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Detection of multiple sclerosis using blood and brain cells transcript profiles: Insights from comprehensive bioinformatics approach

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ABSTRACT

Multiple sclerosis (MS) is a severely disabling disease affecting the brain and spinal cord. The detection of MS at an early stage is difficult. However, detection of MS from blood cell gene expression may ameliorate the early identification of MS. The present study addressed overlapped genes between blood cell and brain tissues of MS patients. We analyzed microarray gene expression brain tissue datasets and eQTL data to identify overlapped genes in the blood and brain of MS patients. We identified 23 overlapped differentially expressed genes (DEGs) (PWP2, UPK3BL, NFATC3, NPIPA5, KNOP1, PIK3C2B, LDLRAP1, HN1L, FAM226B, STAG3L2, ZNF814, BMS1P5, SDCCAG8, CLN8, ARHGEF7, NEAT1, ANKRD42, C5orf34, DOK6, PKN2, SP1, DBF4B, VAMP3) in the blood cells and brain tissues of MS patients. The axon guidance mediated and postsynaptic differentiation associated pathways were enriched by the DEGs. The significant regulatory transcription factors (TFs) including SP1, ARNT, MEF2A, YY1, EGR1, MIF, FOS, NFYA, TFAP2A, MYC, USF, MXI1, JUN, MAX, E2F1, POU3F2, GABPA, TFAP2C, CUX1, 2623, SPI1 and microRNAs (miRNAs) including miR-650, miR-223, miR-9, miR-181b, miR-190, miR-561, miR-520c-3p, miR-658, miR-199b-5p, miR-660, miR-20 were identified in MS. The candidate drugs were determined from drug target overrepresentation analysis. This study provides biomarkers at protein levels (hub proteins and TFs) and RNA levels (mRNAs, miRNAs) in MS subjects that were similarly dysregulated in both blood and brain tissues.

1. Introduction

Multiple sclerosis (MS) is a disabling disease of central nervous systems and can affect the brain and spinal cord. The pathobiology of MS involves damage to the myelin sheath of nerves by autoimmunity and perturbation of communications between the brain and other body parts. The hallmark MS pathophysiology is a complex interplay of the central nervous system (CNS) with genetic, environmental, infectious, and immunological factors [1–4]. To study such complex diseases, it is more efficient to describe the perturbed processes and dysregulated pathways rather to identify individual genes [5]. Genetic and transcript levels in patient tissues can help identify possible new biomarkers to assist in defining the strikingly similar phenotypes seen in MS patients.

While the pathogenesis of MS is likely to be multifactorial in nature, the application of molecular methods to improve its diagnosis and assessment has yet to provide substantiated results, and hence the quest to detect early MS biomarkers in peripheral blood has received increased attention [6–9]. Successful identification of such blood molecular biomarkers could have a high impact on MS diagnosis, care, and treatment [5,6,8–10]. Indeed, recently, there have been a number of studies that have profiled genes in MS to delineate tissue mRNA signatures [11–16], and a number of differentially expressed genes (DEGs) have been reported. However, their findings were limited to the transcript level, since the functional interactions among the gene products were not considered. Since the biological molecules interact with each other to carry out functions in biological processes in cells and tissues in pathways, integrative analyses may better reveal the molecular mechanisms that underlie disease processes and which may also identify

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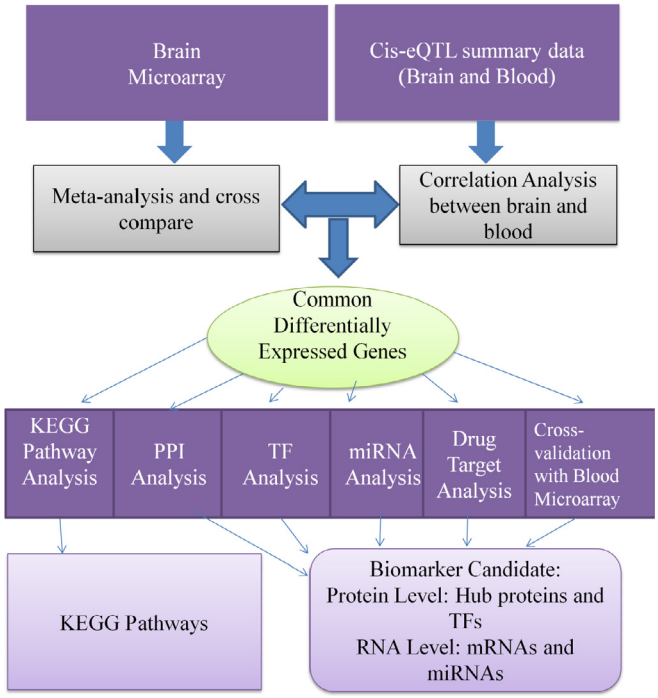

Fig. 1. The overview of the analytical methodology of this study. The microarray gene expression datasets from Multiple Sclerosis (MS) were downloaded from the Gene Expression Omnibus and GTEx portal. The differential gene expression analysis and eQTL datasets and overlapped differentially expressed genes (DEGs) between brain tissues and blood cells were identified in MS. The gene annotation with preferred Gene Ontology (GO) and pathways database revealed significant pathways and GO terms enriched by the identified DEGs. The topological analysis of the protein-protein interaction analysis revealed the central proteins or so-called hub proteins. The regulatory biomolecules (i.e., TFs, miRNAs) were identified from TF-miRNA coregulatory network analysis.

useful biomarkers.

Thus, a systems biology analytical pipeline was applied to identify biomarkers signatures at protein levels (hub proteins, TFs) and RNA levels (mRNAs, miRNAs) for MS from transcriptomics, expressed quantitative loci (cis-eQTL), protein-protein interaction (PPI), DEGs-TFs, and DEGs-miRNAs interactions data. Geneset enrichment analyses were used for gene ontology (GO) and pathways enriched by the DEGs. Candidate drugs targeting identified biomarkers were also identified based on the gene signatures. In sum, the present study employed a systems biology approach to reveal molecular signatures comprising biomolecules at the protein level (hub protein, TFs), RNA levels (mRNAs, miRNAs), with pathways to provide an in-depth understanding of the mechanisms of pathogenesis in MS (Fig. 1).

2. Materials and methods

2.1. Identification of deregulated differentially expressed genes from gene expression transcriptomics data of brain tissues in MS

We downloaded a gene expression microarray dataset of MS brain lesions in comparison to control brain samples with accession number GSE38010 from Gene Expression Omnibus [17]. The microarray gene expression profiling was performed in active MS lesions in the brain compared to control brain samples. We used GEO2R and R Bioconductor packages to identify DEGs. Each sample of the gene expression matrix was normalized using the mean and standard deviation. The expression value of gene i in sample j represented by g_{ij} was transformed into Z_{ij} by computing

$$Z_{ij} = \frac{g_{ij} - mean(g_i)}{SD(g_i)} \quad (1)$$

where SD is the standard deviation. The DEGs were screened based on a p-value < 0.05 and logFC ≥ 2, and the Benjamini-Hochberg (BH) method was utilized to adjust p-values. We demonstrated gene-pathway, gene-TF and gene-miRNA associations using neighborhood-based benchmarking and topological methods.

2.2. eQTL effects between blood and brain tissues

The eQTL data which links gene SNPs to gene expression related to brain tissues and blood cells were obtained from the GTEx portal (https://gtexportal.org/home/). We utilized the GTEx database to identify genes with a similar genetic control of expression. The overlapped genes between these two tissues were identified using meta-analysis approaches.

If we allow $x\sim$ to be the estimated effect of the top-linked cis-eQTL for a gene, we can calculate $x\sim$ based on the method explained in Ref. [18], and as below:

$$x\sim = x + \varrho \quad (2)$$

where x is the true effect and ϱ is the estimated error. The covariance of the effects of cis-eQTL between tissues i and j across genes can be partitioned into the covariance of true cis-QTL effects and the co-variance of estimation errors. We can calculate the correlation of true cis-eQTL effect sizes of genes between tissues i and j.

2.3. Gene ontology and molecular pathways were identified from enrichment analysis

We identified the GO and pathways annotation of the common DEGs via Enrichr [19]. The statistically significant GO and pathways were selected based on an adjusted p-value < 0.05.

2.4. Protein-protein interaction and topological analysis

The PPI networks of the encoded proteins of the DEGs used a STRING database [20]. A high confidence score of 900 was chosen via NetworkAnalyst [21]. The degree $\geq 10^{\circ}$ was selected to screen hub proteins from PPI analysis.

2.5. Regulators of the identified DEGs

We performed the TFs-miRNA coregulatory interactions obtained from the RegNetwork database which is an integrated database of documented interactions of the genes and TF, with human miRNAs queried via NetworkAnalyst [21] to identify regulatory biomolecules (i.e., TFs and miRNAs) to provide insights into the transcriptional and/or post-transcriptional regulator of the DEGs.

2.6. The candidate drugs identified from gene set enrichment analysis

Drug-target over-representation analyses were used via the Drug Signatures Database (DSigDB) [22] using Enrichr, which is a database that relates the drugs/compounds with target genes. Presently, the DSigDB maintains 22,527 gene sets, consists of 17,389 unique compounds covering 19,531 genes.

3. Results

3.1. Identification of overlapped deferentially expressed genes between blood and brain tissues in MS from transcriptomics and eQTL data

The transcriptomics datasets of the brain tissues were analyzed and 1137 DEGs were identified in the brain tissues of MS patients. Then, we

Table 1
The Gene Ontology (GO) terms in Multiple sclerosis. Top 10 significant GO terms summarized.

Category	GO ID	Gene Ontology Term	Adjusted p-value	Genes
Biological Process	GO:1902652	secondary alcohol metabolic process	0.0006	LDLRAP1;CLN8
	GO:0090181	regulation of cholesterol metabolic process	0.0010	SP1;LDLRAP1
	GO:0016125	sterol metabolic process	0.0021	LDLRAP1;CLN8
	GO:0008203	cholesterol metabolic process	0.0028	LDLRAP1;CLN8
	GO:0090068	positive regulation of cell cycle process	0.0067	PKN2;DBF4B
Cellular Component	GO:0030665	clathrin-coated vesicle membrane	0.0039	LDLRAP1;VAMP3
	GO:0030136	clathrin-coated vesicle	0.0059	LDLRAP1;VAMP3
	GO:0030121	AP-1 adaptor complex	0.0080	LDLRAP1
	GO:0055037	recycling endosome	0.0083	LDLRAP1;VAMP3
	GO:0030130	clathrin coat of trans-Golgi network vesicle	0.0103	LDLRAP1
Molecular Function	GO:0035612	AP-2 adaptor complex binding	0.0091	LDLRAP1
	GO:0035005	1-phosphatidylinositol-4-phosphate 3-kinase activity	0.0091	PIK3C2B
	GO:0035615	clathrin adaptor activity	0.0125	LDLRAP1
	GO:0098748	endocytic adaptor activity	0.0125	LDLRAP1
	GO:0016307	phosphatidylinositol phosphate kinase activity	0.0182	PIK3C2B

identified 673 blood-brain co-expressed genes (BBCG) using the correlation and meta-analysis approach. Thus, 23 genes were commonly dysregulated between MS blood and brain compared to control tissues. In total, transcriptomics and eQTL analysis revealed a list of common DEGs (PWP2, UPK3BL, NFATC3, NPIPA5, KNOP1, PIK3C2B, LDLRAP1, HN1L, FAM226B, STAG3L2, ZNF814, BMS1P5, SDCCAG8, CLN8, ARHGEF7, NEAT1, ANKRD42, C5orf34, DOK6, PKN2, SP1, DBF4B, VAMP3) in both blood cells and brain tissues in MS subjects.

In order to cross validate the 23 identified genes from brain and cis-eQTL data, we performed cross validations with genes identified from a blood dataset of MS (GSE43591). GSE43591 is the microarray expression analysis of mRNA and miRNA in peripheral blood T-cells of controls and MS patients. Ten significant genes (p-value < 0.05) (PWP2, UPK3BL, NFATC3, NPIPA5, PIK3C2B, LDLRAP1, STAG3L2, ZNF814, BMS1P5, ANKRD42) of the 23 identified genes were found similarly expressed in blood tissues with GSE43591 dataset.

Gene enrichment analysis was done to provide insights into the biological roles of the identified DEGs. The significant GO terms were identified (Table 1). The pathways analysis revealed significant pathways (Table 2).

3.2. Hub proteins were identified from protein-protein interaction analysis

The hub proteins are considered as drug targets. Thus, the hub proteins (SP1, PIK3C2B, VAMP3, PWP2, ARHGEF7) were identified from the PPI analysis (Fig. 2).

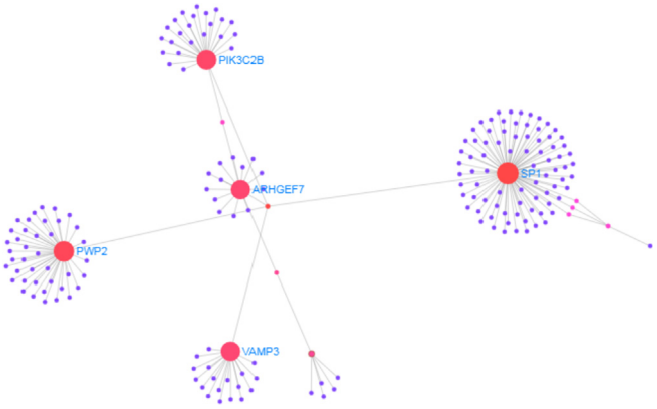

Fig. 2. The protein-protein interactions (PPI) were done around the proteins encoded by the overlapped differentially expressed genes using a comprehensive PPI database called STRING, queried via NetworkAnalyst. The nodes represent the proteins where edges represented the interactions among the proteins.

3.3. Transcriptional and/or post-transcriptional regulator of the identified differentially expressed genes

The regulatory biomolecules i.e., TFs and miRNAs were identified (Fig. 3). TFs (SP1, ARNT, MEF2A, YY1, EGR1, MIF, FOS, NFYA, TFAP2A, MYC, USF, MXI1, JUN, MAX, E2F1, POU3F2, GABPA, TFAP2C, CUX1, 2623, SPI1) and microRNAs (miR-650, miR-223, miR-9, miR-181b, miR-190, miR-561, miR-520c-3p, miR-658, miR-199b-5p, miR-660, miR-20) were identified as the regulator of the DEGs.

3.4. Identification of drug target and candidate drugs

The potential candidate drugs (dorzolamide, iohexol, naringin, benzylpenicillin, cicloheximide, mycophenolic acid, GSK461364, disopyramide, H-89, 0175029–0000) enriched by the DEGs were identified from the DSigDB database (Table 3).

4. Discussion

Much research is directed towards identifying peripheral blood

Table 2
Molecular pathways were identified based on the differentially expressed genes in Multiple sclerosis.

Category	Molecular Pathways	Adjusted p-value	Gene
KEGG	SNARE interactions in vesicular transport	0.038	VAMP3
PANTHER	Axon guidance mediated by netrin	0.021	NFATC3;PIK3C2B
	Muscarinic acetylcholine receptor 1 and 3 signaling pathway	0.021	PKN2;VAMP3
BioCarter	Agrin in Postsynaptic Differentiation	0.01	SP1;ARHGEF7
	Human Cytomegalovirus and Map Kinase Pathways	0.029	SP1
	Effects of calcineurin in Keratinocyte Differentiation	0.029	SP1
	Overview of telomerase protein component gene hTert Transcriptional Regulation	0.029	SP1
	Overview of telomerase RNA component gene hTerc Transcriptional Regulation	0.029	SP1
Wikipathways	Initiation of transcription and translation elongation at the HIV-1 LTR	0.022	SP1;NFATC3

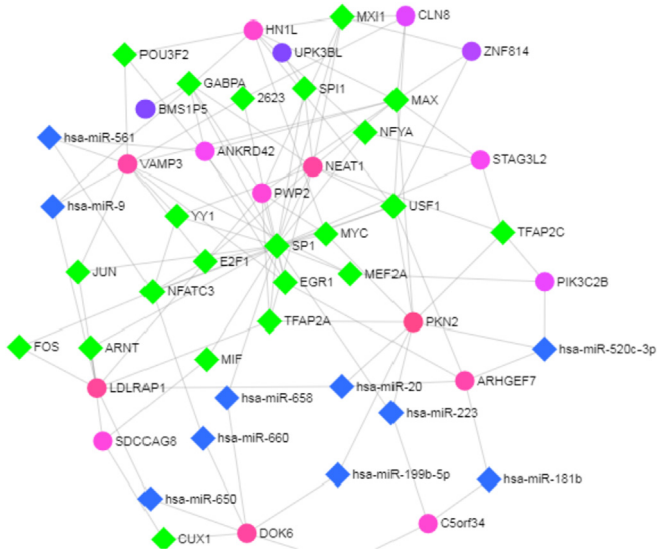

Fig. 3. The analysis of the TFs-miRNAs coregulatory interaction networks from the data obtained from RegNetwork database, queried via NetworkAnalyst, to reveal the transcriptional and/or post-transcriptional regulators of the overlapped differentially expressed genes in Multiple Sclerosis.

Table 3
Top 10 drugs identified from drug-target over-representation analysis.

Drug/Small Molecule	P-value	Genes
Dorzolamide	0.0001	ZNF814;UPK3BL;NEAT1;VAMP3
Iohexol	0.0005	NFATC3;UPK3BL;NEAT1
Naringin	0.0009	NEAT1;VAMP3
Benzylpenicillin	0.0009	UPK3BL;NEAT1
Cicloheximide	0.0009	HN1L;NFATC3;KNOP1;PWP2;NEAT1
Mycophenolic Acid	0.0011	UPK3BL;NEAT1
Gsk461364	0.0012	PKN2;PIK3C2B
Disopyramide	0.0014	HN1L;VAMP3
H-89	0.0021	UPK3BL;VAMP3
0175029–0000	0.0022	ZNF814;NFATC3;KNOP1;PKN2;DBF4B; LDLRAP1;ARHGEF7; LN8;PWP2;PIK3C2B

biomarkers for MS which will help in the early diagnosis. Safari-Alighiarloo et al. identified 3062 genes with FDR < 0.05 whose expression was different in the cerebrospinal fluid (CSF) of MS patients as compared to controls, but none in the respective peripheral blood mononuclear cells [12]. Freiesleben et al. identified 18 differentially expressed miRNAs and 128 DEGs that may contribute to the regulatory alterations behind MS [13]. The miRNAs were linked to immunological and neurological pathways, and their work identified let-7b-5p and miR-345-5p as blood-derived disease biomarkers in MS [13]. Microarray gene expression analyses are typically used for the identification of new disease candidate biomarkers [23–27]. The gene expression datasets generated from brain tissues of MS patients and from blood-brain co-expression studies revealed potential biomarkers. We performed geneset enrichment analysis to identify significant molecular pathways enriched by the DEGs. Emerging evidences suggested SNARE interactions pathways are involved in dendritic cell function [28]. The PPI network was analyzed to identify central hub proteins from the identified DEGs. The gender-based approach of blood gene expression and regulomics data revealed differential mRNA levels of SP1 in MS women and men in *in-silico* analyses [29]. Moreover, Wang et al. have shown a potential role for SP1-mediated transcriptional regulation in MS pathogenesis [30]. The hub gene PIK3C2B inhibition improves function and prolongs survival in myotubular myopathy in animal models. Sabha et al. demonstrated that PIK3C2B inhibition is a potential treatment strategy for myotubular myopathy [31], but the role of this gene is not clear in MS. Chabas et al. observed decreased transcription levels of hub gene vehicle-associated membrane protein-3 (VAMP3) in demyelinating disease, indicating a possible link to MS [32]. In a study, Lei et al. showed the up-regulation of ARHGEF7 in the development and metastasis of colorectal cancer [33], but the involvement of this gene in MS has not been reported until now. Lucchese et al. found PWP2 as epileptic proteins [34], but the involvement of this protein in MS is as yet unclear.

The role of the regulators of the DEGs is being recognized as tentative biomarkers in different neurodegenerative diseases [13,35,36]. The TFs were identified. ARNT2 is a neuronal transcription factor whose expression is linked to neuronal and axonal health [37]. Riveros et al. observed transcription repressor YY1 (Yin and Yang 1) are involved in several pathways that may have a plausible role in MS causation [38]. Freiesleben et al., also identified EGR1 (early growth response protein-1) as one of the significant TFs in MS [13] and, indeed, EGR1 plays an important role in brain plasticity and neuropsychiatric disorders [39]. Fagone et al. discussed the significant involvement of the MIF pathway in MS etiopathogenesis [40]. Baranzini et al. observed MXI1 as one of the top markers associated with MS phenotype [41]. MAX was identified as one of the significant TFs in TF-miRNA coregulatory networks in MS pathogenesis [35]. Other regulatory TFs in MS includes E2F1 [13] and POU3F2 (which is mainly expressed in the central nervous system and plays a role in brain development) [42].

Evidence suggesting miRNAs might be potential biomarkers of MS [13]. Relevant miRNAs were identified as potential biomarkers for MS. Freiesleben et al. identified 18 differentially expressed miRNAs [13]. miR-20b has been reported as a miRNA with decreased expression in blood cells of MS patients [43]. Sondergaard et al. identified miR-145, miR-660, and miR-939 as significantly and differentially distributed in the plasma of MS patients as compared with healthy controls [44]. Wu et al. also indicated that miR-199b-5p may be a novel alternative therapeutic target for triple negative breast cancer [45] while miR-520c-3p was also differentially expressed in MS [46]. miR-190 has been identified as inactive multiple sclerosis lesions [47]. The miR-181a and miR-181a-b have been studied in the context of autoimmune neuroinflammation, making it a plausible candidate for MS pathogenesis, which involves inflammation [48]. Up-regulation of miR-9-5p and down-regulation of miR-106a-5p in the relapsing phase of MS patients were also observed as compared to healthy controls [49]. miR-223 has been reported to play a critical role in MS suggesting, that it can be used as a biomarker for diagnosis of MS and for discovering novel therapeutics for MS treatment [50]. Despite the significance of the present systems biology study of gene expression of MS to identify the putative biomarkers, we propose wet-lab experimentation to validate these bioinformatics based candidate biomarkers, and to study in-depth to elucidate the roles of the identified biomolecules in-vivo to establish them as new biomarkers in MS. Potential candidate drugs were identified targeting the putative MS markers. The gene-drug interactions were obtained from the drug-target enrichment analysis of the identified DEGs in MS. In this way, we have disclosed a link between drugs/compounds with genes, but it should be noted that the effect of the drugs/compounds on the MS markers is not clear from this study.

5. Conclusion

In the present study, MS transcriptomics and eQTL data relating to blood and brain cells were analyzed to identify MS-affected gene expression in the brain that may be altered in blood cells which are more amenable to clinical analysis. The overlapped MS-DEGs and blood/brain eQTL were integrated to look for common pathway analysis, to identify other potential blood biomarkers, as well as protein-protein interactions, and TFs and miRNAs to look for common regulators. Twenty three genes were identified as common to the MS DEGs identified from microarray and the eQTL datasets in MS. Relevant TFs (SP1, ARNT, MEF2A, YY1, EGR1, MIF, FOS, NFYA, TFAP2A, MYC, USF,

MXI1, JUN, MAX, E2F1, POU3F2, GABPA, TFAP2C, CUX1, 2623, SPI1) and miRNAs (miR-650, miR-223, miR-9, miR-181b, miR-190, miR-561, miR-520c-3p, miR-658, miR-199b-5p, miR-660, miR-20) were identified as putative transcriptional and post-transcriptional regulators of the DEGs. We propose further basic biology experiments to establish these as biomarkers of MS.

Conflicts of interest

The authors declare no conflict of interest.

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Ethical statement

No ethical clearance is required for this study.

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References

[1] Ascherio A. Environmental factors in multiple sclerosis. Expert Rev Neurother 2013;13:3–9. https://doi.org/10.1586/14737175.2013.865866.
[2] Svejgaard A. The immunogenetics of multiple sclerosis. Immunogenetics 2008;60:275–86. https://doi.org/10.1007/s00251-008-0295-1.
[3] Brynedal B, Khademi M, Wallstrom E, Hillert J, Olsson T, Duvefelt K. Gene expression profiling in multiple sclerosis: a disease of the central nervous system, but with relapses triggered in the periphery? Neurobiol Dis 2010;37:613–21. https://doi.org/10.1016/j.nbd.2009.11.014.
[4] Goris A, Liston A. The immunogenetic architecture of autoimmune disease. Cold Spring Harb Perspect Biol 2012;4. https://doi.org/10.1101/cshperspect.a007260.
[5] Kim Y-A, Wuchty S, Przytycka TM. Identifying causal genes and dysregulated pathways in complex diseases. PLoS Comput Biol 2011;7:e1001095https://doi.org/10.1371/journal.pcbi.1001095.
[6] D'Ambrosio A, Pontecorvo S, Colasanti T, Zamboni S, Francia A, Margutti P. Peripheral blood biomarkers in multiple sclerosis. Autoimmun Rev 2015;14:1097–110. https://doi.org/10.1016/j.autrev.2015.07.014.
[7] Malkki H. Blood-based biomarkers provide insight into progressive MS. Nat Rev Neurol 2014;10:612.
[8] Harris VK, Tuddenham JF, Sadiq SA. Biomarkers of multiple sclerosis: current findings. Degener Neurol Neuromuscul Dis 2017;7:19–29. https://doi.org/10.2147/DNND.S98936.
[9] Raphael I, Webb J, Stuve O, Haskins W, Forsthuber T. Body fluid biomarkers in multiple sclerosis: how far we have come and how they could affect the clinic now and in the future. Expert Rev Clin Immunol 2015;11:69–91. https://doi.org/10.1586/1744666X.2015.991315.
[10] Robelin L, Gonzalez De Aguilar JL. Blood biomarkers for amyotrophic lateral sclerosis: myth or reality? BioMed Res Int 2014;2014:525097. https://doi.org/10.1155/2014/525097.
[11] Srinivasan S, Severa M, Rizzo F, Menon R, Brini E, Mechelli R, et al. Transcriptional dysregulation of interferome in experimental and human multiple sclerosis. Sci Rep 2017;7:8981. https://doi.org/10.1038/s41598-017-09286-y.
[12] Safari-Alighiarloo N, Rezaei-Tavirani M, Taghizadeh M, Tabatabaei SM, Namaki S. Network-based analysis of differentially expressed genes in cerebrospinal fluid (CSF) and blood reveals new candidate genes for multiple sclerosis. PeerJ 2016;4:e2775https://doi.org/10.7717/peerj.2775.
[13] Freiesleben S, Hecker M, Zettl UK, Fuellen G, Taher L. Analysis of microRNA and gene expression Profiles in multiple sclerosis: integrating interaction data to uncover regulatory mechanisms. Sci Rep 2016;6:34512. https://doi.org/10.1038/srep34512.
[14] Tajouri L, Fernandez F, Griffiths LR. Gene expression studies in multiple sclerosis. Curr Genom 2007;8:181–9.
[15] Hendrickx DAE, van Scheppingen J, van der Poel M, Bossers K, Schuurman KG, van Eden CG, et al. Gene expression profiling of multiple sclerosis pathology identifies early patterns of demyelination surrounding chronic active lesions. Front Immunol 2017;8:1810. https://doi.org/10.3389/fimmu.2017.01810.
[16] Yazdanpanahi N, Etemadifar M, Kardi M-T, Shams E, Shahbazi S. Investigation of ERG gene expression in Iranian patients with multiple sclerosis. Immunol Investig 2018;47:351–9. https://doi.org/10.1080/08820139.2018.1433203.
[17] Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al. NCBI GEO: archive for functional genomics data sets - Update. Nucleic Acids Res 2013;41:991–5. https://doi.org/10.1093/nar/gks1193.
[18] Qi T, Wu Y, Zeng J, Zhang F, Xue A, Jiang L, et al. Identifying gene targets for brain-related traits using transcriptomic and methylomic data from blood. Nat Commun 2018;9:2282. https://doi.org/10.1038/s41467-018-04558-1.
[19] Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res 2016;44:W90–7. https://doi.org/10.1093/nar/gkw377.
[20] Szklarczyk D, Morris JH, Cook H, Kuhn M, Wyder S, Simonovic M, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res 2017;45:D362–8. https://doi.org/10.1093/nar/gkw937.
[21] Xia J, Gill EE, Hancock REW. NetworkAnalyst for statistical, visual and network-based meta-analysis of gene expression data. Nat Protoc 2015;10:823–44. https://doi.org/10.1038/nprot.2015.052.
[22] Yoo M, Shin J, Kim J, Ryall KA, Lee K, Lee S, et al. DSigDB: drug signatures database for gene set analysis. Bioinformatics 2015;31:3069–71. https://doi.org/10.1093/bioinformatics/btv313.
[23] Rahman MR, Islam T, Turanli B, Zaman T, Faruquee HM, Rahman MM, et al. Network-based approach to identify molecular signatures and therapeutic agents in Alzheimer's disease. Comput Biol Chem 2019;78:431–9. https://doi.org/10.1016/j.compbiolchem.2018.12.011.
[24] Rahman MR, Islam T, Shahjaman M, Quinn JMW, Holsinger RMD, Moni MA. Identification of common molecular biomarker signatures in blood and brain of Alzheimers disease. bioRxiv 2019:482828. https://doi.org/10.1101/482828.
[25] Rahman MR, Islam T, Shahjaman M, Zaman T, Faruquee HM, Jamal MAHM, et al. Discovering biomarkers and pathways shared by alzheimer's disease and ischemic stroke to identify novel therapeutic targets. Medicina (Kaunas) 2019;55. https://doi.org/10.3390/medicina55050191.
[26] Rahman MR, Islam T, Gov E, Turanli B, Gulfidan G, Shahjaman M, et al. Identification of prognostic biomarker signatures and candidate drugs in colorectal cancer: insights from systems biology analysis. Medicina (Kaunas) 2019;55. https://doi.org/10.3390/medicina55010020.
[27] Islam T, Rahman MR, Gov E, Turanli B, Gulfidan G, Haque MA, et al. Drug targeting and biomarkers in head and neck cancers: insights from systems biology analyses. OMICS 2018;22:422–36. https://doi.org/10.1089/omi.2018.0048.
[28] Collins LE, DeCourcey J, Soledad di Luca M, Rochfort KD, Loscher CE. An emerging role for SNARE proteins in dendritic cell function. Front Immunol 2015;6:133. https://doi.org/10.3389/fimmu.2015.00133.
[29] Menon R, Di Dario M, Cordiglieri C, Musio S, La Mantia L, Milanese C, et al. Gender-based blood transcriptomes and interactomes in multiple sclerosis: involvement of SP1 dependent gene transcription. J Autoimmun 2012;38:J144–55. https://doi.org/10.1016/j.jaut.2011.11.004.
[30] Wang L, Liu R, Li D, Lin S, Fang X, Backer G, et al. A hypermorphic SP1-binding CD24 variant associates with risk and progression of multiple sclerosis. Am J Transl Res 2012;4:347–56.
[31] Sabha N, Volpatti JR, Gonorazky H, Reifler A, Davidson AE, Li X, et al. PIK3C2B inhibition improves function and prolongs survival in myotubular myopathy animal models. J Clin Investig 2016;126:3613–25. https://doi.org/10.1172/JCI86841.
[32] Chabas D, Baranzini SE, Mitchell D, Bernard CC, Rittling SR, Denhardt DT, et al. The influence of the proinflammatory cytokine, osteopontin, on autoimmune demyelinating disease. Science 2001;294:1731–5. https://doi.org/10.1126/science.1062960.
[33] Lei X, Deng L, Liu D, Liao S, Dai H, Li J, et al. ARHGEF7 promotes metastasis of colorectal adenocarcinoma by regulating the motility of cancer cells. Int J Oncol 2018;53:1980–96. https://doi.org/10.3892/ijo.2018.4535.
[34] Lucchese G, Spinosa JP, Kanduc D. The peptide network between tetanus toxin and human proteins associated with epilepsy. Epilepsy Res Treat 2014;2014:236309. https://doi.org/10.1155/2014/236309.
[35] Nuzziello N, Vilardo L, Pelucchi P, Consiglio A, Liuni S, Trojano M, et al. Investigating the role of MicroRNA and transcription factor Co-regulatory networks in multiple sclerosis pathogenesis. Int J Mol Sci 2018;19. https://doi.org/10.3390/ijms19113652.
[36] Chen C, Zhou Y, Wang J, Yan Y, Peng L, Qiu W. Dysregulated MicroRNA involvement in multiple sclerosis by induction of T helper 17 cell differentiation. Front Immunol 2018;9:1256. https://doi.org/10.3389/fimmu.2018.01256.
[37] Rahim T, Becquart P, Baeva M-E, Quandt J. Expression of the neuroprotective protein aryl hydrocarbon receptor nuclear translocator 2 correlates with neuronal stress and disability in models of multiple sclerosis. J Neuroinflammation 2018;15:270. https://doi.org/10.1186/s12974-018-1290-6.
[38] Riveros C, Mellor D, Gandhi KS, McKay FC, Cox MB, Berretta R, et al. A transcription factor map as revealed by a genome-wide gene expression analysis of whole-blood mRNA transcriptome in multiple sclerosis. PLoS One 2010;5:e14176https://doi.org/10.1371/journal.pone.0014176.
[39] Duclot F, Kabbaj M. The role of early growth response 1 (EGR1) in brain plasticity and neuropsychiatric disorders. Front Behav Neurosci 2017;11:35. https://doi.org/10.3389/fnbeh.2017.00035.
[40] Fagone P, Mazzon E, Cavalli E, Bramanti A, Petralia MC, Mangano K, et al. Contribution of the macrophage migration inhibitory factor superfamily of cytokines in the pathogenesis of preclinical and human multiple sclerosis: in silico and in vivo evidences. J Neuroimmunol 2018;322:46–56. https://doi.org/10.1016/j.jneuroim.2018.06.009.
[41] Baranzini SE, Wang J, Gibson RA, Galwey N, Naegelin Y, Barkhof F, et al. Genome-wide association analysis of susceptibility and clinical phenotype in multiple sclerosis. Hum Mol Genet 2009;18:767–78. https://doi.org/10.1093/hmg/ddn388.
[42] Hashizume K, Yamanaka M, Ueda S. POU3F2 participates in cognitive function and adult hippocampal neurogenesis via mammalian-characteristic amino acid repeats. Genes Brain Behav 2018;17:118–25. https://doi.org/10.1111/gbb.12408.
[43] Wu T, Chen G. miRNAs participate in MS pathological processes and its therapeutic

response. Mediat Inflamm 2016;2016:4578230. https://doi.org/10.1155/2016/4578230.
[44] Sondergaard HB, Hesse D, Krakauer M, Sorensen PS, Sellebjerg F. Differential microRNA expression in blood in multiple sclerosis. Mult Scler 2013;19:1849–57. https://doi.org/10.1177/1352458513490542.
[45] Wu A, Chen Y, Liu Y, Lai Y, Liu D. miR-199b-5p inhibits triple negative breast cancer cell proliferation, migration and invasion by targeting DDR1. Oncol Lett 2018;16:4889–96. https://doi.org/10.3892/ol.2018.9255.
[46] Quintana E, Ortega FJ, Robles-Cedeno R, Villar ML, Buxo M, Mercader JM, et al. miRNAs in cerebrospinal fluid identify patients with MS and specifically those with lipid-specific oligoclonal IgM bands. Mult Scler 2017;23:1716–26. https://doi.org/10.1177/1352458516684213.
[47] Junker A, Krumbholz M, Eisele S, Mohan H, Augstein F, Bittner R, et al. MicroRNA profiling of multiple sclerosis lesions identifies modulators of the regulatory protein CD47. Brain 2009;132:3342–52. https://doi.org/10.1093/brain/awp300.
[48] Ghorbani S, Talebi F, Chan WF, Masoumi F, Vojgani M, Power C, et al. MicroRNA-181 variants regulate T cell phenotype in the context of autoimmune neuroinflammation. Front Immunol 2017;8:758. https://doi.org/10.3389/fimmu.2017.00758.
[49] Majd M, Hosseini A, Ghaedi K, Kiani-Esfahani A, Tanhaei S, Shiralian-Esfahani H, et al. MiR-9-5p and miR-106a-5p dysregulated in CD4(+) T-cells of multiple sclerosis patients and targeted essential factors of T helper17/regulatory T-cells differentiation. Iran J Basic Med Sci 2018;21:277–83. https://doi.org/10.22038/ijbms.2018.25382.6275.
[50] Gharibi S, Moghimi B, Tahoori TM, Mahmudi MB, Shahvazian E, Yazd EF. The mir-223: an inflammatory MicroRNA involved in pathogenesis of multiple sclerosis TT -—. Int J Med Lab 2018;5:237–45.