

Biomarkers in Autism Spectrum Disorder: From Traditional Constraints to AI-Driven Horizons in Precision Medicine

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Received: 2025-08-23

Accepted: 2025-11-14

Published Online: 2025-11-27

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ABSTRACT

Objective: The aim of this review is to comprehensively evaluate the potential of biomarkers in the diagnosis, prognosis, and individualized treatment of Autism Spectrum Disorder (ASD), considering its complex nature and current diagnostic limitations.

Methods: The article provides an updated literature review focusing on blood- and urine-based biomarkers (oxidative stress, inflammation, neurotransmitters, microbiome), neuroimaging, genetic, and physiological markers. It also examines key challenges, ethical considerations, and promising future directions such as artificial intelligence (AI)-assisted multi-omics data integration.

Results: Biomarkers measured in blood and urine (e.g., isoprostanes, 8-OHdG, and inflammatory cytokines) highlight the role of oxidative stress and chronic inflammation in ASD pathophysiology. Neuroimaging and genetic markers show promise for early risk identification and biological subtyping. However, most current studies suffer from small sample sizes, replication issues, and lack of standardization, limiting their clinical applicability.

Conclusion: Biomarker-based approaches hold promise for making ASD diagnosis more objective and for facilitating earlier intervention by reducing diagnostic delays. In the future, AI-driven multi-omics integration may provide deeper insights into ASD heterogeneity and support the development of personalized treatment strategies. Managing ethical concerns such as privacy and discrimination through a neurodiversity-oriented perspective will be crucial in this process.

Keywords: autism spectrum disorder, biomarkers, oxidative stress, inflammation, artificial intelligence

INTRODUCTION

Autism Spectrum Disorder (ASD) is a multifaceted neurodevelopmental condition characterized by deficits in social communication and interaction, accompanied by restricted interests and repetitive behaviors. According to 2020 data from the U.S. Centers for Disease Control and Prevention, ASD affects approximately one in every 36 children [1]. Despite extensive research, no definitive cure has been identified, nor have reliable biomarkers for diagnosis and follow-up been established [2].

The socioeconomic burden of ASD is substantial. A review synthesizing data from around 50 studies estimated that the lifetime cost for a single autistic individual (aged 3–66 years) due to productivity loss alone amounts to approximately 971,072 USD [3-4]. When diagnostic procedures, inpatient and outpatient care, special education, pharmacological interventions, and parental loss of productivity are considered, overall costs rise significantly. Consequently, worldwide efforts to investigate ASD from multiple perspectives continue to expand.

The literature consistently emphasizes the interplay of genetic and environmental factors in ASD pathogenesis. More recently, growing evidence has pointed toward the contribution of biological pathways such as inflammation and oxidative stress. Meta-analyses have identified these processes as potential biomarkers, highlighting their role in ASD pathophysiology [5-6].

Main Points

- ASD diagnosis still depends on subjective behavioral observations, often causing delays with adverse developmental and socioeconomic consequences.
- Biomarkers (e.g., oxidative stress, cytokines, neurotransmitters) may provide insights into ASD biology and support more objective diagnostics.
- Given ASD's heterogeneity, multi-omics approaches integrating genetic, biochemical, and neuroimaging data are essential for personalized treatment.
- AI and machine learning enhance diagnostic accuracy and help identify homogeneous ASD subgroups.
- Ethical concerns such as privacy and genetic discrimination must be addressed within a neurodiversity framework.

The aim of this review is to critically evaluate the current state of ASD biomarker research, highlight promising candidates, and assess the potential of AI-driven approaches for clinical translation.

MATERIALS AND METHODS

This review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A comprehensive literature search was performed across major electronic databases, including PubMed, Scopus, Web of Science, and Google Scholar. The search strategy combined Medical Subject Headings (MeSH) and free-text terms related to *ASD* and *biomarkers*, in alignment with the thematic sections of this review. Keywords included, but were not limited to: “*autism spectrum disorder*,” “*biomarker*,” “*genetic markers*,” “*epigenetics*,” “*metabolomics*,” “*neuroimaging*,” “*EEG*,” “*microstates*,” “*gut microbiota*,” and “*machine learning*.” Full electronic search strings for PubMed, Scopus, Web of Science, and Google Scholar are provided in the Supplementary Appendix.”

To ensure contemporary relevance, the search was restricted to studies published in the last five years (2020–2025), with a particular emphasis on systematic reviews, meta-analyses, and original research articles that provided data on diagnostic, prognostic, or therapeutic biomarkers in ASD. Only articles published in English or Turkish and indexed in peer-reviewed journals were included. Conference abstracts, editorials, and case reports were excluded. Titles and abstracts were screened independently by two reviewers, and potentially relevant articles were retrieved for full-text assessment. Studies were included if they directly evaluated biomarker-related outcomes (molecular, neuroimaging, physiological, or AI-based). Exclusion criteria included sample size <10, non-human studies, or lack of biomarker-related endpoints. Discrepancies were resolved through consensus. Data extracted included study design, sample size, biomarker type, analytical methods, and major findings. The synthesis was organized according to biomarker categories (e.g., blood and urine biomarkers, neuroimaging, electrophysiology, multi-omic approaches, and artificial intelligence-based integration). A conceptual roadmap outlining the progression from biomarker discovery to clinical implementation is presented in Figure 1, which depicts the sequential stages required for validation, integration, and translational application.

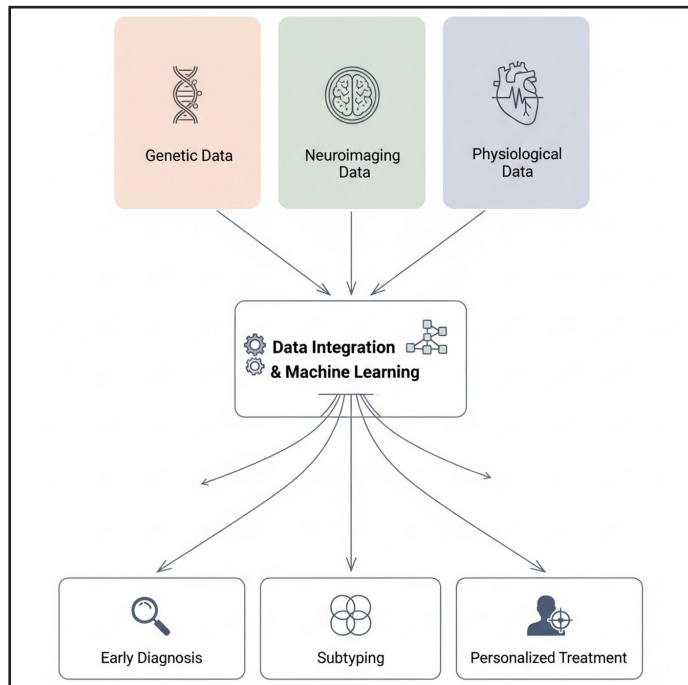


Figure 1. From Biomarker Discovery to Clinical Application: A Roadmap for ASD Research

RESULTS

The Concept of Biomarkers and Their Potential Role in ASD

Biomarkers are measurable indicators used to identify, monitor, or predict biological processes or diseases. In the context of ASD, biomarkers hold promise for identifying at-risk individuals, improving diagnostic accuracy compared to current subjective tools, monitoring treatment response, and stratifying patients into more homogeneous subgroups. Given the high heterogeneity of ASD, multiple classes of biomarkers—including oxidative stress, immunological, genetic, neuroimaging, and physiological markers—are being investigated to delineate the biological underpinnings of the disorder. A summary of major

biomarker domains investigated in ASD, including oxidative stress, inflammation, genetic, metabolomic, neuroimaging, and physiological markers, is presented in Table 1.

Oxidative Stress and DNA Damage Biomarkers in ASD

A significant relationship has been demonstrated between increased oxidative stress and mitochondrial dysfunction in ASD, which exerts profound effects on gastrointestinal and mitochondrial health [7]. These biochemical abnormalities disrupt synaptic plasticity and trigger chronic neuroinflammation, ultimately impairing neurodevelopmental processes and contributing to ASD pathology. Growing evidence supports that oxidative stress biomarkers are elevated in individuals with ASD compared to typically developing controls. Such markers hold promise for early diagnosis, with glutathione and its derivatives, as well as isoprostanes, emerging as particularly relevant candidates [8].

F2-isoprostanes are generated through non-enzymatic free radical-mediated oxidation of arachidonic acid within phospholipids. Among them, 8-isoprostane and its metabolites are chemically stable and unaffected by dietary lipid intake. 8-isoprostane is regarded as one of the most sensitive indicators of lipid peroxidation and redox system dysfunction [9]. Studies have shown higher levels of 8-isoprostane in children with ASD compared to controls [10-11]. Furthermore, prenatal oxidative stress, as reflected by elevated maternal 8-iso-PGF2 α , has been modestly associated with reduced cognitive scores and increased autism-related traits in offspring, highlighting the need for larger cohort studies to confirm oxidative stress as a mechanistic pathway in ASD.

Table 1. Categories of Biomarkers in Autism Spectrum Disorder and Their Clinical Potential

Biomarker Category	Examples	Clinical Potential
Oxidative Stress & DNA Damage	8-isoprostane, 8-OHdG, Glutathione	Early diagnosis, risk stratification
Immunological & Inflammatory	IL-17C, CCL19, Autoantibodies	Risk assessment, personalized therapy
Neurotransmitters & Hormones	Serotonin, Dopamine, Oxytocin, Melatonin	Biomarkers for social/behavioral symptoms
Proteomic & Metabolomic	ApoE, VCL, Hippuric acid, Amino acids	Molecular subtyping, precision medicine
Gut Microbiome	Firmicutes/Bacteroidetes ratio, Bifidobacterium	Novel therapeutic target (e.g., probiotics)
Neuroimaging & EEG	MRI volumetry, fMRI activation, EEG microstates	Early risk detection, subtype classification
Genetic & Genomic	SHANK3, NLGN3, CNVs, miRNAs	Personalized medicine, genetic counseling
Physiological & Behavioral	N170 ERP, HRV, Eye-tracking, Retinal markers	Non-invasive screening, treatment monitoring

8-hydroxy-2-deoxyguanosine (8-OHdG) is another sensitive biomarker of oxidative DNA damage. Findings have been inconsistent: while Ming et al. [12] reported higher urinary levels without statistical significance, other studies found significantly elevated 8-OHdG in children with ASD [13-14]. These findings suggest that reduced antioxidant and methylation capacity may be characteristic of ASD. However, contradictory results across studies, particularly regarding urinary 8-OHdG, highlight the need for replication and standardization. Recent studies also emphasize dynamic thiol/disulfide homeostasis as a novel oxidative stress marker, warranting inclusion in future biomarker panels.

Immunological and Inflammatory Markers

Inflammatory responses, comprising acute and chronic forms, are increasingly recognized as playing a role in ASD [15-17]. Maternal immune activation during early pregnancy has been linked to ASD risk in offspring, with animal models showing that even a single exposure to an inflammatory agent can induce ASD-like behaviors. For instance, valproic acid administration has been shown to reliably trigger ASD-like neurobehavioral phenotypes through inflammatory and oxidative pathways [18]. Maternal autoantibodies targeting fetal brain antigens, as well as folate receptor α autoantibodies, have been identified in association with ASD [19]. Proteomic analyses further revealed altered expression of 18 inflammatory proteins in ASD children, including elevated plasma levels of interleukin-17C (IL-17C), chemokine ligand-19 (CCL19), and CCL20, with diagnostic AUC values exceeding 0.7, underscoring their potential as inflammatory biomarkers [20].

Together, these findings support a strong role for inflammatory processes in ASD and highlight the potential of immune-related biomarkers for risk assessment and diagnosis.

Other Potential Biochemical Markers

Neurotransmitters and Hormonal Markers

ASD has been associated with imbalances in several neurotransmitter systems, including disrupted GABA–glutamate balance, altered serotonin and dopamine pathways, and deficits in neuropeptides such as oxytocin and arginine-vasopressin, all of which are implicated in social and repetitive behaviors [21-23]. Endocrine alterations, including reduced thyroid hormone levels [24], as well as abnormalities in melatonin, serotonin, and orexin, contribute to the sleep disturbances frequently observed

in ASD [25]. Vitamin D deficiency has also been suggested to increase early ASD risk [26]. Importantly, combined analysis of serotonin, N-acetylserotonin, and melatonin has demonstrated high diagnostic performance, distinguishing ASD cases with 80% sensitivity and 85% specificity [27].

Metabolomic and Proteomic Biomarkers

Proteomic and metabolomic approaches in ASD represent an increasingly important area of research, although their integration into clinical practice remains limited. These methods enable the identification of candidate biomarkers in both central nervous system and peripheral biological samples, thereby providing insights into underlying pathophysiological mechanisms. A comprehensive meta-analysis has demonstrated that proteins such as flotillin-2 (FLOT2), apolipoprotein E (ApoE), EH domain-containing protein 3 (EHD3), and vinculin (VCL), along with metabolites including hippuric acid and salicylic acid, are significantly associated with ASD across brain tissue, blood, and urine samples [28].

The analysis of proteins and metabolites in biological fluids such as blood, urine, and saliva may yield both diagnostic and etiological insights. One study using untargeted serum analysis implicated complement pathways, cholesterol metabolism, and apoptosis-related proteins in ASD pathology. Similarly, another study reported that a five-protein panel (C3, C5, GC, ITGA2B, and TLN1) could discriminate ASD individuals from controls with a remarkably high area under the curve (AUC) value of 0.982. Urine, unlike blood, is not subject to homeostatic regulation and therefore may better reflect dynamic biological changes, making it a promising matrix for biomarker discovery. A proteomic study demonstrated differential expression of 118 proteins in the urine of children with ASD compared to healthy controls, many of which were linked to neurodevelopmental pathways such as axonal guidance signaling [29].

Plasma metabolomic profiling has also revealed marked differences between ASD children and neurotypical peers. Specific metabolites, including FAHFA (18:1(9Z)/9-O-18:0), DL-2-hydroxystearic acid, and derivatives of docosapentaenoic acid, have been identified as strong discriminative biomarker candidates [30]. Likewise, SWATH-MS (Sequential Windowed Acquisition of All Theoretical Fragment Ion Mass Spectra)–based proteomic studies have reported distinct protein signatures associated with immune response, complement activation, and

coagulation pathways, highlighting proteins such as PPBP, APCS, FGG, and PF4V1 as potential diagnostic indicators for ASD screening [31].

Metabolomic investigations further illuminate the biological underpinnings of ASD. Systematic reviews underscore consistent disruptions in amino acid metabolism, glutathione cycling, and energy pathways [32]. Urinary metabolomic analyses have identified links between ASD and metabolites such as prostaglandin E2, lysine, threonine, and phenylalanine, as well as alterations in phosphatidylinositol and inositol phosphate metabolism [33]. Blood-based analyses have further revealed abnormalities in amino acid metabolism, including alterations in carnosine, glutamic acid, tryptophan, and histidine [34]. Additionally, serum-based metabolomic studies have associated bile acids, dicarboxylic acids, and omega-6 polyunsaturated fatty acids with cognitive and social impairments.

Taken together, these findings suggest that proteomic and metabolomic profiling holds substantial promise for early diagnosis and personalized therapeutic strategies in ASD. Nonetheless, heterogeneity of existing data, small sample sizes, and methodological discrepancies underscore the urgent need for larger, multi-center, and standardized studies before these biomarkers can be translated into clinical use.

The Microbiome

Significant alterations in the gut microbiota (GM) have been identified in individuals with ASD, and such dysbiosis is thought to contribute to both gastrointestinal and behavioral manifestations. Studies in children have reported imbalances in bacterial phyla such as Firmicutes, Bacteroidetes, and Proteobacteria, along with reduced levels of beneficial genera including *Bifidobacterium* and *Prevotella* [35]. By contrast, increases in *Fusobacteriota* and *Veillonella* have been associated with social and behavioral impairments [36]. Similar findings have been observed in adults with ASD, where altered microbial profiles and reduced microbial diversity were inversely correlated with the severity of autistic traits [37].

Meta-analytic evidence suggests that while the overall microbial composition of individuals with ASD resembles that of their healthy siblings, certain taxa—particularly Bacteroidetes, Firmicutes, and Fusobacteria—exhibit inconsistent differences [38]. These discrepancies may be influenced by environmental exposures and demographic factors such as age and sex.

Importantly, the gut microbiome is thought to influence brain function through the production of short-chain fatty acids, modulation of neurotransmitter pathways, and regulation of immune responses, thereby contributing to neuroinflammation and ASD pathophysiology [39-40].

In conclusion, gut microbiota dysregulation in ASD is emerging as both a promising biomarker source and a potential therapeutic target. While interventions such as probiotics, dietary modifications, and fecal microbiota transplantation show promise, further research is essential to establish their long-term efficacy and safety [41].

Neuroimaging and Neurophysiological Biomarkers in ASD

Techniques such as magnetic resonance imaging (MRI), functional MRI (fMRI), and electroencephalography (EEG) have provided valuable insights into the structural and functional abnormalities underlying ASD.

Structural MRI (sMRI) studies have consistently demonstrated alterations in young children with ASD, including increased total brain volume, enlarged frontal and temporal lobes, greater cortical thickness, and reduced corpus callosum volume, particularly in those under the age of six [29]. These early brain changes coincide with the developmental window when autistic behaviors first emerge. Remarkably, a deep learning model trained on MRI scans from 6–12-month-old infants was able to predict ASD diagnosis at 24 months with a positive predictive value of 81% and a sensitivity of 88% [42].

Meta-analyses of task-based fMRI studies further highlight atypical activation patterns in social cognition. For instance, an activation likelihood estimation (ALE) meta-analysis of facial emotion processing showed that although the fusiform face area was activated in both ASD and typically developing individuals, amygdala involvement was significantly stronger in the latter group [43]. Multimodal MRI approaches have revealed additional heterogeneity, such as variations in gray matter volume and low-frequency fluctuations, suggesting the presence of neurobiological subtypes [44-45]. Importantly, ensemble machine learning (ML) models applied to MRI data have shown high discriminative accuracy in classifying ASD, underscoring their diagnostic potential [46].

EEG provides complementary insights into brain function by capturing electrical activity in a non-invasive manner,

making it particularly suitable for infants and young children. Between 2014 and 2024, studies reported epileptiform or non-epileptiform abnormalities in 23–80% of ASD cases. However, their association with core ASD symptoms such as cognitive impairment, language delay, or behavioral difficulties remains inconsistent. Current evidence does not support routine EEG screening for all ASD cases; instead, its use is most justified in cases with suspected epilepsy [47].

More refined analyses of EEG microstates—stable topographical patterns lasting 60–120 ms—have identified differences in ASD, particularly prolonged duration and coverage of microstate B and reduced occurrence of microstate C, though age-related heterogeneity complicates interpretations [48]. Large-scale resting-state EEG studies integrating multiple datasets ($n = 776$) suggest that many apparent group differences may be driven by small-sample bias, as bootstrap analyses revealed limited reproducibility [49].

Together, MRI and EEG biomarkers provide crucial insights into the neurobiological underpinnings of ASD, though challenges in reproducibility, heterogeneity, and clinical translation remain.

Genetic and Genomic Biomarkers

ASD is a polygenic and highly heterogeneous disorder, making it rare for specific genetic variants to recur at high frequency across individuals. Nevertheless, large-scale genomic studies have implicated hundreds of risk genes related to brain development, synaptic function, and neuronal signaling. Recent reviews emphasize the interplay of genetic susceptibility and environmental exposures as critical drivers of ASD risk, highlighting the importance of developing reliable early genetic biomarkers [50]. RNA sequencing studies have identified genes such as hydroxycarboxylic acid receptor 3 (HCAR3), linking ASD to immune and signaling pathways [51]. MicroRNAs (miRNAs) have emerged as particularly promising due to their regulatory role in gene expression and their stability when carried in exosomes, suggesting both diagnostic and therapeutic applications [52].

Cutting-edge techniques such as optical genome mapping have uncovered novel structural variants undetectable by conventional methods, thereby expanding our understanding of ASD's genetic architecture [53]. Moreover, proteogenomic approaches have identified inflammatory pathways, with cytokines like IL-17C, CCL19, and CCL20 showing potential as diagnostic candidates

[20]. Well-known synaptic genes such as SHANK3 and NLGN3 remain central to ASD research, while copy number variations (CNVs) and single nucleotide polymorphisms (SNPs) hold promise for identifying at-risk individuals. However, challenges such as population stratification, ethnic variability, and ethical concerns (e.g., prenatal screening and potential discrimination) necessitate cautious interpretation and application.

Overall, genetic and genomic biomarkers are indispensable to unraveling ASD's heterogeneity and lay the foundation for personalized medicine approaches.

Physiological and Behavioral Biomarkers

Beyond molecular and genetic findings, physiological and behavioral biomarkers offer important tools for early diagnosis and stratification in ASD. Methods such as magnetoencephalography (MEG), electrodermal activity (EDA), and eye-tracking are increasingly employed to assess social communication deficits. Among electrophysiological measures, the EEG/ERP N170 component—a marker of face processing—has emerged as one of the most widely studied and holds potential as a predictor of treatment response [54]. Retinal biomarkers are also gaining interest, as the retina provides a direct window into central nervous system function. Altered levels of A Disintegrin and Metalloproteinase 10 (ADAM10) and ciliary neurotrophic factor (CNTF) have been associated with ASD severity, linking visual and cognitive processes [55]. Alterations in retinal thickness, as well as associations with ADAM10 and CNTF levels, have been reported, further supporting the retina as a window to CNS dysfunction in ASD.

Physiological signals also reveal novel biomarker avenues. Electrodermal responses to olfactory stimuli indicate differences in emotional regulation in ASD [56], while heart rate variability (HRV) serves as a non-invasive index of autonomic function. Meta-analyses have shown that individuals with ASD exhibit reduced HRV reactivity under social stress, making it a promising biomarker for both diagnosis and monitoring intervention outcomes [57-58].

Salivary biomarkers provide another promising, minimally invasive modality, encompassing cytokines, hormones, oxidative stress markers, and miRNAs [59]. In addition, maternal and environmental biomarker studies highlight the role of prenatal and perinatal exposures in ASD risk, paving the way for early prediction and tailored preventive strategies [60].

Eye-tracking technologies, capable of detecting atypical visual attention patterns, may serve as cost-effective and scalable screening tools for infants. Similarly, electroretinogram (ERG) studies have reported delayed light response in ASD individuals, potentially reflecting broader autonomic and vagal dysfunction. Collectively, physiological and behavioral biomarkers expand the biomarker landscape beyond molecular assays, providing dynamic, functional, and non-invasive tools for both research and clinical translation.

DISCUSSION

Autism research faces significant challenges in integrating biomarkers into clinical practice. The pronounced genotypic and phenotypic heterogeneity of ASD makes the search for a single, universal biomarker impractical. This heterogeneity implies that distinct biological pathways may be implicated in different individuals, resulting in inconsistent or non-replicable findings—often exacerbated by small sample sizes and methodological variability [61]. Reported high classification accuracies may also be artificially inflated due to circular analyses and underpowered studies, thereby limiting generalizability. Furthermore, current clinical assessment tools are inherently subjective and may fail to capture the wide spectrum of ASD symptoms comprehensively [62]. Although systematic in approach, no meta-analysis or pooled effect size estimates were performed. Therefore, distinctions between replicated findings and preliminary results are highlighted where possible.

The frequent presence of comorbidities such as anxiety and depression complicates diagnosis and challenges the ability of biomarkers to differentiate ASD-specific pathophysiology. Beyond technical and methodological limitations, ethical concerns surrounding the use of genetic information—including privacy risks, fears of genetic discrimination, and eugenic implications of prenatal testing—add further complexity. In this regard, the neurodiversity perspective advocates reframing ASD not as a “disorder” but as a natural variation in human evolution, urging researchers to focus on reducing stigma and adapting environments to better accommodate neurodivergent individuals.

To overcome these challenges, future studies must embrace multimodal data integration, combining genetic, biochemical, metabolic, and neuroimaging datasets. Such integrative approaches hold the potential to map the complex biological networks underlying ASD more accurately, advancing the

concept of a comprehensive “biological signature.” AI and ML models are particularly well suited to extract meaningful patterns from these high-dimensional datasets, enhancing diagnostic accuracy and stratification. However, issues of interpretability and transparency remain central; the adoption of explainable AI frameworks is essential for clinical trust and the generation of biologically meaningful hypotheses. Finally, larger, multicenter, and ethnically diverse studies are needed to ensure that biomarker applications are equitable and generalizable across populations.

Artificial Intelligence and Multi-Omics Integration: A New Era in ASD Biomarker Discovery

The extraordinary heterogeneity of ASD has limited efforts to identify a universal biomarker anchored in a single biological system. Instead, recent research aims to define a holistic “biological signature” encompassing genetic susceptibility, molecular alterations, neural network functionality, and behavioral phenotypes. In this context, AI—particularly ML algorithms—offers transformative potential by uncovering complex, non-linear patterns in multidimensional datasets that traditional statistical approaches cannot resolve.

Diagnosis and Classification

ML models trained on structural and functional MRI, EEG, blood metabolomic profiles, and genetic data can distinguish individuals with ASD from healthy controls with high accuracy [42, 46]. Deep learning methods, especially when applied to raw neuroimaging or EEG time-series, can capture subtle features imperceptible to human observers, facilitating earlier diagnosis. Notably, Shaban [63] reported >99% classification accuracy; however, such results require cautious interpretation due to risks of overfitting and limited external validation. In EEG-based classification, support vector machines (SVMs) with radial basis kernels and regularized logistic regression remain competitive on modest sample sizes, particularly when combined with artifact-cleaned band-power features and microstate metrics. For example, ensemble ML models integrating MRI and genetic data [46], as well as EEG-based CNN approaches [47], have demonstrated robust classification performance. Similarly, prognostic models [64] predict treatment response, underscoring clinical applicability. By contrast, convolutional neural networks (CNNs)—including 2D/3D variants—have shown advantages for sMRI/fMRI by leveraging spatial and spatiotemporal structure. Feature selection (e.g., RFE/LASSO), nested cross-validation, and external validation on multi-site

Table 2. Summary of AI Models Applied in ASD Biomarker Research

Study / Reference	AI Model	Input Data	Performance / Notes
Shaban et al. (2025) [63]	CNN (Deep Learning)	fMRI connectivity matrices	>99% accuracy (caution: possible overfitting)
Zhang et al. (2025) [53]	Support Vector Machine	EEG features (band power, microstates)	85–90% accuracy, validated on external dataset
Liu et al. (2024) [30]	Random Forest	Metabolomic + proteomic markers	AUC 0.87, identified key metabolites
Zhang-James et al. (2021) [70]	Ensemble (XGBoost + Logistic Regression)	Genetic variants + MRI data	Improved classification vs. single-modality models
Li et al. (2021) [71]	Graph Neural Network	Structural MRI + functional connectivity graphs	Detected ASD subgroups, good interpretability

Note: Performance metrics are reported as described in the respective studies. High reported accuracy (e.g., >99%) should be interpreted with caution due to possible overfitting and limited external validation.

cohorts are essential to prevent optimistic bias and to ensure generalizability. Representative studies applying AI models to biomarker and neuroimaging data, along with their performance metrics, are summarized in Table 2.

Subtype Identification

One of AI's most valuable contributions lies in stratifying the heterogeneous ASD spectrum into biologically meaningful subgroups. Clustering algorithms integrating genetic, biochemical, and neuroimaging data have successfully delineated patient subgroups sharing common mechanisms, paving the way for personalized medicine. For example, anti-inflammatory therapies may be targeted toward subgroups with elevated inflammatory profiles, while individuals with synaptic gene mutations may benefit from precision pharmacological interventions [44–45]. On a molecular level, Mehmetbeyoglu et al. [65] emphasized the diagnostic relevance of miR-126-3p, whereas Nahas et al. [66] identified genes such as HOXB3, NR2F2, and MAPK8IP3 as well as high-risk SNPs, reinforcing the biological basis of ASD subtyping.

Beyond supervised models, unsupervised approaches (e.g., Gaussian mixture models, spectral clustering) and graph-based methods (e.g., graph neural networks applied to connectivity matrices) can reveal mechanistic subgroups by jointly modeling imaging and molecular features. Stability selection and consensus clustering should be reported to demonstrate the reproducibility of inferred subtypes.

Treatment Response Prediction

ML approaches are increasingly being applied to predict

individual responses to therapeutic interventions based on baseline biomarker profiles. This predictive capacity not only optimizes resource allocation but also enables prioritization of the most effective interventions [67]. Longitudinal evidence from Usta et al. [64] demonstrated that ML algorithms integrating early diagnostic variables, baseline symptom severity, and comorbidities could reliably predict long-term outcomes. For prognostic modeling, a longitudinal framework that mixes baseline biomarker profiles with repeated measures improves performance and clinical interpretability. Elastic-net or gradient-boosting models paired with SHAP-based explanations can prioritize candidate biomarkers while quantifying individual-level effects. Time-split validation (training on early enrollees, testing on later ones) reduces temporal leakage.

Multi-Omics Integration

The most transformative potential lies in multi-omics integration, wherein genomic, proteomic, metabolomic, microbiome, neuroimaging, and behavioral data are jointly analyzed. This approach allows modeling of cross-level interactions, such as how specific genetic variants influence gut microbiota composition, which in turn modulates metabolite levels and neural connectivity, ultimately shaping social behavior. For instance, [68] demonstrated that AI algorithms identified *Bifidobacterium* abundance as a microbiome biomarker associated with ASD, while [31] validated plasma proteins such as PPBP, APCS, FGG, and PF4V1 as promising proteomic biomarkers through ML-driven analyses. Multi-omics can be integrated via early fusion (feature-level concatenation after harmonization), intermediate fusion (representation learning per modality followed by joint embedding), or late fusion (model-level ensembling).

Batch-effect correction, confounder adjustment (age/sex/site), and missing-data handling (e.g., multiple imputation) are prerequisites for clinically reliable inferences.

Challenges and Ethical Considerations

Despite these advances, significant barriers persist. Data quality and cross-center standardization remain critical for reproducibility. The “black-box” nature of many AI models underscores the urgent need for explainable AI to ensure clinical trust and biological interpretability. Overfitting, especially in studies with limited sample sizes, continues to hinder generalizability. Finally, ethical concerns must be addressed, as AI systems risk perpetuating biases inherent in training datasets, potentially reinforcing health disparities across socioeconomic or ethnic groups [69].

Future Research Agenda

To accelerate clinical translation, we propose: (i) prospective, multi-center studies with harmonized acquisition protocols for imaging and biospecimens; (ii) pre-registered analysis plans with nested cross-validation and external test sites; (iii) multi-omics integration linking high-impact variants (e.g., synaptic genes such as SHANK3) to proteomic/metabolomic profiles using deep learning-based fusion; (iv) explainability (e.g., SHAP, saliency) to derive testable biological hypotheses; (v) fairness audits (subgroup performance by sex/age) and cost-effectiveness analyses to anticipate real-world impact.

CONCLUSION

ASD is a highly prevalent and complex neurodevelopmental condition that poses substantial diagnostic and management challenges. The subjectivity and delayed nature of current diagnostic approaches hinder early intervention opportunities, negatively impacting developmental outcomes. Peripheral biomarkers (e.g., oxidative stress markers, inflammatory cytokines, neurotransmitters), alongside neuroimaging, genetic, and physiological indicators, offer promising avenues for addressing these limitations and elucidating the biological underpinnings of ASD.

Nevertheless, current research remains in its infancy, constrained by replication challenges, methodological heterogeneity, and ethical considerations. Overcoming these barriers will require innovative strategies, including the integration of multi-omics datasets and the application of AI/ML methodologies

to disentangle ASD’s heterogeneity into biologically defined subgroups. Such approaches hold the potential to advance personalized medicine strategies, tailoring interventions to the unique biological profiles of individuals.

Future progress depends on large-scale, multicenter, and internationally collaborative studies that ensure the robustness, generalizability, and equity of biomarker applications. Importantly, research must remain grounded in ethical frameworks, incorporating safeguards around privacy, discrimination, and the neurodiversity perspective. Pragmatic trials that embed biomarker-driven decision support into routine pathways will be crucial to demonstrate improvements in time-to-diagnosis and patient-centered outcomes. By embracing these integrated and ethically conscious approaches, biomarker research can evolve into a transformative tool—reducing diagnostic delays, guiding precision therapies, and ultimately enhancing the quality of life and developmental potential of individuals with ASD.

Ethics and Conflict of Interest Statement

This article is a review study and does not involve human participants or animal subjects; therefore, ethics committee approval was not required. The authors declare that there is no conflict of interest regarding the publication of this manuscript.

Acknowledgments: The authors would like to thank Giresun University Faculty of Medicine for providing institutional support during the preparation of this review.

Funding: No funding required.

Conflict of Interest: The authors declare no conflict of interest.

Informed Consent: Not applicable, as this is a review article.

Ethical Approval: Ethics committee approval was not required for this review article.

Author Contributions: Conception: Berkan Şahin, Cansu Çobanoğlu Osmanlı; Design: Berkan Şahin; Supervision: Berkan Şahin; Literature Review: Berkan Şahin, Cansu Çobanoğlu Osmanlı; Writing: Berkan Şahin, Cansu Çobanoğlu Osmanlı; Critical Review: All authors.

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How to Cite;

Sahin B, Cobanoglu Osmanli C (2025) Biomarkers in Autism Spectrum Disorder: From Traditional Constraints to AI-Driven Horizons in Precision Medicine. *Eur J Ther.* 31(6):463-476. <https://doi.org/10.58600/eurjther2832>

Supplementary Appendix – Search Strategies**PubMed**

("Autism Spectrum Disorder"[MeSH] OR "ASD" OR "autism")

AND ("biomarkers"[MeSH] OR biomarker OR "oxidative stress" OR "inflammation" OR "genetic markers" OR "neurotransmitters" OR "metabolomics" OR "proteomics" OR "neuroimaging")

AND ("artificial intelligence"[MeSH] OR "machine learning" OR "deep learning" OR "neural network" OR "support vector machine" OR "AI" OR "ML")

Filters: Humans, English, 2015–2025

Scopus

(TITLE-ABS-KEY("autism spectrum disorder" OR ASD OR autism))

AND (TITLE-ABS-KEY(biomarker OR "oxidative stress" OR inflammation OR cytokine OR neurotransmitter OR metabolomic* OR proteomic OR neuroimaging))

AND (TITLE-ABS-KEY("artificial intelligence" OR "machine learning" OR "deep learning" OR "support vector machine" OR "convolutional neural network" OR AI OR ML))

AND (LIMIT-TO(LANGUAGE, "English"))

AND (PUBYEAR > 2014)

Web of Science (Core Collection)

TS=("autism spectrum disorder" OR ASD OR autism)

AND TS=(biomarker OR "oxidative stress" OR inflammation OR cytokine OR neurotransmitter OR metabolomic OR proteomic OR neuroimaging)

AND TS=("artificial intelligence" OR "machine learning" OR "deep learning" OR "support vector machine" OR "convolutional neural network" OR AI OR ML)

Refined by: English language, 2015–2025

Google Scholar

"autism spectrum disorder" AND (biomarker OR "oxidative stress" OR cytokine OR metabolomic OR proteomic OR neuroimaging) AND ("artificial intelligence" OR "machine learning" OR "deep learning" OR "support vector machine" OR "convolutional neural network")

Date range: 2015–2025