BJMG 28 (2), 2025 • 85-92
DOI: 10.2478/bjmg-2025-00023

ORIGINAL ARTICLE

IDENTIFICATION OF SERUM PROTEOME IN CHILDREN WITH AUTISM SPECTRUM DISORDER

Popov T N[1,3], Minchev S D[2], Vachev I T[4,*]

[*]**Corresponding Author:** Tihomir I. Vachev Department of Molecular Biology, University of Plovdiv "Paisii Hilendarski", 24 Tzar Assen Str., Plovdiv, Bulgaria; Tel.: +359 896 026 004; E-mail: tihomirvachev@uni-plovdiv.bg

ABSTRACT

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition characterized by impaired social interaction, communication deficits, and restricted, repetitive behaviors. Early and accurate diagnosis is essential for timely intervention and improved outcomes. While numerous efforts have been made to identify reliable biomarkers, no clinically validated protein biomarkers for ASD are currently available. The identification of such biomarkers could aid in diagnosis, subtyping, treatment monitoring, and the discovery of novel therapeutic targets. In this study, we analyzed the serum proteome profiles of children with ASD compared to typically developing children (TDC) using an iTRAQ-based quantitative proteomic approach. Pooled serum samples were assessed to identify potential ASD-associated protein biomarkers. A total of 59 differentially expressed proteins were identified between the ASD and TDC groups. These proteins are implicated in several biological pathways, including cholesterol metabolism, complement and coagulation cascades, tight junctions, regulation of the actin cytoskeleton, and extracellular matrix (ECM)-receptor interactions. Notably, levels of complement C4A, APOC2, and PFN1 were significantly elevated in the ASD group, while proteins involved in ECM-cell interactions - HSPG2, HPSE, and NID1 - were markedly decreased. These findings highlight a distinct proteomic signature in ASD and suggest that the identified proteins may serve as promising candidates for molecular biomarkers. Further validation in larger, independent cohorts is warranted to establish their diagnostic and clinical utility.

Keywords: Autism Spectrum Disorder (ASD), biomarker discovery, differential protein expression, iTRAQ, serum, proteomics

INTRODUCTION

Autism spectrum disorder (ASD) comprises a heterogeneous group of neurodevelopmental conditions characterized by impaired social communication, restricted interests, and repetitive behaviors or activities [1]. Over recent decades, the prevalence of ASD has risen markedly in many countries, affecting approximately 1–2% of the global population. In the United States, the reported male-to-female ratio is approximately 4:1 [2]. The etiology and pathogenesis of ASD are complex and remain incompletely understood. Numerous studies suggest that ASD is a multifactorial disorder involving both genetic predispositions and environmental influences [3]. Immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction, and exposure to environmental toxicants have all been implicated in ASD-related abnormalities [4]. Currently, there are no approved pharmacological treatments that effectively address the core symptoms of ASD. However, early behavioral interventions have shown promise in improving developmental outcomes in children with ASD [5]. As such, early diagnosis is critical. Presently, ASD diagnosis relies on clinical evaluations of behavioral signs and symptoms according to criteria outlined in the Diagnostic and Statis-

[1] Medical center Spectar – Plovdiv; 107 Macedonia Street; Plovdiv, Bulgaria; nikpopov@abv.bg

[2] Department of Human Anatomy and Physiology; University of Plovdiv "Paisii Hilendarski", 24 Tzar Asen Street; Plovdiv, Bulgaria; dminchev@uni-plovdiv.bg

[3] Department of Psychology; University of Plovdiv "Paisii Hilendarski", 24 Tzar Asen Street; Plovdiv, Bulgaria; nikpopov@abv.bg

[4] Department of Molecular Biology; University of Plovdiv "Paisii Hilendarski", 24 Tzar Asen St.; Plovdiv, Bulgaria; tihomirvachev@uni-plovdiv.bg

tical Manual of Mental Disorders, Fifth Edition (DSM-5). The absence of objective biological markers makes early detection difficult and highly subjective, with the average age of diagnosis estimated at approximately 4 - 5 years [6]. The identification of reliable, early-detection biomarkers for ASD remains a critical unmet clinical need. While genetic testing has been explored, the genetic heterogeneity of ASD limits the predictive power of current genetic approaches [7]. To address this gap, various biomarker discovery strategies have been employed, including neuroimaging, genomics, transcriptomics, metabolomics, and proteomics. Nevertheless, these methods have yet to yield diagnostic tools with sufficient sensitivity and specificity [8]. Proteomics, which complements genomic research, offers a promising avenue for identifying protein-level alterations that reflect underlying pathophysiological mechanisms. In the present study, we applied an iTRAQ-based quantitative proteomic approach to investigate serum protein expression patterns in children with ASD compared to typically developing controls (TDC). Our objective was to identify candidate protein biomarkers with potential utility for ASD diagnosis.

MATERIALS AND METHODS

Patient Recruitment and Serum Collection

A total of 60 children were included in this study, comprising 30 children diagnosed with autism spectrum disorder (ASD) (24 males and 6 females; aged 3 to 11 years) and 30 age- and sex-matched typically developing children (TDC) serving as controls. All participants were randomly selected from general medical practices in the Plovdiv region, Bulgaria. The diagnosis of ASD was established by certified psychiatrists based on clinical assessments using the Gilliam Autism Rating Scale (GARS), the Childhood Autism Rating Scale (CARS), and the Autism Diagnostic Interview – Revised (ADI-R), in accordance with the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria. GARS was used as a norm-referenced tool to evaluate the severity of ASD symptoms. CARS assisted in distinguishing ASD from other developmental delays. ADI-R, a structured interview with the parents, served as the gold standard for comprehensive ASD assessment. The control group (TDC) was matched by age and sex to the ASD group. Clinical evaluations and CARS assessments were performed to confirm the absence of autistic traits in all control participants. None of the participants had received any medication prior to blood collection. Children with a history of infectious, oncological, metabolic, or genetic disorders were excluded from the study. Blood samples were collected in the morning prior to food intake (fasting state). To minimize pre-analytical variability, samples were processed according to standard operating procedures. Briefly, blood was allowed to clot for 1 hour at 37 °C and subsequently centrifuged at 3000 g for 20 minutes at 4 °C. The resulting serum was aliquoted and immediately stored at −80 °C until further analysis. All procedures were reviewed and approved by the Institutional Review Board of the Ethics Committee of the Medical University of Plovdiv. Written informed consent was obtained from the parents or legal guardians of all participants prior to inclusion in the study.

Protein Sample Preparation

Equal volumes of serum from each individual were pooled to generate composite samples for the ASD and TDC groups, respectively. To reduce sample complexity and remove highly abundant proteins, the pooled serum samples were processed using the ProteoMiner™ Protein Enrichment Kit (Bio-Rad, Hercules, CA, USA), following the manufacturer's instructions. After enrichment, the samples were buffer-exchanged into a denaturing sample buffer containing 7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 40 mM dithiothreitol (DTT), and 40 mM Tris base. Protein concentrations were quantified using the Bradford assay. For each pooled sample, 100 µg of total protein was enzymatically digested using Trypsin Gold (Promega, Madison, WI, USA) at a protein-to-trypsin ratio of 20:1. Digestion was carried out in two steps: the first for 4 hours at 37 °C, followed by a second digestion under the same conditions for an additional 8 hours.

iTRAQ Labeling, SCX Fractionation, and LC-ESI-MS/MS Analysis

Following digestion, peptides were dried by vacuum centrifugation and reconstituted in 0.5 M triethylammonium bicarbonate (TEAB) for isobaric tagging. Peptide labeling was conducted using 8-plex iTRAQ reagents (AB Sciex, Framingham, MA, USA) according to the manufacturer's protocol. The ASD group peptides were labeled with iTRAQ tag 115, and the TDC group with tag 118. The labeled peptides were then pooled and dried again by vacuum centrifugation. Peptide fractionation was performed using Strong Cation Exchange (SCX) chromatography, following established protocols [9]. The resulting fractions were subjected to liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) using a Triple TOF 5600 mass spectrometer (AB SCIEX, Concord, ON, Canada).

Data Processing and Functional Analysis

Peptide and protein identification was conducted by searching the acquired MS/MS data against the human protein database (IPI: human_v3.87) using the Mascot search engine (version 2.3.02; Matrix Science, London, UK). Protein identification and quantification procedures were carried

out as described by An et al. [9]. For protein quantification, only proteins containing at least two unique peptide spectra were considered. Quantitative protein ratios were normalized and weighted by the median ratio using Mascot's integrated quantification tools. Proteins with p-values < 0.05 were considered to be significantly differentially expressed. To investigate the biological significance of the differentially expressed proteins (DEPs), functional annotation and enrichment analysis were performed using the Enrichr web-based tool(https://amp.pharm.mssm.edu/Enrichr/) [10]. Gene Ontology (GO) enrichment analysis was performed across three categories: biological process (BP), molecular function (MF), and cellular component (CC). Additionally, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was conducted to explore relevant signaling and metabolic pathways. Enrichment results with p-values < 0.05 were considered statistically significant.

RESULTS

iTRAQ-Based Comparative Proteomics Analysis

Using iTRAQ proteomic profiling, a total of 843 proteins were identified with 95% confidence and a 1% false discovery rate (FDR). Of these, 417 proteins were successfully quantified based on 3,699 unique peptides, corresponding to 413,032 MS/MS spectra. Among the quantified proteins, 59 were differentially expressed proteins (DEPs) between the autism spectrum disorder (ASD) group and the typically developing children (TDC) group (Table 1). Of these DEPs, 24 proteins were significantly upregulated, while 35 were downregulated in the ASD group relative to controls (Figure 1).

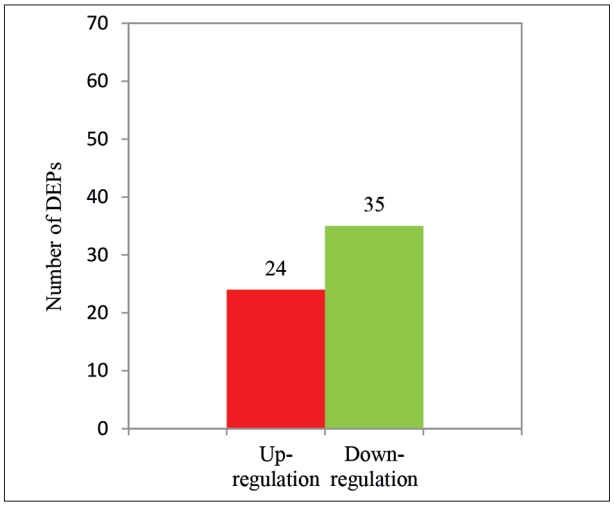

Figure 1. Differentially Expressed Proteins between ASD and control samples: names of the comparable group; Y-axis: the number of the differentially expressed protein. Red stands for up-regulated proteins, green stands for the number of down-regulated proteins.

Bioinformatics Analysis of Differentially Expressed Proteins

To elucidate the biological roles of the identified DEPs, we performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses using Enrichr (https://amp.pharm.mssm.edu/Enrichr/). Biological Process (BP): The DEPs were significantly associated with processes such as protein processing regulation, activation cascades, complement activation, humoral immune response, immune effector processes, acute inflammatory response, and Fc-gamma receptor signaling pathway (Figure 2A). Cellular Component (CC): Enrichment was observed in structures such as actin filament bundles, stress fibers, platelet alpha granules, focal adhesions, very-low-density lipoprotein (VLDL) particles, actomyosin complexes, and the endoplasmic reticulum lumen (Figure 2B). Molecular Function (MF): Significant enrichment was found for functions including serine-type endopeptidase activity, general endopeptidase activity, immunoglobulin receptor binding, integrin binding, complement component C1q binding, and metal ion binding (Figure 2C).

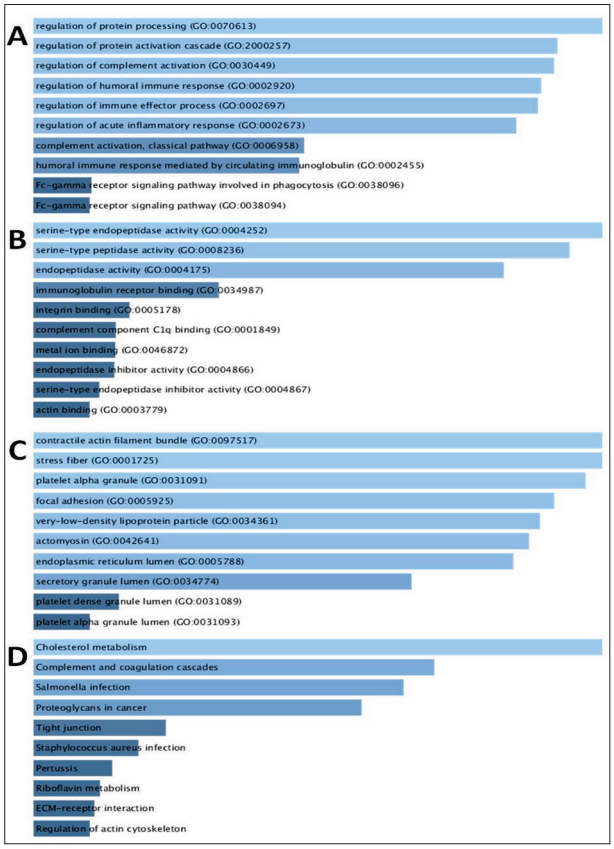

Figure 2. GO and KEGG pathway enrichment analyses performed using Enrichr on DEPs identified from ASD and TDC.
(A) The top 10 enriched biological processes for DEPs.
(B) The top 10 enriched molecular functions for DEPs.
(C) The top 10 enriched cellular components for DEPs.
(D) The top 10 enriched KEGG pathways for DEPs.

Table 1. Differentially expressed proteins identified in blood serum samples from children with ASD and healthy controla

No	Protein name	Accession number	Gene name	Peptides (95%)	% Cov	Fold change
1	A disintegrin and metalloproteinase with thrombospondin motifs 13 (-)	Q76LX8	ADAMTS13	8	6.5	0.78
2	Angiogenin (-)	P03950	ANG	3	21.8	0.81
3	Anthrax toxin receptor 2 (+)	P58335	ANTXR2	5	16.6	1.41
4	Apolipoprotein C-II (+)	P02655	APOC2	4	49.5	1.26
5	Apolipoprotein C-IV (+)	P55056	APOC4	6	39.4	1.72
6	Apolipoprotein F (+)	Q13790	APOF	2	6.7	1.30
7	Apolipoprotein L1 (+)	O14791	APOL1	15	40.6	1.40
8	Apolipoprotein(a) - precursor (+)	P08519	LPA/APOA	13	3.7	1.75
9	Basement membrane-specific heparan sulfate proteoglycan core protein (-)	P98160	HSPG2	5	1.3	0.73
10	Calponin-2 (-)	Q5RFN6	CNN2	3	14.2	0.72
11	Calsyntenin-1 (-)	O94985	CLSTN1	3	3.8	0.75
12	Carboxypeptidase N catalytic chain (-)	P15169	CPN1	7	17.5	0.77
13	Carboxypeptidase N subunit 2 (-)	P22792	CPN2	12	28.1	0.80
14	Chromogranin-A (-)	P10645	CHGA	5	13.1	0.66
15	Complement C1q subcomponent subunit A (+)	P02745	C1QA	6	29	1.29
16	Complement C4-A (+)	P0C0L4	C4A	1	62.3	7.36
17	C-reactive protein (-)	P02741	CRP	6	23.7	0.83
18	Extracellular matrix protein 1 (-)	Q16610	ECM1	13	31.3	0.76
19	Fibronectin 1 (-)	Q28275	FN1	2	45.5	0.76
20	Fibulin-5 (-)	Q9UBX5	FBLN5	6	15.4	0.79
21	Flavin reductase (+)	P30043	BLVRB	3	18.9	1.59
22	Glia-derived nexin (-)	P07093	SERPINE2	6	15.6	0.74
23	Heparanase (-)	Q9Y251	HPSE	5	8.8	0.80
24	Histone H1.3 (+)	P43277	H1-3	2	13.6	1.39
25	Histone H2B 1/2/3/4/6 (+)	P0C1H3	H2B-I	3	23	1.33
26	Histone H4 (+)	Q6WV73	H4	6	51.5	1.52
27	Immunoglobulin heavy constant gamma 1 (+)	P01857	IGHG1	1	36.5	1.40
28	Immunoglobulin heavy constant gamma 2 (+)	P01859	IGHG2	1	33.6	1.35
29	Immunoglobulin heavy constant gamma 4 (+)	P01861	IGHG4	3	21	1.63
30	Immunoglobulin heavy variable 3-23 (-)	P01764	IGHV3-23	2	26.1	0.46
31	Immunoglobulin J chain (-)	P01591	JCHAIN	4	26.4	0.82
32	Immunoglobulin kappa variable 1-5 (-)	P01602	IGKV1-5	3	40.5	0.82
33	Immunoglobulin kappa variable 3-20 (-)	P01619	IGKV3-20	2	31.5	0.80
34	Immunoglobulin lambda variable 3-1 (-)	P01715	IGLV3-1	1	27.4	0.78
35	Immunoglobulin lambda variable 3-21 (-)	P80748	IGLV3-21	1	24.5	0.79
36	Immunoglobulin lambda variable 3-25 (-)	P01717	IGLV3-25	1	36.6	0.77
37	Insulin-like growth factor-binding protein 5 (-)	P24593	IGFBP5	1	3.7	0.76
38	Intelectin-1 / Omentin (-)	Q8WWA0	ITLN1	5	19.8	0.73
39	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial (+)	P50213	IDH3A	1	2.7	1.41
40	Kallistatin (+)	P29622	SERPINA4	17	44.7	1.44
41	Keratin, type I cytoskeletal 14 (-)	P02533	KRT14	4	15.3	0.34
42	Keratin, type I cytoskeletal 9 (-)	P35527	KRT9	12	26.3	0.34
43	Latent-transforming growth factor beta-binding protein 1 (-)	Q14766	LTBP1	9	7.8	0.73
44	Lipopolysaccharide-binding protein (+)	P18428	LBP	11	26.2	1.32
45	Lipoprotein lipase (-)	P06858	LPL	4	13.3	0.70
46	Mimecan (-)	P20774	OGN	6	13.8	0.80
47	Myosin-9 (+)	P35579	MYH9	13	8.7	1.47
48	Nidogen-1 (-)	P14543	NID1	11	10.1	0.69
49	Periostin isoform 8 precursor (+)	Q15063	POSTN	17	28.8	1.21
50	Plasma serine protease inhibitor (-)	P05154	SERPINA5	12	30.3	0.77
51	Plastin-2 (+)	P13796	LCP1	10	19.1	1.37
52	Platelet basic protein (-)	P02775	PPBP	7	45.3	0.77
53	Platelet factor 4 variant (-)	P10720	PF4V1	2	48.1	0.79
54	Profilin-1 (+)	P07737	PFN1	4	35.7	1.43
55	Protein disulfide-isomerase A3 (-)	Q5RDG4	PDIA3	4	8.1	0.67
56	Transforming growth factor beta-1 proprotein (-)	P01137	TGFB1	7	19.7	0.78
57	Tropomyosin alpha-4 chain (-)	P67936	TPM4	5	28.2	0.80
58	Tubulin alpha-1A chain (+)	P68362	TUBA1A	4	28.6	1.35
59	Vasodilator-stimulated phosphoprotein (+)	P50552	VASP	2	6.3	1.31

(+), protein increased in abundance; (−), protein decreased in abundancet. b) Fold change (log2 ratio), $p < 0.05$ versus the control.

KEGG Pathway Analysis

A total of 80 KEGG pathways were enriched among the DEPs. The top 10 significantly enriched pathways included: Cholesterol metabolism, Complement and coagulation cascades, Salmonella infection, Proteoglycans in cancer, Tight junction, Staphylococcus aureus infection, Pertussis, Riboflavin metabolism, ECM-receptor interaction, Regulation of actin cytoskeleton (Figure 2D).

COG Functional Classification

Clusters of Orthologous Groups (COG) classification revealed the functional distribution of DEPs: Class O (Posttranslational modification, protein turnover, chaperones): 98 proteins, Class R (General function prediction only): 73 proteins, Class Z (Cytoskeleton): 28 proteins, Class T (Signal transduction mechanisms): 21 proteins, Additional COG classes included smaller numbers of proteins across various functional categories (Figure 3).

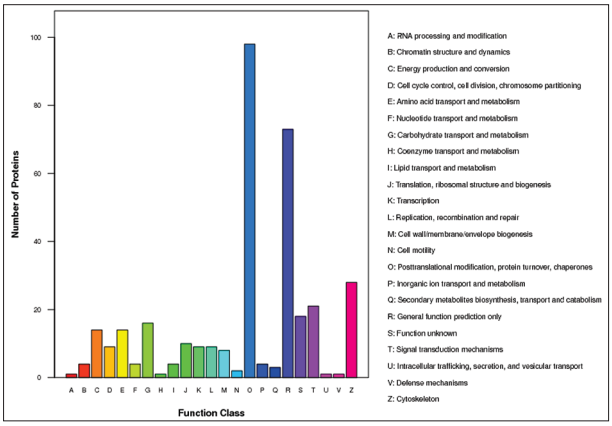

Figure 3. Histogram of the GOG Analysis. The X-axis displays the COG term; Y-axis displays the corresponding protein count illustrating the protein number of different functions.

DISCUSSION

In this study, iTRAQ-based LC-MS/MS proteomic profiling revealed 59 differentially expressed proteins (DEPs) in the serum of children with autism spectrum disorder (ASD) compared to typically developing children (TDC). These DEPs provide insights into the molecular mechanisms underlying ASD and suggest potential candidate biomarkers for diagnosis or subtyping. Among the identified DEPs, five apolipoproteins (APOC2, APOC4, APOF, APOL1, and LPA) were upregulated in the ASD group. Apolipoproteins (Apos) are essential for the transport of lipids, cholesterol, and fat-soluble vitamins in the bloodstream and play critical roles in maintaining lipid homeostasis [11]. Notably, APOC2, LPL, and LPA were enriched in the cholesterol metabolism pathway, which is consistent with previous reports indicating lipid metabolism disturbances in individuals with ASD. Dysregulated cholesterol and lipid pathways have been proposed to contribute to neurological development and function, and such disruptions may play a role in ASD pathogenesis. Furthermore, we observed elevated levels of complement-related proteins, including C4A, C1QA, and SERPINA5, suggesting immune system dysregulation in ASD. Both C4A and C1QA participate in the classical complement activation pathway, and their upregulation has also been documented in prior ASD studies [12]. This reinforces the hypothesis that innate immune responses, particularly those involving the complement system, are implicated in the pathophysiology of ASD. SERPINA5, a multifunctional serine protease inhibitor, regulates hemostasis, fibrinolysis, and extracellular matrix (ECM) remodeling, as well as embryonic development. While elevated SERPINA5 levels have been previously reported in ASD [13], our findings confirm its increased expression, supporting its potential involvement in disease-specific physiological processes. An interesting observation in our study was the downregulation of C-reactive protein (CRP). CRP is a key inflammatory marker that can bind to C1Q and trigger complement activation. Elevated CRP levels have been reported in children with ASD in several studies [14]. However, our results contradict these findings, highlighting a possible heterogeneity in inflammatory profiles among ASD patients. This discrepancy may stem from differences in sample size, methodology, disease subtypes, or environmental influences, and warrants further investigation. Overall, our data support previous findings while providing novel insights into lipid metabolism and immune dysregulation in ASD. These differentially expressed serum proteins—particularly apolipoproteins and complement components—could serve as valuable candidate biomarkers for ASD. However, further validation studies in larger, independent cohorts and functional studies are necessary to elucidate their precise roles in ASD pathophysiology. Another set of five proteins - VASP, TUBA1A, MYH9, FN1, and PFN1—were found to be involved in tight junction formation and the regulation of the actin cytoskeleton, key processes in neurodevelopment and synaptic plasticity. Vasodilator-stimulated phosphoprotein (VASP), a member of the Ena/VASP family, regulates actin filament elongation and dynamics, and is critical in cytoskeletal organization, platelet aggregation, and cellular motility [15]. Profilin-1 (PFN1), an actin-binding protein, facilitates actin polymerization by binding to G-actin and enhancing filament elongation. PFN1 works in concert with VASP, forming a Profilin-Actin-VASP complex that promotes dynamic cytoskeletal remodeling [16]. Notably, this study is the first to report elevated PFN1 levels in ASD patient serum, although in-

creased PFN1 expression has previously been observed in mouse models of Fragile X syndrome, a condition with overlapping symptoms and molecular pathways with ASD [17]. TUBA1A, a tubulin alpha-1A chain, is a key component of the microtubule cytoskeleton, highly expressed during brain development and involved in neuronal migration and axonal outgrowth. Mutations in TUBA1A have been associated with human brain malformations and neurodevelopmental syndromes [18]. Together, Profilins and Ena/VASP proteins, such as VASP, play essential roles in neuritogenesis by coordinating the cross-talk between actin filaments and microtubules, critical for the formation and maintenance of neuronal connections [19]. Fibronectin 1 (FN1) is an extracellular matrix glycoprotein involved in cell adhesion, migration, and tissue repair, and is known to interact with the complement system. Previous studies reported increased FN1 levels in ASD patients [12, 20]; however, in our study, FN1 was down-regulated, indicating possible heterogeneity in ECM remodeling or inflammation in ASD, or differences in cohort characteristics and analytical platforms. This discrepancy warrants further investigation. MYH9 (myosin-9), also known as non-muscle myosin heavy chain IIA, plays a critical role in cell migration, signal transduction, and cytokinesis. MYH9 was previously reported as down-regulated in the BTBR mouse model of autism, supporting its potential involvement in the cytoskeletal abnormalities seen in ASD [21]. Interestingly, MYH10, a closely related paralog and MYH9 antagonist, has been classified as a high-confidence ASD candidate gene (score 2) in the SFARI Gene database (https://gene.sfari.org/). Collectively, these findings emphasize the potential involvement of cytoskeletal regulatory proteins and ECM-interacting components in the molecular pathology of ASD, particularly in processes underlying neuronal structure, plasticity, and synaptic function. Moreover, two differentially expressed proteins—TGFB1 and LTBP1—were found to be involved in the TGF-β signaling pathway, a pathway known to regulate development, immune modulation, and neuronal plasticity. Transforming growth factor beta-1 (TGFB1) is a multifunctional cytokine implicated in various neurodevelopmental and neurodegenerative conditions. In agreement with previous studies, we observed decreased levels of TGFB1 in ASD patients, which has been correlated with reduced adaptive behavior and more severe behavioral symptoms [22]. Latent-transforming growth factor beta-binding protein 1 (LTBP1) functions as a structural component of the extracellular matrix (ECM) and plays a role in regulating TGFB1 bioavailability. Accumulating biological, genetic, and clinical evidence suggests that heparan sulfate (HS) metabolism abnormalities may play a key role in the pathogenesis of ASD and other neurodevelopmental disorders. In our study, heparan sulfate proteoglycan 2 (HSPG2), a major component of the basement membrane, was significantly decreased in ASD individuals. HSPG2 contributes to basal lamina integrity, and acts as a reservoir for growth factors and cytokines, influencing key signaling pathways [23]. Heparanase (HPSE), an enzyme that regulates HS degradation and remodeling, was also found to be reduced in ASD patients, suggesting dysregulation in ECM turnover and signaling homeostasis. In addition, Nidogen-1 (NID1), another basement membrane protein known to bind HSPG2 and stabilize the ECM, was significantly down-regulated in ASD serum samples. Together, these three ECM-associated proteins—HSPG2, HPSE, and NID1—participate in cell-ECM interactions, growth factor signaling, and maintenance of ECM structure. Their concurrent down-regulation suggests a disruption in ECM integrity and signaling, which may contribute to abnormal neurodevelopment in ASD. Proteomic biomarkers not only offer promise for early and objective ASD diagnosis, but also hold potential for monitoring treatment responses and furthering our understanding of the molecular mechanisms underlying ASD. In summary, the iTRAQ-based proteomic analysis conducted in this study identified multiple differentially expressed proteins involved in pathways such as the complement cascade, tight junction and actin cytoskeleton regulation, TGF-β signaling, ECM interactions, and cholesterol metabolism. These findings demonstrate the utility of quantitative proteomics in ASD research and provide a valuable foundation for future biomarker validation and mechanistic studies, especially using accessible biospecimens such as peripheral blood.

ACKNOWLEDGMENTS

The authors are most grateful to the families of the participants for their collaboration. Additionally, we would like to acknowledge Prof. V. Stoyanova, Asst. Prof. H. Ivanov and Prof. I. Ivanov (Department of Pediatrics and Medical Genetics Medical University Plovdiv, Bulgaria) for their technical insights and support of this work.

Funding

This work was supported by the European Unions Horizon 2020 research and innovation program under grant agreement No 739582 (Project PlantaSYST)

Declaration of interests

The authors have no competing personal or financial interests. The authors alone are responsible for the content and writing of this manuscript.

Conflicts of interest

The authors declare no conflicts of interest

REFERENCES

1. Masi A., M.M. DeMayo, N. Glozier, A.J. Guastella. An Overview of Autism Spectrum Disorder, Heterogeneity and Treatment Options. Neuroscience bulletin 2017; 33(2), 183–193.
2. Maenner M.J., K.A. Shaw, J. Baio, A. Washington, M. Patrick, et al. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years — Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2016. MMWR Surveill Summ, 2020; 69(4):1–12.
3. Tordjman S., E. Somogyi, N. Coulon, S. Kermarrec, D. Cohen, et al. Gene × environment interactions in autism spectrum disorders: Role of epigenetic mechanisms. Front Psychiatry. 2014; 4:5:53.
4. Kaur K., V. Chauhan, F. Gu, A. Chauhan Bisphenol A induces oxidative stress and mitochondrial dysfunction in lymphoblasts from children with autism and unaffected siblings. Free Radic Biol Med. 2014; 76:25-33.
5. Fernell E., M.A. Eriksson, C. Gillberg. Early diagnosis of autism and impact on prognosis: A narrative review. Clin Epidemiol. 2013; 5:33-43.
6. Brett D., F. Warnell, H. McConachie, J.R. Parr. Factors Affecting Age at ASD Diagnosis in UK: No Evidence that Diagnosis Age has Decreased Between 2004 and 2014. J Autism Dev Disord. 2016; 46(6):1974-1984.
7. Gurrieri F. Working up autism: The practical role of medical genetics. Am J Med Genet C Semin Med Genet. 2012; 160C (2):104-110.
8. Skafidas E., R. Testa, D. Zantomio, G. Chana, I.P. Everall, et al. Predicting the diagnosis of autism spectrum disorder using gene pathway analysis. Mol Psychiatry. 2014; 19(4):504-510.
9. An D., X. Wei, H. Li, H. Gu, T. Huang, et al. (2015) Identification of PCSK9 as a novel serum biomarker for the prenatal diagnosis of neural tube defects using iTRAQ quantitative proteomics. Sci Rep. 2015; 5:17559.
10. Kuleshov M.V., M.R. Jones, A.D. Rouillard, N.F. Fernandez, Q. Duan, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 2016; 44(W1):W90-W97.
11. Woods A.G., I. Sokolowska, R. Taurines, M. Gerlach, E. Dudley, et al. Potential biomarkers in psychiatry: Focus on the cholesterol system. J Cell Mol Med. 2012; 16(6):1184–95.
12. Corbett B.A., A.B. Kantor, H. Schulman, W.L. Walker, L. Lit, et al. A proteomic study of serum from children with autism showing differential expression of apolipoproteins and complement proteins. Mol Psychiatry 2007; 12(3):292-306.
13. Yang J., Y. Chen, X. Xiong, X. Zhou, L. Han, et al. Peptidome Analysis Reveals Novel Serum Biomarkers for Children with Autism Spectrum Disorder in China. Proteomics - Clin Appl. 2018; 12(5):1–30.
14. Nadeem R., T. Hussain, H. Sajid. C reactive protein elevation among children or among mothers‘ of children with autism during pregnancy, a review and meta-analysis. BMC Psychiatry 2020; 20(1):251.
15. Kwiatkowski A.V., F.B. Gertler, J.J. Loureiro Function and regulation of Ena/VASP proteins. Trends Cell Biol. 2003; 13(7):386-392.
16. Basu S., R. Lamprech. The role of actin cytoskeleton in dendritic spines in the maintenance of long-term memory. Front Mol Neurosci, 2018; 11:143.
17. Michaelsen-Preusse K., S. Zessin, G. Grigoryan, F. Scharkowski, J. Feuge, et al. Neuronal profilins in health and disease: Relevance for spine plasticity and Fragile X syndrome. Proc Natl Acad Sci USA 2016; 113(12):3365-3370.
18. Gardner J.F., T.D. Cushio, G. Niotakis, H.E. Olson, P.E. Grant, et al. Clinical and functional characterization of the recurrent TUBA1A p.(ARG2HIS) mutation. Brain Sci. 2018; 8(8):145.
19. Pinto-Costa R., S.C. Sousa, S.C. Leite, J. Nogueira-Rodrigues, T.F. da Silva, et al. Profilin 1 delivery tunes cytoskeletal dynamics toward CNS axon regeneration. J Clin Invest. 2020; 130(4):2024–40.
20. Shen L., K. Zhang, C. Feng, Y. Chen, S. Li, et al. (2018) iTRAQ-Based Proteomic Analysis Reveals Protein Profile in Plasma from Children with Autism. Proteomics - Clin Appl. 2018; 12(3):1–45.
21. Wei H., Y. Ma, J. Liu, C. Ding, F. Hu, et al. Proteomic analysis of cortical brain tissue from the BTBR mouse model of autism: Evidence for changes in STOP and myelin-related proteins. Neuroscience 2016; 312:26–34.
22. El Gohary T.M., N.A. El Aziz, M. Darweesh, E.S. Sadaa Plasma level of transforming growth factor β 1 in children with autism spectrum disorder. Egypt J Ear, Nose, Throat Allied Sci, 2015; 16(1):69–73.
23. Condomitti G., J. De Wit Heparan sulfate proteoglycans as emerging players in synaptic specificity. Front Mol Neurosci 2018; 11:1–14.