Discovery and Development of Autism Biomarkers: The State of the Science

Dost Muhammad Halepoto, Shahid Bashir and Laila Y. AL-Ayadhi

Abstract—Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by impairments in social interaction, difficulty with communication, and restrictive and repetitive behaviors appearing during the first 3 years of life. Autism is currently affecting as many as 1 out of 91 individuals in the United States. There is growing evidence that autism may be influenced by genetic, neurological, environmental and immunological factors, however its exact pathophysiology is unknown. The use of potential biomarkers that point to specific mechanism of ASD will help to diagnosis and tailor treatment or prevention strategies for disease rather than solely to a symptom category. Several studies have been carried out to find a candidate biomarker linked to the development of ASD, but up to date no reliable biomarker is available. The aim of this article is to provide an overview of the various potential autism biomarkers reported in literature, and consider the future development of this area of research.

Keywords—Autism spectrum disorder (ASD),

I. INTRODUCTION

AUTISM spectrum disorder (ASD) is prevalent neurodevelopmental disorder which is characterized by impairments in social relatedness and communication, repetitive behaviours, abnormal movement patterns, and sensory dysfunction [1]. The prevalence of ASD has increased by over 600% in the past few decades [2]. The current prevalence in the United States is estimated, at 1 in 91 children [3]. Few reports have been published about the occurrence of autism in developing countries; however studies from the Middle East on this topic have been particularly rare [4]. ASD in Saudi Arabia is slightly higher than reported in developed countries. One report estimated that in Saudi Arabia there were 42,500 confirmed cases of autism in 2002 and that many more remained undiagnosed [5].

Although there is no known unique cause of autism, there is growing evidence that autism can be caused by a variety of disorders, however its exact pathophysiology is unknown [6]. To date, the diagnosis of autism is solely based on the patient’s history and the observation of behavioral abnormalities. The pathophysiology of autism is not fully understood and no disease markers for the diagnosis of autism have been validated. A reliable biomarker, however, could significantly contribute to an early and more exact autism diagnosis, a crucial prerequisite for an early behavior modifying therapeutic intervention.

Novel biomarker identification in autism disorder will facilitate the achievement of these goals, first, by providing sensitive and selective clinical correlates for the evaluation and diagnosis of those affected and, second, by providing insights into disease mechanisms that can be used to identify therapeutic targets and to develop efficacious compounds to target them [7].

Biomarkers are believed as key molecular or cellular measures that link a specific environmental exposure to a health outcome. Biomarkers play an important role in understanding the relationships between exposure to environmental chemicals, the development of chronic human diseases, and the identification of subgroups that are at increased risk for disease. Much progress has been made in identifying and validating new biomarkers that can be used in population based studies of environmental disease [8].

It has been suggested that molecules involved in serotonin metabolism [9] and several cytokines and chemokines [10, 11] are associated with ASD. Altered blood levels of neurotrophic factors have also been reported [12, 13].

Biomarkers that will be useful for either disease prediction or treatment should have one or more of several properties, including: (i) specific and selective association with illness in a population; (ii) heritability; (iii) state independence and presence, whether or not the clinical phenotype of the disease is present; (iv) co-segregation with disease within families; and (v) presence in relatives of affected individuals at a higher rate than in the general population [14].

Accordingly, biomarkers have been classified as antecedent biomarkers (identifying the risk of developing an illness), screening biomarkers (screening for subclinical disease), diagnostic biomarkers (recognizing overt disease), staging biomarkers (categorizing disease severity), or prognostic
biomarkers (predicting future disease course, including recurrence and response to therapy, and monitoring efficacy of therapy) [15]. Biomarkers for neurological disorders can also be classified as genetic, neuroimaging, clinical, or biochemical markers [16].

Classical research on the identification of biochemical biomarkers in blood and cerebrospinal fluid (CSF) for neurological disorders has been aimed at assaying single metabolites. Often this search has been based on research hypotheses. Unfortunately, none of the single biomarkers identified to date have the desired sensitivity and specificity for diagnosis or have sufficient power to identify disorders at an early stage [17].

The first biomarker described in ASD was elevated whole blood 5-HT, or hyper-serotonemia, was identified 50 years ago [18] and unique to autism among developmental disorders [19]. Biomarker research has been focused on various autism linked factors including genetic, neurological, environmental and immunological factors. Peripheral biomarkers related to the immune system have also generated considerable interest. There are numerous studies have been carried out to find autism biomarkers in Saudi autistic children related to role for immune dysfunction [20].

The immune and nervous systems interact in health and disease, with both systems able to impact the development and function of the other. Abnormalities of the immune cells in brain - the microglia - and abnormalities in the peripheral immune system, including cytokines, leukocytes, and antibodies, have been reported in autism [21].

The contribution of inflammatory processes to the etiology of individuals with autism is supported by an increasing number of studies [22]. Cytokines are possible biomarker for evaluating inflammatory processes in autism. Many studies reveal increased levels of pro-inflammatory cytokines in brain, cerebrospinal fluid (CSF), and blood from children with autism [23]. Gastrointestinal inflammation has also been described in many children with ASD [24].

Various approaches including structural neuroimaging were used to identify biomarkers of autism [25]. Recent epidemiological studies, conducted in different regions of the world, have indicated that at least one in every 100 people has some form of autism. [26].

A. Important studies on Autism Biomarkers

Bailey et al [27] explored the possible use of peripheral biomarkers in the early diagnosis of autism. They measured plasma secreted amyloid precursor protein alpha (sAPP-α) levels in autistic and aged-matched control blood samples and found a significantly increased level of sAPP-α in 60% of the known autistic children.

A review article [28] appeared which examines recognized clinically available biomarkers such as Porphyrin, Transsulphuration, Mitochondrial dysfunction, Oxidative stress, Hormonal and Genetic biomarkers as testing for the evaluation and treatment of ASDs.

Researchers in Canada [29] found that all of the autistic subjects had significantly elevated plasma levels of long chain fatty acids (VLCFA)-containing phosphatidylethanolamines (PtdEtms) and in DHA-containing ethanalamine plasmalogen (PlsEtms). Results “indicated that chronic mitochondrial stress is pervasive in autism and that elevated levels of fatty acid products are useful metabolic biomarkers of both mitochondrial stress and autism.”

DA. Geier and et al [30 - 33] assessed urinary porphyrins levels and also evaluate transsulfuration metabolites in autistic children and suggested that they can play important role as potential markers for autism.

Protein biomarker discovery from biological fluids, such as serum, has been widely applied to neuro-psychiatric disorders with relatively clear biological causes. The application of the associated technologies for the identification of protein biomarker signatures in ASD is comparatively less well established.[34]. Candidates for biomarkers of autism i.e. Gastrointestinal, Immunologic, Neurologic, Toxicologic and Ubiquitous biomarkers were reviewed and discussed [35]. Woods et al, [36] have reported recognized serum biomarkers for psychiatric disorders including ASD and the role of cholesterol and Apos in central nervous system (CNS). Much research indicated that cholesterol, in particular, may be altered in ASD [37]. Recently Momeni et al [38] undertook a proteomic approach using mass spectrometry to discover novel peptide biomarkers with diagnostic utility and to understand the role of these in the pathophysiology of ASD.

In the Social Attention and Communication Study (SACS), Barbaro, and Dissanayake [39] identified the most discriminating and predictive behavioral markers of ASD at 12, 18 and 24 months of age. A systematic literature review [40] was also carried out to clarify and quantify the relationship between oxidative stress-related blood biomarkers and ASDs.

Fatty acids were suggested as diagnostic markers, altered in the plasma of autistic patients, specifically showing an increase in most of the saturated fatty acids except for propionic acid, and a decrease in most of polyunsaturated fatty acids in Saudi autistic patients [41].

Recognized autism urinary biomarkers were identified [42] and categorized according to the key theories that exist regarding the etiology of autism: gastrointestinal factors, immune dysregulation, heavy metal toxicity, neurotransmitter abnormalities, and oxidative stress. Using the 1-Year Well-Baby Check-Up Approach [43], examination of early biomarkers was suggested related to early brain overgrowth, cerebellar development, gene expression patterns and immune system function for the early diagnosis efforts under 3 years. Liquid chromatography coupled with mass spectrometry (LC-MS/MS) flow injection analyses method was used to identify Novel plasma phospholipid Biomarkers [29].


B. Systematic Approach and Technologies used to Biomarker Identification

A systematic approach to biomarker identification have been involved multiple technologies to investigate a disease process at all levels. For biomarker identification in ASD,
unique challenges are required for the simultaneous use of each of these technologies; common technologies used in ASD biomarker discovery are summarized in Table 1. There have been numerous successful applications of combinations of these technologies have also been reported [7].

II. DISCUSSION

There is a long tradition of biomarker research in ASD. Biomarkers may point toward ASD susceptibility factors in different ways. In theory, a biomarker could contribute directly to susceptibility, but a biomarker may also represent an endophenotype, or a heritable trait resulting from an underlying factor that is the prime contributor to ASD susceptibility [25].

It is assumed that biomarker may be a secondary result of ASD itself or of ASD treatment. Deciding among these possibilities has therapeutic relevance in narrowing down potential targets and/or using the particular measure as a diagnostic or treatment aid.

Biomarkers need to be assessable, linked with the particular condition and stable or predictable across and within individuals. Biomarkers can be measured using various biological samples, including blood, urine or saliva. There is increased interest among researchers in so-called neuro-markers, which are biomarkers based on measures of neurochemicals in the cerebrospinal fluid, brain structure measured using MRI, and/or brain function.

Biomarkers have several applications. First, they can be viewed as risk factors that increase an individual’s susceptibility for a condition, and as such can be used to identify individuals who are at high risk for the condition. Biomarkers that can be detected before disease symptoms occur could be used to improve early detection of a condition. Second, they can be used to improve diagnosis, as they may enable better prediction of the nature and severity of disease outcomes in an individual. Third, they may be used to develop personalized treatments and, if monitored over time, can be used to evaluate treatment outcomes. A wide range of autism biomarkers have been proposed, but as of yet none has been validated for clinical use.

There is an intensive search for biological markers for autism. Such biomarkers could not only reveal causes of the condition but could also be clinically useful in complementing or improving the behavioral diagnosis of autism and in enabling earlier detection of the condition. Biomarkers would thereby assist in the validation of very early, targeted and individualized intervention programmes.

A slight hope is that single biomarkers can capture the complex process underlying an illness. Rather, by looking as perturbations of biochemical networks, it becomes clear that a multiparameter analysis (panel of markers or multiple metabolites) may provide better insight into disease diagnosis, prognosis, and treatment [51].

Ethical issues regarding the implementation and clinical recommendations from the outcomes of biomarker tests require greater examination; however, future discussion and research into the use of biomarkers for screening behaviorally defined disorders are of high importance for researchers, clinicians, and families [47].

<table>
<thead>
<tr>
<th>Biomarker type</th>
<th>Tissue source</th>
<th>Technology</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene expression profile</td>
<td>Blood samples</td>
<td>Genomics (CGH arrays)</td>
<td>2,45</td>
</tr>
<tr>
<td>Proteomic profile</td>
<td>Serum samples</td>
<td>Mass spectrometry, 2DE MS, LC-MS</td>
<td>34,38</td>
</tr>
<tr>
<td>Metabolomic profile</td>
<td>Urine, blood, saliva and CSF</td>
<td>ELISA, NMR, MS</td>
<td>20,23,42,46</td>
</tr>
<tr>
<td>Brain size and structure</td>
<td>Neural tracts through the brain</td>
<td>MRI, DTI</td>
<td>47</td>
</tr>
<tr>
<td>Brain function</td>
<td>Neural activity</td>
<td>Functional MRI, EEG, ERPs</td>
<td>48</td>
</tr>
<tr>
<td>Head size</td>
<td>Head-size</td>
<td>Head circumference trajectory</td>
<td>49</td>
</tr>
<tr>
<td>Eye movement</td>
<td>Looking measures, saccadic reaction time</td>
<td>Looking measures, saccadic reaction time</td>
<td>50</td>
</tr>
</tbody>
</table>

CGH Array, comparative genomic hybridization; ELISA, Enzyme-linked immunosorbent assay; NMR, Nuclear magnetic resonance MRI, Magnetic resonance imaging; DTI, diffusion tensor imaging; EEG, electroencephalography; ERPs, event-related potentials.

III. CONCLUSION

Biomarkers should ideally be quantitative biological measures with an accurate indication of a specific mechanism and ideally are not invasive. An increasing prevalence of ASD shows the importance of several biomarkers in the disease diagnosis and treatment. Identifying biomarkers will almost certainly lead to a better understanding of the pathogenesis required to design the most effective treatments of autism.

Alterations in neurotransmitters, oxidative damage, neuroinflammation, mitochondrial dysfunction, neurological abnormalities in brain and gastrointestinal disturbances play a promising in the pathology of disease. Periodic diagnosis of these biomarkers in biological samples will provide a basement for effective and efficient therapy.

Although initial reports look promising, the application of biomarkers in the clinical setting currently remains a vision for the future. Several crucial questions have to be addressed first before these methods find their way into clinical practice. Collaborative approaches involving scientists and other stakeholders must combine the search for valid, clinically useful autism biomarkers with efforts to ensure that individuals
with autism and their families are treated with respect and understanding.

ACKNOWLEDGMENT

We thank Autism Research and Treatment Centre, Al-Amodi Autism research chair, King Abdul Aziz city for science and technology (KACST), and Health Research Studies program at (NPST), at King Saud University for sponsorship and financial support.

REFERENCES


