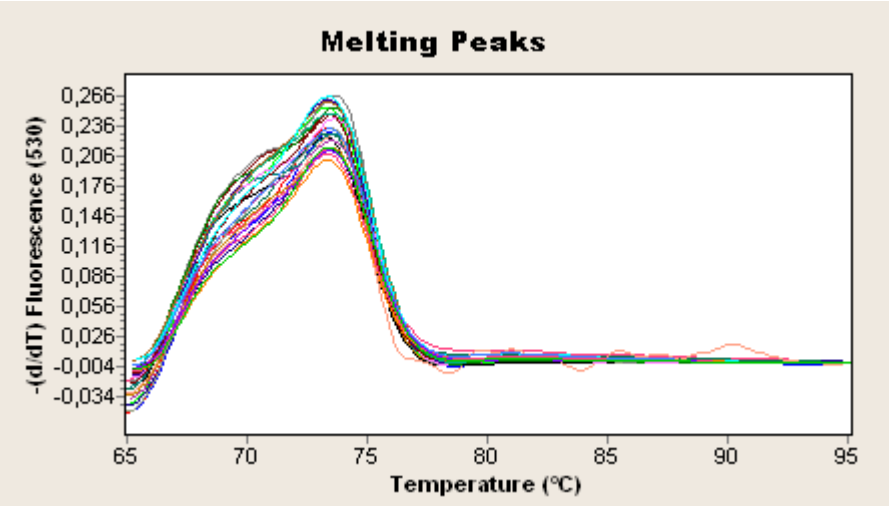

Figure A.10 Melting Peaks of WIF1

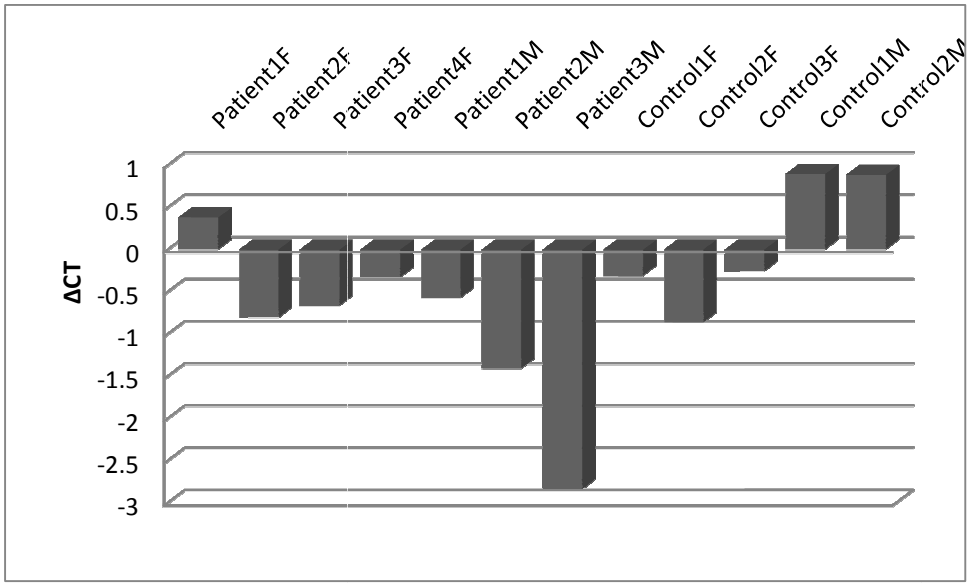

Figure A.11 IFI44 ΔC_T values (F: Female, M: Male)

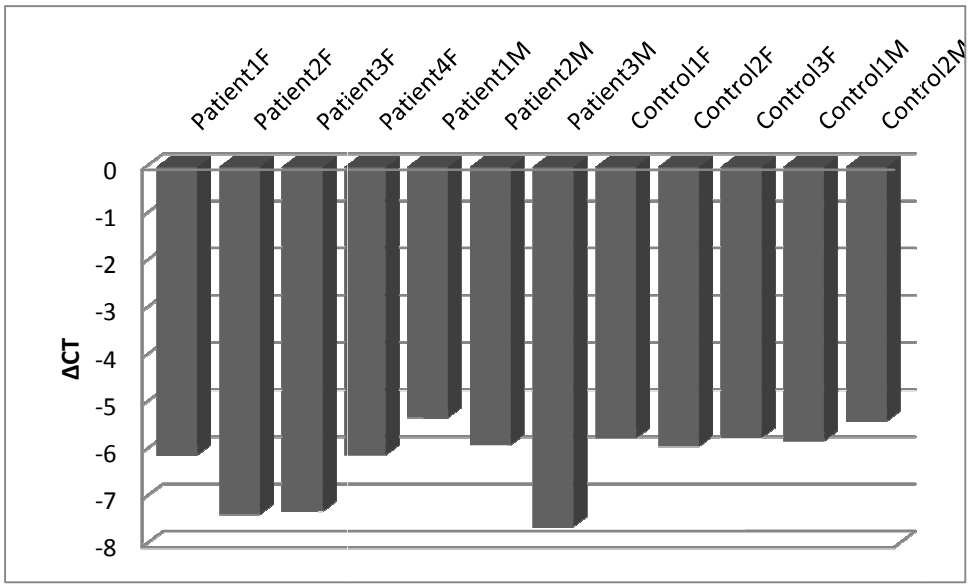

Figure A.12 IFIT1 ΔC_T values (F: Female, M: Male)

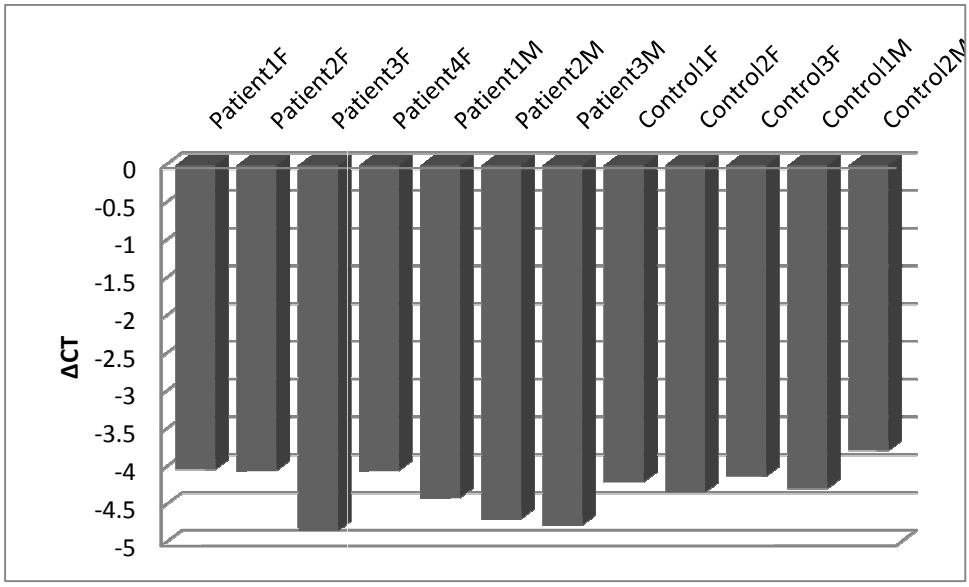

Figure A.13 IRF9 ΔC_T values (F: Female, M: Male)

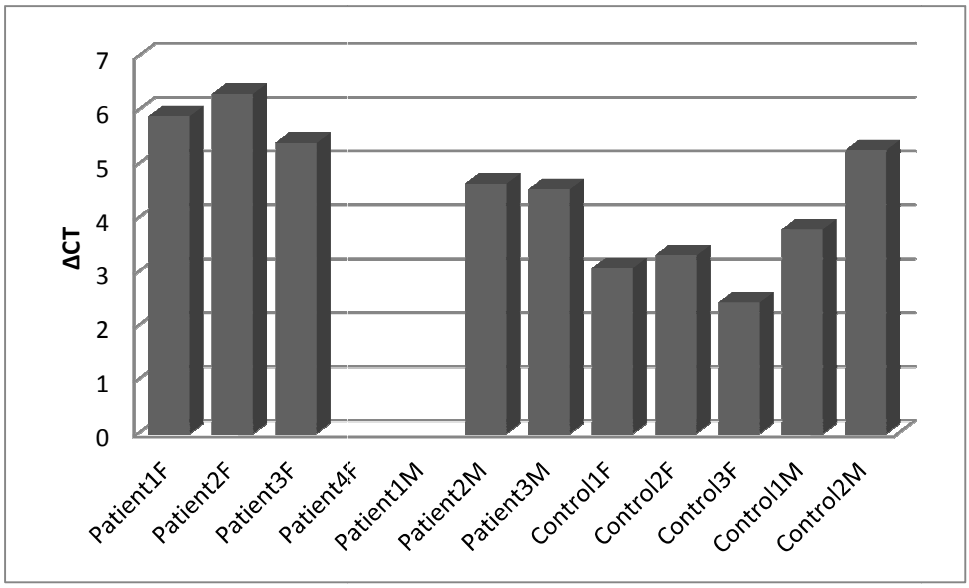

Figure A.14 NMI ΔC_T values (F: Female, M: Male)

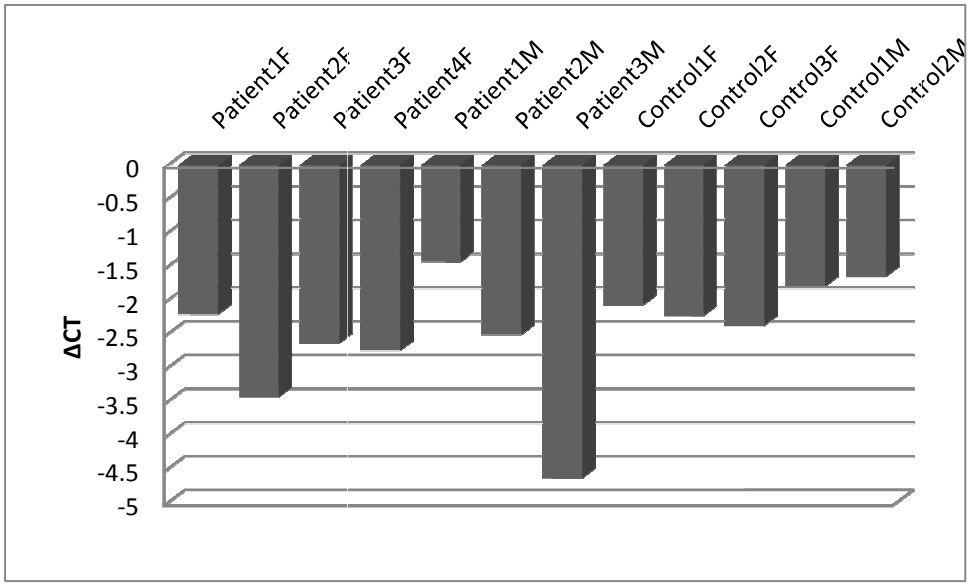

Figure A.14 OAS2 ΔC_T values (F: Female, M: Male)

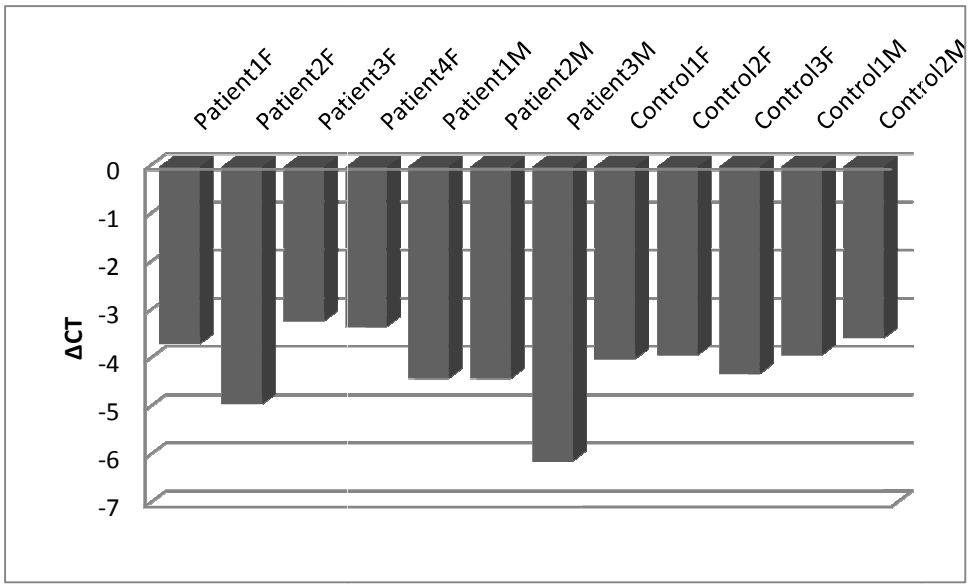

Figure A.15 PI3 ΔC_T values (F: Female, M: Male)

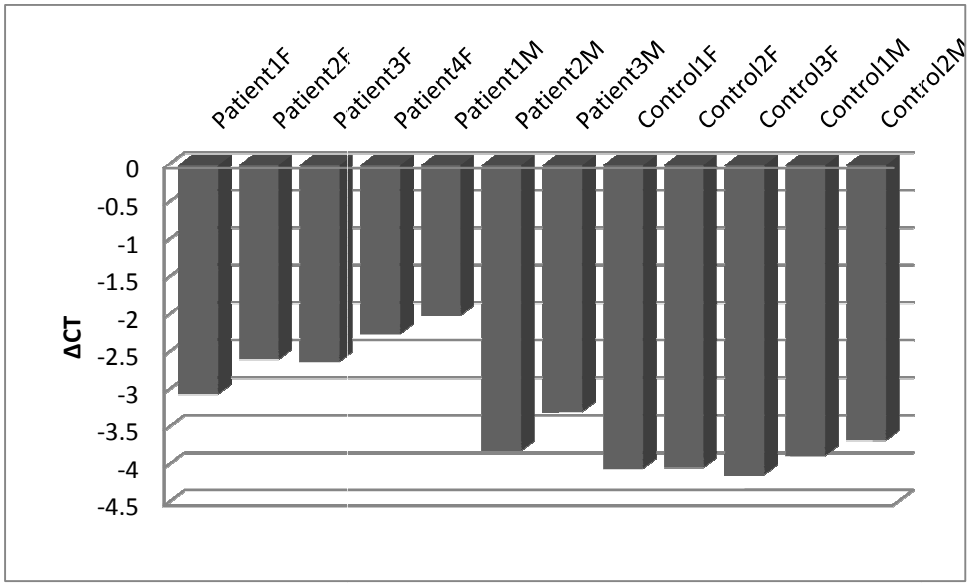

Figure A.16 RSAD2 ΔC_T values (F: Female, M: Male)

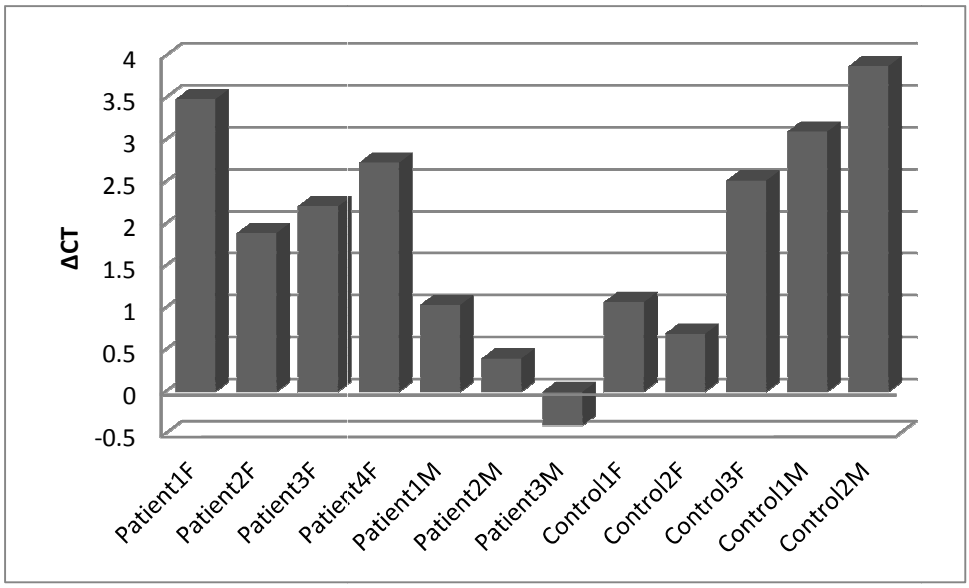

Figure A.17 SUB1 ΔC_T values (F: Female, M: Male)

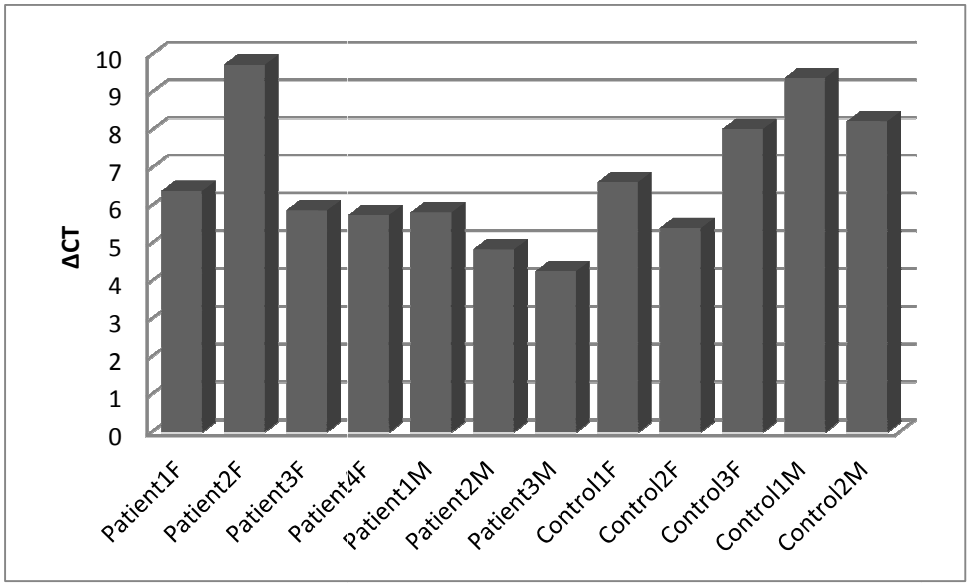

Figure A.18 WIF1 ΔC_T values (F: Female, M: Male)

Appendix B Supplementary results associated with computational analyses

Table B.1 Differentially expressed genes of rheumatoid arthritis (Core DEGs are marked with a *)

Protein Symbol	**Protein Name**
STAT1*	Signal transducer and activator of transcription 1
BTN3A3*	Butyrophilin, subfamily 3, member A3
CD52*	CD52 molecule
MAFF*	V-maf avian musculoaponeurotic fibrosarcoma oncogene homolog F
ACP5	acid phosphatase 5, tartrate resistant
ADM	Adrenomedullin
ADORA3	adenosine A3 receptor
AQP3	aquaporin 3 (Gill blood group)
ATF3	activating transcription factor 3
BLNK	B-cell linker
CCL20	chemokine (C-C motif) ligand 20
CD27	CD27 molecule
CD33	CD33 molecule
CD37	CD37 molecule
CD4	CD4 molecule
CD48	CD48 molecule
CD53	CD53 molecule
CDK14	cyclin-dependent kinase 14
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
CEBPD	CCAAT/enhancer binding protein (C/EBP), delta
KIAA1199	KIAA1199 ortholog
CLK1	CDC-like kinase 1
CORO1A	coronin, actin binding protein, 1A
CPVL	carboxypeptidase, vitellogenic-like
CREB5	cAMP responsive element binding protein 5
CRIP1	cysteine-rich protein 1 (intestinal)
CTSK	cathepsin K
CTSZ	cathepsin Z
DDIT3	DNA-damage-inducible transcript 3
DDIT4	DNA-damage-inducible transcript 4

DPYSL3	dihydropyrimidinase-like 3
DUSP4	dual specificity phosphatase 4
EGR2	early growth response 2
ETS2	v-ets avian erythroblastosis virus E26 oncogene homolog 2
FBP1	fructose-1,6-bisphosphatase 1
FCGR1A	Fc fragment of IgG, high affinity Ia, receptor (CD64)
FCGR3A	Fc fragment of IgG, low affinity IIIa, receptor (CD16a)
FCGRT	Fc fragment of IgG, receptor, transporter, alpha
FOLR2	folate receptor 2 (fetal)
FOXO3	forkhead box O3
FUCA1	fucosidase, alpha-L- 1, tissue
G0S2	G0/G1switch 2
GADD45A	growth arrest and DNA-damage-inducible, alpha
GADD45B	growth arrest and DNA-damage-inducible, beta
GALC	Galactosylceramidase
GLB1	GLNB1-like protein
GM2A	GM2 ganglioside activator
H1FX	H1 histone family, member X
HCK	hemopoietic cell kinase
HEG1	heart development protein with EGF-like domains 1
HEXA	hexosaminidase A (alpha polypeptide)
HLA-DMB	major histocompatibility complex, class II, DM beta
HLA-DQB1	major histocompatibility complex, class II, DQ beta 1
HMOX1	heme oxygenase (decycling) 1
HSPA1B	heat shock 70kDa protein 1B
HSPA6	heat shock 70kDa protein 6 (HSP70B')
IFI27	interferon, alpha-inducible protein 27
IFRD1	interferon-related developmental regulator 1
IL10RA	interleukin 10 receptor, alpha
ITGAM	integrin, alpha M (complement component 3 receptor 3 subunit)
KIAA0101	KIAA0101 ortholog
KLF9	Kruppel-like factor 9
LAIR1	leukocyte-associated immunoglobulin-like receptor 1
LCP2	lymphocyte cytosolic protein 2 (SH2 domain containing leukocyte protein of 76kDa)
LILRB4	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 4
LPL	lipoprotein lipase

LY86	lymphocyte antigen 86
MMP1	matrix metallopeptidase 1 (interstitial collagenase)
MMP3	matrix metallopeptidase 3 (stromelysin 1, progelatinase)
MNDA	myeloid cell nuclear differentiation antigen
MT1X	metallothionein 1X
MTHFD2	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase
MYOF	Myoferlin
NAMPT	nicotinamide phosphoribosyltransferase
NFIL3	nuclear factor, interleukin 3 regulated
NREP	neuronal regeneration related protein
OLR1	oxidized low density lipoprotein (lectin-like) receptor 1
PHKB	phosphorylase kinase, beta
PIM1	pim-1 oncogene
PIM2	pim-2 oncogene
PLA2G15	phospholipase A2, group XV
PLEK	Pleckstrin
PLIN2	perilipin 2
PLOD2	procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2
PLXNC1	plexin C1
PPARG	peroxisome proliferator-activated receptor gamma
PSD3	pleckstrin and Sec7 domain containing 3
PSMB9	proteasome (prosome, macropain) subunit, beta type, 9
PTGER3	prostaglandin E receptor 3 (subtype EP3)
PTPN22	protein tyrosine phosphatase, non-receptor type 22 (lymphoid)
PTX3	pentraxin 3, long
RAC2	ras-related C3 botulinum toxin substrate 2 (rho family, small GTP binding protein Rac2)
RARRES3	retinoic acid receptor responder (tazarotene induced) 3
RB1	retinoblastoma 1
RGS19	regulator of G-protein signaling 19
RPA1	replication protein A1, 70kDa
RPS4Y1	ribosomal protein S4, Y-linked 1
RRAD	Ras-related associated with diabetes
RTN1	reticulon 1
S100A8	S100 calcium binding protein A8
SEL1L3	sel-1 suppressor of lin-12-like 3 (C. elegans)
SEMA3C	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C

SEPT6	septin 6
SFPQ	splicing factor proline/glutamine-rich
SLC16A3	solute carrier family 16 (monocarboxylate transporter), member 3
SLC2A3	solute carrier family 2 (facilitated glucose transporter), member 3
SLC5A3	solute carrier family 5 (sodium/myo-inositol cotransporter), member 3
SLC7A5	solute carrier family 7 (amino acid transporter light chain, L system), member 5
SLCO2B1	solute carrier organic anion transporter family, member 2B1
TNFAIP3	tumor necrosis factor, alpha-induced protein 3
TSC22D3	TSC22 domain family, member 3
TTC3	tetratricopeptide repeat domain 3
UCP2	uncoupling protein 2 (mitochondrial, proton carrier)
VCAN	Versican
VEGFA	vascular endothelial growth factor A
ZFP36L2	ZFP36 ring finger protein-like 2
FCGR1B	Fc fragment of IgG, high affinity Ib, receptor (CD64)
HSPA1A	heat shock 70kDa protein 1A
LOC401317	uncharacterized LOC401317
TTC3P1	tetratricopeptide repeat domain 3 pseudogene 1
FCGR1C	Fc fragment of IgG, high affinity Ic, receptor (CD64), pseudogene

Table B.2 Differentially expressed genes of atopic dermatitis

Protein Symbol	Protein Name
ACOT2	acyl-CoA thioesterase 2
C5orf20	dendritic cell-associated nuclear protein
DEFB4B	defensin, beta 4B
KRT6B	keratin 6B, type II
KRT6C	keratin 6C, type II
MIR155HG	MIR155 host gene
MIR1908	microRNA 1908
PIK3R2	phosphoinositide-3-kinase, regulatory subunit 2 (beta)
SLC35F6	solute carrier family 35, member F6
UGT1A1	UDP glucuronosyltransferase 1 family, polypeptide A1
UGT1A10	UDP glucuronosyltransferase 1 family, polypeptide A10
UGT1A3	UDP glucuronosyltransferase 1 family, polypeptide A3
UGT1A4	UDP glucuronosyltransferase 1 family, polypeptide A4

UGT1A5	UDP glucuronosyltransferase 1 family, polypeptide A5
UGT1A6	UDP glucuronosyltransferase 1 family, polypeptide A6
UGT1A7	UDP glucuronosyltransferase 1 family, polypeptide A7
UGT1A8	UDP glucuronosyltransferase 1 family, polypeptide A8
UGT1A9	UDP glucuronosyltransferase 1 family, polypeptide A9
VMP1	vacuole membrane protein 1
ACAA2	acetyl-CoA acyltransferase 2
ACADL	acyl-CoA dehydrogenase, long chain
ACOT1	acyl-CoA thioesterase 1
ACOX2	acyl-CoA oxidase 2, branched chain
ACSL1	acyl-CoA synthetase long-chain family member 1
ACSS3	acyl-CoA synthetase short-chain family member 3
ADAM19	ADAM metallopeptidase domain 19
ADAM8	ADAM metallopeptidase domain 8
ADRB1	adrenoceptor beta 1
AKR1B10	aldo-keto reductase family 1, member B10 (aldose reductase)
ALDH1A1	aldehyde dehydrogenase 1 family, member A1
AMICA1	adhesion molecule, interacts with CXADR antigen 1
AMMECR1	Alport syndrome, mental retardation, midface hypoplasia and elliptocytosis chromosomal region gene 1
APOBEC3A	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A
APOBEC3B	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B
APOL6	apolipoprotein L, 6
AQP3	aquaporin 3 (Gill blood group)
ARHGAP29	Rho GTPase activating protein 29
ARHGAP9	Rho GTPase activating protein 9
ARNTL2	aryl hydrocarbon receptor nuclear translocator-like 2
ASPM	asp (abnormal spindle) homolog, microcephaly associated (Drosophila)
ATP1A2	ATPase, Na+/K+ transporting, alpha 2 polypeptide
BAIAP2	BAI1-associated protein 2
BCL3	B-cell CLL/lymphoma 3
BIRC3	baculoviral IAP repeat containing 3
BUB1	BUB1 mitotic checkpoint serine/threonine kinase
CACNB4	calcium channel, voltage-dependent, beta 4 subunit
CAPG	capping protein (actin filament), gelsolin-like
CARD9	caspase recruitment domain family, member 9
CASC5	cancer susceptibility candidate 5

CCL18	chemokine (C-C motif) ligand 18 (pulmonary and activation-regulated)
CCL2	chemokine (C-C motif) ligand 2
CCL22	chemokine (C-C motif) ligand 22
CCL26	chemokine (C-C motif) ligand 26
CCNB1	cyclin B1
CCNE2	cyclin E2
CCR7	chemokine (C-C motif) receptor 7
CD1B	CD1b molecule
CD274	CD274 molecule
CD3D	CD3d molecule, delta (CD3-TCR complex)
CD47	CD47 molecule
CD96	CD96 molecule
CDC25B	cell division cycle 25B
CDH3	cadherin 3, type 1, P-cadherin (placental)
CDK5R1	cyclin-dependent kinase 5, regulatory subunit 1 (p35)
CENPA	centromere protein A
CEP55	centrosomal protein 55kDa
CFB	complement factor B
CH25H	cholesterol 25-hydroxylase
CHEK1	checkpoint kinase 1
CIDEA	cell death-inducing DFFA-like effector a
CIDEC	cell death-inducing DFFA-like effector c
CLEC10A	C-type lectin domain family 10, member A
CLEC7A	C-type lectin domain family 7, member A
CLMP	CXADR-like membrane protein
COL4A4	collagen, type IV, alpha 4
COL6A5	collagen, type VI, alpha 5
COL6A6	collagen, type VI, alpha 6
COTL1	coactosin-like F-actin binding protein 1
CTLA4	cytotoxic T-lymphocyte-associated protein 4
CTSC	cathepsin C
CXCL1	chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)
CYB5A	cytochrome b5 type A (microsomal)
CYLD	cylindromatosis (turban tumor syndrome)
CYP7B1	cytochrome P450, family 7, subfamily B, polypeptide 1
DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58
DDX60L	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60-like

DEFB4A	defensin, beta 4A
DGAT2	diacylglycerol O-acyltransferase 2
DHCR7	7-dehydrocholesterol reductase
DIO2	deiodinase, iodothyronine, type II
DLGAP5	discs, large (Drosophila) homolog-associated protein 5
DNASE1L3	deoxyribonuclease I-like 3
DOCK10	dedicator of cytokinesis 10
DSG3	desmoglein 3
DTX3L	deltex 3 like, E3 ubiquitin ligase
DUOX1	dual oxidase 1
DUOXA1	dual oxidase maturation factor 1
ECT2	epithelial cell transforming 2
ELOVL3	ELOVL fatty acid elongase 3
ELOVL5	ELOVL fatty acid elongase 5
EPSTI1	epithelial stromal interaction 1 (breast)
F12	coagulation factor XII (Hageman factor)
FABP7	fatty acid binding protein 7, brain
FADS1	fatty acid desaturase 1
FAM110C	family with sequence similarity 110, member C
FAM83D	family with sequence similarity 83, member D
FAR2	fatty acyl CoA reductase 2
FBP1	fructose-1,6-bisphosphatase 1
FCER1A	Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide
FGL2	fibrinogen-like 2
G0S2	G0/G1 switch 2
GAL	galanin/GMAP prepropeptide
GALNT6	polypeptide N-acetylgalactosaminyltransferase 6
GBP1	guanylate binding protein 1, interferon-inducible
GNA15	guanine nucleotide binding protein (G protein), alpha 15 (Gq class)
GPAM	glycerol-3-phosphate acyltransferase, mitochondrial
GPD1	glycerol-3-phosphate dehydrogenase 1 (soluble)
GPR171	G protein-coupled receptor 171
GPR68	G protein-coupled receptor 68
GSDMC	gasdermin C
GZMB	granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)
HAS3	hyaluronan synthase 3
HERC6	HECT and RLD domain containing E3 ubiquitin protein ligase family member 6

HIVEP3	human immunodeficiency virus type I enhancer binding protein 3
HN1	hematological and neurological expressed 1
HRH1	histamine receptor H1
HS3ST3A1	heparan sulfate (glucosamine) 3-O-sulfotransferase 3A1
HS3ST3B1	heparan sulfate (glucosamine) 3-O-sulfotransferase 3B1
HSD11B1	hydroxysteroid (11-beta) dehydrogenase 1
IFI16	interferon, gamma-inducible protein 16
IFI27	interferon, alpha-inducible protein 27
IFI30	interferon, gamma-inducible protein 30
IFI44	interferon-induced protein 44
IFI6	interferon, alpha-inducible protein 6
IFIH1	interferon induced with helicase C domain 1
IL12RB2	interleukin 12 receptor, beta 2
IL13RA2	interleukin 13 receptor, alpha 2
IL26	interleukin 26
IL32	interleukin 32
IL4I1	interleukin 4 induced 1
IL4R	interleukin 4 receptor
IL7R	interleukin 7 receptor
INPP1	inositol polyphosphate-1-phosphatase
INSIG1	insulin induced gene 1
IRF1	interferon regulatory factor 1
IRF7	interferon regulatory factor 7
ISG15	ISG15 ubiquitin-like modifier
ITGAL	integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)
ITK	IL2-inducible T-cell kinase
JAK3	Janus kinase 3
JUNB	jun B proto-oncogene
KANK4	KN motif and ankyrin repeat domains 4
KCNK6	potassium channel, two pore domain subfamily K, member 6
KIF14	kinesin family member 14
KIF20A	kinesin family member 20A
KLB	klotho beta
KREMEN2	kringle containing transmembrane protein 2
KRT16	keratin 16, type I
KRT6A	keratin 6A, type II

KRT79	keratin 79, type II
KYNU	Kynureninase
LAMP3	lysosomal-associated membrane protein 3
LCE3D	late cornified envelope 3D
LCK	LCK proto-oncogene, Src family tyrosine kinase
LINC00518	long intergenic non-protein coding RNA 518
LOC101929623	LINC01215/long intergenic non-protein coding RNA 1215
LTB	lymphotoxin beta (TNF superfamily, member 3)
LY6D	lymphocyte antigen 6 complex, locus D
MACF1	microtubule-actin crosslinking factor 1
MAP3K14	mitogen-activated protein kinase kinase kinase 14
MAPK1	mitogen-activated protein kinase 1
ME1	malic enzyme 1, NADP(+)-dependent, cytosolic
MFHAS1	malignant fibrous histiocytoma amplified sequence 1
MGST1	microsomal glutathione S-transferase 1
MIAT	myocardial infarction associated transcript (non-protein coding)
MICB	MHC class I polypeptide-related sequence B
MIR155	microRNA 155
MIR21	microRNA 21
MKI67	marker of proliferation Ki-67
MMP1	matrix metallopeptidase 1
MMP12	matrix metallopeptidase 12
MPZL2	myelin protein zero-like 2
MSMO1	methylsterol monooxygenase 1
MX1	MX dynamin-like GTPase 1
MYO5A	myosin VA (heavy chain 12, myoxin)
N4BP1	NEDD4 binding protein 1
NABP1	nucleic acid binding protein 1
NAPSB	napsin B aspartic peptidase, pseudogene
NCAPG	non-SMC condensin I complex, subunit G
NLRC5	NLR family, CARD domain containing 5
NMI	N-myc (and STAT) interactor
NOD2	nucleotide-binding oligomerization domain containing 2
OAS1	2'-5'-oligoadenylate synthetase 1, 40/46kDa
OAS3	2'-5'-oligoadenylate synthetase 3, 100kDa
OASL	2'-5'-oligoadenylate synthetase-like
ODF3B	outer dense fiber of sperm tails 3B

OSMR	oncostatin M receptor
PANX1	pannexin 1
PARP12	poly (ADP-ribose) polymerase family, member 12
PARP14	poly (ADP-ribose) polymerase family, member 14
PARP9	poly (ADP-ribose) polymerase family, member 9
PCDH7	protocadherin 7
PCK1	phosphoenolpyruvate carboxykinase 1 (soluble)
PDZK1	PDZ domain containing 1
PECR	peroxisomal trans-2-enoyl-CoA reductase
PGM2	phosphoglucomutase 2
PLA2G16	phospholipase A2, group XVI
PLA2G3	phospholipase A2, group III
PLAU	plasminogen activator, urokinase
PLIN4	perilipin 4
PLSCR1	phospholipid scramblase 1
PNPLA3	patatin-like phospholipase domain containing 3
POLR3G	polymerase (RNA) III (DNA directed) polypeptide G (32kD)
PPARG	peroxisome proliferator-activated receptor gamma
PPP1R1A	protein phosphatase 1, regulatory (inhibitor) subunit 1A
PPP4R1	protein phosphatase 4, regulatory subunit 1
PRR11	proline rich 11
PRRG4	proline rich Gla (G-carboxyglutamic acid) 4 (transmembrane)
PRSS53	protease, serine, 53
PSMB10	proteasome (prosome, macropain) subunit, beta type, 10
PSMB8	proteasome (prosome, macropain) subunit, beta type, 8
PSMB9	proteasome (prosome, macropain) subunit, beta type, 9
PTGES	prostaglandin E synthase
PYCARD	PYD and CARD domain containing
RAB27A	RAB27A, member RAS oncogene family
RAB31	RAB31, member RAS oncogene family
RASGRP1	RAS guanyl releasing protein 1 (calcium and DAG-regulated)
RBP4	retinol binding protein 4, plasma
RGS1	regulator of G-protein signaling 1
RRM2	ribonucleotide reductase M2
RTP4	receptor (chemosensory) transporter protein 4
SAMD9	sterile alpha motif domain containing 9
SAMSN1	SAM domain, SH3 domain and nuclear localization signals 1

SBNO2	strawberry notch homolog 2 (Drosophila)
SCN7A	sodium channel, voltage gated, type VII alpha subunit
SCO2	SCO2 cytochrome c oxidase assembly protein
SECTM1	secreted and transmembrane 1
SELE	selectin E
SELPLG	selectin P ligand
SERPINB13	serpin peptidase inhibitor, clade B (ovalbumin), member 13
SFN	Stratifin
SLAMF1	signaling lymphocytic activation molecule family member 1
SLAMF7	SLAM family member 7
SLAMF8	SLAM family member 8
SLC16A14	solute carrier family 16, member 14
SLC35E4	solute carrier family 35, member E4
SNX20	sorting nexin 20
SOAT1	sterol O-acyltransferase 1
SOCS3	suppressor of cytokine signaling 3
SORBS1	sorbin and SH3 domain containing 1
SOX7	SRY (sex determining region Y)-box 7
C12orf39	spexin hormone
ST14	suppression of tumorigenicity 14 (colon carcinoma)
STAT1	signal transducer and activator of transcription 1, 91kDa
STYK1	serine/threonine/tyrosine kinase 1
TAP1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)
TAP2	transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)
TEAD4	TEA domain family member 4
TF	Transferin
THRSP	thyroid hormone responsive
TIFAB	TRAF-interacting protein with forkhead-associated domain, family member B
C12orf5	Chromosome 12 Open Reading Frame 5
TMC5	transmembrane channel-like 5
TMEM56	transmembrane protein 56
TMPRSS4	transmembrane protease, serine 4
TNC	tenascin C
TNFSF10	tumor necrosis factor (ligand) superfamily, member 10
TNMD	Tenomodulin
TPBG	trophoblast glycoprotein
TRBC1	T cell receptor beta constant 1

TSTA3	tissue specific transplantation antigen P35B
TTC39A	tetratricopeptide repeat domain 39A
TYMP	thymidine phosphorylase
UHRF1	ubiquitin-like with PHD and ring finger domains 1
VSNL1	visinin-like 1
WNT5A	wingless-type MMTV integration site family, member 5A
XAF1	XIAP associated factor 1
ZBTB16	zinc finger and BTB domain containing 16
ZC3H12D	zinc finger CCCH-type containing 12D
ZC3HAV1	zinc finger CCCH-type, antiviral 1

Table B.3 Differentiall expressed genes of systemic lupus erythematosus

Protein Symbol	**Protein Name**
AGTRAP	angiotensin II receptor-associated protein
ALPK1	alpha-kinase 1
ANKRD22	ankyrin repeat domain 22
ARNTL2	aryl hydrocarbon receptor nuclear translocator-like 2
BATF2	basic leucine zipper transcription factor, ATF-like 2
C5orf56	chromosome 5 open reading frame 56
CCDC71L	coiled-coil domain containing 71-like
CD274	CD274 molecule
CMPK2	cytidine monophosphate (UMP-CMP) kinase 2, mitochondr
CRIPT	cysteine-rich PDZ-binding protein
CYSLTR1	cysteinyl leukotriene receptor 1
CYSTM1	cysteine-rich transmembrane module containing 1
DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58
DDX60L	DEAD (Asp-Glu-Ala-Asp) box polypeptide 60-like
DPH3	diphthamide biosynthesis 3
E2F2	E2F transcription factor 2
EFHD2	EF-hand domain family, member D2

EPSTI1	epithelial stromal interaction 1 (breast)
FAR2	fatty acyl CoA reductase 2
FBXO6	F-box protein 6
FNDC3B	fibronectin type III domain containing 3B
GBP1	guanylate binding protein 1, interferon-inducible
GBP2	guanylate binding protein 2, interferon-inducible
GBP5	guanylate binding protein 5
GPR84	G protein-coupled receptor 84
HELZ2	helicase with zinc finger 2, transcriptional coactiva
IFIT2	interferon-induced protein with tetratricopeptide rep
IFIT3	interferon-induced protein with tetratricopeptide rep
KLHDC7B	kelch domain containing 7B
LINC00487	long intergenic non-protein coding RNA 487
LINC00537	long intergenic non-protein coding RNA 537
C19orf59	mast cell-expressed membrane protein 1
MOV10	Mov10 RISC complex RNA helicase
MRPS18C	mitochondrial ribosomal protein S18C
NEXN	nexilin (F actin binding protein)
OAS2	2'-5'-oligoadenylate synthetase 2, 69/71kDa
OAS3	2'-5'-oligoadenylate synthetase 3, 100kDa
ODF3B	outer dense fiber of sperm tails 3B
PARP9	poly (ADP-ribose) polymerase family, member 9
PATL1	protein associated with topoisomerase II homolog 1 (y
PLEKHF2	pleckstrin homology domain containing, family F (with
RNF213	ring finger protein 213
RSAD2	radical S-adenosyl methionine domain containing 2
SAMD9L	sterile alpha motif domain containing 9-like
SIPA1L2	signal-induced proliferation-associated 1 like 2
SLC26A8	solute carrier family 26 (anion exchanger), member 8
SP110	SP110 nuclear body protein
SRGAP2D	SLIT-ROBO Rho GTPase activating protein 2D (pseudogen
STAT1	signal transducer and activator of transcription 1,

	9
TMTC1	transmembrane and tetratricopeptide repeat containing
TRIM25	tripartite motif containing 25
USP25	ubiquitin specific peptidase 25
XAF1	XIAP associated factor 1
ZBP1	Z-DNA binding protein 1
ZNFX1	zinc finger, NFX1-type containing 1
SRGAP2	SLIT-ROBO Rho GTPase activating protein 2

Table B.4 Biological process enrichment results of atopic dermatitis

Term	**PValue**
GO:0002376~immune system process	7.21E-18
GO:0006955~immune response	6.17E-17
GO:0050896~response to stimulus	9.20E-13
GO:0051707~response to other organism	4.31E-11
GO:0009607~response to biotic stimulus	1.04E-10
GO:0006952~defense response	9.31E-10
GO:0009605~response to external stimulus	1.50E-09
GO:0051704~multi-organism process	1.18E-08
GO:0048518~positive regulation of biological process	3.68E-08
GO:0042221~response to chemical stimulus	4.82E-08
GO:0006950~response to stres	5.73E-08
GO:0032787~monocarboxylic acid metabolic process	8.00E-08
GO:0009615~response to virus	1.69E-07
GO:0010033~response to organic substance	4.90E-07
GO:0048583~regulation of response to stimulus	1.63E-06
GO:0006629~lipid metabolic process	2.15E-06
GO:0031347~regulation of defense response	3.18E-06
GO:0006631~fatty acid metabolic process	3.68E-06
GO:0002682~regulation of immune system process	3.88E-06
GO:0048522~positive regulation of cellular process	6.00E-06
GO:0002237~response to molecule of bacterial origin	9.30E-06
GO:0042493~response to drug	1.00E-05
GO:0050776~regulation of immune response	1.68E-05

GO:0002684~positive regulation of immune system process	2.97×10^{-5}
GO:0032868~response to insulin stimulus	3.17×10^{-5}
GO:0001817~regulation of cytokine production	3.49×10^{-5}
GO:0051240~positive regulation of multicellular organismal process	3.92×10^{-5}
GO:0009611~response to wounding	4.29×10^{-5}
GO:0009617~response to bacterium	6.53×10^{-5}
GO:0006954~inflammatory response	7.09×10^{-5}
GO:0007584~response to nutrient	8.48×10^{-5}
GO:0044255~cellular lipid metabolic process	1.12×10^{-4}
GO:0043434~response to peptide hormone stimulus	1.87×10^{-4}
GO:0008610~lipid biosynthetic process	2.24×10^{-4}
GO:0032496~response to lipopolysaccharide	2.28×10^{-4}
GO:0043436~oxoacid metabolic process	2.37×10^{-4}
GO:0019752~carboxylic acid metabolic process	2.37×10^{-4}
GO:0006935~chemotaxis	2.55×10^{-4}
GO:0042330~taxis	2.55×10^{-4}
GO:0006082~organic acid metabolic process	2.64×10^{-4}
GO:0042180~cellular ketone metabolic process	3.12×10^{-4}
GO:0019432~triglyceride biosynthetic process	3.17×10^{-4}
GO:0031667~response to nutrient levels	3.36×10^{-4}
GO:0045087~innate immune response	3.78×10^{-4}
GO:0080134~regulation of response to stres	4.77×10^{-4}
GO:0050778~positive regulation of immune response	5.43×10^{-4}
GO:0043065~positive regulation of apoptosis	5.69×10^{-4}
GO:0001819~positive regulation of cytokine production	5.95×10^{-4}
GO:0043330~response to exogenous dsRNA	6.08×10^{-4}
GO:0043068~positive regulation of programmed cell death	6.15×10^{-4}
GO:0033273~response to vitamin	6.43×10^{-4}
GO:0010942~positive regulation of cell death	6.47×10^{-4}
GO:0006917~induction of apoptosis	6.57×10^{-4}
GO:0008283~cell proliferation	6.71×10^{-4}
GO:0012502~induction of programmed cell death	6.77×10^{-4}
GO:0001775~cell activation	7.41×10^{-4}
GO:0046460~neutral lipid biosynthetic process	8.01×10^{-4}
GO:0046463~acylglycerol biosynthetic process	8.01×10^{-4}
GO:0009991~response to extracellular stimulus	8.48×10^{-4}
GO:0042110~T cell activation	9.49×10^{-4}
GO:0046504~glycerol ether biosynthetic process	0.001029

GO:0051239~regulation of multicellular organismal process	0.001041
GO:0031349~positive regulation of defense response	0.001098
GO:0042981~regulation of apoptosis	0.001128
GO:0043067~regulation of programmed cell death	0.001295
GO:0010941~regulation of cell death	0.001362
GO:0046649~lymphocyte activation	0.001393
GO:0019319~hexose biosynthetic process	0.001434
GO:0032680~regulation of tumor necrosis factor production	0.001434
GO:0055114~oxidation reduction	0.001448
GO:0048584~positive regulation of response to stimulus	0.001492
GO:0008202~steroid metabolic process	0.001557
GO:0007626~locomotory behavior	0.001558
GO:0034097~response to cytokine stimulus	0.001655
GO:0006633~fatty acid biosynthetic process	0.001655
GO:0045321~leukocyte activation	0.001833
GO:0042127~regulation of cell proliferation	0.001838
GO:0007155~cell adhesion	0.001963
GO:0022610~biological adhesion	0.001998
GO:0002683~negative regulation of immune system process	0.002132
GO:0009719~response to endogenous stimulus	0.002259
GO:0009725~response to hormone stimulus	0.002402
GO:0046364~monosaccharide biosynthetic process	0.002792
GO:0070201~regulation of establishment of protein localization	0.003318
GO:0016053~organic acid biosynthetic process	0.003505
GO:0046394~carboxylic acid biosynthetic process	0.003505
GO:0040011~locomotion	0.00405
GO:0002673~regulation of acute inflammatory response	0.004353
GO:0033189~response to vitamin A	0.004447
GO:0046165~alcohol biosynthetic process	0.005261
GO:0016125~sterol metabolic process	0.00565
GO:0002697~regulation of immune effector process	0.00565
GO:0044419~interspecies interaction between organisms	0.005971
GO:0051716~cellular response to stimulus	0.00633
GO:0009628~response to abiotic stimulus	0.006432
GO:0032880~regulation of protein localization	0.006785
GO:0006094~gluconeogenesis	0.007182
GO:0043331~response to dsRNA	0.007182
GO:0050727~regulation of inflammatory response	0.007402

GO:0006066~alcohol metabolic process	0.009322
GO:0002757~immune response-activating signal transduction	0.009502
GO:0051050~positive regulation of transport	0.009854
GO:0007159~leukocyte adhesion	0.009876
GO:0051223~regulation of protein transport	0.010028
GO:0009266~response to temperature stimulus	0.010637
GO:0019882~antigen processing and presentation	0.010637
GO:0002822~regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	0.011535
GO:0006694~steroid biosynthetic process	0.011714
GO:0050707~regulation of cytokine secretion	0.011957
GO:0002819~regulation of adaptive immune response	0.01227
GO:0002764~immune response-regulating signal transduction	0.01227
GO:0014070~response to organic cyclic substance	0.01319
GO:0008285~negative regulation of cell proliferation	0.013588
GO:0016337~cell-cell adhesion	0.013631
GO:0050708~regulation of protein secretion	0.013828
GO:0032101~regulation of response to external stimulus	0.014127
GO:0050718~positive regulation of interleukin-1 beta secretion	0.015154
GO:0008630~DNA damage response, signal transduction resulting in induction of apoptosis	0.015516
GO:0008203~cholesterol metabolic process	0.016064
GO:0000278~mitotic cell cycle	0.016253
GO:0007267~cell-cell signaling	0.01641
GO:0051241~negative regulation of multicellular organismal process	0.0165
GO:0002253~activation of immune response	0.017481
GO:0002694~regulation of leukocyte activation	0.017584
GO:0002286~T cell activation during immune response	0.017722
GO:0050706~regulation of interleukin-1 beta secretion	0.017722
GO:0016126~sterol biosynthetic process	0.018185
GO:0002521~leukocyte differentiation	0.018831
GO:0002263~cell activation during immune response	0.019609
GO:0002366~leukocyte activation during immune response	0.019609
GO:0043123~positive regulation of I-kappaB kinase/NF-kappaB cascade	0.019757
GO:0030217~T cell differentiation	0.020248
GO:0050716~positive regulation of interleukin-1 secretion	0.02046
GO:0032731~positive regulation of interleukin-1 beta production	0.02046
GO:0002252~immune effector process	0.020802

GO:0048585~negative regulation of response to stimulus	0.022219
GO:0051222~positive regulation of protein transport	0.022368
GO:0050865~regulation of cell activation	0.022657
GO:0050704~regulation of interleukin-1 secretion	0.023362
GO:0032760~positive regulation of tumor necrosis factor production	0.023362
GO:0032869~cellular response to insulin stimulus	0.023477
GO:0031646~positive regulation of neurological system process	0.02424
GO:0007259~JAK-STAT cascade	0.02424
GO:0002429~immune response-activating cell surface receptor signaling pathway	0.02424
GO:0048534~hemopoietic or lymphoid organ development	0.024396
GO:0034637~cellular carbohydrate biosynthetic process	0.024618
GO:0031099~regeneration	0.024618
GO:0019221~cytokine-mediated signaling pathway	0.025793
GO:0050714~positive regulation of protein secretion	0.027627
GO:0043122~regulation of I-kappaB kinase/NF-kappaB cascade	0.028715
GO:0002768~immune response-regulating cell surface receptor signaling pathway	0.02941
GO:0006090~pyruvate metabolic process	0.02941
GO:0051591~response to cAMP	0.02941
GO:0032655~regulation of interleukin-12 production	0.029633
GO:0032732~positive regulation of interleukin-1 production	0.029633
GO:0042035~regulation of cytokine biosynthetic process	0.030825
GO:0006641~triglyceride metabolic process	0.031253
GO:0051249~regulation of lymphocyte activation	0.031847
GO:0048519~negative regulation of biological process	0.032257
GO:0002285~lymphocyte activation during immune response	0.032991
GO:0002520~immune system development	0.03397
GO:0019216~regulation of lipid metabolic process	0.034021
GO:0032270~positive regulation of cellular protein metabolic process	0.034241
GO:0000038~very-long-chain fatty acid metabolic process	0.036491
GO:0045089~positive regulation of innate immune response	0.037135
GO:0043281~regulation of caspase activity	0.037874
GO:0042770~DNA damage response, signal transduction	0.039386
GO:0007610~behavior	0.039469
GO:0050863~regulation of T cell activation	0.039898
GO:0032651~regulation of interleukin-1 beta production	0.040126
GO:0032570~response to progesterone stimulus	0.040126
GO:0051726~regulation of cell cycle	0.040676
GO:0042108~positive regulation of cytokine biosynthetic process	0.041349

GO:0051247~positive regulation of protein metabolic process	0.042014
GO:0009314~response to radiation	0.042087
GO:0052548~regulation of endopeptidase activity	0.042512
GO:0007049~cell cycle	0.043264
GO:0006639~acylglycerol metabolic process	0.043542
GO:0009409~response to cold	0.043891
GO:0019835~cytolysis	0.043891
GO:0006638~neutral lipid metabolic process	0.045792
GO:0051049~regulation of transport	0.047523
GO:0006662~glycerol ether metabolic process	0.048099
GO:0051301~cell division	0.048183
GO:0052547~regulation of peptidase activity	0.049171

Table B.5 Biological process enrichment results of differentially expressed genes of rheumatoid arthritis

Term	**PValue**
GO:0050896~response to stimulus	4.76×10^{-11}
GO:0002376~immune system process	1.33×10^{-9}
GO:0006955~immune response	1.14×10^{-8}
GO:0042221~response to chemical stimulus	7.86×10^{-8}
GO:0006950~response to stres	2.42×10^{-7}
GO:0010033~response to organic substance	9.50×10^{-6}
GO:0048871~multicellular organismal homeostasis	4.92×10^{-5}
GO:0009605~response to external stimulus	6.64×10^{-5}
GO:0001894~tissue homeostasis	1.23×10^{-4}
GO:0042592~homeostatic process	2.03×10^{-4}
GO:0006952~defense response	2.68×10^{-4}
GO:0002682~regulation of immune system process	7.84×10^{-4}
GO:0048771~tissue remodeling	9.07×10^{-4}
GO:0010035~response to inorganic substance	0.00105
GO:0044092~negative regulation of molecular function	0.001117
GO:0046479~glycosphingolipid catabolic process	0.00121
GO:0019377~glycolipid catabolic process	0.00121
GO:0060249~anatomical structure homeostasis	0.001367
GO:0051789~response to protein stimulus	0.001425

GO:0030097~hemopoiesis	0.002348
GO:0002684~positive regulation of immune system process	0.002462
GO:0005975~carbohydrate metabolic process	0.002608
GO:0009611~response to wounding	0.002608
GO:0000302~response to reactive oxygen species	0.002684
GO:0048518~positive regulation of biological process	0.002807
GO:0044242~cellular lipid catabolic process	0.002817
GO:0008015~blood circulation	0.003186
GO:0003013~circulatory system process	0.003186
GO:0006954~inflammatory response	0.003675
GO:0048534~hemopoietic or lymphoid organ development	0.004013
GO:0006672~ceramide metabolic process	0.004131
GO:0051716~cellular response to stimulus	0.004188
GO:0070482~response to oxygen levels	0.004716
GO:0046519~sphingoid metabolic process	0.005021
GO:0002520~immune system development	0.005542
GO:0045453~bone resorption	0.00581
GO:0006909~phagocytosis	0.006019
GO:0051348~negative regulation of transferase activity	0.006485
GO:0048522~positive regulation of cellular process	0.006641
GO:0009991~response to extracellular stimulus	0.007164
GO:0046466~membrane lipid catabolic process	0.007449
GO:0030149~sphingolipid catabolic process	0.007449
GO:0048872~homeostasis of number of cells	0.007478
GO:0032868~response to insulin stimulus	0.007478
GO:0042127~regulation of cell proliferation	0.007555
GO:0019915~lipid storage	0.008339
GO:0042542~response to hydrogen peroxide	0.009227
GO:0008219~cell death	0.009442
GO:0016265~death	0.009943
GO:0050867~positive regulation of cell activation	0.010711
GO:0046849~bone remodeling	0.011274
GO:0050865~regulation of cell activation	0.011473
GO:0051239~regulation of multicellular organismal process	0.012944
GO:0009719~response to endogenous stimulus	0.013139
GO:0014070~response to organic cyclic substance	0.014333
GO:0008284~positive regulation of cell proliferation	0.014826
GO:0050793~regulation of developmental process	0.014932

GO:0007275~multicellular organismal development	0.015189
GO:0031099~regeneration	0.01623
GO:0065009~regulation of molecular function	0.016914
GO:0006687~glycosphingolipid metabolic process	0.017017
GO:0031100~organ regeneration	0.017017
GO:0048878~chemical homeostasis	0.017525
GO:0031667~response to nutrient levels	0.018293
GO:0008283~cell proliferation	0.019611
GO:0043066~negative regulation of apoptosis	0.019811
GO:0001666~response to hypoxia	0.020088
GO:0006665~sphingolipid metabolic process	0.020236
GO:0043086~negative regulation of catalytic activity	0.020412
GO:0043069~negative regulation of programmed cell death	0.021206
GO:0060548~negative regulation of cell death	0.021492
GO:0006916~anti-apoptosis	0.021721
GO:0045768~positive regulation of anti-apoptosis	0.022327
GO:0007584~response to nutrient	0.023161
GO:0034097~response to cytokine stimulus	0.023182
GO:0006664~glycolipid metabolic process	0.023748
GO:0001775~cell activation	0.023802
GO:0006643~membrane lipid metabolic process	0.024738
GO:0006351~transcription, DNA-dependent	0.025632
GO:0032501~multicellular organismal process	0.025924
GO:0042493~response to drug	0.025993
GO:0019882~antigen processing and presentation	0.026349
GO:0046942~carboxylic acid transport	0.027091
GO:0032774~RNA biosynthetic process	0.027161
GO:0015849~organic acid transport	0.027683
GO:0009607~response to biotic stimulus	0.029214
GO:0006469~negative regulation of protein kinase activity	0.029736
GO:0050790~regulation of catalytic activity	0.029892
GO:0006689~ganglioside catabolic process	0.030534
GO:0033554~cellular response to stres	0.030945
GO:0043434~response to peptide hormone stimulus	0.031397
GO:0065008~regulation of biological quality	0.032133
GO:0033673~negative regulation of kinase activity	0.032421
GO:0045767~regulation of anti-apoptosis	0.034675
GO:0006366~transcription from RNA polymerase II promoter	0.034965

GO:0051704~multi-organism process	0.037574
GO:0006979~response to oxidative stres	0.038212
GO:0031325~positive regulation of cellular metabolic process	0.038712
GO:0031328~positive regulation of cellular biosynthetic process	0.03886
GO:0051251~positive regulation of lymphocyte activation	0.039159
GO:0045321~leukocyte activation	0.0395
GO:0002694~regulation of leukocyte activation	0.039669
GO:0006090~pyruvate metabolic process	0.041635
GO:0009891~positive regulation of biosynthetic process	0.042206
GO:0006915~apoptosis	0.043146
GO:0016042~lipid catabolic process	0.045016
GO:0051329~interphase of mitotic cell cycle	0.045458
GO:0007050~cell cycle arrest	0.045458
GO:0012501~programmed cell death	0.046643
GO:0043433~negative regulation of transcription factor activity	0.047168
GO:0043085~positive regulation of catalytic activity	0.048019
GO:0051325~interphase	0.048784
GO:0002696~positive regulation of leukocyte activation	0.048784

Table B.6 Biological process bi-comparison of rheumatoid arthritis, atopic dermatitis and systemic lupus erythematosus

RA – SLE	GO:0002376~immune system process GO:0006955~immune response
SLE - AD	GO:0002376~immune system process GO:0006955~immune response GO:0009615~response to virus
RA-AD	GO:0002376~immune system process GO:0006955~immune response GO:0050896~response to stimulus GO:0009607~response to biotic stimulus GO:0006952~defense response GO:0009605~response to external stimulus GO:0051704~multi-organism process GO:0048518~positive regulation of biological process GO:0042221~response to chemical stimulus GO:0006950~response to stress

	GO:0010033~response to organic substance GO:0002682~regulation of immune system process GO:0048522~positive regulation of cellular process GO:0042493~response to drug GO:0002684~positive regulation of immune system process GO:0032868~response to insulin stimulus GO:0009611~response to wounding GO:0006954~inflammatory response GO:0007584~response to nutrient GO:0043434~response to peptide hormone stimulus GO:0031667~response to nutrient levels GO:0008283~cell proliferation GO:0001775~cell activation GO:0009991~response to extracellular stimulus GO:0051239~regulation of multicellular organismal process GO:0034097~response to cytokine stimulus GO:0045321~leukocyte activation GO:0042127~regulation of cell proliferation GO:0009719~response to endogenous stimulus GO:0051716~cellular response to stimulus GO:0019882~antigen processing and presentation GO:0014070~response to organic cyclic substance GO:0002694~regulation of leukocyte activation GO:0050865~regulation of cell activation GO:0048534~hemopoietic or lymphoid organ development GO:0031099~regeneration GO:0006090~pyruvate metabolic process GO:0002520~immune system development

Table B.7 Comparison of Biological Processes for psoriasis versus other autoimmune diseases

Psoriasis -SLE	GO:0002376~immune system process GO:0006955~immune response GO:0009615~response to virus
Psoriasis – RA	GO:0009607~response to biotic stimulus GO:0050896~response to stimulus

	GO:0002376~immune system process GO:0051704~multi-organism process GO:0006955~immune response GO:0006952~defense response GO:0042221~response to chemical stimulus GO:0009605~response to external stimulus GO:0006954~inflammatory response GO:0048518~positive regulation of biological process GO:0009611~response to wounding GO:0006950~response to stress GO:0010033~response to organic substance GO:0048522~positive regulation of cellular process GO:0042127~regulation of cell proliferation GO:0009719~response to endogenous stimulus GO:0008283~cell proliferation GO:0042493~response to drug GO:0034097~response to cytokine stimulus GO:0031667~response to nutrient levels GO:0051716~cellular response to stimulus GO:0009991~response to extracellular stimulus GO:0031099~regeneration GO:0051239~regulation of multicellular organismal process GO:0002682~regulation of immune system process GO:0002684~positive regulation of immune system process GO:0002694~regulation of leukocyte activation GO:0050865~regulation of cell activation GO:0043434~response to peptide hormone stimulus GO:0014070~response to organic cyclic substance GO:0007584~response to nutrient GO:0048534~hemopoietic or lymphoid organ development GO:0002520~immune system development GO:0001775~cell activation GO:0045321~leukocyte activation GO:0032868~response to insulin stimulus GO:0065009~regulation of molecular function GO:0008284~positive regulation of cell proliferation GO:0031100~organ regeneration GO:0050790~regulation of catalytic activity GO:0051251~positive regulation of lymphocyte activation

	GO:0044092~negative regulation of molecular function GO:0051329~interphase of mitotic cell cycle GO:0051325~interphase GO:0002696~positive regulation of leukocyte activation GO:0050867~positive regulation of cell activation GO:0043085~positive regulation of catalytic activity GO:0050793~regulation of developmental process GO:0043066~negative regulation of apoptosis GO:0043069~negative regulation of programmed cell death GO:0060548~negative regulation of cell death GO:0006916~anti-apoptosis GO:0031325~positive regulation of cellular metabolic process GO:0043433~negative regulation of transcription factor activity GO:0007275~multicellular organismal development GO:0065008~regulation of biological quality GO:0042592~homeostatic process GO:0032501~multicellular organismal process GO:0043086~negative regulation of catalytic activity GO:0008219~cell death GO:0016265~death GO:0030097~hemopoiesis GO:0006915~apoptosis GO:0012501~programmed cell death GO:0000302~response to reactive oxygen species GO:0048878~chemical homeostasis GO:0031328~positive regulation of cellular biosynthetic process GO:0009891~positive regulation of biosynthetic process GO:0048872~homeostasis of number of cells GO:0006979~response to oxidative stress GO:0006366~transcription from RNA polymerase II promoter
Psoriasis – AD	GO:0009607~response to biotic stimulus GO:0050896~response to stimulus GO:0002376~immune system process GO:0051704~multi-organism process GO:0006955~immune response GO:0006952~defense response GO:0042221~response to chemical stimulus

	GO:0009605~response to external stimulus GO:0006954~inflammatory response GO:0048518~positive regulation of biological process GO:0009611~response to wounding GO:0006950~response to stress GO:0010033~response to organic substance GO:0048522~positive regulation of cellular process GO:0042127~regulation of cell proliferation GO:0009719~response to endogenous stimulus GO:0008283~cell proliferation GO:0042493~response to drug GO:0034097~response to cytokine stimulus GO:0031667~response to nutrient levels GO:0051716~cellular response to stimulus GO:0009991~response to extracellular stimulus GO:0031099~regeneration GO:0051239~regulation of multicellular organismal process GO:0002682~regulation of immune system process GO:0002684~positive regulation of immune system process GO:0002694~regulation of leukocyte activation GO:0050865~regulation of cell activation GO:0043434~response to peptide hormone stimulus GO:0014070~response to organic cyclic substance GO:0007584~response to nutrient GO:0048534~hemopoietic or lymphoid organ development GO:0002520~immune system development GO:0001775~cell activation GO:0045321~leukocyte activation GO:0032868~response to insulin stimulus GO:0051707~response to other organism GO:0009615~response to virus GO:0009617~response to bacterium GO:0000278~mitotic cell cycle GO:0007049~cell cycle GO:0019221~cytokine-mediated signaling pathway GO:0009725~response to hormone stimulus GO:0048519~negative regulation of biological process GO:0002237~response to molecule of bacterial origin

	GO:0048583~regulation of response to stimulus GO:0042330~taxis GO:0006935~chemotaxis GO:0051726~regulation of cell cycle GO:0032496~response to lipopolysaccharide GO:0007610~behavior GO:0007626~locomotory behavior GO:0007259~JAK-STAT cascade GO:0042981~regulation of apoptosis GO:0043067~regulation of programmed cell death GO:0010941~regulation of cell death GO:0051301~cell division GO:0040011~locomotion GO:0050776~regulation of immune response GO:0051249~regulation of lymphocyte activation GO:0032570~response to progesterone stimulus GO:0050863~regulation of T cell activation GO:0002683~negative regulation of immune system process GO:0001819~positive regulation of cytokine production GO:0001817~regulation of cytokine production GO:0032270~positive regulation of cellular protein metabolic process GO:0002822~regulation of adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains GO:0002819~regulation of adaptive immune response GO:0045087~innate immune response GO:0051247~positive regulation of protein metabolic process GO:0031347~regulation of defense response GO:0048585~negative regulation of response to stimulus GO:0002697~regulation of immune effector process GO:0080134~regulation of response to stress GO:0042770~DNA damage response, signal transduction GO:0043065~positive regulation of apoptosis GO:0002521~leukocyte differentiation GO:0043068~positive regulation of programmed cell death GO:0010942~positive regulation of cell death GO:0051240~positive regulation of multicellular organismal process GO:0042108~positive regulation of cytokine

	biosynthetic process GO:0032655~regulation of interleukin-12 production GO:0009314~response to radiation GO:0019835~cytolysis GO:0032101~regulation of response to external stimulus GO:0044419~interspecies interaction between organisms GO:0008285~negative regulation of cell proliferation GO:0009628~response to abiotic stimulus GO:0033273~response to vitamin GO:0048584~positive regulation of response to stimulus GO:0031349~positive regulation of defense response GO:0042035~regulation of cytokine biosynthetic process GO:0006917~induction of apoptosis GO:0012502~induction of programmed cell death GO:0032680~regulation of tumor necrosis factor production GO:0008630~DNA damage response, signal transduction resulting in induction of apoptosis GO:0002252~immune effector process GO:0050778~positive regulation of immune response GO:0051241~negative regulation of multicellular organismal process

ÖZGEÇMİŞ

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