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Bioinformatics analysis of differentially expressed genes in pediatric multiple sclerosis; identification of key biomarkers and therapeutic targets

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Abstract

Introduction: Pediatric-onset multiple sclerosis (POMS) is a rare but severe form of MS that presents before the age of 18, leading to long-term disability. Despite its clinical significance, the molecular mechanisms driving POMS remain poorly understood.

Objectives: This study aims to identify key genes, pathways, and potential therapeutic targets involved in POMS using integrative bioinformatics approaches.

Materials and Methods: In this in silico study, gene expression profiles from peripheral blood mononuclear cells of POMS patients and healthy controls were analyzed using the GEO2R platform (GSE203241 dataset). Differentially expressed genes (DEGs) were identified using adjusted *P* value < 0.05 and a fold change>1.1 or <-1.1. Gene ontology (GO) and pathway enrichment analyses were conducted using BiNGO and Enrichr to explore biological functions and dysregulated pathways. A protein-protein interaction (PPI) network was constructed using STRING (search tool for the retrieval of interacting genes/proteins) and analyzed by Cytoscape with CytoHubba and CytoCluster plugins to identify hub genes and functional modules. Promoter motif analysis was conducted using Tomtom and GoMo to uncover regulatory elements, while DrugBank was utilized to identify potential drug-target interactions.

Results: A total of 1101 DEGs were identified, with enrichment in immune response pathways, cytokine signaling, and interferon responses. PPI network analysis revealed 15 hub genes, with MYC, HSP90AA1, JUN, HSPA4, and HIF1A identified by two independent algorithms, highlighting their central role in POMS. CytoCluster analysis uncovered functional modules related to RNA metabolism and vesicle transport. Promoter motif analysis revealed transcription factor binding sites that could influence hub gene expression. Drug-target analysis identified Food and Drug Administration (FDA)-approved and investigational drugs that interact with key hub proteins, offering potential therapeutic candidates.

Conclusion: This study provides a comprehensive molecular overview of POMS, identifying critical genes, regulatory elements, and potential drug targets. These findings offer valuable insights into POMS pathogenesis and highlight novel avenues for therapeutic intervention, advancing the prospects for personalized treatment strategies in pediatric MS.

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Introduction

Multiple sclerosis (MS) is a chronic disorder of the nervous system caused by immune system activity, involving inflammation, myelin loss, and axonal injury in the central nervous system (CNS) (1). It primarily affects young adults, leading to a wide range of neurological symptoms, including motor dysfunction, sensory disturbances, and cognitive impairment. Although the exact cause of MS remains elusive, it is widely recognized as a complex disease influenced by genetic susceptibility, environmental triggers, and immune dysregulation (2).

Pediatric-onset multiple sclerosis (POMS), defined by disease onset before the age of 18 years, accounts for approximately 3%-10% of all MS cases (3). While it shares many clinical and pathological features with adult-onset MS, POMS presents distinct challenges, including a higher relapse rate and a potential impact on neurodevelopment (4). The global prevalence of POMS varies geographically, but recent studies suggest an increasing incidence, underscoring the need for targeted research to understand its unique pathophysiology and improve treatment strategies for younger patients (5).

The pathogenesis of POMS involves a complex interplay between immune-

Key point

- Pediatric-onset multiple sclerosis (POMS) is a severe neuroimmune disorder with limited understanding of its molecular mechanisms.
- This study analyzed gene expression data (GSE203241) to identify differentially expressed genes (DEGs) and key biological pathways in POMS.
- Bioinformatics approaches revealed 1101 DEGs, with hub genes (MYC, HSP90AA1, JUN, HSPA4, HIF1A) playing central roles in immune response and neuroinflammation.
- Promoter motif analysis identified regulatory elements, while drug-target interaction analysis highlighted potential therapeutic candidates.
- Study results improve our knowledge of POMS pathogenesis and offer new avenues for biomarker discovery and targeted treatments.

mediated mechanisms and genetic predispositions. Inflammatory processes play a central role, with autoreactive T cells crossing the blood-brain barrier and initiating demyelination (6). Several studies shown that, B cells, microglia activation, and cytokine imbalances further contribute to disease progression. Genetic factors significantly influence susceptibility to MS, with several genome-wide association studies identifying risk alleles in immune-related genes. In POMS, specific genetic variants may modulate immune responses differently than in adults, potentially explaining the distinct clinical course observed in pediatric cases (7).

Protein-protein interaction (PPI) network analysis has emerged as a crucial tool in understanding complex biological systems and disease mechanisms. By mapping the interactions between proteins, researchers can identify key regulatory nodes, known as hub proteins, which often play central roles in disease pathogenesis (8). In neurodegenerative disorders like Alzheimer's disease, PPI network analysis has facilitated the identification of critical molecular pathways and potential therapeutic targets, advancing our understanding of disease progression and treatment development. This systems biology approach allows for a more comprehensive exploration of the molecular underpinnings of complex diseases, beyond single-gene analyses (9,10).

Objectives

Given the significance of network-based approaches in elucidating disease mechanisms, this study aims to apply bioinformatics and PPI network analysis to investigate the molecular landscape of POMS. By identifying differentially expressed genes (DEGs), hub proteins, regulatory motifs, and potential drug targets, this research seeks to uncover novel biomarkers and therapeutic candidates that could contribute to more effective diagnosis and treatment strategies for POMS.

Materials and Methods

Data collection

The data for this in silico study was retrieved from the Gene Expression Omnibus (GEO) database under the accession number GSE203241. The dataset comprises gene expression profiles from peripheral blood mononuclear cells, collected from two distinct groups; case (POMS) and pediatric healthy control. The control group consists of healthy individuals labeled as pediatric healthy control, with an age range of 10 to 17 years, including both male and female participants. The case group includes individuals diagnosed with POMS, with an age range of 8 to 17 years, also comprising both genders (11).

The primary objective of collecting this dataset is to identify DEGs between these two groups, which may contribute to the understanding of POMS pathogenesis. By comparing gene expression patterns, it is possible to uncover genes that are significantly upregulated or downregulated in POMS patients compared to healthy individuals.

The dataset was analyzed using GEO2R, a web-based tool that allows for direct comparison of gene expression between groups. DEGs were selected based on the following criteria; adjusted $P < 0.05$, and a fold change greater than 1.1 or less than -1.1. These thresholds ensure that only statistically significant and biologically relevant genes are considered for further analysis.

Gene ontology and pathway enrichment analysis

To gain a deeper understanding of the biological significance and functional implications of the DEGs identified in POMS, gene ontology (GO) enrichment and pathway enrichment analysis were performed. Meanwhile, gene ontology analysis was conducted using the BiNGO plugin in Cytoscape (12). This tool facilitates the identification of overrepresented gene ontology categories in the set of DEGs, allowing for the exploration of associated biological processes, molecular functions, and cellular components (12). The DEGs were input into BiNGO, and the analysis was performed with a significance threshold of $P<0.05$, using the hypergeometric test with Benjamini-Hochberg correction for multiple testing. The results were visualized in Cytoscape, highlighting significant gene ontology terms that may be involved in POMS pathogenesis (12). Pathway enrichment analysis was performed using the Enrichr database, which integrates various pathway resources, including Kyoto Encyclopedia of Genes and Genomes, Reactome, and WikiPathways. The list of DEGs was uploaded to Enrichr, and pathways significantly enriched (adjusted $P < 0.05$) were identified. This analysis provided insights into the key signaling pathways and biological networks potentially dysregulated in POMS. The results were ranked based on combined scores and adjusted P values, and the most significant pathways were further explored for their potential roles in disease progression and therapeutic targeting.

Hub genes and subnetwork identification

To identify hub genes and subnetworks within the PPI network, we utilized the CytoHubba plugin in Cytoscape.

Initially, DEGs were analyzed using the search tool for the retrieval of interacting genes/proteins database (STRING) to construct the PPI network. The resulting network was then imported into Cytoscape for further analysis (13).

Using the CytoHubba application, four topological analysis algorithms (maximal clique centrality [MCC], maximum neighborhood component [MNC], density of maximum neighborhood component [DMNC], and [Degree]) were applied to rank the nodes based on their importance within the network. Through this process, the top five hub proteins were identified from each algorithm. By intersecting the results of these methods, a set of 15 core hub proteins was obtained. Subsequently, the primary subnetwork, formed by the intersection of these hub proteins, was further examined. Its interactions with other proteins in the broader network were analyzed to explore potential functional modules and key regulatory components involved in the biological processes of interest.

CytoCluster analysis

To identify significant functional modules within the PPI network, CytoCluster analysis was performed using the CytoCluster app in Cytoscape. The ClusterONE algorithm was applied to detect densely connected regions, with parameters set for clusters of at least three nodes and a minimum density score of 0.5 (14). This analysis revealed key clusters enriched with immune-related genes, cytokine signaling components, and inflammatory mediators. These clusters were further examined using gene ontology and pathway enrichment analyses by the Enrichr database, highlighting their potential roles in POMS pathogenesis. The identified modules offer new insights into disease mechanisms and possible therapeutic targets.

Promoter motif analysis

To analyze the regulatory elements in the promoter regions of hub genes, 1000 base pairs from the promoter region upstream of the transcription start site were retrieved from the Ensembl database. This upstream region was selected to capture potential transcription factor binding sites critical for gene regulation. The obtained promoter sequences were analyzed using the Tomtom tool to identify conserved transcription factor binding motifs. Tomtom compares query motifs against known databases, enabling the identification of significant motif matches. Identified motifs were filtered using stringent criteria, selecting only those with an E-value < 0.1 and a *P* value < 0.001 to ensure the reliability of the results. The filtered motifs were then analyzed using the GoMo tool to explore their potential biological functions. GoMo maps motifs to relevant gene ontology terms, offering insights into the regulatory processes potentially involved in POMS. This analysis highlights key transcription factor binding sites within the promoter regions of hub genes, providing a deeper understanding of gene regulation mechanisms implicated in POMS pathogenesis (15).

Drug-target interaction analysis of hub proteins

To identify potential therapeutic compounds targeting the hub proteins associated with POMS, a drug-target interaction analysis was conducted using the DrugBank database. All hub proteins were searched in DrugBank to identify existing drugs and bioactive compounds that directly interact with these proteins (16). For each hub protein, compounds were extracted based on their direct binding interactions, including Food and Drug Administration (FDA)-approved drugs, investigational agents, and experimental compounds. The selection prioritized compounds with known therapeutic relevance, focusing on those acting as inhibitors, activators, or modulators. Identified drug-protein interactions were further evaluated based on their mechanism of action, binding affinity, and clinical status. The resulting list highlights potential candidates for drug repurposing and offers insights into novel therapeutic strategies for POMS. This analysis provides a foundation for exploring existing compounds that could influence key pathways in POMS, particularly those involved in immune regulation, inflammation, and neurodegeneration.

Statistical analysis

Differential gene expression was assessed using the GEO2R tool, applying the Benjamini-Hochberg correction to control for false discovery rate (FDR), with an adjusted *P* value threshold of <0.05. PPI network centrality measures were analyzed using CytoHubba in Cytoscape, employing topological algorithms (MCC, MNC, DMNC and Degree) to identify hub genes. Cluster analysis for functional modules was performed using the ClusterONE algorithm, with statistical significance evaluated based on density scores and *P* values. Promoter motif enrichment was analyzed using Tomtom and GoMo, applying stringent selection criteria (E-value < 0.1, *P* value < 0.001).

Results

Overview of dataset and identification of DEGs

The dataset (GSE203241) included peripheral blood mononuclear cell samples from 27 POMS patients (12 males, 15 females; aged 8–17 years) and 22 healthy pediatric controls (10 males, 12 females; aged 11–13 years). All samples were collected at a single time point without follow-up. Following the analysis, a total of 1101 DEGs were identified based on the criteria of an adjusted *P* value < 0.05 and a fold change threshold of >1.1 or <-1.1 (Figure 1).

The volcano plot visualizes the distribution of DEGs, where genes with significant differential expression are highlighted. Genes that are upregulated in POMS samples are marked in red, while downregulated genes are marked in blue. The black dots represent genes with no significant changes. The MA plot further illustrates the log fold changes against the mean expression levels. It highlights the upregulated (red) and downregulated

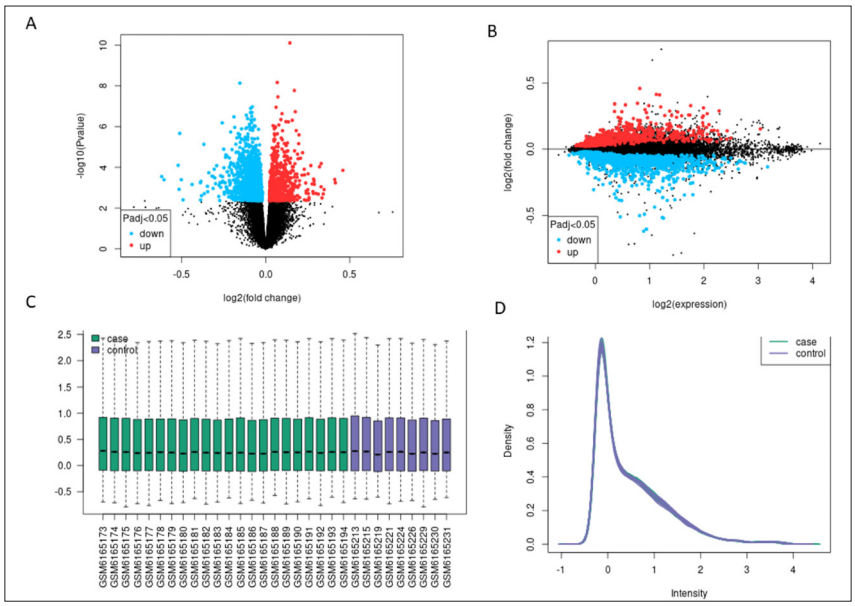


Figure 1. (A) Volcano plot illustrating differential gene expression between two groups. Red dots represent upregulated genes, and blue dots represent downregulated genes with $P < 0.05$. (B) MA plot displaying the average gene expression (log2(expression)) versus log2(fold change). Significantly upregulated and downregulated genes are highlighted in red and blue, respectively. (C) Box plot showing normalized gene expression data across different samples (GSM IDs), comparing distribution between case and control groups. (D) Density plot comparing the intensity distribution of gene expression between case and control groups, indicating proper alignment across samples.

(blue) genes, reinforcing the significance of the identified DEGs. The box plot compares the distribution of gene expression levels across all samples. The uniform distribution post-normalization indicates consistency across the dataset, ensuring the reliability of the differential expression analysis. The density plot shows the overall expression intensity between the case and control groups. The similarity in density curves suggests effective normalization, allowing for accurate comparison between the two groups (Figure 1).

Gene ontology and pathway enrichment analysis of all DEGs

To gain a deeper understanding of the biological functions and pathways associated with the DEGs identified in POMS, gene ontology enrichment and pathway analysis were performed. The top enriched pathways included the immune system, cytokine signaling in the immune system, and interferon signaling (Figure 2A). Additional pathways such as antiviral mechanisms by interferon-stimulated genes, cellular responses to stimuli, and innate immune system were also highly represented. These pathways emphasize the involvement of immune regulation and antiviral responses in POMS pathogenesis Gene ontology enrichment analysis was performed using the BiNGO plugin in Cytoscape, applying a significance threshold of $P < 0.001$, which categorized DEGs based on their functional roles within biological systems. The cellular component (CC) analysis (Figure 2B) showed that the DEGs were predominantly localized to components such as the cytoplasm, nucleus, membrane-bounded organelles, and intracellular organelles. Notably, significant enrichment was observed in components like the cytosol, organelle lumen, nuclear speck, and macromolecular complex, suggesting roles in intracellular transport, transcriptional regulation, and subcellular compartmentalization. The biological process (BP) analysis (Figure 3A) identified key biological processes affected by the DEGs, particularly those related to metabolic processes, transcriptional regulation, RNA processing, and programmed cell death. Notably, enrichment was also observed in pathways such as response to stress, intracellular transport, and response to virus, underscoring the involvement of cellular homeostasis, gene expression control, and antiviral mechanisms in POMS pathophysiology. The molecular function analysis (Figure 3B) highlighted significant functions such as protein binding, RNA binding, and nucleic acid binding, indicating that many of the DEGs are involved in essential molecular interactions. Additional enriched terms included transcription regulator activity, transcription repressor activity, and nucleotide binding, suggesting the involvement of these genes in transcriptional regulation and gene expression control.

Hub genes and subnetwork analysis

The comprehensive PPI network was established using DEGs through the STRING database, followed by visualization in Cytoscape. The network displays complex interactions among proteins, highlighting dense connections that may play critical roles in the biological processes under study (Figure 4A).

Using the CytoHubba plugin, key hub genes were

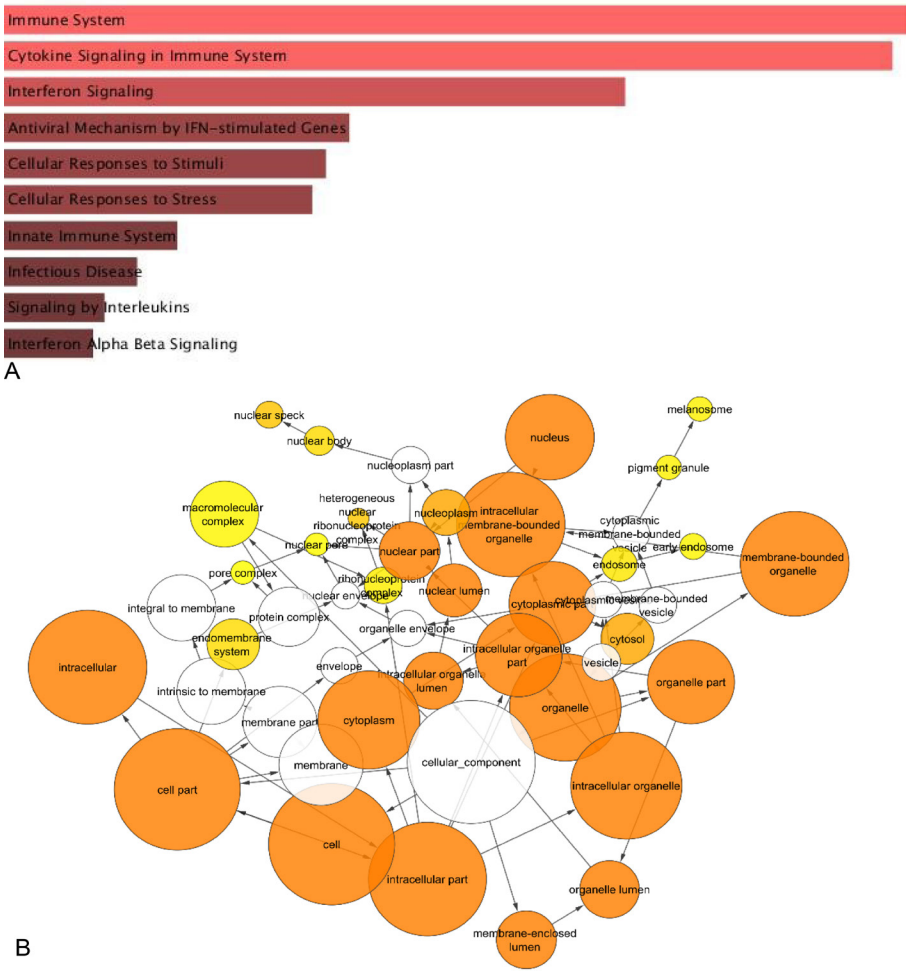


Figure 2. A comprehensive overview of the enrichment analysis results; (A) Pathway enrichment analysis highlighting key immune-related pathways. (B) GO analysis of cellular components, illustrating the primary cellular locations of the DEGs.

identified based on four topological algorithms, MCC, MNC, DMNC, and degree. Initially, five hub proteins were extracted using each algorithm, and their intersection resulted in a set of 15 core hub proteins (highlighted in green). This refined subnetwork reveals the central role of these hub genes within the broader network, as they show high connectivity with numerous interacting proteins (Figure 4B, Table 1).

CytoCluster analysis results

CytoCluster analysis was conducted to identify densely connected protein modules within the PPI network, providing insights into functional clusters relevant to POMS. Using the CytoCluster app with the ClusterONE algorithm, three significant clusters were identified based on node density, quality, and statistical significance. Cluster A consisted of 46 nodes with a density of 0.550, a quality score of 0.430, and a p-value of 1.162e-4. This cluster was primarily enriched in RNA-related biological processes, including processing of capped intron-containing pre-mRNA, metabolism of RNA, mRNA splicing (major pathway), and mRNA 3-end processing. These pathways underscore the role of RNA metabolism and splicing in POMS pathogenesis. Cluster B included 40 nodes, with a higher density of 0.603 and a quality score of 0.406 (P = 0.001). Similar to cluster A, it was significantly enriched in mRNA splicing (major pathway), processing of capped intron-containing pre-mRNA, and metabolism of RNA. The recurrence of splicing-related processes highlights their critical involvement in disease mechanisms (Figure 5).

Cluster C was identified 16 nodes with a density of 0.600 and a quality score of 0.419 (P = 0.025). This cluster was distinct, being primarily associated with vesicle transport and glycosylation pathways. Key enriched pathways included COPI-mediated vesicle transport, ER to Golgi anterograde transport, asparagine N-linked glycosylation, and post-translational protein modification. These findings suggest a role for intracellular trafficking and post-translational modifications in POMS (Figure 5).

Collectively, the CytoCluster analysis revealed core functional modules centered around RNA metabolism and vesicle transport, providing new perspectives on potential molecular mechanisms driving POMS progression.

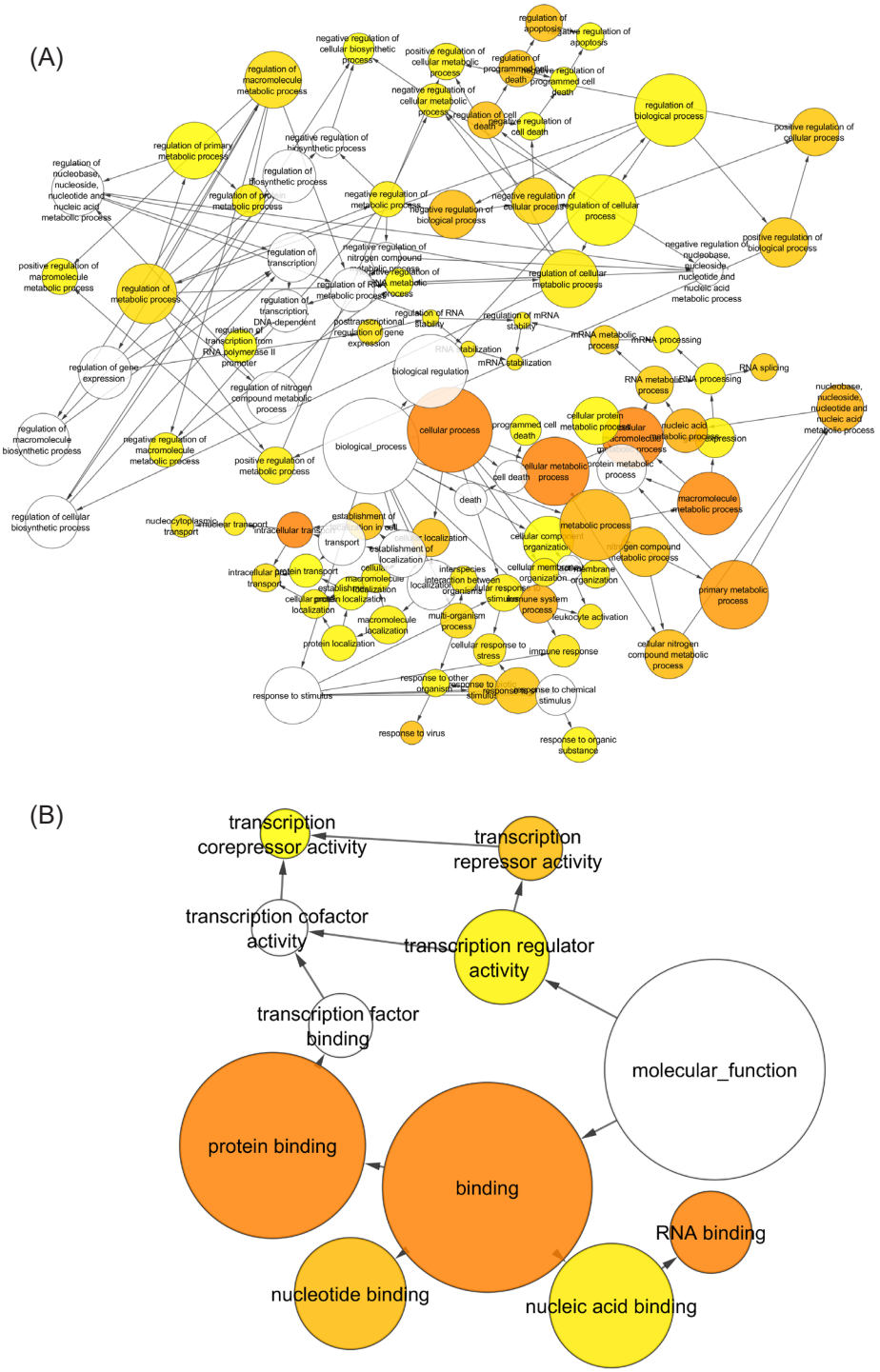


Figure 3. A comprehensive overview of the enrichment analysis results; 3A: gene ontology analysis of biological processes, focusing on immune responses and inflammatory pathways. 3B: gene ontology analysis of molecular functions, identifying critical molecular interactions and activities.

Promoter motif analysis results

The promoter motif analysis aimed to identify regulatory elements within the promoter regions of hub genes involved in POMS. A 1000 base pair upstream region from the transcription start site (TSS) was analyzed for each hub gene to detect conserved transcription factor binding motifs using the Tomtom tool, followed by functional annotation through the GoMo tool. The significant motifs identified were filtered using stringent criteria (E-value < 0.1and *P* value < 0.001) to ensure the accuracy of results. For HSP90AA1 (ENSG00000080824), two distinct motifs were identified. The first, a 9 bp motif, was associated with GTPase activity and ATP binding (molecular function) and was located in the cytosol (cellular component). This motif also plays a role in the negative regulation of signal transduction (biological process) and exhibits transcription corepressor activity. The second motif, a 24 bp sequence, was enriched in the

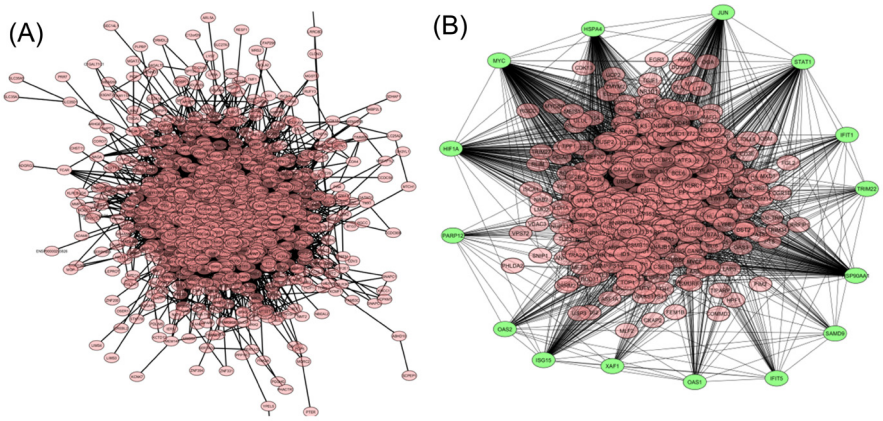


Figure 4. (A) The complete PPI network of DEGs constructed using STRING and visualized in Cytoscape. (B) Identification of 15 hub genes (green) using CytoHubba algorithms (MCC, MNC, DMNC, Degree) and their interactions within the subnetwork.

transcription factor complex (cellular component) and was involved in inner ear morphogenesis and the positive regulation of gene-specific transcription from the RNA polymerase II promoter. Additionally, it showed roles in protein heterodimerization and potassium ion binding. The analysis of XAF1 (ENSG00000132530) revealed a 24 bp motif predominantly linked to developmental processes. This motif was enriched in anterior/posterior pattern formation, lung development, and cartilage development (biological process). It was also involved in both the positive and negative regulation of transcription from the RNA polymerase II promoter, indicating its dual regulatory role in gene expression. For HSPA4 (ENSG00000177606), a 9 bp motif was identified within the transcription factor complex (cellular component), associated with crucial biological processes such as axon guidance and inner ear morphogenesis. It also played a role in the negative regulation of signal transduction and exhibited chromatin binding activity (molecular function), suggesting its involvement in chromatin remodeling and gene expression regulation. The promoter motif analysis of ISG15 (ENSG00000187608) uncovered a 14 bp motif enriched in the transcription factor complex (cellular component) with significant roles in gene regulation. This motif displayed transcription activator activity, zinc ion binding, and protein homodimerization activity (molecular function), and it was involved in the negative regulation of signal transduction (biological process), highlighting its potential role in modulating immune responses (Table 2).

Table 1. Core hub proteins identified from the PPI network using topological ranking methods

Rank	Hub protein	Method
1,1	MYC	MNC/degree
2,2	HSP90AA1	MNC/degree
3,3	JUN	MNC/degree
4,4	HSPA4	MNC/degree
5,5	HIF1A	MNC/degree
1	STAT1	MCC
2	ISG15	MCC
3	OAS1	MCC
4	OAS2	MCC
5	IFIT1	MCC
1	SAMD9	DMNC
2	XAF1	DMNC
3	PARP12	DMNC
4	TRIM22	DMNC
5	IFIT5	DMNC

Overall, the promoter motif analysis identified critical regulatory motifs in the upstream regions of hub genes, each linked to key molecular functions and biological processes relevant to POMS pathogenesis.

Drug-target interaction analysis

The drug-target interaction analysis was conducted to identify potential therapeutic compounds targeting the hub proteins identified in POMS. Using the DrugBank database, several drugs and bioactive compounds were found to interact with the key hub proteins, particularly HSP90AA1, HIF1A, HSPA4, and OAS1. For HSP90AA1, multiple compounds were identified, including FDA-approved drugs such as cromoglicic acid, copper, rifabutin, and Amlexanox. While some of these drugs have an unknown pharmacological action on HSP90AA1, others, like rifabutin, do not exhibit a direct pharmacological effect. Additionally, several experimental compounds demonstrated known interactions with HSP90AA1, such as 2-(1H-pyrrol-1-ylcarbonyl) benzene-1,3,5-triol, CCT-018159, and various purin-6-amine derivatives, all showing confirmed binding activity.

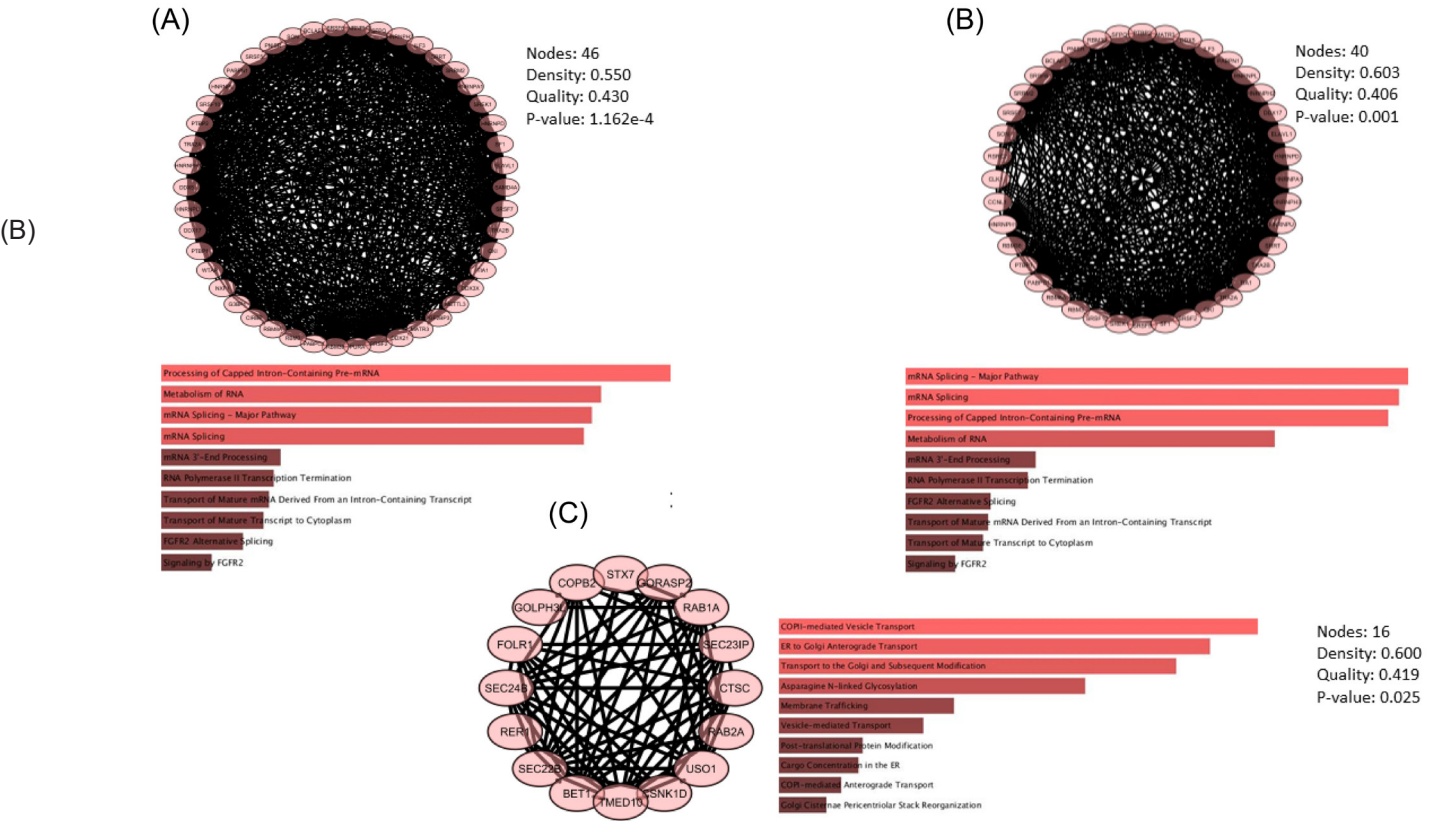


Figure 5. Functional modules identified by Cytocluster analysis. (A) Cluster A (46 nodes, *P* = 1.162e-4) enriched in RNA processing and splicing. (B) Cluster B (40 nodes, *P* = 0.001) similarly enriched in RNA metabolism and mRNA splicing. (C) Cluster C (16 nodes, *P* = 0.025) associated with vesicle transport and glycosylation pathways. Bar plots show pathway enrichment results for each cluster.

The hub protein HSPA4 was found to interact with phenethyl isothiocyanate, an investigational compound, though its pharmacological action remains unknown. For HIF1A, several compounds with varying approval statuses were identified like 2-Methoxyestradiol and PX-478 demonstrated confirmed pharmacological activity, while drugs like carvedilol, hydralazine (both approved), and investigational compounds such as ENMD-1198, FG-2216, PT-2385, and vadadustat were found to target hypoxia-inducible factor 1-alpha (HIF1A), though many of their specific actions remain undefined. Finally, the hub protein oligoadenylate synthetase 1 (OAS1) was linked to the experimental compound cysteine-s-acetamide, though its pharmacological impact on the protein is currently unknown (Table 3).

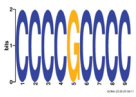
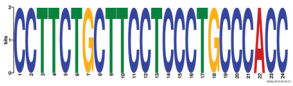
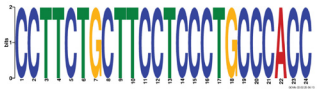
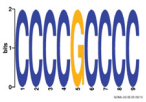
Discussion

This study aimed to investigate the molecular underpinnings of POMS through a comprehensive bioinformatics approach. By integrating differential gene expression analysis, PPI network analysis, promoter motif identification, and drug-target interaction mapping, we identified critical genes and pathways that may contribute to POMS pathogenesis and offer potential therapeutic targets.

The identification of 1101 DEGs between POMS patients and healthy controls highlights the widespread transcriptional alterations contributing to disease progression. Enrichment analyses emphasized pathways related to immune system regulation, cytokine signaling, and interferon responses, aligning with the well-established immune-mediated nature of MS. Particularly, the activation of antiviral defense pathways and cytokine-mediated signaling suggests that environmental triggers, such as viral infections, could interact with genetic predispositions to accelerate disease onset in pediatric populations.

One of the key findings of this study was the identification of 15 hub proteins, namely: MYC, HSP90AA1, JUN, HSPA4, HIF1A, STAT1, ISG15, OAS1, OAS2, IFIT1, SAMD9, XAF1, PARP12, TRIM22, and IFIT5. In addition, MYC, HSP90AA1, JUN, HSPA4, and HIF1A, recognized by two distinct topological algorithms, underscoring their central role in the PPI network. These genes may serve as crucial regulators in POMS pathogenesis, either through direct involvement in immune modulation or by influencing neuroinflammatory processes. MYC is a critical transcription factor involved in cell proliferation, apoptosis, and immune regulation. Although its role in MS is not extensively characterized, MYC has been implicated in the regulation of T-cell activation and differentiation. MYC plays a crucial role in the activation

Table 2. Identified promoter motifs in the upstream regions of hub genes and their associated molecular functions and biological processes

Motif	Logo	Length	Top 5 specific predictions
ENSG00000080824| HSP90AA1		9	MF GTPase activity CC cytosol MF transcription corepressor activity BP negative regulation of signal transduction MF ATP binding
ENSG00000080824|HSP90AA1		24	CC transcription factor complex BP inner ear morphogenesis MF protein heterodimerization activity BP positive regulation of gene-specific transcription from RNA polymerase II promoter MF potassium ion binding
ENSG00000132530|XAF1		24	BP anterior/posterior pattern formation BP positive regulation of transcription from RNA polymerase II promoter BP lung development BP negative regulation of transcription from RNA polymerase II promoter BP cartilage development
ENSG00000177606|HSPA4		9	CC transcription factor complex BP axon guidance BP negative regulation of signal transduction MF chromatin binding BP inner ear morphogenesis
ENSG00000187608|ISG15		14	CC transcription factor complex MF transcription activator activity BP negative regulation of signal transduction MF zinc ion binding MF protein homodimerization activity

of memory T helper (Th) and naive T cells in experimental autoimmune encephalomyelitis (EAE), a model for MS. This activation is mediated through the induction of PRMT5, an arginine methyltransferase that is essential for inflammatory T cell expansion and disease progression. The transcriptional activity of MYC is associated with T cell receptor-independent signaling from activated CD28, leading to inflammatory T cell responses in MS. This process involves phospho-STAT3 and RelA/NF-κB (17). Bioinformatic analysis have identified MYC as a central gene in PPI networks related to MS disease susceptibility. This suggests that MYC is a key regulator in the pathogenesis of CNS autoimmunity (18). HSP90AA1 (heat shock protein 90 alpha family class A member 1) acts as a molecular chaperone, facilitating the proper folding of proteins and preventing their aggregation (19,20). Recent studies have highlighted the involvement of HSP90AA1 in synaptic homeostasis, particularly in the context of Alzheimer's disease. Proteomic analyses have shown that HSP90AA1, along with other proteins like PTK2B and ANXA2, is implicated in disturbed synaptogenesis pathways in the human entorhinal cortex. This suggests that HSP90AA1 may contribute to synaptic dysfunction observed in AD (21). Studies have demonstrated that HSP90 inhibitors have been effective in reducing tau protein hyperphosphorylation and aggregation, which are key features of Alzheimer's disease pathology. Studies have demonstrated that these inhibitors can decrease tau expression and phosphorylation in tau transgenic models, thereby mitigating neurodegenerative processes (22). Jun proto-oncogene, AP-1 transcription factor subunit (JUN), a component of the AP-1 transcription factor complex, plays a vital role in regulating gene expression in response to stress, cytokines, and growth factors. It is involved in the control of cell proliferation, differentiation, and apoptosis. In neurodegenerative diseases, including MS, JUN has been linked to pathways involved in neuronal apoptosis and glial activation, potentially contributing to neuroinflammation and axonal damage (22,23). Heat shock protein family A member 4 (HSPA4) is another molecular chaperone involved in protein folding and cellular stress responses. Although its role in MS has been less studied, HSPA4 has been implicated in immune regulation and neuroinflammatory pathways. Its identification as a hub protein in this study suggests that it may play a role in maintaining neuronal integrity under inflammatory conditions (24). HIF1A (hypoxia-inducible factor 1-alpha) is a transcription factor that responds to hypoxic conditions and regulates genes involved in angiogenesis, metabolism, and immune responses. In MS, hypoxia-like tissue damage has been observed in active lesions, implicating HIF1A in disease pathogenesis. It has been shown that HIF1A can influence immune cell migration and cytokine production, making it a potential target for therapeutic intervention (25,26). The identification of these five hub genes by two independent algorithms strengthens the validity of their central role in POMS and highlights their potential as biomarkers or drug targets.

CytoCluster analysis revealed distinct functional modules within the PPI network, emphasizing key biological processes implicated in POMS. Two major clusters were enriched in pathways related to RNA metabolism and mRNA splicing, processes that are

Table 3. DrugBank-based identification of compounds targeting hub proteins and their pharmacological profiles

Drug	Drug group	Pharmacological action?	Target
Cromoglicic acid	Approved	Unknown	HSP90AA1
Copper	Approved, investigational	Unknown	
Rifabutin		No	
Amlexanox		Unknown	
2-(1H-pyrrol-1-ylcarbonyl)benzene-1,3,5-triol	Experimental	Yes	
4-(2-methoxyethoxy)-6-methylpyrimidin-2-amine		Yes	
6-(3-BROMO-2-NAPHTHYL)-1,3,5-TRIAZINE-2,4-DIAMINE		Yes	
8-BENZO[1,3]DIOXOL-,5-YLMETHYL-9-BUTYL-9H-		Yes	
9-Butyl-8-(3-Methoxybenzyl)-9h-Purin-6-Amine		Yes	
9-Butyl-8-(3,4,5-Trimethoxybenzyl)-9h-Purin-6-Amine		Yes	
9-Butyl-8-(4-Methoxybenzyl)-9h-Purin-6-Amine		Yes	
CCT-018159		Yes	
(3E)-3-[(phenylamino)methylidene]dihydrofuran-2(3H)-one		Unknown	
(5E,7S)-2-amino-7-(4-fluoro-2-pyridin-3-ylphenyl)-4-methyl-7,8-dihydroquinazolin-5(6H)-one oxime		Unknown	
2-[(2-methoxyethyl)amino]-4-(4-oxo-1,2,3,4-tetrahydro-9H-carbazol-9-yl)benzamide		Unknown	
2-AMINO-4-(2,4-DICHLOROPHENYL)-N-ETHYLTHIENO[2,3-D]PYRIMIDINE-6-CARBOXAMIDE		Unknown	
2-amino-4-[2,4-dichloro-5-(2-pyrrolidin-1-ylethoxy)phenyl]-N-ethylthieno[2,3-d]pyrimidine-6-carboxamide		Unknown	
3-({2-[(2-AMINO-6-METHYLPYRIMIDIN-4-YL)ETHYNYL]BENZYL}AMINO)-1,3-OXAZOL-2(3H)-ONE		Unknown	
3,6-DIAMINO-5-CYANO-4-(4-ETHOXYPHENYL)THIENO[2,3-B]PYRIDINE-2-CARBOXAMIDE		Unknown	
4-(1,3-Benzodioxol-5-Yl)-5-(5-Ethyl-2,4-Dihydroxyphenyl)-2h-Pyrazole-3-Carboxylic Acid		Unknown	
Phenethyl Isothiocyanate	Investigational	Unknown	HSPA4
2-Methoxyestradiol	Investigational	Yes	HIF1A
Carvedilol	Approved	Unknown	
ENMD-1198	Investigational	Unknown	
FG-2216	Investigational	Unknown	
Hydralazine	Approved	Unknown	
PT-2385	Investigational	Unknown	
PX-478	Investigational	Yes	
Vadadustat	Approved, investigational	Unknown	
Cysteine-S-acetamide	Experimental	Unknown	OAS1

increasingly recognized for their roles in neurodegenerative and neuroinflammatory diseases. Disruptions in RNA splicing could lead to the production of dysfunctional proteins, exacerbating inflammation and neuronal damage (27). Another identified cluster was enriched in vesicle-mediated transport and post-translational modifications, processes essential for maintaining neuronal communication and synaptic integrity (28). Dysregulation of these pathways may contribute to synaptic dysfunction and neurodegeneration observed in MS (28). These functional modules provide a deeper understanding of the molecular events driving POMS and highlight novel areas for therapeutic exploration, such as targeting RNA splicing machinery or modulating vesicle transport pathways.

The promoter motif analysis revealed potential transcription factor binding sites within the 1000 bp upstream regions of hub genes, providing insights into the regulatory mechanisms that may influence their expression in POMS. Identifying promoter motifs associated with hub genes allows researchers to pinpoint key transcription factors that drive aberrant gene expression in POMS. These transcription factors often act as upstream regulators of pathological processes, including inflammation, immune dysregulation, and neurodegeneration, which are central to MS pathogenesis. By targeting these transcription factors, either through small molecule inhibitors or gene-editing techniques like CRISPR/Cas9, it may be possible to modulate the expression of disease-driving genes, ultimately reducing inflammation and promoting

remyelination (29).

The drug-target interaction analysis revealed several promising therapeutic candidates targeting key hub proteins implicated in POMS. Notably, HSP90AA1 and HIF1A emerged as central targets with multiple interacting compounds, including FDA-approved drugs like rifabutin and hydralazine, as well as investigational agents such as PX-478 and CCT-018159, some of which demonstrated confirmed pharmacological activity. Despite the identification of drugs like cromoglicic acid and phenethyl isothiocyanate, their exact mechanisms of action on respective targets remain unclear, highlighting the need for further pharmacodynamic studies. The interaction of OAS1 with cysteine-S-acetamide also suggests potential for novel therapeutic approaches, although its functional impact remains to be elucidated. Overall, these findings support the potential repurposing and development of targeted compounds for modulating immune responses in pediatric MS (Table 3).

Conclusion

This study provides a comprehensive bioinformatics analysis of POMS, shedding light on key DEGs, biological pathways, and potential therapeutic targets. The identification of 1101 DEGs, along with hub genes such as MYC, HSP90AA1, JUN, HSPA4, and HIF1A, highlights their central roles in immune regulation and neuroinflammation. Functional enrichment analyses underscore the involvement of cytokine signaling, interferon responses, and immune system regulation, reinforcing the notion that POMS is driven by complex immune-mediated mechanisms.

Furthermore, promoter motif analysis reveals regulatory elements that may control the expression of these hub genes, offering insights into gene regulation mechanisms implicated in POMS. Additionally, drug-target interaction analysis identifies FDA-approved and investigational drugs that interact with key hub proteins, suggesting potential avenues for repurposing existing therapeutics for POMS treatment.

Overall, these findings enhance our molecular understanding of POMS and open new possibilities for biomarker discovery and targeted interventions. Future research should focus on experimental validation of these candidate genes and pathways, as well as preclinical investigations into the therapeutic potential of identified drug candidates to pave the way for personalized treatment strategies in pediatric MS.

Future directions

Building on the findings of this study, future research should focus on experimental validation of the identified hub genes and pathways through in vitro and in vivo models of POMS. Functional studies using CRISPR/Cas9 gene editing, RNA interference (RNAi), and overexpression assays can help elucidate the precise roles of MYC, HSP90AA1, JUN, HSPA4, and HIF1A in disease pathogenesis. Additionally, single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics can provide a more detailed understanding of cell-type-specific gene expression alterations in POMS.

To translate these findings into clinical applications, target-based drug screening should be conducted to assess the therapeutic potential of the identified drug candidates interacting with hub proteins. This includes testing FDA-approved and investigational drugs identified in our drug-target interaction analysis, with a focus on their efficacy in modulating immune responses and neuroinflammatory pathways. Organoid models and patient-derived induced pluripotent stem cells could serve as platforms for personalized drug testing, helping to identify optimal treatment strategies for different patient subgroups.

Further exploration of epigenetic modifications and regulatory elements affecting hub gene expression is also warranted. Investigating histone modifications, DNA methylation patterns, and non-coding RNAs (e.g., microRNAs, long non-coding RNAs) associated with POMS may reveal additional therapeutic targets. Additionally, multi-omics integration combining genomics, transcriptomics, proteomics, and metabolomics data can provide a more holistic view of disease mechanisms and identify novel biomarkers for early diagnosis and disease monitoring.

Finally, large-scale prospective cohort studies with longitudinal follow-up should be conducted to validate the clinical relevance of the identified molecular markers. Establishing biomarker-driven risk stratification models will aid in the development of personalized treatment approaches, ultimately improving disease management and outcomes for pediatric MS patients.

Limitations of the study

This study provides valuable insights into the molecular mechanisms of POMS using bioinformatics approaches; however, several limitations should be considered.

One major limitation is the lack of experimental validation. While computational analyses identify potential key genes and pathways, laboratory-based studies, including in vitro and in vivo models, are necessary to confirm their functional relevance in the disease process. Another limitation is the relatively small sample size of the dataset analyzed. Genetic and environmental factors vary across populations, and the findings may not be fully generalizable. Larger multi-cohort studies with diverse patient populations are needed to ensure broader applicability. The study relies on bulk RNA-sequencing data from peripheral blood mononuclear cells, which does not account for cell-type-specific gene expression changes. Single-cell RNA sequencing could provide a more detailed understanding of which immune cell subtypes contribute to disease progression. Confounding factors such as prior treatments, disease duration, and comorbidities were

not controlled for in this analysis. These variables could influence gene expression patterns, and future studies incorporating clinical metadata and longitudinal data will be necessary to address these concerns.

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Conflicts of interest

The authors declare that they have no competing interests.

Data availability statement

The data that support the conclusions of this study can be made available by the corresponding author upon a reasonable request.

Ethical issues

This *in silico* study did not involve human/animal subjects or primary data collection; thus, ethical approval was not required.

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