Weighted Correlation Network Analysis of Multiple Sclerosis: Identifying Key Genes Involved in Disease Progress

Yazdan Rahmati[a], Masoomeh Ghorbani[b], Hasan Mollanoori[a], Fatemeh Ghorbani[c,d*]

[a] Department of Medical Genetics and Molecular Biology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran.

[b] Department of Agronomy and Plant Breeding, Faculty of Sciences, Razi University, Kermanshah, Iran.

[c] Medical Biology Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran

[d] Department of Biology, Faculty of Sciences, University of Sistan and Baluchestan, Zahedan, Iran

CORRESPONDENCE AUTHOR
Fatemeh Ghorbani
E-mail: tima.ghorbani@gmail.com

ABSTRACT
Purpose: Multiple Sclerosis (MS) is an autoimmune inflammatory disease of the white matter of the central nervous system (CNS) characterized by demyelination and axonal loss that finally caused progressive neurodegeneration. In this study, we aimed to apply a new algorithm, weighted gene coexpression network analysis (WGCNA), to carry out a comprehensive expression data analysis.
Methods: We applied this method to datasets of MS patients obtained from the NCBI Gene Expression Omnibus. We identified network modules of co-expressed genes in the healthy subject dataset and verified their preservation in the MS dataset. Then, in the non-preserved modules, we selected hub genes that distinguishing normal from disease conditions and performed functional enrichment analyses of genes involved in these modules.
Results: We identified several genes that discriminate healthy subjects from MS patients that may play essential roles in MS development. Functional enrichment analysis revealed that they regulate leukocytes migration and its related pathways, the endocannabinoid system, and proteasome complex.
Conclusions: Our results indicated several genes essential for MS progress using WGCNA algorithm. These genes may be used as biomarkers for MS diagnosis or potential novel therapeutic targets.

Keywords: Multiple sclerosis, Gene expression, WGCNA, Systems biology, Transcriptional biomarkers

1. INTRODUCTION

Immune system failure in the central nervous system is the main reason of multiple sclerosis (MS) as an inflammatory neurodegenerative disease (1-3). The immune system attacks to myelin sheath and starts the demyelination and axonal damage in central nervous system (CNS) (4, 5). Although many factors are proposed to associate with the MS, this autoimmune disease has unknown etiology (6).

There is evidence that environmental and genetic factors are obviously involved in MS, but their communication is complicated (7). MS is not a single gene disorder and different genes are involves in this multiple gene disease. It seems that MHC genes are the most important genes which are associated with MS. Activation of B and T cells lead to demyelination and sclerotic plaques in the brain cells. Most important cells are CD4+ Th1, Th17, T CD8+, B-lymphocytes, and activated microglia which involves in disease (8, 9). Myelin basic protein (MBP) defect also plays a role in disease (10). Previous researches emphasized on association of HLA allele DRB1*1501 with MS. HLA proteins are involved in the immune system, and this allele is responsible for almost half of the cases of MS by genetic reasons (7, 11).

Today, new approaches in genetic science make it easy to evaluating of the genes involved in the disease. Genome-wide association studies revealed that loci such as interleukin-2 receptor α, interleukin-7 receptor α, CLEC16A, CD6, CD58, IRF8, IL12A, Olig3-TNFAIP3, PTGER4, RGS1 and TNFRSF1A genes seem associated with MS (12). In addition to monitoring the sequence of the genome, the study of the expression of genes in healthy and patients also helps to diagnose the relationship between the genes and the disease. So far, many studies have been conducted on the expression profiles of healthy and MS patients and their results are available in the GEO database as datasets.

It has been recognized that the similar expression patterns may lead to similar biochemical and functional features so that genes with these similarities may create complexes or may act in similar pathways (13-15), however, microarray data just provides the expression profile of individual genes and does not reflect correlations among genes. The weighted gene co-expression network analysis (WGCNA) use differential co-expression network analysis algorithms to supply a general explanation of gene expression data by establishing gene networks based on similarities in expression profiles among genes across microarray samples (16). WGCNA can be used to find modules, the highly co-expressed genes that are closely connected in the network and form a sub-network region. It has been proven that modules: 1- conserved across phylogenies, 2- enriched in protein-protein interactions and 3- often consist of functionally related genes (13, 17-20). After finding the modules, WGCNA also enables to summarizing such modules to the most central and connected genes, that is the so-called hub genes.

In this study, we used WGCNA to constructing 2 gene networks based on normal and MS peripheral blood leukocytes expression data in GSE41890 dataset that make it possible to distinguish modules and hub genes that exhibit different network properties and may be contributed in pathological processes of disease and could serve as diagnostic biomarkers or as potential therapeutic targets.

2. MATERIAL AND METHODS

2.1. Data selection, preprocessing and detection of outlier samples

In the present study, one expression array including GSE41890 obtained from the NCBI Gene Expression Omnibus (GEO). GSE41890 consists of 68 samples including 22 Multiple Sclerosis patients with samples both in remission and relapse and 24 healthy controls, this dataset was produced using Affymetrix Human Gene 1.0 ST Array. To normalize this dataset quantile normalization method in limma package used. For better results, we included only single measure for each gene, by the aggregate

function in S4Vectors package, which gives an average measure of the probes for each gene. To evaluate the comparability of control and relaps samples, the soft Connectivity function in WGCNA package used(21). soft Connectivity evaluates two factors, 1) correlation of expression level of each gene between two datasets, 2) correlation of connectivity of each gene between two datasets. The datasets are comparable if two mentioned correlations are positive and have significance p.value. To remove outlier samples standardized connectivity (Z. K) method used and samples which had Z. K score< −2 deleted from the rest of the analysis.

2.2. Network construction and module detection

Two weighted gene co-expression networks according to control and relapse samples were made. Using pick Soft Threshold function that helps to choose proper soft-thresholding power, we selected soft thresholding power of 6 for providing scale-free topology fit index that reaches values above 0.9. After calculation of adjacencies, to minimize the effect of noise and spurious associations, adjacency results transformed to Topological Overlap Matrix (TOM). TOM of different datasets may have different statistic properties which can affect the results of comparing. Here scaling of Topological Overlap Matrices was used to mitigate the effect of different statistical properties and Quantile-quantile plot of the TOMs in control and relapse datasets to see what scaling is achieved. Results of TOM considered as input to produce dendrogram of genes, and cutree Dynamic function used for branch cutting. Minimum module size of 30, the module detection sensitivity deep Split 2 in block wise Consensus Modules function used for network construction. Finally, network modules of control and relapse datasets detected and control network modules considered as reference. The function module preservation of WGCNA package used to study the preservation of control modules in relapse samples. Zsummary as the output of module preservation function considered for detecting modules that preserved in both mentioned studies. Modules

with Z score > 10 are well preserved between control and relapse samples and removed from the rest of analysis whereas Z score < 10 are less preserved and considered as the result of analysis.

2.3. Detection of hub genes and their functional annotations

Within detected module, the genes with the highest module membership scores are considered hub genes of that module. Next we carried out functional enrichment analyses of known genes in order to facilitate the interpretation of the biological mechanisms related to these genes. KEGG and STRING databases used to functional and biological interpretation of detected genes, using the KEGG database, important signaling pathways with P.value < 0.05 and combined Z value > 10 were identified. PPI network of detected genes was constructed by Search Tool for the Retrieval of Interacting Gene (STRING10.5; https://string-db.org/) with a combined score >0.4 as the cut-off point.

3. RESULTS
3.1. WGCNA prerequisites, outlier samples, batch effects, comparability

One of the key points in using the WGCNA package is the number of samples, the minimum number of samples for construction of weighted gene co-expression network is 15, the number of samples used in this study was 24 and 22 for control and relapse samples respectively, so was sufficient enough. The batch effect is not observed because only one dataset with one platform and one laboratory condition was used. As required for WGCNA package and based on the Z.K score, 5 outlier samples excluded from the control dataset (Figure 1), and also datasets comparability evaluated by the soft Connectivity function that provides positive correlation values when there is high comparability between two datasets. Our datasets were comparable because the overall gene expression correlation was (cor=1, p<1e−200) and the overall gene connectivity was (cor=0.58, p<1e−200) (Figure 2).

A

Sample clustering to detect outliers

B

Sample clustering to detect outliers
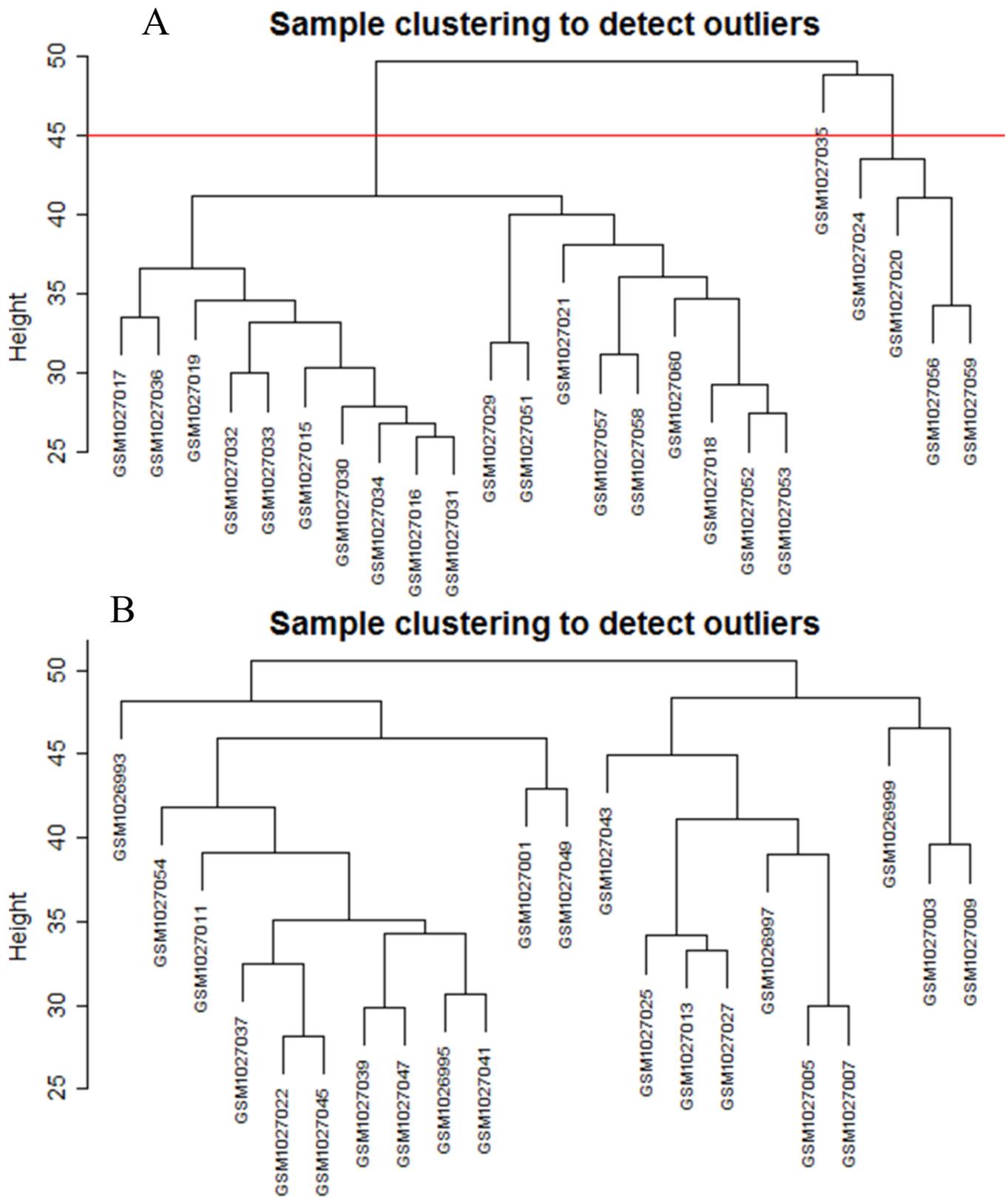
Figure 1: Clustering dendrogram of samples based on their Euclidean distance and detecting outlier samples. A) Control samples, B) Relapse samples.
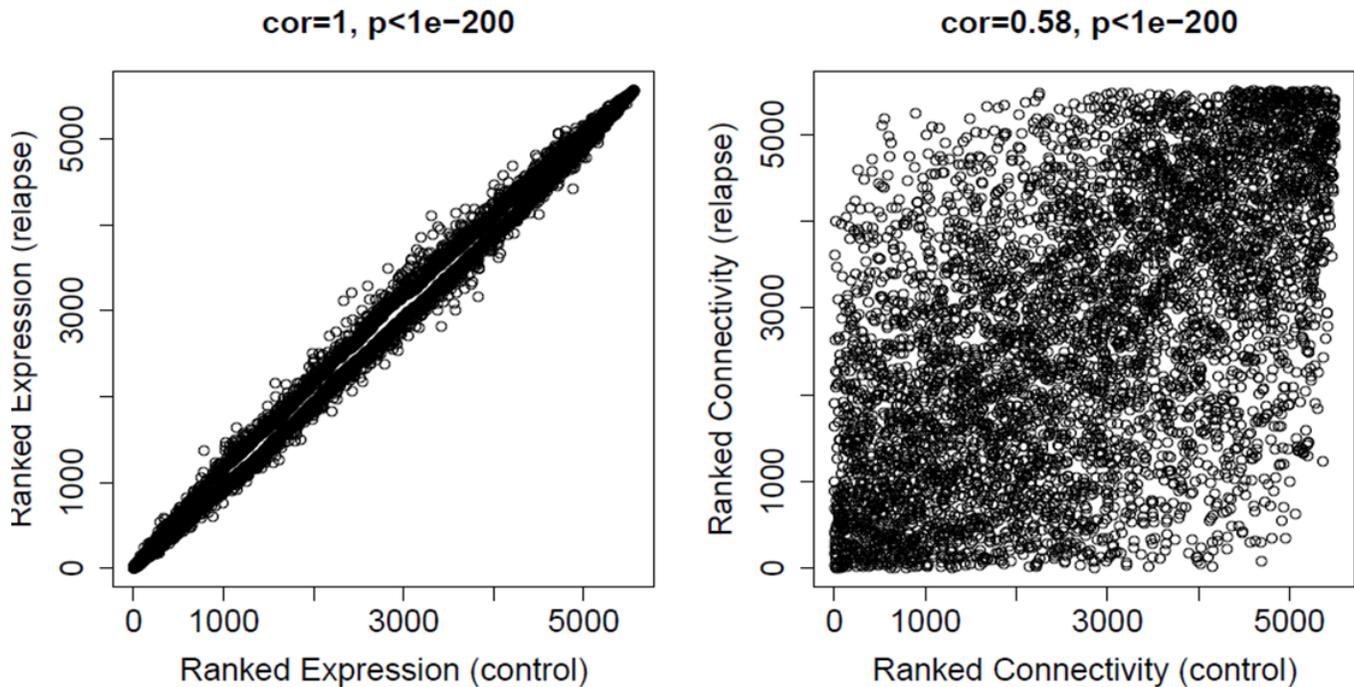
Figure 2: results of softConnectivity. Correlations are positive and the p-values are significant for both datasets. In addition correlations and p-values are better for expression than for connectivity.

3.2. Identification of modules able to distinguish control from relapse datasets

Control and relapse datasets exhibited a scale-free topology because the Scale-free Topology Fit Index reached values above 0.9 for low powers (< 30), the powers were 6 for both control and relapse samples, and these results also show that there is batch-effects were not present in our datasets (Figure 3). After constructing weighted gene co-expression networks for each control and relapse expression datasets, the modules of each of these datasets identified. Based on the WGCNA package, a module is a group of strongly co-expressed genes, these genes have similar biochemical and functional properties or belong to similar pathways. By hierarchical clustering, 23 modules identified for the control dataset as the reference network, these modules have different sizes in term of the number of genes and labelled by the different colors which shows in Figure 1.

By considering relapse dataset as a test network, we evaluated the preservation level across the two networks by finding modules that maintained in two datasets and modules that are not preserved in them. As expected, many of modules well preserved between two mentioned expression datasets and few of them were not preserved between them, so potentially related to the transition from control to the relapse phase of the MS disease. To quantify exactly the module preservation level, module Preservation function from WGCNA package was used. For each module, a Z-score representing the preservation level was calculated, modules with Z-score > 10 are well preserved between two datasets, whereas modules with Z-scores <10 are less preserved and can distinguish control from the relapse phase of MS condition (Figure 5).
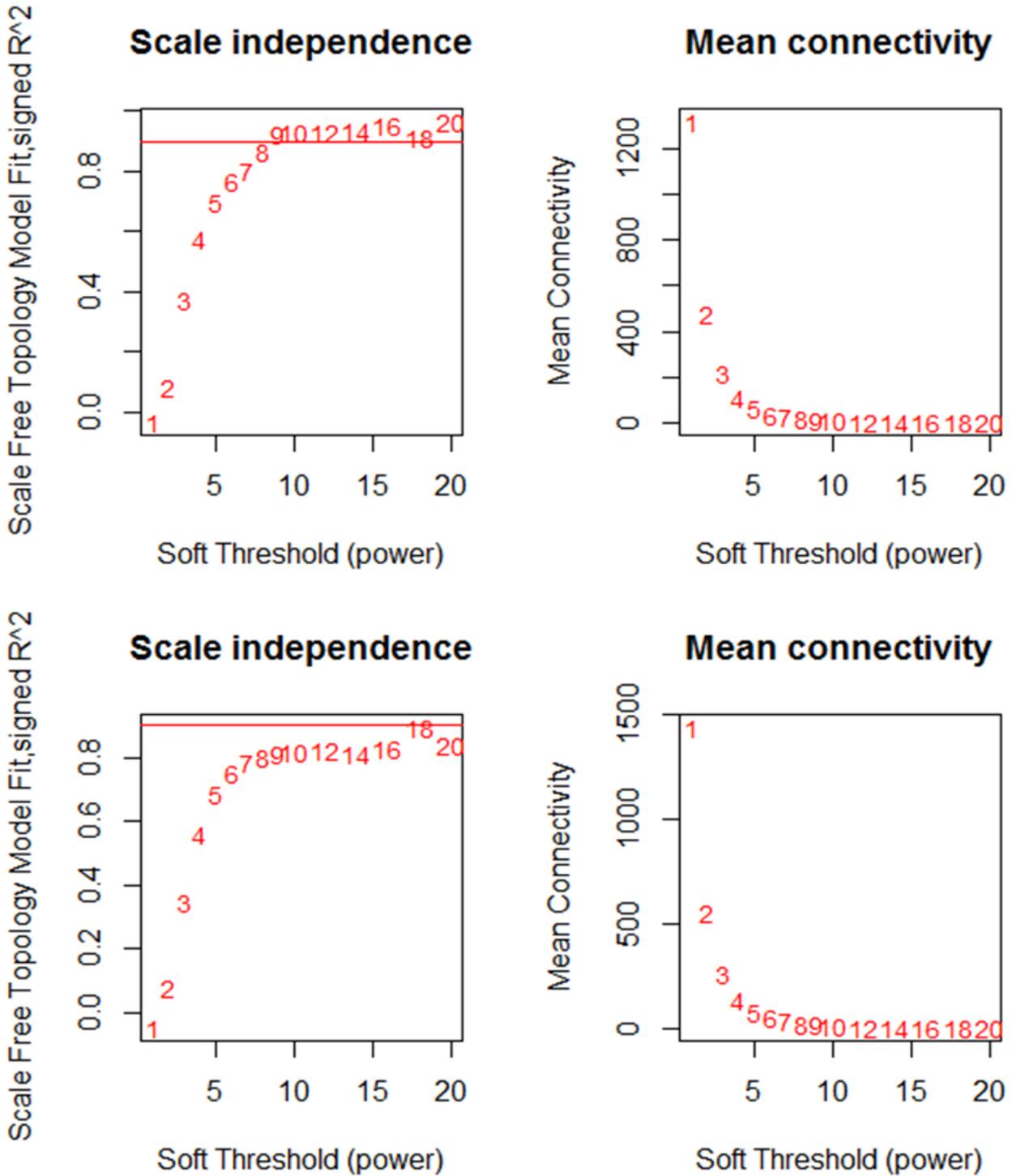
Figure 3: network topology for various soft-thresholding powers, the left panel shows the scale-free fit index and the right panel displays the mean connectivity for A) Control dataset. B) Relapse dataset
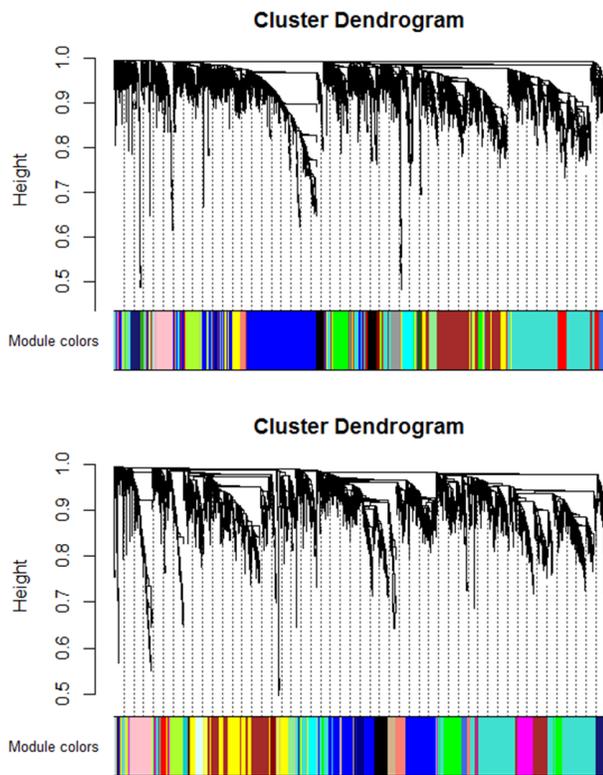
Figure 4: Clustering dendrogram of genes, with dissimilarity based on topological overlap, together with assigned module colors for A) control dataset, B) relapse dataset
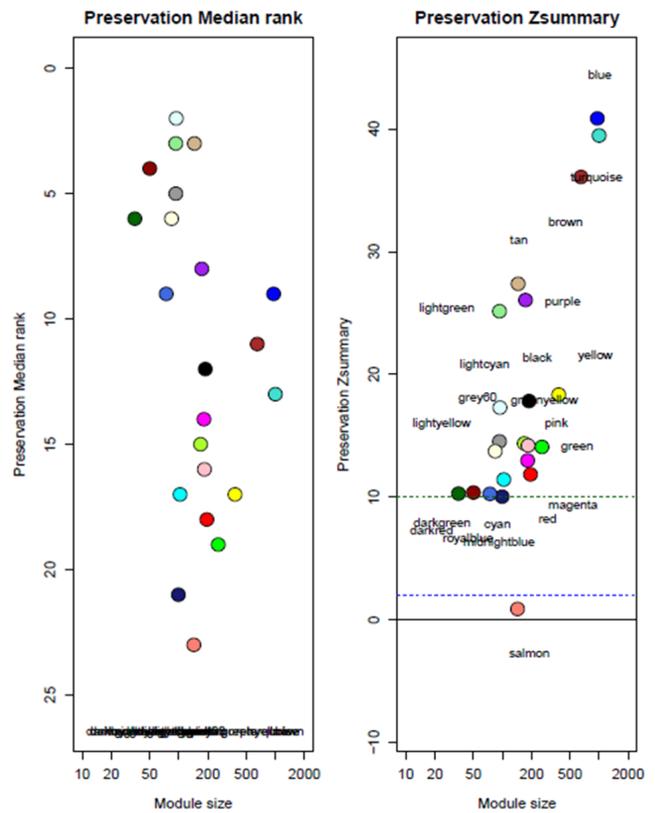
Figure 5: The medianRank and Zsummary statistics of module preservation of control modules in relapse modules (y-axis) vs. module size (x-axis).

3.3. Identification of hub genes and functional analysis

Since we found the salmon module that was not preserved between control and relapse phase of MS, it seems genes related to this module play an important role in the transition from the control to relapse phase. We set out to identify hub genes with the highest module membership scores in this module, these genes can be seen in table 1. To identify the most significant clusters of the detected genes, PPI network of them was constituted by STRING as shown in Figure 6. There are 115 nodes related to hub genes and 42 edged in the PPI network. According to the results of KEGG database analysis, the most important signaling pathways related to the transition between the control and relapse phase of disease are shown in Table 2.

Table 1: Hub genes identified in transition from control to relapse phase

Module	Hub Genes
Salmon	AP3B1, NOTCH1, LSM6, NDUFA12, PSMB2, PTK2, ACTN1, CAPN3, CDC40, NDUFB5, PIK3CD, PLCB2, PSMD14, SNRPE, UTRN, TPX2
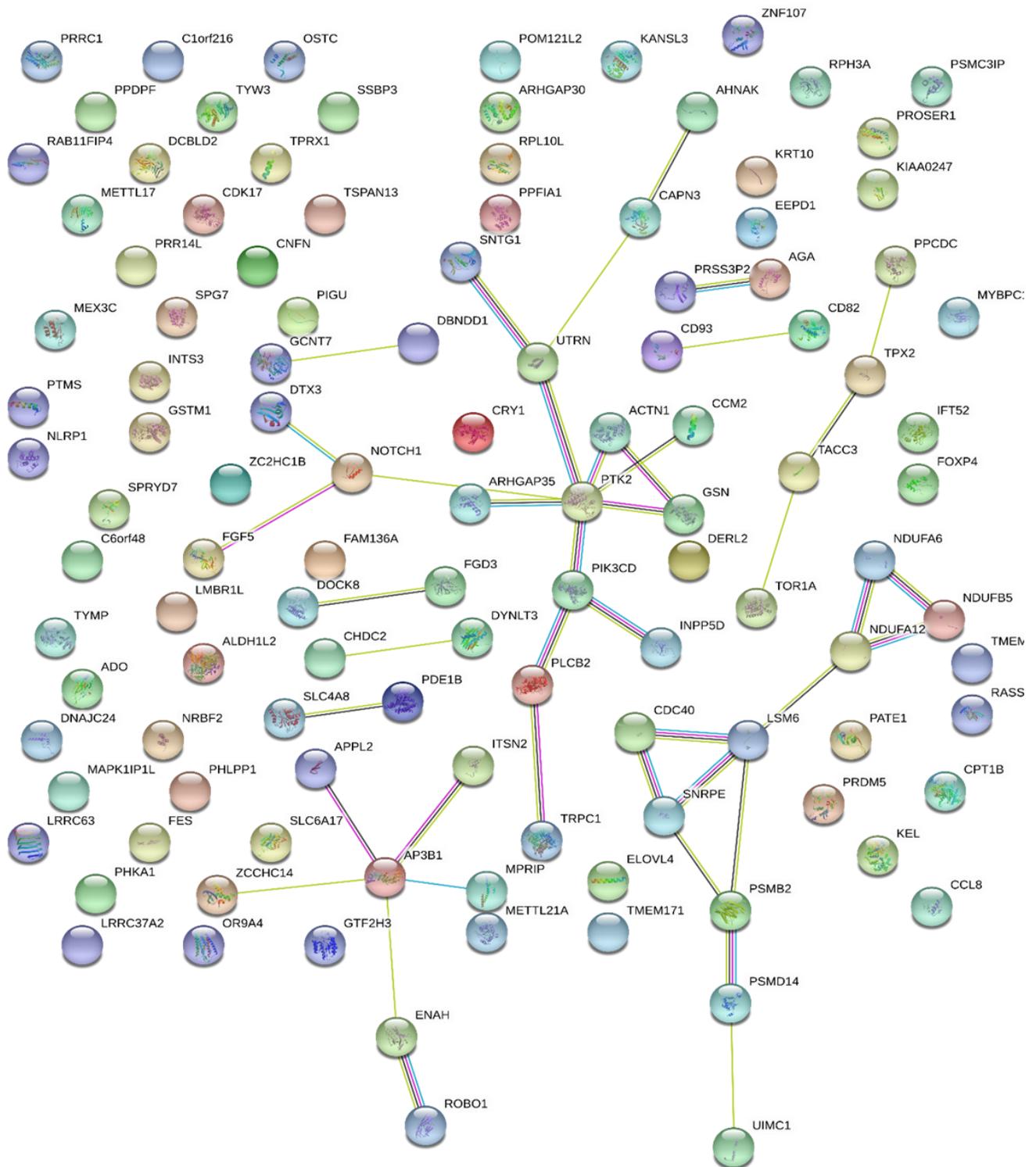
Figure 6: The PPI network of detected hub genes that was analyzed by String software. 115 nodes and 42 edged in the PPI network. Among the shown genes AP3B1, NOTCH1, LSM6, NDUFA12, PSMB2 and PTK2 have the most number of interactions.

Table 2: the list of important signaling pathways related to hub detected genes

Signaling pathway	P-value	Combined Z Score	Genes
Regulation of actin cytoskeleton	1.48E-04	46.41987445	FGF5;ENAH;FGD3;GSN;ACTN1;PIK3CD;ARHGAP35;PTK2
Axon guidance	0.001873989	29.31920975	ENAH;FES;TRPC1;PIK3CD;PTK2;ROBO1
Amoebiasis	0.004884885	31.23010309	ACTN1;PIK3CD;PLCB2;PTK2
Leukocyte transendothelial migration	0.00837394	24.05749877	ACTN1;PIK3CD;ARHGAP35;PTK2
Inositol phosphate metabolism	0.015739187	23.70537687	INPP5D;PIK3CD;PLCB2
Retrograde endocannabinoid signaling	0.021311081	14.64989754	NDUFA6;NDUFA12;NDUFB5;PLCB2
Non-alcoholic fatty liver disease (NAFLD)	0.021781788	14.4689709	NDUFA6;NDUFA12;NDUFB5;PIK3CD
Fc gamma R-mediated phagocytosis	0.027050939	16.76226699	GSN;INPP5D;PIK3CD
Pancreatic secretion	0.032686414	14.74904761	TRPC1;PRSS2;PLCB2
Phosphatidylinositol signaling system	0.033537678	14.49033615	INPP5D;PIK3CD;PLCB2
Alzheimer disease	0.033767455	11.16302716	NDUFA6;NDUFA12;NDUFB5;PLCB2
Glucagon signaling pathway	0.037057307	13.51821301	PHKA1;CPT1B;PLCB2
Carbohydrate digestion and absorption	0.038994651	20.77036361	PIK3CD;PLCB2
Proteasome	0.040622396	20.05280586	PSMD14;PSMB2

4. DISCUSSION

Here we employed weighted gene co-expression network analysis (WGCNA), on expression data obtained from GSE41890 of MS and healthy specimens. By WGCNA, we discovered that one out of twenty network modules (salmon module) in the healthy network were not preserved in the MS network. This gene module may play a significant function in MS progress and, consequently, the hub gens within this module may serve as new diagnostic or therapeutic purposes. Afterward, we summarized the list of genes within this module by distinguishing only the hub genes or the most MS-associated genes according to WGCNA. Functional enrichment analysis of genes in salmon modules revealed that the most significant GO terms related to Leukocyte transendothelial migration and their associated events such as regulation of actin cytoskeleton and amoebiasis (probably amoeboid movement), inositol phosphate metabolism and pathway, and retrograde endocannabinoid signaling.

Leukocyte motility is requisite for trafficking inside lymphoid organs and for launching touch with antigen-presenting cells (22). MS as an autoimmune inflammatory disorder of the CNS, characterized by multifocal infiltration of auto-reactive T lymphocytes (such as CD4+ and Th17 lymphocytes), one type of leukocyte transendothelial migration, from blood circulation into the brain through the blood-brain barrier and currently this accepted as the primary step of pathogenesis in the disease (23-25). For reaching to sites of inflammation or CNS in this condition, auto-reactive T lymphocytes must first overcome endothelial cells (ECs), however, it has been surprisingly revealed that ECs support leukocytes during extravasation (26). The ECS support leukocyte extravasation by reconstruction of the endothelial actin cytoskeleton to confer the morphological alterations and accommodating leukocyte creeping on its apical surface and diapedesis beyond its cell body that facilitates leukocyte diapedesis. Actin-binding proteins (ABPs) play a focal function in the control of the cellular motility and actin cytoskeletal remodeling involved in

diapedesis (27). ACTN1 (Actinin Alpha 1) is an actin-binding protein that involved cell motility and Immune Cell Transmigration (28-30). Also, based on functional enrichment, ACTN1 and some number of identified hub genes (such as PIK3CD, PTK2, and etc.) involved in leukocyte transendothelial migration, axon guidance, and amoeboid movement in addition to regulation of actin cytoskeleton that suggested these pathways are linked together. It seems that the amoeboid movement is an important mechanism in leukocyte migration or other words migration of leukocytes was occurred in a manner alike crawling amoeba because of fast cell shape alterations that are nearly exclusively induced by the cytoskeleton (31-34). UTRN that encode utrophin identified as a hub gene in our study, we suggest that because of its actin-binding activity may be involved in remodeling of the actin cytoskeleton. CAPN3 is a member of the calpain family of proteases that play a role in the regulation of cellular motility by regulation of dynamics of both integrin-mediated adhesion and actin-based membrane protrusion. Generally, adhesion and spreading; detachment of the rear; integrin- and growth-factor-mediated signaling; and membrane protrusion are the key aspects of cellular motility that Calpains family participate in them (35). Recently Kong et al. identified that CDC40 promotes proliferation, cell cycle, and migration in HCC cell lines (36).
The leukocyte migration cascade requires the activation of various signaling pathways, one of them is intracellular signaling through phosphatidylinositol 3-kinase (PI3K) (37-39). PI3K, a key signaling component involved in gradient sensing during amoeboid chemotaxis (such as leukocyte migration), coordinates polarity during cell migration (40). Among identified hub gene PIK3CD (is a class I PI3K found primarily in leukocytes) and PLCB2 (that catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate to the second messengers inositol 1,4,5-trisphosphate (IP3) and diacylglycerol) are involved in PI3K pathway and metabolism (41). Also, It is identified that actin remodeling in cell migration was regulated by non-canonical notch signaling through activating PI3K/AKT/Cdc42 pathway (42). PTK2 (involved in the focal adhesion kinase (FAK) signaling pathway) plays a crucial role in controlling cell

motility, remodeling of the actin cytoskeleton, formation and disassembly of focal adhesions and cell protrusions (39, 43-45).

Currently, it was recognized that the nervous and immune systems share some fundamental biological principles, for example in axonal and leukocyte migration (46, 47). Also, may axon guidance proteins medaite an abnormal immune response against CNS (48). AP3B1 is a member of adaptor proteins (APs) that could mediate microtubule-mediated trafficking of lytic granules to the immune synapse (49). TPX2, a spindle assembly factor, required for normal assembly of microtubules and may participate in association with AP3B1 in microtubule-mediated trafficking of lytic granules (50). Generally, the shared hub gene among the aforementioned pathway suggests the relative communication of them.

Regarding proteasome, we identified PSMB2 and PSMD14 as members of proteasome complex that induce the production of peptides for presentation on MHC class I molecules (that presented peptides derived from endogenous proteins or proteins gained from the extracellular environment during cross-presentation) that also termed as immunoproteasome. It has known that stimulation of cells by pro-inflammatory cytokines lead to the replacement of most of their constitutively expressed proteasomes with immunoproteasomes (51). Dianzani et al indicated that extracellular proteasome-osteopontin circuit involved in MS implications through regulating cell migration. Immunoproteasomes processed osteopontin and release the active OPN fragments that lead to activate the chemotactic activity of it and boost an increased binding to chemotactic receptors that intensify its chemotactic activity towards migrating blood leukocytes (52).

Type-1 cannabinoid receptors (CB1) presented on neuronal mitochondrial membranes activate by exogenous cannabinoids and endocannabinoids and lead to restricting neuronal respiration by soluble adenylate cyclase-dependent modulation of complex I enzymatic activity (53). NDUFA12 and NDUFB5 are subunits of the multisubunit NADH: ubiquinone oxi-

doreductase (complex I) and probably are under the influence of endocannabinoid signaling. Preclinical data propose that cannabinoids and endocannabinoids may decelerate the progression of MS by exerting neuroprotective effects, thus modulating the endocannabinoid signaling may be potentially useful in the treatment of MS (54).

SNRPE and LSM6 gene, are components of U-class of small nuclear RNAs that play a role in pre-mRNA splicing and considering our knowledge MS is also associated with genome-wide defects in mRNA splicing. The Spurlock et al study indicated that mononuclear cells from subjects with RRMS uniquely show prevalent enhancement in the levels of polyadenylated ncRNAs such as Y1 RNA, 18S rRNA, 28S rRNA, U1, U2, and U4 snRNAs (55).

CONCLUSION

In the present study, we attempted to decipher potential molecular mechanisms in MS by using a comprehensive algorithm for system biology. The salmon module identified has been associated with the progression of MS, and functional enrichment analyses demonstrated that leukocyte transendothelial migration and its related pathways, endocannabinoid signaling, and proteasome complex involved in the progression of MS. Also, hub genes may be used as transcriptional biomarkers or potential therapeutic targets.

CONFLICTS OF INTEREST

The authors have indicated that they have no conflicts of interest regarding the content of this article.

REFERENCES

[1] Weiner HL. A shift from adaptive to innate immunity: a potential mechanism of disease progression in multiple sclerosis. Journal of neurology. 2008;255 (1):3-11. PMid:18317671 View Article   PubMed/NCBI

[2] Lucchinetti CF, Popescu BF, Bunyan RF, Moll NM, Roemer SF, Lassmann H, et al. Inflammatory cortical demyelination in early multiple sclerosis. New England Journal of Medicine. 2011;365 (23):2188-97. PMid:22150037 View Article   PubMed/NCBI

[3] Trapp BD, Nave K-A. Multiple sclerosis: an immune or neurodegenerative disorder? Annu Rev Neurosci. 2008;31:247-69. PMid:18558855 View

Article   PubMed/NCBI

[4] Martino G, Franklin RJ, Van Evercooren AB, Kerr DA, Group SCiMSC. Stem cell transplantation in multiple sclerosis: current status and future prospects. Nature Reviews Neurology. 2010;6(5):247. PMid:20404843 View Article   PubMed/NCBI

[5] Steinman L. A molecular trio in relapse and remission in multiple sclerosis. Nature Reviews Immunology. 2009;9(6):440. PMid:19444308 View Article   PubMed/NCBI

[6] Loma I, Heyman R. Multiple sclerosis: pathogenesis and treatment. Current neuropharmacology. 2011;9(3):409-16. PMid:22379455 View Article   PubMed/NCBI

[7] Milo R, Kahana E. Multiple sclerosis: geoepidemiology, genetics and the environment. Autoimmunity reviews. 2010;9(5):A387-A94. PMid:19932200 View Article   PubMed/NCBI

[8] Stys PK, Zamponi GW, Van Minnen J, Geurts JJ. Will the real multiple sclerosis please stand up? Nature Reviews Neuroscience. 2012;13(7):507. PMid:22714021 View Article   PubMed/NCBI

[9] De Oliveira GL, De Lima KW, Colombini AM, Pinheiro DG, Panepucci RA, Palma PV, et al. Bone marrow mesenchymal stromal cells isolated from multiple sclerosis patients have distinct gene expression profile and decreased suppressive function compared with healthy counterparts. Cell transplantation. 2015;24(2):151-65. PMid:24256874 View Article   PubMed/NCBI

[10] Harauz G, Ishiyama N, Hill CM, Bates IR, Libich DS, Fares C. Myelin basic protein-diverse conformational states of an intrinsically unstructured protein and its roles in myelin assembly and multiple sclerosis. Micron. 2004;35(7):503-42. PMid:15219899 View Article   PubMed/NCBI

[11] Ramagopalan SV, Maugeri NJ, Handunnetthi L, Lincoln MR, Orton S-M, Dyment DA, et al. Expression of the multiple sclerosis-associated MHC class II Allele HLA-DRB1* 1501 is regulated by vitamin D. PLoS genetics. 2009;5(2):e1000369. PMid:19197344 View Article   PubMed/NCBI

[12] Hohlfeld R, editor Future challenges in MS. MULTIPLE SCLEROSIS; 2009: SAGE PUBLICATIONS LTD 1 OLIVERS YARD, 55 CITY ROAD, LONDON EC1Y 1SP, ENGLAND.

[13] Carlson MR, Zhang B, Fang Z, Mischel PS, Horvath S, Nelson SF. Gene connectivity, function, and sequence conservation: predictions from modular yeast co-expression networks. BMC genomics. 2006;7:40. PMid:16515682 View Article   PubMed/NCBI

[14] Carter SL, Brechbuhler CM, Griffin M, Bond AT. Gene co-expression network topology provides a framework for molecular characterization of cellular state. Bioinformatics. 2004;20(14):2242-50. PMid:15130938 View Article   PubMed/NCBI

[15] Stuart JM, Segal E, Koller D, Kim SK. A genecoexpression network for global discovery of conserved genetic modules. Science (New York, NY).

2003;302(5643):249-55. PMid:12934013 View Article PubMed/NCBI

[16] Zhang B, Horvath S. A general framework for weighted gene co-expression network analysis. Statistical applications in genetics and molecular biology. 2005;4:Article17. PMid:16646834 View Article PubMed/NCBI

[17] Miller JA, Horvath S, Geschwind DH. Divergence of human and mouse brain transcriptome highlights Alzheimer disease pathways. Proceedings of the National Academy of Sciences of the United States of America. 2010;107(28):12698-703. PMid:20616000 View Article PubMed/NCBI

[18] Oldham MC, Konopka G, Iwamoto K, Langfelder P, Kato T, Horvath S, et al. Functional organization of the transcriptome in human brain. Nature neuroscience. 2008;11(11):1271-82. PMid:18849986 View Article PubMed/NCBI

[19] Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S, et al. Transcriptomic analysis of autistic brain reveals convergent molecular pathology. Nature. 2011;474(7351):380-4. PMid:21614001 View Article PubMed/NCBI

[20] Gargalovic PS, Imura M, Zhang B, Gharavi NM, Clark MJ, Pagnon J, et al. Identification of inflammatory gene modules based on variations of human endothelial cell responses to oxidized lipids. Proceedings of the National Academy of Sciences of the United States of America. 2006;103(34):12741-6. PMid:16912112 View Article PubMed/NCBI

[21] Langfelder P, Horvath S. WGCNA: an R package for weighted correlation network analysis. BMC bioinformatics. 2008;9(1):559. PMid:19114008 View Article PubMed/NCBI

[22] Miller MJ, Wei SH, Parker I, Cahalan MD. Two-photon imaging of lymphocyte motility and antigen response in intact lymph node. Science (New York, NY). 2002;296(5574):1869-73. PMid:12016203 View Article PubMed/NCBI

[23] Prat A, Biernacki K, Lavoie JF, Poirier J, Duquette P, Antel JP. Migration of multiple sclerosis lymphocytes through brain endothelium. Archives of neurology. 2002;59(3):391-7. PMid:11890842 View Article PubMed/NCBI

[24] Engelhardt B. Molecular mechanisms involved in T cell migration across the blood-brain barrier. Journal of neural transmission (Vienna, Austria : 1996). 2006;113(4):477-85. PMid:16550326 View Article PubMed/NCBI

[25] Kermode AG, Thompson AJ, Tofts P, MacManus DG, Kendall BE, Kingsley DP, et al. Breakdown of the blood-brain barrier precedes symptoms and other MRI signs of new lesions in multiple sclerosis. Pathogenetic and clinical implications. Brain : a journal of neurology. 1990;113 (Pt 5):1477-89. PMid:2245307 View Article PubMed/NCBI

[26] Schnoor M. Endothelial actin-binding proteins and actin dynamics in leukocyte transendothelial migration. Journal of immunology (Baltimore, Md :

1950). 2015;194(8):3535-41. PMid:25848070 View Article PubMed/NCBI

[27] Babich A, Burkhardt JK. Coordinate control of cytoskeletal remodeling and calcium mobilization during T-cell activation. Immunological reviews. 2013;256(1):80-94. PMid:24117814 View Article PubMed/NCBI

[28] Shao H, Wang JH, Pollak MR, Wells A. alpha-actinin-4 is essential for maintaining the spreading, motility and contractility of fibroblasts. PLoS One. 2010;5(11):e13921. PMid:21085685 View Article PubMed/NCBI

[29] Kovac B, Makela TP, Vallenius T. Increased alpha-actinin-1 destabilizes E-cadherin-based adhesions and associates with poor prognosis in basal-like breast cancer. PLoS One. 2018;13(5):e0196986. PMid:29742177 View Article PubMed/NCBI

[30] Dupré L, Houmadi R, Tang C, Rey-Barroso J. T Lymphocyte Migration: An Action Movie Starring the Actin and Associated Actors. Front Immunol. 2015;6:586-. PMid:26635800 View Article PubMed/NCBI

[31] Norberg B, Bandmann U, Rydgren L. Amoeboid movement in human leucocytes: basic mechanisms, cytobiological and clinical significance. Journal of mechanochemistry & cell motility. 1977;4(1):37-53.

[32] Barros-Becker F, Lam PY, Fisher R, Huttenlocher A. Live imaging reveals distinct modes of neutrophil and macrophage migration within interstitial tissues. Journal of cell science. 2017;130(22):3801-8. PMid:28972134 View Article PubMed/NCBI

[33] Lämmermann T, Germain RN. The multiple faces of leukocyte interstitial migration. Semin Immunopathol. 2014;36(2):227-51. PMid:24573488 View Article PubMed/NCBI

[34] Wolf K, Muller R, Borgmann S, Brocker EB, Friedl P. Amoeboid shape change and contact guidance: T-lymphocyte crawling through fibrillar collagen is independent of matrix remodeling by MMPs and other proteases. Blood. 2003;102(9):3262-9. PMid:12855577 View Article PubMed/NCBI

[35] Franco SJ, Huttenlocher A. Regulating cell migration: calpains make the cut. Journal of cell science. 2005;118(Pt 17):3829-38. PMid:16129881 View Article PubMed/NCBI

[36] Kong XX, Lv YR, Shao LP, Nong XY, Zhang GL, Zhang Y, et al. HBx-induced MiR-1269b in NF-kappaB dependent manner upregulates cell division cycle 40 homolog (CDC40) to promote proliferation and migration in hepatoma cells. Journal of translational medicine. 2016;14(1):189. PMid:27349221 View Article PubMed/NCBI

[37] Smith MS, Bivins-Smith ER, Tilley AM, Bentz GL, Chan G, Minard J, et al. Roles of phosphatidylinositol 3-kinase and NF-kappaB in human cytomegalovirus-mediated monocyte diapedesis and adhesion: strategy for viral persistence. J Virol. 2007;81(14):7683-94. PMid:17507481 View Arti-

cle   PubMed/NCBI

[38] Fernandez-Borja M, van Buul JD, Hordijk PL. The regulation of leucocyte transendothelial migration by endothelial signalling events. Cardiovasc Res. 2010;86(2):202-10. PMid:20068003 View Article   PubMed/NCBI

[39] Luster AD, Alon R, von Andrian UH. Immune cell migration in inflammation: present and future therapeutic targets. Nature immunology. (12):1182-90. PMid:16369557 View Article   PubMed/NCBI

[40] Deng Q, Huttenlocher A. Leukocyte migration from a fish eye's view. Journal of cell science. 2012;125(Pt 17):3949-56. PMid:23104739 View Article   PubMed/NCBI

[41] Vanhaesebroeck B, Welham MJ, Kotani K, Stein R, Warne PH, Zvelebil MJ, et al. P110delta, a novel phosphoinositide 3-kinase in leukocytes. Proceedings of the National Academy of Sciences of the United States of America. 1997;94(9):4330-5. PMid:9113989 View Article   PubMed/NCBI

[42] Liu L, Zhang L, Zhao S, Zhao X-Y, Min P-X, Ma Y-D, et al. Non-canonical Notch Signaling Regulates Actin Remodeling in Cell Migration by Activating PI3K/AKT/Cdc42 Pathway. Front Pharmacol. 2019;10:370-. PMid:31057403 View Article   PubMed/NCBI

[43] Yu H, Gao M, Ma Y, Wang L, Shen Y, Liu X. Inhibition of cell migration by focal adhesion kinase: Time-dependent difference in integrin-induced signaling between endothelial and hepatoblastoma cells. Int J Mol Med. 2018;41(5):2573-88. PMid:29484384 View Article   PubMed/NCBI

[44] Tomar A, Schlaepfer DD. Focal adhesion kinase: switching between GAPs and GEFs in the regulation of cell motility. Current Opinion in Cell Biology. 2009;21(5):676-83. PMid:19525103 View Article   PubMed/NCBI

[45] Parsons JT, Horwitz AR, Schwartz MA. Cell adhesion: integrating cytoskeletal dynamics and cellular tension. Nat Rev Mol Cell Biol. 2010;11(9):633-43. PMid:20729930 View Article   PubMed/NCBI

[46] Morote-Garcia JC, Napiwotzky D, Kohler D, Rosenberger P. Endothelial Semaphorin 7A promotes neutrophil migration during hypoxia. Proceedings of the National Academy of Sciences of the United States of America. 2012;109(35):14146-51. PMid:22891341 View Article   PubMed/NCBI

[47] Strochlic L, Dwivedy A, van Horck FPG, Falk J, Holt CE. A role for S1P signalling in axon guid-ance in the Xenopus visual system. Development. 2008;135(2):333-42. PMid:18077591 View Article   PubMed/NCBI

[48] Kubo T, Tokita S, Yamashita T. Repulsive guidance molecule-a and demyelination: implications for multiple sclerosis. Journal of neuroimmune pharmacology : the official journal of the Society on NeuroImmune Pharmacology. 2012;7(3):524-8. PMid:22183806 View Article   PubMed/NCBI

[49] Clark RH, Stinchcombe JC, Day A, Blott E, Booth S, Bossi G, et al. Adaptor protein 3-dependent microtubule-mediated movement of lytic granules to the immunological synapse. Nature immunology. 2003;4(11):1111-20. PMid:14566336 View Article   PubMed/NCBI

[50] Wei JH, Zhang ZC, Wynn RM, Seemann J. GM130 Regulates Golgi-Derived Spindle Assembly by Activating TPX2 and Capturing Microtubules. Cell. 2015;162(2):287-99. PMid:26165940 View Article   PubMed/NCBI

[51] Groettrup M, Kirk CJ, Basler M. Proteasomes in immune cells: more than peptide producers? Nature reviews Immunology. 2010;10(1):73-8. PMid:20010787 View Article   PubMed/NCBI

[52] Dianzani C, Bellavista E, Liepe J, Verderio C, Martucci M, Santoro A, et al. Extracellular proteasome-osteopontin circuit regulates cell migration with implications in multiple sclerosis. Scientific reports. 2017;7:43718. PMid:28276434 View Article   PubMed/NCBI

[53] Benard G, Massa F, Puente N, Lourenco J, Bellocchio L, Soria-Gomez E, et al. Mitochondrial CB(1) receptors regulate neuronal energy metabolism. Nature neuroscience. 2012;15(4):558-64. PMid:22388959 View Article   PubMed/NCBI

[54] Chiurchiu V, van der Stelt M, Centonze D, Maccarrone M. The endocannabinoid system and its therapeutic exploitation in multiple sclerosis: Clues for other neuroinflammatory diseases. Progress in neurobiology. 2018;160:82-100. PMid:29097192 View Article   PubMed/NCBI

[55] Spurlock CF, 3rd, Tossberg JT, Guo Y, Sriram S, Crooke PS, 3rd, Aune TM. Defective structural RNA processing in relapsing-remitting multiple sclerosis. Genome Biol. 2015;16(1):58-. PMid:25885816 View Article   PubMed/NCBI