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Original Article

Evaluation of key genes and biological pathways that play a role in primary Sjogren syndrome by using a systems biology approach

Hanieh Validad[a], Parvin Parvaie[b], Samira Nomiri[c], Ebrahim Miri-Moghaddam[d, e], Hossein Safarpour[f*]

[a]Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran. [b]Department of oral and maxollofacial medicine, School of dentistry, Birjand university of medical sciences, Birjand, Iran. [c]Department of Clinical Biochemistry, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran. [d]Cardiovascular Diseases Research Center, School of Medicine, Birjand University of Medical Sciences, Birjand Iran. [e]Department of Molecular Medicine, School of Medicine, Birjand University of Medical Sciences, Birjand Iran. [f]Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran.

Abstract

Primary Sjogren syndrome (PSS) is one of the most common systemic autoimmune diseases. Lymphocytic infiltration of exocrine glands, especially lacrimal and salivary in PSS, causes ocular and oral dryness. Dry mouth may lead to difficulty in speaking, chewing, and swallowing and result in reduced quality. The pathogenesis of PSS involves multiple factors, such as genetic, environmental, and immunological factors. Despite extensive research over the last few decades, the exact etiology and progression of PSS and its inflammatory lesions is still unknown. Gene co-expression network analysis (WGCNA) is a system biology method that can be used to describe the correlation between different genes and find modules of highly correlated genes and key genes. Also, by using these modules, we can get gene ontology information and biological pathways. In this study, we used WGCNA to analyze the GSE40611 dataset, which consists of 17 PSS patients and 18 healthy controls. We construct a co-expression network for mRNA expression data of patients and control groups and then find the most significant module and hub genes that play important roles in PSS. We also identify biological pathways and related miRNA for hub genes. Among all the modules, turquoise had the most correlation with PSS and some of the hub genes, including *GPR18*, *FCRL1*, *VNN2* and etc. Also, a large number of pathways were identified in the turquoise module, most of them related to immune system activity, like T-cell activation, lymphocyte differentiation, leukocyte and lymphocyte activation, regulation of immune system processes, regulation of immune response, and cell-cell adhesion. External validation using bulk RNA sequencing data also confirmed the presence of selected hub-genes in pathogenicity of PSS. Finally, these results can lead to finding key players in treatment of PSS.

Keywords: Primary Sjogren syndrome; Systems biology; WGCNA; Microarray; Bulk RNA sequencing.

Corresponding Author: Hossein Safarpour, Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran, E-mail: safarpour701@yahoo.com

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1. Introduction

Primary Sjogren's syndrome (PSS) is one of the most common systemic autoimmune diseases; its prevalence ranges from 0.1 to 0.6 percent, and it affects women more than men [1].

Lymphocytic infiltration of exocrine glands, especially lacrimal and salivary in PSS, causes ocular and oral dryness. It can also affect extra-glandular sites like joints, vessels, lungs, nerves, and kidneys, and even cause an enhanced risk of lymphoma [2-4]. Dry mouth may lead to difficulty in speaking, chewing, and swallowing and may result in a reduced quality of life and unpleasant feelings. Hyposalivation also contributes to increased dental caries risk, rampant caries and problems in prosthetic replacement [5, 6].

The pathogenesis of PSS involves multiple factors such as genetic predisposition, environmental factors, and immunological disorders [7]. Both innate and adaptive immune responses play a role in PSS. In genetically susceptible people, environmental stimuli can trigger salivary gland epithelial cells to express ligands, receptors, and cytokines and result in the activation of many innate immune cells like Natural killer cells (NKs), Type 3 innate lymphoid cells (ILC3s), Dendritic cells (DCs), and macrophages [3]. Hyperactivation of B cells has been shown in PSS to lead to the production of auto-antibodies such as anti-Ro/SSA and anti-La/SSB antibodies, which are considered as biomarkers for disease classification and diagnosis [8, 9]. Evidence also shows T cells' activity in PSS. They can be involved in a loss of self-tolerance, mediating B cell hyperactivity and secreting several pro-inflammatory cytokines associated with inflammation in PSS, including IFN-γ, IL-17, and IL-21 [10]. Despite extensive research over the last several decades, the exact etiology and progression of PSS and its inflammatory lesions is still unknown [11, 12].

Autoimmunity is a complicated interaction between genes and the environment, and using system biology to understand this complex and multifactorial disease like PSS can be useful [13-15]. System biology uses a systematic and holistic view and connects molecular components on one single scale and also different scales, like cells and tissues, to functional pathways, so we can understand the relationships between different scales better [16-18]. Weighted gene co-expression network analysis (WGCNA) is a system biology method that can be used to describe correlation between different genes, find modules of highly correlated genes and key genes. Also, by using this module, we can get to gene ontology information and biological pathways [19-21]. Hillen and coworkers used WGCNA analysis to study interactions between genes which play roles in PSS and also construct gene co-expression networks and find key pathways altered in PSS patient plasmacytoid dendritic cells (pDCs). As a result, they found some genes with the most interactions known as hub genes, which were associated with the IFN-α/β receptor, antiviral processes, pDC activation, and encoding ribosomal proteins. They also showed that genes associated with type-I IFN-activity and pDC activation had the largest changes in PSS patients compared to healthy controls [22]. In another study, Yoa and teammates also used WGCNA to analyze the microarray data of Sjogren patients and healthy controls to create a holistic view of Sjogren syndrome and identify potential pathways and hub genes that may be involved in Sjogren syndrome. As a result of the analysis, they found some hub genes which were related to the immune response, defense response, the

response to cytokine stimulus, and the inflammatory response [23].

Recording to the recent statements using WGCNA may help us to understand PSS much better, so in this study we use systems biology and WGCNA to identify key genes and biological interactions that play a role in PSS as a unique system.

2. Materials and Methods

2.1. Dataset and preprocessing

In the present study, the GSE40611 dataset was procured from the NCBI Gene Expression Omnibus (GEO) database and utilized for subsequent analysis (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE40611). This particular dataset was predicated upon the GPL-570 platform and comprised of mRNA expression profiles of 18 healthy individuals as controls, 17 patients afflicted with PSS, and 14 patients with non-PSS sicca. Subsequently, PSS patients and the control group were selected for further analysis. Thereafter, data correction and normalization were carried out using the affy package in R studio. Moreover, the called package was also employed to annotate the probe names and acquire gene IDs and symbols for the same. In instances where the IDs were not obtainable, they were excluded. Finally, the average data of identifiers was computed for each sample.

2.2. WGCNA network construction and module extraction

To ascertain the comparative significance of genes and the involvement of potential modules, a network analysis was conducted utilizing the WGCNA package in R [24]. In a nutshell, the GSE40611 dataset was subjected to WGCNA. Co-expression networks were produced by using a soft threshold power to differentiate modules with varied expression patterns. The weighted co-expression connections contained in the adjacency matrix were then evaluated using the Pearson correlation coefficient. The co-expression relationships between genes were subsequently determined using a similarity function for topological overlap matrices. The networks were developed by clustering genes with exceedingly similar co-expression patterns. Therefore, the modules were acquired, which consisted of the requisite crucial genes and their co-expressed modular genes.

2.3. Identification of key or modules of interest

The present study entailed an analysis of the correlation between module eigengenes and clinical traits, with the aim of identifying modules that manifested a significant association with clinical traits. The correlation values were visually represented on a heatmap. The modules that were found to be most significantly correlated with PSS were deemed the key modules of PSS. Gene significance (GS) was defined as the correlation between gene expression and each trait. Furthermore, module membership (MM) was defined as the association between gene expression and each module eigengene. Thereafter, the correlation between GS and MM were examined in order to substantiate certain module-trait associations. It is noteworthy that the correlation analyses in this study were performed utilizing the Pearson correlation, as outlined in the 'WGCNA' package [19].

2.4. Function and Pathway Enrichment Analysis of modules

To explicate the biological import of the differentially expressed genes (DEGs), an enrichment analysis predicated on Gene Ontology and KEGG pathways [25] was carried out. The overrepresented Gene Ontology (GO) was delineated using ClueGO (version 2.5.7) plugin of Cytoscape (version 3.8.0). ClueGO is a Cytoscape plug-in that portrays the non-repetitive biological terminologies for voluminous gene sets. Furthermore, ClueGO amalgamates the GO terms to fabricate a GO/pathway network [26].

2.5. Differential Expression Analysis

The utilization of the R package 'limma' was employed to screen out differentially expressed genes (DEGs) within two sets of comparisons between PSS and Control. The criteria for DEGs were set at a |fold change (FC)|>1.5 and P<0.05. The DEGs were visually represented using the R package 'ggplot2' (https://cran.r-project.org/web/packages/ggplot2/) as a volcano plot (**Fig. S1**). Subsequently, the DEGs were cross-referenced with the modules of interest to identify potential connections. The results of this analysis were illustrated as a Venn diagram using the R package 'Venn Diagram' (https://cran.r-project.org/web/packages/VennDiagram/).

2.6. External validation on another GEO dataset

The dataset GSE154926, which comprised of 43 PSS and 7 control minor salivary gland samples, was subjected to high-throughput sequencing. In order to standardize the data and acquire the standardized matrix file, the R Bioconductor package DESeq2 was utilized. Subsequently, genes with an Adj. p. value of less than 0.05 and LogFC exceeding |1| were chosen for further analysis.

2.7. Construction protein-protein interaction network

The integration of targeted hub-gene protein-protein interaction (PPI) networks is a crucial step in understanding the intricate relationships between molecules, specifically in the realm of organismal and cellular biochemistry. With this in mind, our investigation delves into the connections of hub-genes using PPI networks, which we constructed by including proteins that interacted with targeted genes via the reliable and widely-used STRING website.

3. Results and Discussion

3.1 Network construction and modules analysis

Data preprocessing including quantile-normalization (**Figure 1A**), probe ID conversion, and averaging of probes was performed. Sample clustering identified 12 outliers which were excluded from downstream analysis (**Figure 1B**). Afterwards, as shown in **Fig. 2A**, a β value equal to 7 was selected as the soft-thresholding power. As this power follow the powerlaw distribution and the network is closer to the true biological network state. Then, weighted co-expression network from PSS patients and normal samples was reconstructed. As a result, the hierarchical clustering dendrogram identified 27 modules (**Fig. 2B**). The number of genes in each module varied from 141 (purple) to 952 (turquoise).

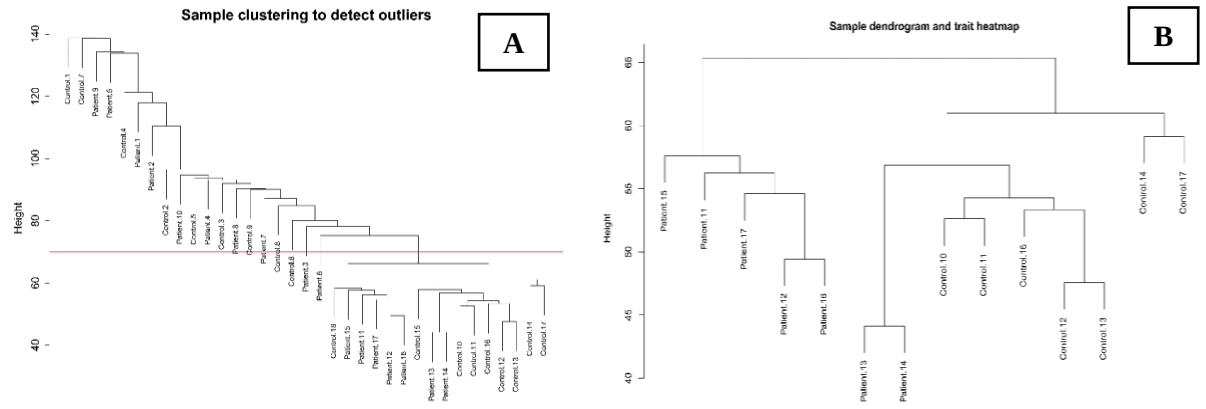

Figure 1: Sample clustering to detect outliers; **A**. Before removing outliers; **B**. After removing one outlier.

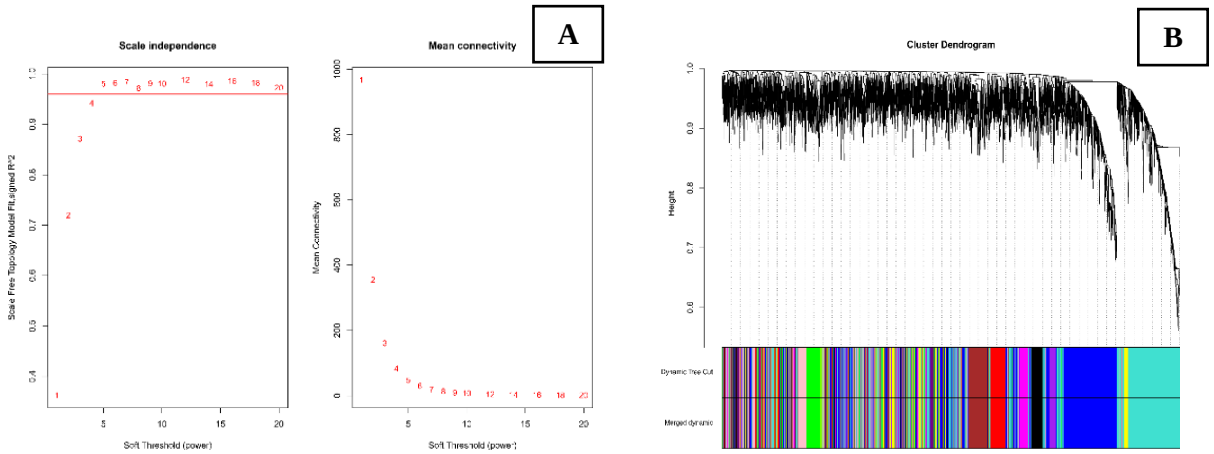

Figure 2: Identification of WGCNA Modules; **A**. Selection of the soft-thresholding powers. **B**. Cluster dendrogram and module assignment from WGCNA. The branches correspond to highly interconnected groups of genes. Colors in the horizontal bar represent the modules**.**

3.2 Module-trait and module-module association

In order to understand the association of modules with the presence of disease in samples and also module-module relationships, eigengenes for each module were calculated and turquoise module (r = 0.97, P-value = 2.00E-08) was selected for more analysis (**Fig. 3A and Table 1**). Indeed, the eigengene network contains the clustering tree and heatmap that show the relationships between modules and the PSS trait (**Fig. 3A**). This plot indicates that the turquoise module and PSS trait are the most correlated.

Table 1: Module colors characteristics.

Modulecolors	Genes	Correlation	P-value
Turquoise	952	0.97	0.9
Purple	141	0.34	0.8
Black	235	0.25	0.8
Magenta	174	0.19	0.8
Green	249	0.041	0.5
Red	238	-0.062	0.4
Pink	183	-0.088	0.2
Grey	282	-0.088	0.2
Blue	949	-0.36	0.2
Brown	337	-0.39	0.01
Yellow	260	-0.65	2.00E-08

3.3 Enrichment analysis of turquoise module

We used the ClueGO tool to visualize the significant pathways of interested module genes with their interactions. There are a large number of pathways identified in the Turquoise Module related to immune system activity, like T-cell migration, G-protein coupled receptor signaling, neuropeptide signaling pathways, and receptor regulator activity (**Fig. 3B**).

3.4 Network analysis of the selected modules and Hub genes

First, genes with the maximum MM (Module Membership) and GS (Gene Significance) values in turquoise module were compared with their DEG list counterparts, and the similar genes were then considered the final hub genes. We build co-expression networks of selected hub genes from the previous step by using the GeneMANIA database (https://genemania.org/). The selected hub-genes were including: *CXCL3*, *UHRF1*, *ICOS*, *VNN2*, *MS4A1*, *GPR18*, *FCRL1*, *LINC01215*, *FCRL4*, and *NLRC3* (**Fig. 4A-C**). **Table 2** shows the LogFCs and adjusted p. values of these hub-genes.

Table 2: Common Hub-genes characteristics.

Genes	Log FC	P-value
CXCL13	5.930151	5.51E-08
MS4A1	5.3814	2.26E-05
ICOS	3.258313	5.61E-06
GPR18	3.118624	7.19E-05
FCRL1	3.038282	2.70E-08
VNN2	2.896468	1.87E-06
UHRF1	2.877767	2.77E-05
FCRL4	2.756927	2.60E-05
NLRC3	2.567454	8.37E-05
LINC01215	2.44512	8.26E-06

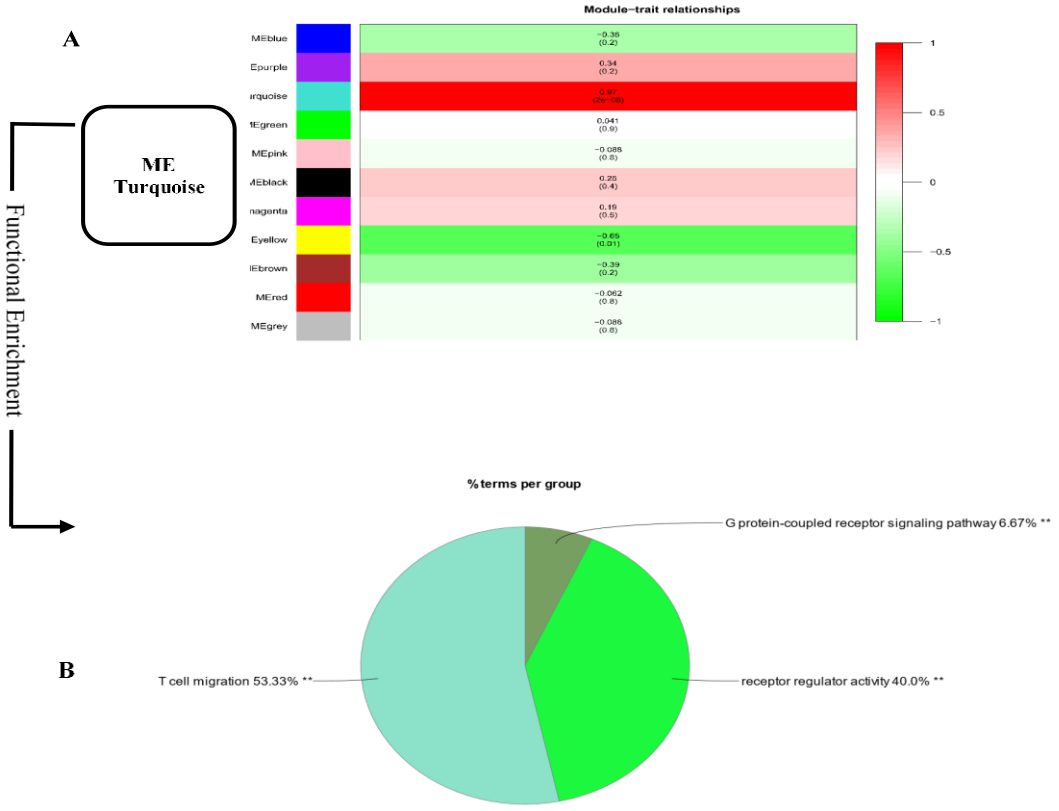

Figure 3: Module-Trait association analysis; **A.** Module-trait relationship. Each row corresponds to a module Eigen gene, and the column corresponds to Pss status. The numbers in each cell represent the corresponding correlation and p-value. **B.** Functional enrichment analysis of the turquoise module.

3.5 External validation of hub-genes on bulk RNA sequencing data

In order to validate selected hub-genes in previous section, DEG analysis of bulk RNA sequencing data of PSS patients was performed. Interestingly 5 of 10 hub-genes were common between 3 independent lists (**Fig. 4A and 4B**). These genes were including *UHRF1*, *ICOS*, *MS4A1*, *FCRL4*, and *NLRC3*.

3.6 Examination of the Protein - Protein Interacting Networks

As evidenced by the PPI analysis, it was observed that pre-existing and anticipated associations were present among GFR18, FCRL1, and VNN2 with an additional 30 proteins, which are clearly depicted in **Figure 4C**.

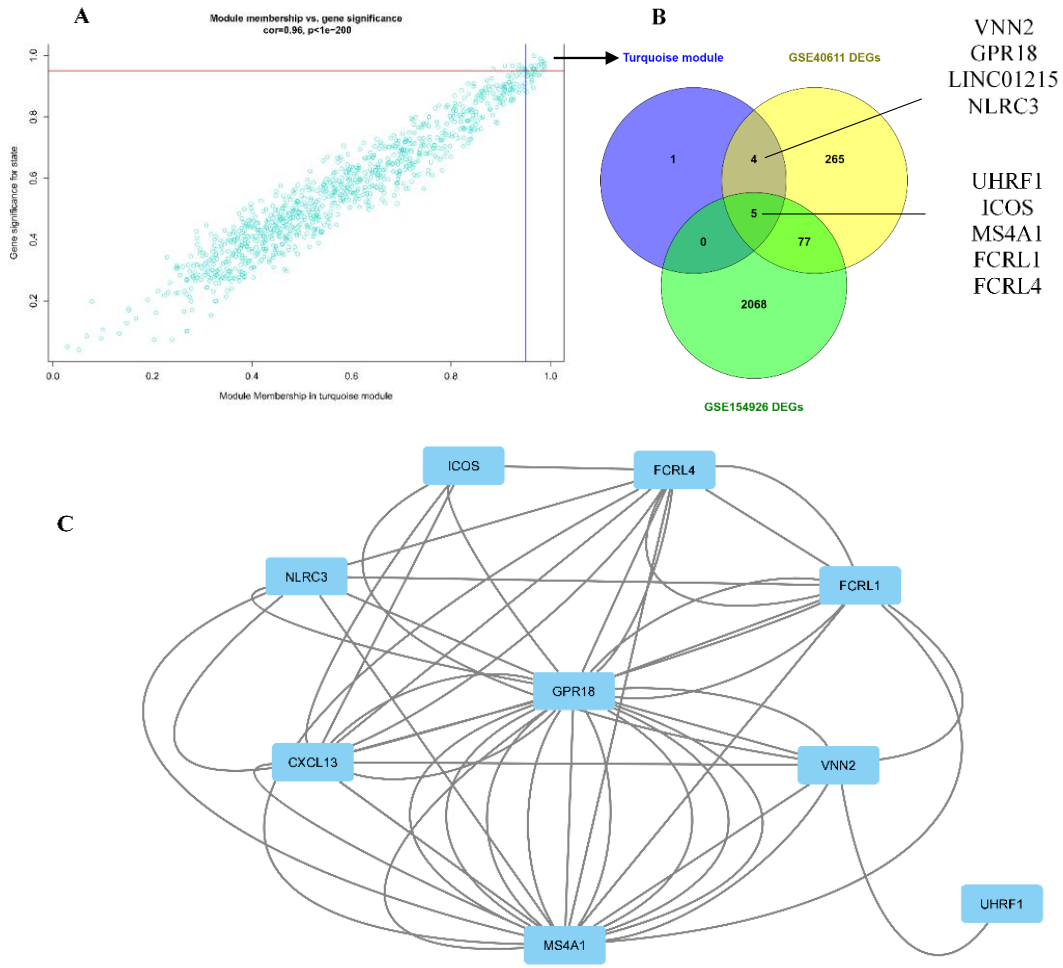

Figure 4: Hub-genes detection; **A.** Turquoise module features of GS and MM, which were significantly correlated with pSS status (adjacent normal vs. pSS). Each point represents an individual gene within each module, which was plotted by GS on the y-axis and MM on the x-axis. **B.** Evaluation of similarity between DEGs and hub-genes lists using a Venn diagram. **C.** The similar genes were imported to Gene MANIA to construct a co-expression network.

4. Discussion

PSS is a systemic autoimmune disease that is characterized by dryness, widespread pain, and severe fatigue in patients and is also considered an etiologic factor for lymphoma. Despite the progress in understanding the underlying mechanisms of PSS, there is a lack of effective treatment, and experts agree that we still need to identify new biomarkers which may allow better diagnosis and treatment of PSS [27-29].

The WGCNA algorithm is a novel and popular systems biology method that is used to identify highly correlated gene modules and hub genes, predict gene function, and discover new disease biomarkers [30-32].

The aim of this study was to reconstruct PSS gene co-expression networks and identify key genes and biological interactions that play a role in PSS by using the WGCNA package. Once we found the GSE40611 dataset from the GEO database and analyzed it with the WGCNA package, we understood that the turquoise module is the most correlated module with PSS (r = 0.97, P-value = 2.00E-08). Based on enrichment analysis on DEGs and this module, they play a role in T-cell migration, G-protein coupled receptor signaling, neuropeptide signaling pathways, and receptor regulator activity. We also chose three hub genes, including *GFR18*, *FCRL1*, and *VNN2* based on their LogFC and novelty for further evaluation.

First hub gene we discussed about is *GPR18*, in humans, the *GPR18* gene is located on chromosomal region 13q32.2 and encodes the GPR18 protein [33]. *GPR18* is an orphan G-protein receptor that was discovered in 1997. It highly expressed in different tissues and cells of the immune system, like spleen, thymus, lymph nodes, and leukocytes, including monocytes, polymorphonuclear neutrophils (PMNs) and macrophages [34, 35]. Studies have identified that regulation of *GPR18* is related to physio-pathological processes like pain, sperm physiology, hemodynamic responses, cell migration, intraocular pressure, metabolism, and immunomodulation, including resolving inflammation, leucocyte migration, and regulating macrophage apoptosis [34, 36]. *GPR18* is involved in neurodegenerative processes like Alzheimer's disease and multiple sclerosis as well [37, 38]. Yuchen et al. reported that *GPR18*, *MS4A1*, and TIL-B are reliable biomarkers for intratumorally B-cell assessments and can be used for prognostic purposes in cancers like head and neck cancer. They also show significant prognostic power of *GPR18* mRNA levels in breast and lung cancers with microarray expression data [39].

The FCRL immunoglobulin gene superfamily is located in the human chromosome region 1q21–23 [40]. This gene family encodes six transmembrane receptors, including FCRL1-6. FCRL1-5 are expressed on B cells' plasma membranes in different stages and can regulate development, differentiation, activation, antibody secretion and isotype switching [41]. Among FCRL receptors, in this study we focused on *FCRL1*. Several studies have found that this receptor is expressed abnormally in a variety of B-cell-related disorders, including hematological malignancies, disorders, and infection diseases [42, 43]. Rostamzadeh et al. reported an overexpression of *FCRL1* in some

autoimmune diseases like MS, Takayasu's arteritis, lupus anticoagulants, and von Willebrand [41]. Liu et al. also suggested the usage of *FCRL1-5* molecules as biomarkers to predict the prognosis of patients with cutaneous [44].

The Vanin gene family in humans includes *VNN1*, *VNN2* and *VNN3*. *VNN1* and *VNN2* are expressed in several tissues, such as the kidney, spleen, and blood leukocytes. *VNN2* expression increases in neutrophil progenitors during differentiation and maturation. Glycosylphosphatidylinositol anchored surface protein Vanin-2 (*VNN2*) has an important role in leukocyte adhesion and migration to inflammatory sites. Although *VNN2* is mostly found in the plasma membrane, it can be found in secretory vesicles as a soluble form [45-47]. In addition, the soluble *VNN2* is detected in synovial fluids of rheumatoid arthritis patients and serum derived from the coronary sinus of patients with isolated atherosclerotic coronary artery disease, suggesting that *VNN2* can indicate the severity of inflammation [46]. Studies also showed that *VNN2* is related to chemoresistance and relapse in patients with acute lymphoblastic leukemia (ALL) [47, 48].

5. Conclusion

In this investigation, an examination of the expression profile of PSS disease genes, GSE40611, revealed three noteworthy genes, namely *GFR18*, *FCRL1*, and *VNN2*. These genes are known to have a significant impact on inflammatory pathways and the regulation of the immune system. Further review of studies pertaining to immune system diseases and autoimmune disorders has shown that any disruption in the gene expression or protein function of these aforementioned genes can lead to dysfunction in the immune system and the development of autoimmune disorders.

Conflict of interest

The authors declare to have no conflict of interest.

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References

[1] Holdgate, N. and E.W.S. Clair, Recent advances in primary Sjogren's syndrome, *F1000Research* (2016) 5.

[2] Chen, X., et al., Elevated cytokine levels in tears and saliva of patients with primary Sjögren's syndrome correlate with clinical ocular and oral manifestations, Sci Rep (2019) 9(1): 1-10.

[3] Rizzo, C., et al., Primary Sjogren Syndrome: Focus on Innate Immune Cells and Inflammation, Vaccines (Basel) (2020) 8(2): 272.

[4] Retamozo, S., P. Brito-Zerón, and M. Ramos-Casals, Prognostic markers of lymphoma development in primary Sjögren syndrome, Lupus (2019) 28(8): 923-936.

[5] Bolstad, A.I. and K. Skarstein, Epidemiology of Sjögren's Syndrome—from an Oral Perspective, Curr Oral Health Rep (2016) 3(4): 328-336.

[6] Molano-González, N., et al., Anti-citrullinated protein antibodies and arthritis in Sjögren's syndrome: a systematic review and meta-analysis, Scand J Rheumatol (2019) 48(2): 157-163.

[7] Wang, J., L. Zhou, and B. Liu, Update on disease pathogenesis, diagnosis, and management of primary Sjögren's syndrome, Int J Rheum Dis (2020).

[8] Nocturne, G. and X. Mariette, B cells in the pathogenesis of primary Sjögren syndrome, *Nat Rev Rheumatol* (2018) 14(3): 133.

[9] Martín-Nares, E. and G. Hernández-Molina, Novel autoantibodies in Sjögren's syndrome: a comprehensive review, Autoimmun Rev (2019) 18(2): 192-198.

[10] Verstappen, G.M., F.G. Kroese, and H. Bootsma, T cells in primary Sjögren's syndrome: targets for early intervention, Rheumatology (2019).

[11] Groups, S.s.I.C.C.A.R., Natural History and Predictors of Progression to Sjögren's Syndrome Among Participants of the Sjögren's International Collaborative Clinical Alliance Registry, Arthritis Care Res (2018) 70(2): 284-294.

[12] Xin, M., et al., Mirt2 functions in synergy with miR-377 to participate in inflammatory pathophysiology of Sjögren's syndrome, Artif Cells Nanomed Biotechnol (2019) 47(1): 2473-2480.

[13] Sarkar, P.K., et al., Pulmonary manifestations of primary Sjogren's syndrome, Indian J Chest Dis Allied Sci (2009) 51(2): 93-101.

[14] Alberghina, L. and H.V. Westerhoff, Systems biology: definitions and perspectives, Vol. 13. 2007: Springer Science & Business Media.

[15] Tandon, M., The Immune-Epithelial Interaction in the Pathogenesis of Primary Sjögren's Syndrome, Thomas Jefferson University (2017)

[16] Zou, Y. and M.D. Laubichler, From systems to biology: A computational analysis of the research articles on systems biology from 1992 to 2013, PLoS One (2018) 13(7): e0200929.

[17] Tavassoly, I., J. Goldfarb, and R. Iyengar, Systems biology primer: the basic methods and approaches, Essays Biochem (2018) 62(4): 487-500.

[18] Klassen, A., et al., Metabolomics: Definitions and significance in systems biology, in Metabolomics: From Fundamentals to Clinical Applications, Springer (2017): 3-17.

[19] Langfelder, P. and S. Horvath, WGCNA: an R package for weighted correlation network analysis, BMC Bioinformatics (2008) 9(1): 559.

[20] Hu, S., et al., Systems biology analysis of sjögren's syndrome and mucosa-associated lymphoid tissue lymphoma in parotid glands, Arthritis & Rheumatism (2009) 60(1): 81-92.

[21] Presson, A.P., et al., Integrated weighted gene co-expression network analysis with an application to chronic fatigue syndrome, BMC Syst Biol (2008) 2(1): 95.

[22] Hillen, M.R., et al., Plasmacytoid DCs From Patients With Sjögren's Syndrome Are Transcriptionally Primed for Enhanced Pro-inflammatory Cytokine Production, Front immunol (2019) 10: 2096.

[23] Yao, Q., et al., Identifying Key Genes and Functionally Enriched Pathways in Sjögren's Syndrome by Weighted Gene Co-Expression Network Analysis, Front Genet (2019) 10: 1142.

[24] Livak, K.J. and T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method, methods (2001) 25(4): 402-408.

[25] Kanehisa, M., et al., New approach for understanding genome variations in KEGG, Nucleic Acids Res (2019) 47(D1): D590-D595.

[26] Bindea, G., et al., ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks, Bioinformatics (2009) 25(8): 1091-1093.

[27] Seror, R., G. Nocturne, and X. Mariette, Current and future therapies for primary Sjögren syndrome, Nat Rev Rheumatol (2021): 1-12.

[28] Vitali, C., et al., Management of Sjögren's syndrome: present issues and future perspectives, Front Med (2021) 8.

[29] Travaglino, A., et al., Sjögren syndrome in primary salivary gland lymphoma: a systematic review and meta-analysis, Am J Clin Pathol (2020) 153(6): 719-724.

[30] Wu, Z., et al., Using WGCNA (weighted gene co-expression network analysis) to identify the hub genes of skin hair follicle development in fetus stage of Inner Mongolia cashmere goat, *PloS one* (2020) 15(12): e0243507.

[31] Yin, X., et al., Identification of key modules and genes associated with breast cancer prognosis using WGCNA and ceRNA network analysis, Aging (Albany NY) (2021) 13(2): 2519.

[32] He, Y., et al., Tumor infiltrating lymphocytes associated competitive endogenous RNA networks as predictors of outcome in hepatic carcinoma based on WGCNA analysis, PloS one (2021) 16(7): e0254829.

[33] Nourbakhsh, F., R. Atabaki, and A. Roohbakhsh, The role of orphan G protein-coupled receptors in the modulation of pain: A review, Life Sci (2018) 212: 59-69.

[34] Morales, P., et al., Therapeutic Exploitation of GPR18: Beyond the Cannabinoids? Miniperspective, J Med Chem (2020) 63(23): 14216-14227.

[35] Zhang, L., et al., GPR18 expression on PMNs as biomarker for outcome in patient with sepsis, Life sciences (2019) 217: 49-56.

[36] Guerrero-Alba, R., et al., Some prospective alternatives for treating pain: the endocannabinoid system and its putative receptors GPR18 and GPR55, Front pharmacol (2019) 9: 1496.

[37] Neumann, A., et al., Computational investigations on the binding mode of ligands for the cannabinoid-activated G protein-coupled receptor GPR18, Biomolecules (2020) 10(5): 686.

[38] Reyes-Resina, I., et al., Molecular and functional interaction between GPR18 and cannabinoid CB2 G-protein-coupled receptors. Relevance in neurodegenerative diseases, Biochem Pharmacol (2018) 157: 169-179.

[39] Liu, Y., et al., Omics-wide quantitative B-cell infiltration analyses identify GPR18 for human cancer prognosis with superiority over CD20, Commun Biol (2020) 3(1): 1-11.

[40] Liu, H.-W., et al., The rs6427384 and rs6692977 Single Nucleotide Polymorphisms of the Fc Receptor-Like 5 (FCRL5) Gene and the Risk of Ankylosing Spondylitis: A Case Control Study in a Single Center in China. Med Sci Monit (2020) 26: e920956-1.

[41] Rostamzadeh, D., et al., Update on Fc receptor-like (FCRL) family: new immunoregulatory players in health and diseases, Expert Opin Ther Targets (2018) 22(6): 487-502.

[42] Yousefi, Z. and N. Eskandari, Prognostic significance of Fc receptor-like 1 in patients with chronic lymphocytic leukemia, hairy cell leukemia, and various B-cell non-Hodgkin's lymphoma, *Leuk Res* (2019) 12: 100181.

[43] Yousefi, Z., et al., Fc Receptor-Like 1 as a Promising Target for Immunotherapeutic Interventions of B-Cell-Related Disorders, Biomarker insights (2019) 14: 1177271919882351.

[44] Liu, Y., et al., Development and Validation of the B Cell-Associated Fc Receptor-like Molecule-Based Prognostic Signature in Skin Cutaneous Melanoma, Biomed Res Int (2020) 2020: 8509805.

[45] Chen, Y., et al., MicroRNA‐106a regulates the proliferation and invasion of human osteosarcoma cells by targeting VNN2, Oncol Rep (2018) 40(4): 2251-2259.

[46] Nitto, T. and K. Onodera, The linkage between coenzyme a metabolism and inflammation: roles of pantetheinase, *J Pharmacol Sci* (2013) 123(1): 1-8.

[47] Guimarães, A.F.M., Vanin-2, a potential prognostic marker of resistance in acute lymphoblastic leukemia, (2015).

[48] Bornhauser, B., et al., The hematopoietic stem cell marker VNN2 is associated with chemoresistance in pediatric B-cell precursor ALL, *Blood advances* (2020) 4(17): 4052-4064.