

# Comparative genomic analysis in autoimmune clusters with PV

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## INTRODUCTION

Pemphigus Vulgaris (PV) is a rare autoimmune skin disease in which multiple genetic and also nongenetic elements contribute to the dysregulation of immune tolerance leading to autoantibody formation and blistering of the skin.

Individuals affected by one autoimmune disease are at increased risk for developing other autoimmune diseases.

The **common gene hypothesis** states common genetic and transcriptional factors along with activation of common biological pathways may contribute to multiple autoimmune conditions.

The Sinha Laboratory has recently shown that **PV forms a distinct cluster with thyroid disease (AITD), rheumatoid arthritis (RA), and type 1 diabetes (T1D)**, as well as another cluster with systemic lupus erythematosus (SLE), RA, and AITD [1].

In order to better understand why these diseases cluster with each other, and provide support for the common gene hypothesis, we aimed to identify: (i) overlapping genes at the genetic (DNA) and transcriptional (RNA) levels that are have been associated with the clustering diseases, and (ii) common functional pathways linked to disease cluster overlapping genes.

## OBJECTIVES

To identify common genes derived from genome wide association (GWAS) studies of the PV-associated diseases and compare pathways derived from these genes to known functional pathways in PV in order to determine biological mechanisms linked to common genetic variations across PV clustering diseases.

To identify differentially expressed genes (DEGs) derived from microarray studies that are overlapping between PV and PV-associated diseases in order to elucidate common biological mechanisms that are occurring at the transcriptional level across PV clustering diseases.

To lay the groundwork for future studies on common biological networks operational in clustering autoimmune diseases and to identify potential new therapeutic targets effective in autoimmune diseases with genetic/genomic similarities.

## METHODS

A literature search for GWAS studies on AITD, RA, and T1D, and SLE was performed using the national GWAS database (<https://www.genome.gov/2652384>).

To date, only one GWAS study is available on PV (limited to 100 patients). Due to the lack of extensive GWAS data on PV, an in depth comparison of GWAS studies in clustering autoimmune disease with PV could not be done. Instead, genes revealed by GWAS on clustering diseases were compared to each other and then compared to our lab's gene expression data on PV [2].

Common genes revealed by GWAS for AITD, RA, T1D, and SLE were compared to PV gene expression data from our own lab in order to identify shared genes and pathways in disease clusters.

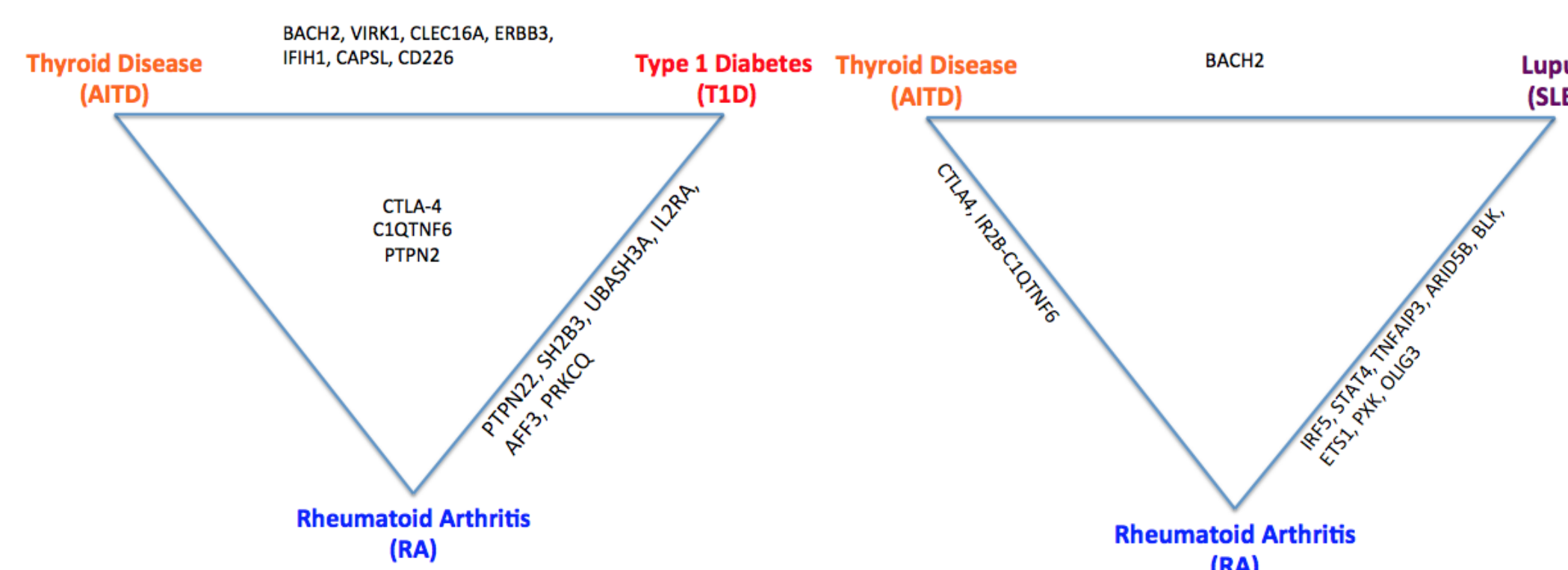
A literature search was performed for microarray studies in AITD, RA, T1D, and SLE [3-18]. Differentially expressed genes (DEGs) derived from microarray studies in AITD, RA, T1D, and SLE were compared with our laboratory's own list for DEGs in PV [2]. Note: Only microarray studies in which DEGs were derived from blood samples were included in the comparison.

Disease	Number of Microarray Studies Included
Rheumatoid Arthritis (RA)	Five [3-7]
Systemic Lupus Erythematosus (SLE)	Four [8-11]
Type 1 Diabetes (T1D)	Four [12-15]
Thyroid Disease (AITD)	Three [16-18]

Canonical pathway analysis using Ingenuity Pathway Analysis software was conducted on dysregulated genes found to overlap between clustering disease and PV.

## RESULTS

### 1. Overlapping genes from GWAS studies



**Figure 1.** Disease associated SNPs derived from GWAS on diseases clustering with PV show few overlapping genes: 7 genes between AITD and T1D; 6 genes between RA and T1D; 6 genes between RA and SLE; 2 genes between RA and AITD; and 1 gene between AITD and lupus. Of note, 3 immune function related genes (CTLA-4, C1QTNF6, and PTPN22) overlapped between AITD, T1D and RA. Due to the lack of an extensive GWAS data on PV, these genes were not compared to PV at the genetic level. However, we hypothesize that, functionally, these overlapping genes may be involved in canonical pathways similar to the ones arising in PV.

### 3. Overlapping DEGs from microarray studies



**Figure 2.** (a) Overlap of DEGs derived from DNA microarray studies between the individual diseases clustering with PV compared to the Sinha Laboratory's list of DEGs in PV. (b) Number of DEGs overlapping in 3 diseases. Only three microarray studies which met our inclusion criteria were available for AITD. Although AITD and PV are known to have a high rate of comorbidity, the low number of overlapping DEGs could be due to a lack of reliable microarray studies. Among the genes found to be overlapping between RA, T1D, and PV is **ARPC5**, an actin related protein 2/3 complex involved heavily in RhoA signaling pathway; between SLE, T1D, and PV are **SOD2** involved in oxidative stress, and **GNA13** involved in RhoA Signaling pathway.

### 2. Top canonical pathways derived from GWAS studies

Disease Cluster	Pathway	p-value
AITD, T1D, RA	Role of JAK1, JAK2 and TYK2 in Interferon Signaling	3.58E-03
T1D, RA	Role of JAK2 in Hormone-like Cytokine signaling	1.04E-02
SLE, RA	Tec Kinase Signaling	1.67E-03

**Table 1a.** Pathway analysis of common genes overlapping between disease specific GWAS studies shows a strong role of JAK2 which is involved with JAK-STAT signaling. JAK-STAT signaling is known as highly involved with autoimmunity. Interestingly, genes associated with JAK-STAT signaling have been found by our group by microarray to be dysregulated in patients with PV as compared HLA-matched controls [2].

Gene	Pathway	p-value
GWAS- PV DEG overlapping genes	Regulation of the epithelial-mesenchymal transition pathway	3.58E-03
	GM-CSF Signaling	1.04E-02
	CTLA4 Signaling in Cytotoxic T Lymphocytes	1.67E-03

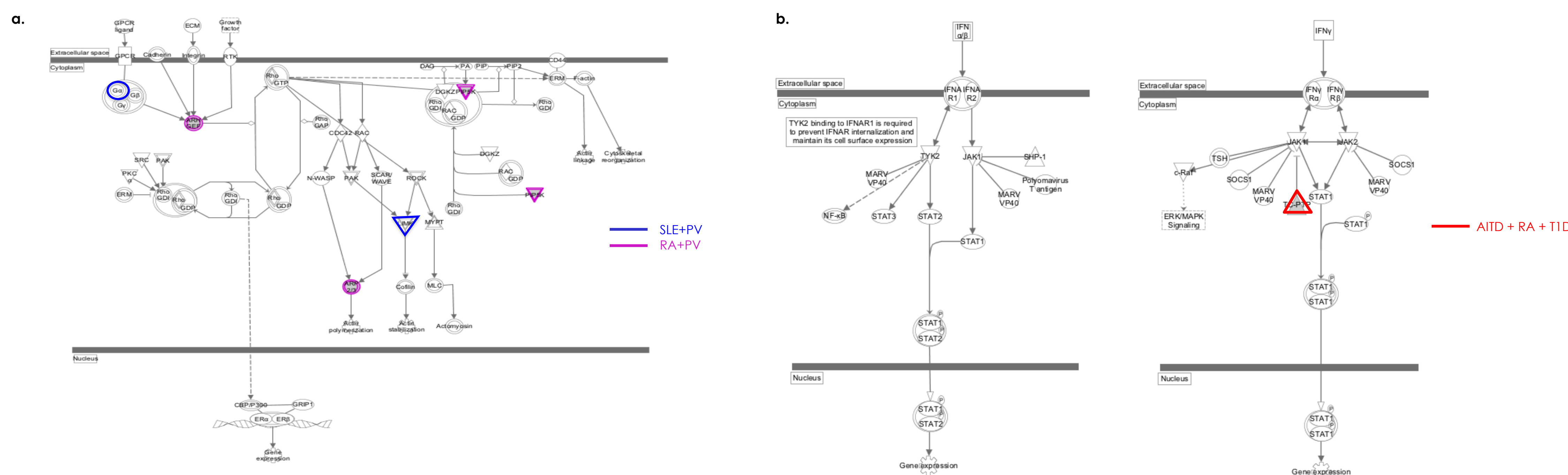
**Table 1b.** Pathway analysis of genes collected from GWAS in clustering diseases that were found to overlap with DEGs from our lab's microarray study in PV. Top canonical pathways show dysregulation of: •The epithelial-mesenchymal transition which is known to be involved in the loss of cell-cell adhesion. •GM-CSF, a cytokine that functions as a white blood cell growth factor and is involved with basic immune response, specifically with JAK2 and STAT5 in stimulating expression of STAT5 target genes. •CTLA-4, which is expressed on the surface of T helper cells and transmits inhibitory signals to T cells.

### 4. Top Canonical Pathways derived from microarray studies

Disease Cluster	Pathway	p-value
RA - PV	RhoA Signaling	1.8E-05
SLE - PV	RhoGDI Signaling	1.14E-03
T1D - PV	Superoxide Radicals Degradation	4.15E-04
AITD - PV	Polyamine Regulation in Colon Cancer	4.41E-03

**Table 2.** Pathway analysis of overlapping DEGs derived from disease specific microarray studies show strong overlap with RhoA signaling pathway between RA, SLE, and PV, and involvement of oxidative stress with T1D and PV. RhoA signaling and oxidative stress have both been suggested as factors in PV pathogenesis. RhoA signaling is key in the coordination of immune responses and activation of T cells and interference with the pathway contributes to the development of PV. A triggering effect of oxidative stress on SLE, RA, and T1D has been described before, and our lab has recently found an upregulation of oxidative stress in PV as well (unpublished data).

## RhoA signaling pathway and JAK-STAT signaling pathway



**Figure 3** (a) The pathway on the left represents the RhoGDI signaling pathway which is a branch of the RhoA signaling family. Complex or group genes (indicated by a circle) and kinases (indicated by an inverted triangle) highlighted in **blue** overlap in SLE and PV, while those highlighted in **pink** overlap in RA and PV. RhoA belongs to a family of G proteins known as Rho GTPases that are involved with the regulation of the actin cytoskeleton and the maintenance of desmosomes, intercellular adhesion molecules expressed in keratinocytes and targeted in PV. Alterations in RhoA signaling effect desmosomal adhesion and cause dysregulation in related mechanisms that lead to blistering. (b) The pathway on the right represents the JAK/STAT signaling pathway. The phosphatase highlighted in **red** (indicated by triangle) overlaps in the role of JAK1, JAK2 and TYK2 in interferon signaling pathway in AITD, RA, and T1D. JAK/STAT signaling regulates a variety of biological responses such as cell proliferation, differentiation, oncogenesis, and immune response. Rho kinases can be activated by JAK/STAT kinases and signal transducers. IL-13 activates JAK-STAT and then the phosphorylated STAT can bind with the DNA site and eventually cause up-regulation of RhoA.

## SUMMARY AND CONCLUSIONS

- Our lab has previously shown that Pemphigus vulgaris (PV) forms distinct clusters with other autoimmune diseases, in particular autoimmune thyroid disease (AITD), type 1 diabetes (T1D), rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). The reason for the observed clustering could be due to common genes expressed or common pathways utilized that underlie pathogenesis of these diseases.
- A comparative analysis of data on the clustering diseases reveals a modest degree of genomic overlap at the DNA level with 1-8 GWAS identified genes per comparison. Pathway analysis of the common genes in the PV-associated diseases reveals predominant involvement of canonical pathways involved in innate and adaptive immunologic processes.
- Comparison of differentially expressed genes (DEGs) derived from microarray studies in the clustered autoimmune conditions with our own list of DEGs in PV reveals an overlap of a somewhat larger group of genes (5-109). Pathway analysis of these common genes reveals a significant involvement of RhoA signaling in RA, SLE and PV, as well as significant effects of oxidative stress in RA, SLE, T1D and PV.
- Our data indicate that autoimmune diseases that cluster with PV share biological pathways linked to genetic and transcriptional alterations.
- While our previous studies indicate that AITD and PV cluster with each other, this is not strongly supported by the presence of a large number of overlapping genes in the currently available studies. Thus, future work will focus on using an interactome-based platform to provide more insight into shared pathways of autoimmune development and expression.

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