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 with activation of common biological pathways may contribute to multiple autoimmune conditions.
- The Sinha Laboratory has recently showed 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 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 studies (GWAS) studies of the PV-associated diseases and compare pathways from these genes to known functional pathways in PV in order to elucidate common biological mechanisms that are occurring at the transcriptional level 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/2652354).
- 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 list of DEGs in PV [2].
- Canonical pathway analysis with 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](https://example.com/figure1.png)

Figure 1. Disease associated SNPs derived from GWAS on diseases clustering with PV show few overlapping genes; genes between AITD and T1D, 6 genes between RA and T1D, 5 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 CTLA4, CD14, and TNFRSF1A overlapped between AITD, T1D and RA. Due to the lack of 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.

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

<table>
<thead>
<tr>
<th>Disease Cluster</th>
<th>Pathway</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AITD, T1D, RA</td>
<td>Rule of JAK1, JAK2, and TYK2 in Interferon Signaling</td>
<td>1.3E-02</td>
</tr>
<tr>
<td>RA, T1D, 5 genes between RA and T1D</td>
<td>Role of JAK2 in Hormone-like Cytokine Signaling</td>
<td>1.4E-02</td>
</tr>
<tr>
<td>RA, T1D, 5 genes between RA and T1D</td>
<td>Role of JAK2 in Hormone-like Cytokine Signaling</td>
<td>1.4E-02</td>
</tr>
<tr>
<td>RA, SLE, 3 genes between RA and SLE</td>
<td>Tec Kinase Signaling</td>
<td>1.67E-05</td>
</tr>
<tr>
<td>RA, AITD, 2 genes between RA and AITD</td>
<td>Tec Kinase Signaling</td>
<td>1.67E-05</td>
</tr>
</tbody>
</table>

2. Top canonical pathways derived from GWAS studies

- GWAS PV overlap: genes

![Figure 2](https://example.com/figure2.png)

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 few 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 ROCK2, an actin related protein 3 complex involved heavily in RhoA signaling pathway, between SLE, T1D, and PV are SQE2 involved in oxidative stress, and GNA3 involved in Rhodopsin Signaling pathway.

<table>
<thead>
<tr>
<th>Disease Cluster</th>
<th>Pathway</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE, PV</td>
<td>RhoA Signaling</td>
<td>1.8E-05</td>
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<tr>
<td>SLE, RA</td>
<td>RhoA Signaling</td>
<td>1.1E-05</td>
</tr>
<tr>
<td>SLE, T1D</td>
<td>Superoxide Dismutation</td>
<td>4.1E-04</td>
</tr>
<tr>
<td>AITD, PV</td>
<td>Polyamine Regulation</td>
<td>4.4E-03</td>
</tr>
</tbody>
</table>

- RhoA signaling pathway and JAK-STAT signaling pathway

![Figure 3](https://example.com/figure3.png)

Figure 3. (a) Pathway on the left represents the Rhodopsin signaling pathway which is a branch of the RhoA signaling family. Complex or group genes (indicated by a circle) and lesions (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 actin cytoskeleton and the maintenance of microdomains, intercellular adhesion molecules expressed in keratinocytes and targeted in PV. Alterations in RhoA signaling effect de novo 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 JAK2, JAK3, and TYK2 in interferon signaling pathway in AITD, RA, and T1D. JAK-STAT signaling regulates a variety of biological response such as cell proliferation, differentiation, oncogenesis, and immune responses. RhoA 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 (5-10). 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 larger 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.

REFERENCES