Journal of Experimental and Basic Medical Sciences 2021;2(1):80-85

Review

Autism Diagnosis and Biomarkers

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ABSTRACT

Autism spectrum disorder (ASD) is a group of complex neurodevelopmental disorders characterized by social interaction and communication deficiencies and repetitive, stereotypical behaviors. Since the etiology and pathogenesis of the disease have not been elucidated yet, specific treatment and reliable diagnostic biomarkers are not available. Early behavioral interventions have been shown to significantly improve symptoms in children with ASD. Given the rapidly increasing prevalence of ASD, there is an urgent need to identify relevant diagnostic biomarkers. Although specific diagnostic markers for ASD have not been identified, related research has advanced in different directions. This review summarizes recent findings on the use of several more biomarker candidate molecules, such as FABP4, an adipokine, Vascular Endothelial Growth Factor (VEGF), Brain-Derived Neurotrophic Factor (BDNF) molecules, as diagnostic biomarkers for ASD.

Keywords: Autism spectrum disorder, brain-derived neurotrophic factor, biomarkers, vascular endothelial growth factor.

Autism Spectrum Disorder (ASD) is a neurodevelopmental difference that is recognized early in life and is characterized by marked inadequacies in social and communicative areas, stereotypical behaviors and/or limited areas of interest. A group of cases with developmental problems, stereotyped behavioral patterns and loss of speech among the symptoms specific to ASD were defined as infancy dementia in 1930 by Heller.[1] In the literature, autism symptoms were first evaluated under the concept of psychosis, and it was defined as "Childhood Schizophrenia" by Potter in 1933 and diagnostic criteria were proposed.[2]

The term "autism" was first used in 1912 by Eugene Bleuler to describe patients with schizophrenia.[3]

It is derived from the ancient Greek words autos (own) and ismos (a suffix of state or movement). In a case series of 11 cases (8 boys and 3 girls) published in 1943, Leo Kanner mentioned the definition of "infantile autism", and he described the common characteristics of the cases as an inability to communicate with other people, echolalia, improper use of pronouns, repetitive behaviors, and persistence in sameness has described.[4]

Later, these children with similar characteristics were classified under different definitions such as "Autistic Psychopathy" by Hans Asperger in 1944 and "Atypical Child" by Degree in 1955.[5,6]

The term "Autism Spectrum Disorder" was first mentioned by Wing and Gould in 1979, and they emphasized that the symptoms they define as the affected areas (social reciprocity, communication and interests and/or repetitive behaviors) can occur with varying intensity and variations.[7]

The Diagnostic and Statistical Manual of Mental Disorders, first published in 1952 by the American Psychiatric Association, is a diagnostic measure for mental illnesses. According to DSM-V, the last edition of which was published in 2013, the Diagnostic Criteria of Autism Spectrum Disorders are as follows:[8]

Inability to respond to social-emotional responses (e.g., unusual social intimacy, difficulty in mutual conversation, inability to share interests,

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Cite this article as: Erdik S, Güneş B, Erbaş O. Autism Diagnosis and Biomarkers. JEB Med Sci 2021;2(1):80-85.

doi: 10.5606/jebms.2021.75641

Received : February 23, 2021
Accepted : March 16, 2021
Published online : May 31, 2021

feelings or affections, inability to respond to social interaction).

Inability in non-verbal communicative behaviors used for social interaction (e.g., inadequacy in verbal and non-verbal communication, unusual eye contact, inability to understand and use body language or gestures; obvious deficiencies in facial expression and body language).

Difficulty developing, maintaining and understanding relationships; such as inability to behave under different social environments, inability to play imaginary, not being able to make friends and not showing interest in friends.

Stereotypes or repetitive motor movements, use of objects or speech (Simple motor stereotypes, arranging or turning toys, echolalia, specific sentences.) Persistence in uniformity, strict adherence to routines, or ritualized verbal and non-verbal behavior (such as excessive anxiety in trivial changes, difficulty in transitions, strict thinking, greeting rituals, choosing the same way or the same food every day).

Unusually limited, fixed interests in terms of subject or intensity (abnormal excessive attachment to unusual objects, excessive repetitive or limited interests.) Sensory over-or under-sensitivity or excessive interest in the sensory dimension of stimuli (hypersensitivity to pain/heat, unexpected response to certain sounds or touches, excessive sniffing or over touching objects, being too visually preoccupied with light or movement).

THE BIOMARKER CONCEPT

The term "biomarker", which is the combination of the words "biological marker" and their meanings, refers to a broad subcategory of medical symptoms that can be accurately and reproducibly measured - that is, objective indicators of the patient's externally observed medical condition.[9]

A biomarker is a property that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.[10] Biomarkers are measurements used to make a clinical assessment, such as blood pressure or cholesterol level, and are used to monitor and predict health status in individuals or populations so that appropriate therapeutic intervention can be planned.[9,10]

Everything from pulse and blood pressure to essential chemicals to more complex laboratory tests of blood and other tissues can be considered biomarkers. Biomarkers can be used alone or in combination to assess an individual's health or disease status.

In 1998, the National Health Biomarkers Definitions Working Group defined a biomarker as "a property that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention".[11]

An even broader definition takes into account not only the incidence and outcome of the disease, but also the effects of treatments, interventions, and even unwanted environmental exposures such as chemicals or nutrients. In its report on the validity of biomarkers in environmental risk assessment, WHO stated that the true definition of biomarkers includes almost any measurement that reflects an interaction between a biological system and a potential hazard, which may be chemical, physical or biological.[12]

The measured response can be a functional and physiological, biochemical or molecular interaction at the cellular level. Biomarkers have a long history of use in clinical practice as old as the medical practice itself, and modern laboratory science allows us to measure reproducibly. The use of biomarkers, particularly laboratory-measured biomarkers, in clinical research is somewhat new, and the best approaches for this application are still being developed. The main issue is to determine the relationship between any measurable biomarker and relevant clinical endpoints.[9]

BIOMARKERS AND METHODS THAT CAN BE USED FOR AUTISM SPECTRUM DISORDER

Fat cells synthesize the adipokine biomolecule, some of which are important in regulating brain activity. Fatty acid-binding protein 4 (FABP4), also known as adipocyte FABP (A-FABP) or aP2, is mainly expressed in adipocytes and macrophages. Despite the absence of a typical secretory signal peptide, FABP4 is released from adipocytes in a nonclassical pathway associated with lipolysis, possibly acting as an adipokine.[13] FABP4 is known to be an adipokine that can regulate brain function, especially during development.[14]

Adipokines known to be associated with ASD and the protein FABP4 were investigated in a study

comparing adipokine levels from blood samples taken from preschool-aged children with and without Autism Spectrum Disorder (ASD).[14]

The results showed that preschool-aged children with ASD had much lower FABP4 levels in their blood than other children, but other adipokines were not different between groups. A second test in the other two groups of children confirmed these results. This result makes FABP4 a potential early biomarker for ASD. In a further study on the subject, similar comparisons were made in older children and postmortem brains, showing equal FABP4 levels between ASD and non-ASD groups. This means that FABP4 levels differ at a critical time during brain development, making it more than just a biomarker.

Its deficiency can be a disease-causing factor rather than just a by-product. To confirm the importance of FABP4, knockout mice lacking the FABP4 gene were generated. Compared with wild-type mice, behavioral testing showed that these mice interacted less with unknown mice and had more difficulties with spatial learning and memory.

In addition, examining neurons in mouse brains found the shape and structural features that matched those found in posthumous brains of people with ASD. Consequently, FABP4 has been identified as a biomarker capable of detecting ASD in children aged four to six, and a prospective cohort study of neonates is planned to determine whether FABP4 levels at birth can predict the future manifestation of ASD.[14]

Vascular Endothelial Growth Factor (VEGF) is a signal protein produced by cells that stimulate vasculogenesis and angiogenesis, namely VEGF, a type of vascular growth factor. VEGF is a signal protein that promotes the growth of new blood vessels. VEGF forms part of the mechanism that restores blood flow to cells and tissues when deprived of oxygenated blood due to impaired blood circulation.[15]

There are various studies on the relationship between VEGF and ASD. One study found lower serum VEGF levels in ASD cases compared to controls.[16] Another study examined neonatal blood samples from patients diagnosed with ASD, Attention-Deficit/Hyperactivity Disorder (ADHD), schizophrenia, affective or bipolar disorder, and a trend for low VEGF-A concentrations in the ASD group was found.[17]

Growth factors, cytokines, and chemokines are important mediators of forebrain cortical development, and researchers have observed consistent neurodevelopmental features, particularly atypical forebrain cortical development in ASD patients.[18] In a study in which an integrative analysis of RNA and protein expression was performed using frontopolar cortex tissues cut from individuals and controls with ASD to examine the abnormal expression of growth factors, cytokines, and chemokines critical for neurodevelopment in ASD patients, 11 differently expressed in ASD versus control brains. Gene was revealed and the most important of these encoded vascular endothelial growth factors (VEGF-A). In the study, both RNA and protein levels of VEGF-A increased in ASD brains. The increased expression of VEGF-A observed in ASD, combined with the enrichment of genes differentially expressed in microglia, demonstrated that VEGF-A may be a biomarker in ASD.[18]

Another study examining vascular endothelial growth factor (VEGF) and its soluble receptors sVEGFR-1 and -2 in autism measured serum angiogenic molecule levels in 22 patients with severe autism and 28 controls.[16] The results indicate that patients and controls had similar sVEGFR-2 levels, but individuals with autism had lower VEGF levels and higher sVEGFR-1 levels. Also, the imbalance between VEGF and its receptor sVEGFR-1 may play a role in the pathophysiology of autism,[16] so VEGF is a candidate molecule to be identified as a biomarker in the diagnosis of autism with further studies.

Brain-derived neurotrophic factor (BDNF) is a secretory protein synthesized from the BDNF gene and is a growth factor from the neurotrophin family and is found in the brain and periphery. It plays an important role in neuron development, survival, and maintenance.[19] Brain-derived neurotrophic factor (BDNF) is one of the neurotrophic factors promoting differentiation,[20] maturation[21] and survival[22] of neurons in the nervous system, and glutamatergic stimulation exerts a neuroprotective effect under adverse conditions such as cerebral ischemia, hypoglycemia, and neurotoxicity.[23]

Brain-derived neurotrophic factor stimulates and controls the growth of new neurons from neural stem cells,[24,25] and also BDNF protein and mRNA have been identified in most brain regions, including the olfactory bulb, cortex, hippocampus, basal forebrain, mesencephalon, hypothalamus, brainstem, and spinal cord. BDNF is emerging as a potential molecule that could help better understand various neurodevelopmental and neurodegenerative disorders. Associations of BDNF with behavioral changes such as hyperactivity,

increased depression and psychiatric disorders including schizophrenia and bipolar disorder have been reported.[26]

Since BDNF easily crosses the Blood-Brain barrier, serum concentrations are directly related to brain concentration, so BDNF is thought to accurately reflect BDNF levels in the brain. Specifically high BDNF expression has been observed in the brain and blood.

In a study in which forty-eight children with autism spectrum disorder and mental retardation were tested with serum BDNF concentrations to evaluate the usability of serum BDNF for the diagnosis of patients with neurodevelopmental disorders, autistic children according to the severity and presentation of the disease phenotype, Group A typical autistic children according to DSM-IV criteria. cases) and Group B (cases with atypical autism).

In general, no significant difference was observed in serum BDNF concentrations in autistic children compared to age-appropriate controls, but serum BDNF levels in Group A did not differ significantly from controls, while BDNF levels were found to be significantly higher in Group B with a milder phenotype compared to normal controls ($p<0.001$).[27] In another study involving 1242 participants, a meta-analysis of BDNF levels in autism was performed to evaluate the role of BDNF.

The higher peripheral BDNF value in autism is consistent with various neurological and psychological theories about the causes and symptoms of this condition and contrasts with the low levels of BDNF found in schizophrenia, bipolar disorder, and depression in particular.

Immunological risk factors may play a critical role in the pathogenesis of ASD during the prenatal, neo- and postnatal periods, including maternal infection and immune activation, immune dysregulation, inflammation, and microbial dysbiosis during pregnancy.[28] Cytokine abnormalities have been observed in the blood, CSF (cerebrospinal fluids) and brains of children with ASD.[29,30]

These abnormalities may be related to disease severity and behavioral disturbances in individuals with ASD.[31] Changes in peripheral cytokine profiles at birth, particularly high levels of interleukin (IL) -1β and IL-4, have been associated with a higher risk of diagnosing ASD later in life.[32] Therefore, peripheral cytokines can be useful as potential biomarkers to predict or diagnose ASD. A systematic review and meta-analysis showed that the pro-inflammatory cytokines interferon (IFN)-γ, IL-1β and IL-6 increased in the blood of children with ASD, while anti-inflammatory cytokine-converting growth factor (TGF) -β1 decreased. stated.[33]

Levels of various chemokines associated with the recruitment of inflammatory cells, including eotaxin, IL-8, and monocyte chemotactic protein-1 (MCP-1), are elevated.[34] Another meta-analysis showed that autistic patients had lower anti-inflammatory cytokine IL-10 and IL-1Ra levels than controls.[35] A recent study found that levels of cytokines, including eotaxin, TGF-1 and TNF-, are increased in Chinese children with ASD compared to levels in typically developing children.[28] Inflammation has been observed in the brains of individuals with ASD, including microglial activation, anti-brain antibody reactivity, and astroglial activation.[36]

High levels of IFN-γ, IL-8, MCP-1, TGF-β2[37] and TNF-α[38] have been reported in the CSF of ASD patients. These findings increase the possibility of peripheral immune and inflammatory factors as potential markers for ASD.

In a study comparing the protein expression levels of peripheral blood mononuclear cells (PBMCs) in autistic children with healthy controls using isobaric tags to apply the relative and absolute quantitation (iTRAQ-Isobaric tag for relative and absolute quantitation) proteomic approach, it was expressed differently in the autistic group compared to the control A total of 41 proteins were shown.[33] These proteins have been associated with metabolic pathways, endoplasmic reticulum (ER) stress and protein folding, endocytosis, immune and inflammatory response, plasma lipoprotein particle organization, and cell adhesion, and are also associated with enzyme-linked immunosorbent assay (ELISA) analysis with previously reported autism. Associated proinflammatory cytokines [interferon-γ (IFN-γ), interleukin-1β (IL-1β), IL-6, IL-12, and tumor necrosis factor-α (TNF-α)] have been detected in plasma[31,32,39,40] and values were found to be noticeably higher in individuals with autism than the normal control group.

These reported proteins can serve as potential biomarkers for early detection of autism, as well as the simultaneous detection of three proteins [complement C3 (C3), calreticulin (CALR) and SERPINA1] in plasma and PBMCs, increasing the accuracy of detection.[41]

Conclusion

Since the etiology and pathogenesis of ASD have not been clarified yet, there is no objective, effective and specific early diagnostic biomarkers and therapeutic drugs for the main symptoms of ASD. However, the effect of early intervention is important. Given the increasing prevalence of ASD, the search for diagnostic markers has received great interest.

ASD is a multifactorial disease that involves the interaction between genetic and environmental factors. Biomarker research focuses on genes, proteins, peptides, metabolites, cytokines, and inflammatory factors in peripheral body fluids (blood, urine, saliva).

New technologies include focusing on signal transduction pathways associated with biomarkers and ohmic methods such as genomics, proteomics, metabolomics, and transcriptomics; continues to make progress in these areas of research.

Multiple biomarker panels covering biomarkers derived from different levels of biological analysis, such as genetics, epigenetics, gene expression and miRNAs, proteomics, metabolomics, will likely have the largest amount of information content and predictive power compared to biomarker panels that allow one-size-fits-all measurement. In recent years, research on metabolites has progressed rapidly, especially in connection with metabolites of the intestinal flora.

Additionally, it is important to look for genes, proteins or metabolites that show consistent changes in different studies. There are probably many genetic and environmental factors underlying autism, it can be predicted that these factors will combine in a relatively limited number of intracellular biochemical pathways, and neurodevelopmental mechanisms can be corrected by applying personalized molecular therapies after they have been labeled and identified using appropriate biomarkers. Even the currently available behavioral interventions, when administered before and after the occurrence of behavioral abnormalities, can reduce their severity and even help prevent a fully developed autistic disorder in at least some children.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The authors received no financial support for the research and/or authorship of this article.

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