Research Article

Bioinformatic Analysis Identifies Biomarkers and Treatment Targets in Primary Sjögren’s Syndrome Patients with Fatigue

Guangshu Chen,1 Li Che,2 Xingdong Cai,2 Ping Zhu,1 and Jianmin Ran1

1Department of Endocrinology, Guangzhou Red Cross Hospital, Jinan University, Guangzhou 510220, China
2Department of Pulmonary and Critical Care Medicine, The First Affiliated Hospital of Jinan University, Guangzhou 510630, China

Correspondence should be addressed to Jianmin Ran; ran_jianmin@ext.jnu.edu.cn

Received 3 November 2021; Accepted 23 December 2021; Published 15 January 2022

Academic Editor: Paul Harrison

Copyright © 2022 Guangshu Chen et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

We aim to identify the common genes, biological pathways, and treatment targets for primary Sjögren’s syndrome patients with varying degrees of fatigue features. We select datasets about transcriptomic analyses of primary Sjögren’s syndrome (pSS) patients with different degrees of fatigue features and normal controls in peripheral blood. We identify common differentially expressed genes (DEGs) to find shared pathways and treatment targets for pSS patients with fatigue and design a protein-protein interaction (PPI) network by some practical bioinformatic tools. And hub genes are detected based on the PPI network. We perform biological pathway analysis of common genes by Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway. Lastly, potential treatment targets for pSS patients with fatigue are found by the Enrichr platform.

We discovered that 27 DEGs are identified in pSS patients with fatigue features and the severe fatigued pSS-specific gene is RTP4. DEGs are mainly localized in the mitochondria, endosomes, endoplasmic reticulum, and cytoplasm and are involved in the biological process by which interferon acts on cells and cells defend themselves against viruses. Molecular functions mainly involve the process of RNA synthesis. The DEGs of pSS are involved in the signaling pathways of viruses such as hepatitis C, influenza A, measles, and EBV. Acetohexamide PC3 UP, sulocidil HL60 UP, pranolamine HL60 UP, and chlorophyllin CTD 00000324 are the four most polygenic drug molecules. PSS patients with fatigue features have specific gene regulation, and chlorophyllin may alleviate fatigue symptoms in pSS patients.

1. Introduction

Primary Sjögren’s syndrome (pSS) is an all-body autoimmune disease that mainly affects middle-aged women [1]. The main clinical feature of the disease is dryness of the mouth and eyes, and the pathophysiology is characterized by focal lymphocyte infiltration in exocrine glands [2, 3]. Fatigue is commonly seen in pSS patients as an extraglandular manifestation and closely links with poor life quality [4–6]. Fatigue affects approximately 70% of pSS patients [7, 8]. Normally, fatigue and depression are considered manifestations of psychological disorders and interact with physical pain and discomfort, which creates a vicious cycle. Fatigue in pSS is induced and regulated by genetic and molecular mechanisms, with the innate immune system playing an important role in the production of fatigue [9–11]. Although pSS always comes with fatigue, not all patients exhibit fatigue, which provides a good model for exploring the underlying biological mechanisms.

High-throughput methods play an increasingly essential role in biology spheres, and microarray data analysis highlights its advantage in large-scale analysis of gene expression among high-throughput applications [12, 13]. Former studies [14, 15] have shown the high-throughput sequencing analysis result for pSS patients with fatigue features but do not offer further analysis based on varying degrees of fatigue. This study tries to present characteristic genes and biological pathways in pSS patients with manifestations of fatigue, as well as drugs of potential benefit.

The GSE66795 dataset from the GPL10558 platform on the GEO database is selected for gene expression of pSS with
fatigue. The GSE66795 dataset was first identified for differentially expressed genes (DEGs) in pSS patients with different levels of fatigue, and based on the coexpressed genes, further analyses including Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway are performed to understand the biological process. The top ten target genes from the protein-protein interaction (PPI) network will be obtained to identify potential drugs that may alleviate fatigue in pSS patients.

2. Materials and Methods

2.1. Dataset Collection. We search “Primary Sjögren’s syndrome” and “fatigue” in the GEO database [16] and select the dataset (GSE66795) demonstrating gene expression in pSS patients with varying degrees of fatigue characteristics and normal controls. The GSE66795 dataset is extracted from the GPL10558 platform (Illumina HumanHT-12 V4.0 expression microbead chip) for RNA sequence analysis. The data of GSE66795 is obtained from the UK registry of primary Sjögren’s syndrome. It includes whole genome microarray profiles of pSS patients with varying degrees of fatigue characteristics and normal controls in peripheral blood. One hundred and thirty-one patients with pSS are involved, including 21 patients with mild fatigue, 74 patients with moderate fatigue, 36 patients with severe fatigue, and 29 normal controls.

2.2. Differential Expression Analysis. Differential expression analysis is performed using the online analysis tool GEO2R; gene expression profiles of pSS patients with mild, moderate, and severe fatigue were compared with normal controls separately to identify DEGs. P values and adjusted P values are calculated using t-tests. Genes with the following criteria were retained for each sample: (1) log2-fold change (log2FC) absolute value greater than 1 and (2) adjusted P value less than 0.05. After identifying DEGs in pSS patients with varying degrees of fatigue, the online website (https://www.xiantao.love/gds) is used to plot a Venn diagram.

2.3. Gene Ontology and Pathway Discovery in Gene Set Enrichment Analysis. Gene set enrichment analysis is used to understand the general biological function and the chromosomal location of a gene [17]. For gene product annotation, the terms of Gene Ontology (GO) are used, including biological process (BP), molecular function (MF), and cellular component (CC) [18]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways are commonly used to describe metabolic pathways [19]. GO terms and KEGG pathways were gotten through the platform Enrichr (https://amp.pharm.mssm.edu/Enrichr/) platform. Enrichr is primarily used as an enrichment analysis platform, providing extensive visual details of the common functions of inputted genes [24].

3. Results

3.1. DEG Identifications. We use the GSE66795 dataset to identify the DEGs of pSS with fatigue. 37, 29, and 33 DEGs are obtained for pSS with mild, moderate, and severe fatigue, respectively. The collected DEGs are further compared by using the online website (https://www.xiantao.love/gds) for gathering common genes in pSS with varying degrees of fatigue. And 27 (OASI, OAS2, GBP1, IRF7, EIF2AK2, IFIT2, USP18, SAMD9L, HES4, IFI44L, SERPING1, IFIT3, IFITM3, IFI6, XAF1, MX1, OASL, OTOF, HERC5, LY6E, EPTI1, OAS3, ISG15, IFIT1, RSAD2, IFI44, and IFI27) common DEGs are identified. The specific genes to pSS with mild fatigue are DDX60, IFIH1, GBP5, LAP3, and TIMM10. The specific genes to pSS with moderate fatigue are HLA-DRB4 and HLA-DRB6, and that to pSS with severe fatigue is RTP4. The Venn diagram (Figure 1) shows that common DEGs accounted for 67.5% out of a total of 40 DEGs.

3.2. GO Terms and KEGG Pathways. We analyzed 27 common DEGs for both GO and KEGG pathways. Both of the results are taken from the top 10 GO entries. GO terms in Table 1 suggest that DEGs are mainly localized in the mitochondria, endosomes, endoplasmic reticulum, and cytoplasm. They are involved in the biological processes of interferon action on cells and cellular defense against viruses. And the molecular functions are mainly engaged in the process of RNA synthesis. KEGG pathways in Table 2 suggest that the DEGs of pSS with fatigue are involved in the signaling pathways of viruses such as hepatitis C, influenza A, measles, and EBV. Both are seen in Figures 2(a) and 2(b).

3.3. Identification of Hub Genes by PPI Networks. We put common DEGs into the STRING website, and the files generated after analysis are further entered into Cytoscape software for visual analysis. PPI networks are designed to detect hub genes for identifying drug molecules for pSS with cytoscape.org/) to further present the network and identify target genes.

2.5. Transcription Factor- (TF-) Gene Interactions. We use NetworkAnalyst (https://www.networkanalyst.ca/) to identify interactions of TF-genes with DEGs [22]. NetworkAnalyst plays a comprehensive network platform for gene expression across a wide range of species and enables them to be subjected to a meta-analysis [23].
fatigue. PPI networks involve 24 nodes and 552 edges, which are shown in Figure 3(a). We present the top 20 genes in Figure 3(b) and Table 3.

3.4. TF-Gene Interactions. The interactions of TF and genes are shown in Figure 4. The network has 60 nodes and 108 edges. Sixteen TF-genes regulate IFIT1, and IFIT3 is handled by 14 TF-genes. The network involves 60 TF-genes. Figure 4 shows the network of TF-gene interactions.

3.5. Identification of Drug Candidates. We identify drug molecules for the top 10 hub genes on the Enrichr platform. We collect drug candidates judged on adjusted P values. The analysis reveals that acetohexamide PC3 UP, sulocidil HL60 UP, prenylamine HL 60 UP, and chlorophyllin CTD 00000324 are the four most polygenic drug molecules that interact with genes. Figure 5 and Table 4 present the drug candidates in DSigDB.

4. Discussion

Fatigue is an annoying experience that means physical and mental tiredness [25]. Mengshoel et al. [26] reveal that most pSS patients literally suffer from fluctuating fatigue out of control regardless of their health condition. Fatigue has a significant influence on patients’ daily life, and patients must adapt to their behavior and lives. Although the underlying mechanisms are still unclear, former studies take depression and pain as the prominent factors associated with fatigue [5, 27]. Currently, growing evidence suggests that fatigue has a molecular and genetic basis on its production and regulation. Therefore, most scholars view fatigue as a biological and brain phenomenon [9–11].

IL-1β tends to increase rapidly secreted from macrophages to activate the immune system when meeting tissue injury or infection. IL-1β plays its role by binding with the IL-1 receptor coming with the downstream of IL-1 response [28]. Then, immune and inflammation systems are activated, which induce the behavior of disease, with fatigue being involved as an important component [29]. All these inflammatory signaling pathways go on working and turn fatigue into a chronic state. In the brain, IL-1β signaling pathways may explain the ultimate pathway of fatigue [30, 31], and IL-1 blocker treatment may effectively release fatigue [32, 33]. Thus, fatigue and other unpleasant mood in those patients with autoimmune disease not only should be understood by the unfortunate development of chronic illness but also may be related to some signaling pathways and activation of genes that regulate the mood in the cerebral system.

Genome-wide association analysis of pSS patients has been conducted, and a gene (RTP4) is identified as highly relevant. Similarly, we confirm that RTP4 is highly expressed in pSS patients with severe fatigue through bioinformatic analysis, suggesting that this gene is critical in the mechanisms of fatigue. RTP4 encodes a protein associated with the expression of opioid receptors on the cell surface. These receptors are also expressed in the lymph system and pain-regulated pathways in the brain [34]. However, the former study did not stratify pSS based on the degree of fatigue, and it is unclear which degree of fatigue expresses the RTP4 gene. Our study finds that pSS patients with severe fatigue specifically express the RTP4 gene, providing clues for further studies on the genomics of fatigue features in pSS patients.

OAS1, a coexpressed gene for pSS in our study, has been established in previous studies as a risk locus of pSS and impacts the flaw of virus clearance because of the altering response of IFN [35]. Our gene pathway analysis points out that DEGs for pSS with fatigue are mainly localized intracellularly and involved in signaling pathways of common viruses in the respiratory and digestive tracts, suggesting that pSS is a systemic disease with an uncertain etiology and that viral infection may be a predisposing factor.

Fatigue always accompanies pSS patients, but it is hard work to manage these bad feelings [36]. The clinical practice guidelines (CPG) committee emphasizes the many causes of fatigue in pSS; therefore, the comprehensive evaluation for diagnosis is essential. So far, the treatment for fatigue in pSS with solid recommendation is mere taking exercise, which is also practical in other autoimmune diseases [37]. In America, hydroxychloroquine (HQC) is the most widely used drug therapy for pSS with fatigue, but the recommendation strength is not strong enough [34]. It is not recommended to release fatigue in pSS using dehydroepiandrosterone (DHEA) [34]. Both the tumor necrosis factor inhibitor is discouraged for the treatment of fatigue in pSS [38, 39]. Our bioinformatic study reveals that besides chloroquine and testosterone drugs that help improve fatigue, chlorophyllin, the sulphphonylurea hypoglycaemic drug acetyltruxene, and the antiallergic drug terfenadine may have improved fatigue in pSS. However, chloroquine and testosterone are not strongly recommended as we mentioned before. Acetohexamide has been discontinued in the American market due to its significant hypoglycaemic risk. Terfenadine is not suitable for long-term use since its
### Table 1: Top 10 GO pathways and their corresponding P values and genes.

| GO       | GO_ID           | Description                                           | P-value      | Genes                                      |
|----------|-----------------|-------------------------------------------------------|--------------|--------------------------------------------|
| MF       | GO:0001730      | 2'-5'-oligoadenylate synthetase activity              | 1.305e-12    | OAS2 OAS3 OASL OAS1                       |
|          | GO:0003725      | double-stranded RNA binding                           | 1.225e-09    | OAS1 OAS2 EIF2AK2 OAS3 OASL               |
|          | GO:0016779      | nucleotidyltransferase activity                       | 2.993e-06    | OAS1 OAS3 OAS2 OASL                       |
|          | GO:0003723      | RNA binding                                           | 2.089e-04    | EIF2AK2 OAS3 HERC5 IFIT2 IFIT3 IFIT1 OASL OAS2 |
|          | GO:0035639      | purine ribonucleoside triphosphate binding           | 8.781e-03    | EIF2AK2 MX1 OAS1 OAS3 GBP1 OASL OAS2     |
| CC       | GO:0001883      | purine nucleoside binding                            | 9.156e-03    | EIF2AK2 MX1 OAS1 OAS3 GBP1 OASL OAS2     |
|          | GO:0032549      | ribonucleoside binding                                | 9.156e-03    | EIF2AK2 MX1 OAS1 OAS3 GBP1 OASL OAS2     |
|          | GO:0001882      | nucleoside binding                                    | 9.363e-03    | EIF2AK2 MX1 OAS1 OAS3 GBP1 OASL OAS2     |
|          | GO:0017076      | purine nucleotide binding                             | 1.032e-02    | EIF2AK2 MX1 OAS1 OAS3 GBP1 OASL OAS2     |
|          | GO:0044822      | poly(A) RNA binding                                   | 5.088e-02    | EIF2AK2 HERC3 IFIT2 OASL                  |
|          | GO:0048471      | perinuclear region of cytoplasm                       | 3.888e-03    | EIF2AK2 MX1 HERC5 OAS2                    |
|          | GO:0005829      | cytosol                                               | 9.919e-06    | EIF2AK2 OAS1 OAS3 IRF7 HERC5 ISG15 OAS2 MX1 USP18 OTOF IFIT3 GBP1 IFIT1 OASL XAF1 |
|          | GO:0005739      | mitochondrion                                         | 4.810e-03    | OAS1 RSAD2 IFIT3 IFIT1 IFI6 XAF1 OAS2     |
|          | GO:0005783      | endoplasmic reticulum                                 | 1.583e-02    | MX1 OAS1 IFIT2 RSAD2 OTOF OAS2            |
|          | GO:0012505      | endomembrane system                                   | 4.168e-03    | SAMD9L OAS1 SERPING1 IRF7 RSAD2 IFIT3 OAS2 MX1 IFIT2 OTOF IFIT1 GBP1 |
|          | GO:0005737      | cytoplasm                                             | 1.488e-02    | IFIT1 OAS3 MX1 IFIT2 RSAD2 OTOF OAS2      |
|          | GO:0035455      | response to interferon-alpha                          | 1.907e-12    | IFITM3 IFIT3 OAS1 IFIT2 EIF2AK2          |
|          | GO:0071357      | cellular response to type I interferon               | 2.702e-30    | IFIT1 OAS3 MX1 USP18 IFIT2 IRF7 OAS1 OAS2 |
|          | GO:0060337      | type I interferon signaling pathway                   | 2.702e-30    | IFIT1 OAS3 MX1 USP18 IFIT2 IRF7 OAS1 OAS2 |
|          | GO:0045071      | negative regulation of viral genome replication      | 2.204e-18    | OAS1 EIF2AK2 IFIT1 OAS3 MX1 IFITM3 OASL ISG15 RSAD2 |
|          | GO:0034340      | response to type I interferon                         | 3.905e-30    | IFIT1 OAS3 MX1 USP18 IFIT2 IRF7 OAS1 OAS2 |
|          | GO:0045069      | regulation of viral genome replication               | 9.301e-17    | EIF2AK2 IFIT1 OAS3 MX1 OAS1 IFITM3 OASL ISG15 RSAD2 |
|          | GO:0048525      | negative regulation of viral process                  | 4.916e-16    | EIF2AK2 IFIT1 OAS3 MX1 OAS1 IFITM3 OASL ISG15 RSAD2 |
|          | GO:0019079      | viral genome replication                               | 9.276e-16    | EIF2AK2 IFIT1 OAS3 MX1 OAS1 IFITM3 OASL ISG15 RSAD2 |
|          | GO:0060333      | interferon-gamma-mediated signaling pathway           | 1.854e-10    | OAS3 GBP1 IRF7 OAS1 OAS2 OASL             |
|          | GO:0051607      | defense response to virus                              | 6.646e-09    | HLA-DPA1 GBP2 IFNGR1 VCAm1 HLA-DRA CCL22 ICAM1 |
central depression as an antiallergy drug. And chlorophyllin appears to hold some promise for reducing fatigue in pSS.

Chlorophyll is an ingredient of the deril drug which is available as an over-the-counter medicine [40]. And chlorophyllin, obtained by hydrolyzing chlorophyll to remove phytyl alcohol, is a water-soluble derivative. Chlorophyll has been shown to exert its anticancer properties by playing a role as an antioxidant [41], a CYP inhibitor [42], an apoptosis inducer [43], a phase II enzyme stimulator [44], and a carcinogen transport modulator [45].

Currently, COVID-19 has swept the world and may last for a long time because of its rapid mutation. Almost 5,000,000 people have died in this epidemic [46], and the reduction of lymphocytes in COVID-19 patients is considered an important risk factor for poor prognosis [47–49].

Recent studies suggest that the chlorophyll derivative sodium copper chlorophyllin (SCC) may improve survival in critically ill COVID-19 patients by increasing the total number of lymphocytes [50]. Increasing consumers choose dietary chlorophyll which is derived from SCC for diet supplements for the sake of keeping healthy [51, 52]. Dietary chlorophyll is safe and has been shown to have a higher absorption rate in the human body, which may trigger ionic compound chelation [53, 54]. Zeng et al. [55] cognize one functional food called barley grass.
powder which is rich in chlorophyll, and other nutrients can effectively alleviate fatigue in chronic patients. The mechanism of chlorophyll’s role in relieving fatigue in pSS patients is unclear. It may be related to the nature of the hepatic enzyme inhibitors that increase the concentrations of immunosuppressant like hydroxychloroquine, which has better control of fatigue. And the capacity of scavenging the oxygen radical as an antioxidant may somewhat improve the fatigue of body.

We have identified gene expression profiles in peripheral blood specific to pSS with fatigue characteristics. The analysis of identified DEGs and pathways in this study will

| Hub gene | Degree | Stress | Closeness | Betweenness | Eccentricity | Clustering coefficient |
|----------|--------|--------|-----------|-------------|--------------|-----------------------|
| MX1      | 23     | 20     | 23        | 1.09235     | 1            | 0.96047               |
| IFIT1    | 23     | 20     | 23        | 1.09235     | 1            | 0.96047               |
| ISG15    | 23     | 20     | 23        | 1.09235     | 1            | 0.96047               |
| RSAD2    | 23     | 20     | 23        | 1.09235     | 1            | 0.96047               |
| IFI44L   | 23     | 20     | 23        | 1.09235     | 1            | 0.96047               |
| IFI44    | 23     | 20     | 23        | 1.09235     | 1            | 0.96047               |
| IFIT3    | 23     | 20     | 23        | 1.09235     | 1            | 0.96047               |
| OAS2     | 23     | 20     | 23        | 1.09235     | 1            | 0.96047               |
| OAS1     | 23     | 20     | 23        | 1.09235     | 1            | 0.96047               |
| IFI6     | 23     | 20     | 23        | 1.09235     | 1            | 0.96047               |
| XAF1     | 23     | 20     | 23        | 1.09235     | 1            | 0.96047               |
| IFIT2    | 23     | 20     | 23        | 1.09235     | 1            | 0.96047               |
| GBP1     | 23     | 20     | 23        | 1.09235     | 1            | 0.96047               |
| OAS3     | 23     | 20     | 23        | 1.09235     | 1            | 0.96047               |
| HERC5    | 22     | 14     | 22.5      | 0.75624     | 0.5          | 0.9697                |
| IRF7     | 22     | 10     | 22.5      | 0.51637     | 0.5          | 0.97835               |
| OASL     | 22     | 10     | 22.5      | 0.51637     | 0.5          | 0.97835               |
| IFI27    | 22     | 14     | 22.5      | 0.77598     | 0.5          | 0.9697                |
| EIF2AK2  | 22     | 10     | 22.5      | 0.51637     | 0.5          | 0.97835               |
| LY6E     | 21     | 10     | 22        | 0.55833     | 0.5          | 0.97619               |
Table 4: Candidate drug for pSS with fatigue.

| Name of drugs                      | $P$ value | Adjusted $P$ value | Target genes                                                                 |
|------------------------------------|-----------|--------------------|-------------------------------------------------------------------------------|
| Acetohexamide PC3 UP               | $3.47e-23$| $8.16e-21$         | RSAD2; OAS1; OAS2; MX1; IFI6; IFI44; IFIT1; IFI44L                             |
| Suloctidil HL60 UP                 | $2.19e-22$| $2.57e-20$         | RSAD2; OAS1; OAS2; MX1; IFI6; IFI44; ISG15; IFIT1; IFI44L; IFIT3              |
| Prenylamine HL60 UP                | $3.46e-21$| $2.71e-19$         | OAS1; MX1; IFI6; IFI44; ISG15; IFIT1; IFI44L                                  |
| Chlorophyllin CTD 00000324         | $9.59e-19$| $5.64e-17$         | OAS1 OAS2 MX1 IFI6 ISG15 IFIT1 IFIT3                                       |
| Terfenadine HL60 UP                | $1.81e-16$| $8.52e-15$         | OAS1 MX1 IFI6 IFI44 ISG15 IFIT1 IFIT3                                       |
| 3'-Azido-3'-deoxythymidine CTD 00007047 | $6.05e-13$| $2.37e-11$         | RSAD2 OAS1 OAS2 MX1 IFI6 ISG15 IFIT1 IFI44L                                |
| Clioquinol PC3 UP                  | $1.79e-11$| $6.00e-10$         | OAS1 MX1 IFI44 IFIT1 IFIT3                                                   |
| Etoposide HL60 UP                  | $2.92e-11$| $8.56e-10$         | OAS1 MX1 IFI6 IFI44 ISG15 IFIT1 IFIT3                                       |
| Testosterone enanthate CTD 00000155 | $1.03e-09$| $2.68e-08$         | RSAD2 OAS2 MX1 IFI6 ISG15 IFI44L IFIT3                                     |
| Gadodiamide hydrate CTD 00002623   | $3.85e-09$| $9.05e-08$         | RSAD2 IFIT1 IFI44L IFIT3                                                    |

Figure 4: Network for TF-gene interaction with common DEGs. The highlighted 10 red color nodes represent the common genes, and other nodes represent TF-genes. The network has 60 nodes and 108 edges.

Figure 5: Acetohexamide PC3 UP, suloctidil HL60 UP, prenylamine HL 60 UP, and chlorophyllin CTD 00000324 are the four most polygenic drug molecules that connect with the top 10 hub genes.
deepen our understanding of the essence of fatigue in pSS. The discovery that chlorophyllin may improve fatigue symptoms provides a theoretical basis for better improving the quality of life in pSS patients. And a preprint has previously been published [56].

Data Availability

The dataset supporting the conclusions of this article is available in the UK registry of primary Sjögren's syndrome repository and in the hyperlink (https://www.ncbi.nlm.nih.gov/geo/geo2r/?acc=GSE66795).

Ethical Approval

GEO belongs to public databases. The patients we choose involved in the database have obtained ethical approval. It is available for all users to download relevant data for free. Our study is based on open-source data, so there is no need to offer ethics approval.

Consent

There is no need for consent to participate.

Conflicts of Interest

The authors declare that they have no competing interests.

Authors’ Contributions

Guangshu Chen and Li Che contributed equally to this work.

Acknowledgments

We sincerely acknowledge the GEO database for providing their platforms and contributors for uploading their meaningful datasets. This study was financially supported by the Guangdong Science and Technology Project Fund for Key Scientific Research Base under Grant no. 2019B020230001.

References

[1] R. I. Fox, F. V. Howell, R. C. Bone, and P. E. Michelson, “Primary Sjögren syndrome: clinical and immunopathologic features,” Seminars in Arthritis and Rheumatism, vol. 14, no. 2, pp. 77–105, 1984.

[2] S. E. Gabriel and K. Michaud, “Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases,” Arthritis Research & Therapy, vol. 11, no. 3, p. 229, 2009.

[3] K. Asmussen, V. Andersen, G. Bendixen, M. Schiodt, and P. Oxholm, “A new model for classification of disease manifestations in primary Sjögren’s syndrome: evaluation in a retrospective long-term study,” Journal of Internal Medicine, vol. 239, no. 6, pp. 475–482, 1996.

[4] K. Haldorsen, I. Bjelland, A. I. Bolstad, R. Jonsson, and J. Brun, “A five-year prospective study of fatigue in primary Sjögren’s syndrome,” Arthritis Research & Therapy, vol. 13, no. 5, p. R167, 2011.

[5] T. Karageorgas, S. Fragioudaki, A. Nezos, D. Karaikos, H. M. Moutsopoulos, and C. P. Mavragani, “Fatigue in primary Sjögren’s syndrome: clinical, laboratory, psychometric, and biologic associations,” Arthritis Care & Research, vol. 68, no. 1, pp. 123–131, 2016.

[6] C. L. Overman, M. B. Kool, J. A. P. da Silva, and R. Geenen, “The prevalence of severe fatigue in rheumatic diseases: an international study,” Clinical Rheumatology, vol. 35, no. 2, pp. 409–415, 2016.

[7] A. Lerdal, A. Wahl, T. Rustoen, B. R. Hanestad, and T. Moum, “Fatigue in the general population: a translation and test of the psychometric properties of the Norwegian version of the fatigue severity scale,” Scandinavian Journal of Public Health, vol. 33, no. 2, pp. 123–130, 2005.

[8] N. Howard Tripp, J. Tarn, A. Natasari et al., “Fatigue in primary Sjögren’s syndrome is associated with lower levels of pro-inflammatory cytokines,” RMD Open, vol. 2, no. 2, article e000282, 2016.

[9] K. Brække Norheim, J. Ingenberg-Kreuz, K. Jonsdottir et al., “Epigenome-wide DNA methylation patterns associated with fatigue in primary Sjögren’s syndrome,” Rheumatology, vol. 55, no. 6, pp. 1074–1082, 2016.

[10] B. Bärtsch, M. M. Nilsen, J. T. Kvaløy, K. B. Norheim, G. Jonsson, and R. Omdal, “Heat shock proteins and chronic fatigue in primary Sjögren’s syndrome,” Innate Immun. vol. 22, no. 3, pp. 162–167, 2016.

[11] R. Dantzer, C. J. Heijnen, A. Kavelaars, S. Laye, and L. Capuron, “The neuroimmune basis of fatigue,” Trends in Neurosciences, vol. 37, no. 1, pp. 39–46, 2014.

[12] A. S. Stum, J. Quackenbush, and Z. Trajanoski, “Genes: cluster analysis of microarray data,” Bioinformatics, vol. 18, no. 1, pp. 207–208, 2002.

[13] M. L. Lee, F. C. Kuo, G. A. Whitmore, and J. Sklar, “Importance of replication in microarray gene expression studies: statistical methods and evidence from repetitive cDNA hybridizations,” Proceedings of the National Academy of Sciences of the United States of America, vol. 97, no. 18, pp. 9834–9839, 2000.

[14] L. Zhang, P. Xu, X. Wang et al., “Identification of differentially expressed genes in primary Sjögren’s syndrome,” Journal of Cellular Biochemistry, vol. 120, no. 10, pp. 17368–17377, 2019.

[15] G. G. Song, J. H. Kim, Y. H. Seo, S. J. Choi, J. D. Ji, and Y. H. Lee, “Meta-analysis of differentially expressed genes in primary Sjögren’s syndrome by using microarray,” Human Immunology, vol. 75, no. 1, pp. 98–104, 2014.

[16] E. Clough and T. Barrett, “The Gene Expression Omnibus database,” Methods in Molecular Biology, vol. 1418, pp. 93–110, 2016.

[17] A. Subramanian, P. Tamayo, V. K. Mootha et al., “Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles,” Proceedings of the National Academy of Sciences of the United States of America, vol. 102, no. 43, pp. 15545–15550, 2005.

[18] A. Doms and M. Schroeder, “GoPubMed: exploring PubMed with the Gene Ontology,” Nucleic Acids Research, vol. 33, no. - Web Server, pp. W783–W786, 2005.

[19] M. Kanehisa and S. Goto, “KEGG: Kyoto Encyclopedia of Genes and Genomes,” Nucleic Acids Research, vol. 28, no. 1, pp. 27–30, 2000.

[20] M. V. Kuleshov, M. R. Jones, A. D. Rouillard et al., “Enrichr: a comprehensive gene set enrichment analysis web server 2016
update,” Nucleic Acids Research, vol. 44, no. W1, pp. W90–W97, 2016.

[21] A. Ben-Hur and W. S. Noble, “Kernel methods for predicting protein-protein interactions,” Bioinformatics, vol. 21, Suppl 1, pp. i38–i46, 2005.

[22] Z. Ye, F. Wang, F. Yan et al., “Bioinformatic identification of candidate biomarkers and related transcription factors in nasopharyngeal carcinoma,” World Journal of Surgical Oncology, vol. 17, no. 1, p. 60, 2019.

[23] G. Zhou, O. Soufan, J. Ewald, R. E. W. Hancock, N. Basu, and J. Xia, “NetworkAnalyzer 3.0: a visual analytics platform for comprehensive gene expression profiling and meta-analysis,” Nucleic Acids Research, vol. 47, no. W1, pp. W234–W241, 2019.

[24] E. Y. Chen, C. M. Tan, Y. Kou et al., “Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool,” BMC Bioinformatics, vol. 14, no. 1, p. 128, 2013.

[25] L. B. Krupp and D. A. Pollina, “Mechanisms and management of fatigue in progressive neurological disorders,” Current Opinion in Neurology, vol. 9, no. 6, pp. 456–460, 1996.

[26] A. M. Mengshoel, K. B. Norheim, and R. Omdal, “Primary Sjögren's syndrome: fatigue is an ever-present, fluctuating, and uncontrollable lack of energy,” Arthritis Care & Research, vol. 66, no. 8, pp. 1227–1232, 2014.

[27] L. C. Pollard, E. H. Choy, J. Gonzalez, B. Khoshaba, and D. L. Scott, “Fatigue in rheumatoid arthritis reflects pain, not disease activity,” Rheumatology, vol. 45, no. 7, pp. 885–889, 2006.

[28] C. A. Dinarello, A. Simon, and J. W. van der Meer, “Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases,” Nature Reviews. Drug Discovery, vol. 11, no. 8, pp. 633–652, 2012.

[29] B. L. Hart, “Biological basis of the behavior of sick animals,” Neuroscience and Biobehavioral Reviews, vol. 12, no. 2, pp. 123–137, 1988.

[30] S. Rossi, V. Studer, C. Motta et al., “Neuroinflammation drives anxiety and depression in relapsing-remitting multiple sclerosis,” Neurology, vol. 89, no. 13, pp. 1338–1347, 2017.

[31] J. M. Kim, H. J. Kang, J. W. Kim et al., “Associations of tumor necrosis factor-α and interleukin-1β levels and polymorphisms with post-stroke depression,” The American Journal of Geriatric Psychiatry, vol. 25, no. 12, pp. 1300–1308, 2017.

[32] R. Omdal and R. Gunnarsson, “The effect of interleukin-1 blockade on fatigue in rheumatoid arthritis—a pilot study,” Rheumatology International, vol. 25, no. 6, pp. 481–484, 2005.

[33] C. Caveliti-Weder, R. Furrer, C. Keller et al., “Inhibition of IL-1beta improves fatigue in type 2 diabetes,” Diabetes Care, vol. 34, no. 10, article e158, 2011.

[34] S. E. Carsons, F. B. Vivino, A. Parke et al., “Treatment guidelines for rheumatologic manifestations of Sjögren’s syndrome: use of biologic agents, management of fatigue, and inflammatory musculoskeletal pain,” Arthritis Care & Research, vol. 69, no. 4, pp. 517–527, 2017.

[35] H. Li, T. R. Reksten, J. A. Ice et al., “Identification of a Sjögren’s syndrome susceptibility locus at OAS1 that influences isoform switching, protein expression, and responsiveness to type I interferons,” PLoS Genetics, vol. 13, no. 6, article e1006820, 2017.

[36] B. Segal, “Fatigue in primary Sjögren’s syndrome,” in Sjögren’s Syndrome: Diagnosis and Therapeutics, M. Ramos-Casals, Ed., pp. 129–143, Springer Verlag, London, 2012.

[37] B. E. Strombeck, E. Theander, and L. T. Jacobsson, “Effects of exercise on aerobic capacity and fatigue in women with primary Sjögren’s syndrome,” Rheumatology, vol. 46, no. 5, pp. 868–871, 2007.

[38] V. Sankar, M. T. Brennan, M. R. Kok et al., “Etanercept in Sjögren’s syndrome: a twelve-week randomized, double-blind, placebo-controlled pilot clinical trial,” Arthritis and Rheumatism, vol. 50, no. 7, pp. 2240–2245, 2004.

[39] X. Mariette, P. Ravaud, S. Steinfeld et al., “Inefficacy of infliximab in primary Sjögren’s syndrome: results of the randomized, controlled Trial of Remicade in Primary Sjögren’s Syndrome (TRIPS),” Arthritis and Rheumatism, vol. 50, no. 4, pp. 1270–1276, 2004.

[40] S. Suryavanshi, D. Sharma, R. Cheeker et al., “Amelioration of radiation-induced hematopoietic syndrome by an antioxidant chlorophyllin through increased stem cell activity and modulation of hematopoiesis,” Free Radical Biology & Medicine, vol. 85, pp. 56–70, 2015.

[41] K. K. Boloor, J. P. Kamat, and T. P. Devasagayam, “Chlorophyllin as a protector of mitochondrial membranes against γ-radiation and photosensitization,” Toxicology, vol. 155, no. 1-3, pp. 63–71, 2000.

[42] C. H. Yun, H. G. Jeong, J. W. Ihoun, and F. P. Guengerich, “Non-specific inhibition of cytochrome P450 activities by chlorophyllin in human and rat liver microsomes,” Carcinogenesis, vol. 16, no. 6, pp. 1437–1440, 1995.

[43] L. C. Chiu, C. K. Kong, and V. E. Ooi, “The chlorophyllin-induced cell cycle arrest and apoptosis in human breast cancer MCF-7 cells is associated with ERK deactivation and cyclin D1 depletion,” International Journal of Molecular Medicine, vol. 16, no. 4, pp. 735–740, 2005.

[44] J. W. Fahey, K. K. Stephenson, A. T. Dinkova-Kostova, P. A. Egner, T. W. Kessler, and P. Talalay, “Chlorophyll, chlorophyllin and related tetrapyrroles are significant inducers of mammalian phase 2 cytoprotective genes,” Carcinogenesis, vol. 26, no. 7, pp. 1247–1255, 2005.

[45] J. E. Mata, Z. Yu, J. E. Gray, D. E. Williams, and R. Rodriguez-Proteau, “Effects of chlorophyll on transport of dibenz(a,h)pyrene, 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine, and aflatoxin B1 across Caco-2 cell monolayers,” Toxicology, vol. 196, no. 1-2, pp. 117–125, 2004.

[46] Coronavirus Resource Center, Johns Hopkins University, “COVID-19 Dashboard,” November 2021, https://coronavirus.jhu.edu/map.html.

[47] E. Terpos, I. Ntanasis-Stathopoulos, I. Elalamy et al., “Hematological findings and complications of COVID-19,” American Journal of Hematology, vol. 95, no. 7, pp. 834–847, 2020.

[48] Q. Zhao, M. Meng, R. Kumar et al., “Lymphopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: a systemic review and meta-analysis,” International Journal of Infectious Diseases, vol. 96, pp. 131–135, 2020.

[49] M. Zheng, Y. Gao, G. Wang et al., “Functional exhaustion of antiviral lymphocytes in COVID-19 patients,” Cellular & Molecular Immunology, vol. 17, no. 5, pp. 533–535, 2020.

[50] N. F. Clark and A. W. Taylor-Robinson, “COVID-19 therapy: could a copper derivative of chlorophyll be used to treat lymphopenia associated with severe symptoms of SARS-CoV-2 infection?,” Frontiers in Medicine, vol. 8, article 620175, 2021.

[51] C. Ulbricht, R. Bramwell, M. Catapang et al., “An evidence-based systematic review of chlorophyll by the Natural
Standard Research Collaboration,” Journal of Dietary Supplements, vol. 11, no. 2, pp. 198–239, 2014.

[52] B. Mysliwa-Kurdziel and K. Solymosi, “Phycobilins and phycobiliproteins used in food industry and medicine,” Mini Reviews in Medicinal Chemistry, vol. 17, no. 13, pp. 1173–1193, 2017.

[53] M. G. Ferruzzi, M. L. Failla, and S. J. Schwartz, “Sodium copper chlorophyllin: in vitro digestive stability and accumulation by Caco-2 human intestinal cells,” Journal of Agricultural and Food Chemistry, vol. 50, no. 7, pp. 2173–2179, 2002.

[54] S. Bhatia, P. N. Prabhu, A. C. Benefiel et al., “Galacto-oligosaccharides may directly enhance intestinal barrier function through the modulation of goblet cells,” Molecular Nutrition & Food Research, vol. 59, no. 3, pp. 566–573, 2015.

[55] Y. Zeng, X. Pu, J. Yang et al., “Preventive and therapeutic role of functional ingredients of barley grass for chronic diseases in human beings,” Oxidative Medicine and Cellular Longevity, vol. 2018, Article ID 3232080, 15 pages, 2018.

[56] G. Chen, L. Che, X. Cai, P. Zhu, J. Ran, and S. Liu, Bioinformatic analysis identifies biomarkers and treatment targets in primary Sjögren’s syndrome patients with fatigue, Research Square, 2021.