PS-1

Identification of Genetic Biomarkers for Ankylosing Spondylitis and Multiple Sclerosis

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OBJECTIVE

Ankylosing spondylitis (AS) is a persistent inflammatory condition that predominantly impacts the spine and sacroiliac joints. However, it may also affect other joints and organs. It is a form of arthritis, characterized by inflammation, pain, and stiffness in affected areas. Multiple sclerosis (MS) is a disorder that can impact the brain and spinal cord, leading to a variety of symptoms such as issues with vision, movement of limbs, sensation, and balance. Both MS and AS are autoimmune diseases characterized by inflammation. In this study, we examined single-cell RNA sequencing data for AS and MS obtained from a public database, collected and analyzed the datasets, and conducted gene expression analyses across various cell types.

1. Data download
   - 5 data
   ↓
2. Data QC
   - gene expression count
   - MT percentage
   - By Phython Scanpy
   ↓
3. Data Cluster
   - Each cluster
   - By UMAP
   ↓
4. Cell typing
   - Data annotation
   - By celltypist
   - Machine learning (ML)
   ↓
5. Data merge
   - Data integration
   - By BBKNN, PANDAS
   - By Scanpy
   ↓
6. DEG analysis
   - Data integration
   - By t-test
   - Control vs. AS vs. MS in CSF and PBMC
   - Control vs. AS vs. MS in PBMC

Figure 1. Study design

Abbreviations: MT, mitochondria; QC, quality control; UMAP, Uniform Manifold Approximation and Projection; DEG, differentiated expressed gene; AS, ankylosing spondylitis; MS, multiple sclerosis; PBMC, peripheral blood monocytes; CSF, cerebrospinal fluid

METHOD

Single-cell datasets were obtained from the cellxgene and Gene Expression Omnibus databases. Two different datasets related to AS and MS in humans were analyzed. The study included 10 samples for controls, 10 for patients with AS, and 23 for patients with MS. Single-cell mRNA sequencing data were analyzed. Statistical significance was determined through the Student's t-test between each group.

RESULTS

Quality control was performed for each dataset, considering the percentage of mitochondrial genes and total number of genes. The Uniform Manifold Approximation and Projection program was used to divide the integrated data into regions based on the genes for each dataset (control, AS, and MS groups). Cluster analysis confirmed that the integrated data were divided into 16 zones. The number of genes expressed in the 16 zones was analyzed and compared between peripheral blood mononuclear cell (PBMC) and cerebrospinal fluid (CSF). Cluster and heat map analyses were performed, including only genes that were significantly expressed in each cell. Significant gene expression in the final 10 cells differed between the AS and MS groups compared to the control group.

Table 1. Information of the Dataset Used in the Analysis

	Control	AS	MS	MS
Dataset ID	EGAD00001007718	GSE194315	GSE138266	GSE163005
Technology	Illumina NovaSeq 6000	Illumina NovaSeq 6000	Illumina NextSeq 500	Illumina NextSeq 500, Illumina NextSeq 6000
Source	PBMC	PBMC	CSF, PBMC	CSF, PBMC
Samples	268	48	22	38
Overall Design	Single-cell data gene expression data set (5'Chromium 10X) of healthy paediatric volunteers, and paediatric and adult COVID-19 patients. Gene expression was determined from samples of PBMCs. In addition to gene expression, PBMC's were assayed by CITE-seq. A subset of samples has VDJ sequencing data for TCR and BCR.	RNA and surface epitope sequencing of peripheral blood mononuclear cells in 28 patients with PSA, 10 patients with AS, 24 patients with cutaneous PSO, 14 psoriasis patients with unclear PSX, and 29 healthy subjects. StudyInfo_20220124.xlsx describes the samples, as well as raw and processed data used for separate studies of (1) AS and healthy subjects and (2) PSA, PSO, PSX, and healthy subjects	5 vs 5 Case-Control design for the single-cell RNA seq experiment. Each donor provided 2 samples from the CSF and PBMC.	Single-cell RNA sequencing of cerebrospinal fluid-derived leukocytes from patients with Neuro-COVID (n=8), non-inflammatory (n = 9) and autoimmune (n=9) neurological diseases and viral encephalitis (n=5).

Abbreviations: AS, ankylosing spondylitis, MS, multiple sclerosis; PBMC, peripheral blood monocytes; COVID-19, coronavirus disease-2019; CITE-seq, cellular indexing of transcriptomes and epitopes-sequencing; TCR, T cell receptors; BCR, B cell receptors; RNA, ribonucleic acid; PSA, psoriatic arthritis; PSO, psoriasis; PSX, PSA diagnosis; CSF, cerebrospinal fluid

Table 2. Classification of Cell Types Including Significant Genes in the AS and MS Groups

Cell types	AS group	MS group
Naive B cell	Significant	Significant
Platelet	No significant	No significant
Plasmacytoid dendritic cell	No significant	No significant
Classical monocyte	Significant	Significant
Non-classical monocyte	Significant	Significant
Central memory CD8-positive, alpha-beta T cell	Significant	No significant
Regulatory T cell	Significant	Significant
CD16-negative, CD56-bright natural killer cell, human	No significant	No significant
CD8-positive, alpha-beta cytotoxic T cell	Significant	Significant
Natural killer cell	Significant	Significant

Abbreviations: AS, ankylosing spondylitis; MS, multiple sclerosis

CONCLUSION

Naive B cells; classical and non-classical monocytes; regulatory T cells; CD8-positive, alpha-beta cytotoxic T cells; and NK cells expressed numerous significant genes involved in the development of AS and MS. Although central memory CD8-positive, alpha-beta T cells are not significantly associated with MS, they expressed genes involved in the pathogenesis of AS.

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