

# Scientific challenges in developing biological markers for autism

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## Abstract

### Introduction

Autism is a highly heterogeneous disorder, with a strong genetic basis and many associated medical and behavioural comorbidities. Current diagnostic methods and screening tools are somewhat subjective and are difficult to assess in younger children, which often results in missed opportunities for early intervention. A biological marker that could predict autism risk, assist in early diagnosis or even identify potential therapeutic targets would have great clinical utility. While research in this field has greatly increased in recent years, progress has been limited and no biological markers for autism have been found to be universal. In this review, the major scientific challenges associated with the identification of biological markers for autism are described. Factors that may play a role in impeding progress in autism biomarker research, including the heterogeneity of autism, the presence of comorbid conditions, gender bias and the availability of appropriate research samples will be carefully reviewed.

### Conclusion

The search for autism biomarkers in the laboratory is an important research endeavour that is fraught with many challenges, yet the translation of such findings into the clinic may be the real challenge and requires

the investigation of large, well-characterized sample cohorts with appropriate controls. Only when these issues are addressed prior to implementing new studies will robust and reliable biomarkers for autism be identified.

### Introduction

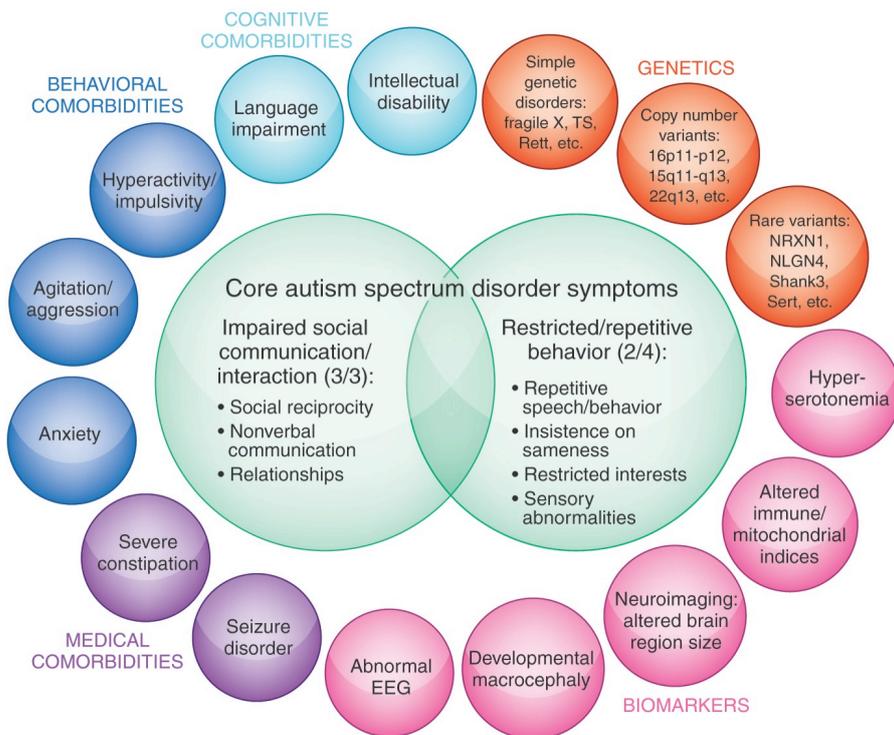
Autism spectrum disorders (ASDs) are a group of pervasive developmental disorders characterized by impairments in social interaction and communication and associated with repetitive and stereotyped patterns of behaviour. As defined in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV), autistic disorder, also referred to as autism, is the most severe form of ASDs. Other conditions along the spectrum include a milder form known as Asperger's syndrome<sup>1</sup> and pervasive developmental disorder not otherwise specified (PDD-NOS)<sup>2</sup>. The latter is considered a 'catch-all' diagnosis sometimes used until a more precise diagnosis can be made<sup>3</sup>. Childhood disintegrative disorder<sup>4</sup> and Rett syndrome<sup>5</sup> are also classified by the DSM-IV under ASDs but are extremely rare. Although ASD varies significantly in character and severity, it occurs in all ethnic and socioeconomic groups and affects every age group. The Centres for Disease Control (CDC) estimate that 1 out of 88 children at the age of 8 have an ASD<sup>6</sup>. There is also a strong gender bias, with males being four times more likely to have an ASD than females<sup>7</sup>, except in the case of Rett syndrome, which only affects girls. For the purpose of this review, the term autism is synonymous with ASD.

Typically, the first signs of autism occur within the first 3 years of life. However, the developmental trajectories in the core deficits seen in autism are so variable in infants and toddlers that the mean age at earliest diagnosis is approximately 5.7 years<sup>8</sup>, and about 27% of children remain undiagnosed at the age of 8 years<sup>8</sup>. Furthermore, a diagnosis of autism is currently based on observations of behaviour occurring years after the complex interactions among genetic factors and environmental influences may have taken their toll, potentially bypassing early intervention and/or treatment opportunities during the critical first few years of life<sup>9</sup>.

Research into the cause of autism has been hampered by the inability to identify biologically meaningful subtypes within the autism population<sup>10</sup> and by the traditional boundaries between the disciplines of psychiatry, psychology, neurology and paediatrics<sup>11</sup>. This is further complicated by the comorbid, health-related medical conditions that are frequently associated with autism<sup>12</sup>, which, if left untreated, can negatively impact behaviour and progress<sup>13,14</sup>. These include seizure disorders<sup>15</sup>, sleep disorders<sup>16</sup>, gastrointestinal disorders<sup>17</sup>, metabolic disorders<sup>18</sup> and immune dysfunction<sup>19</sup>, as well as behavioural and cognitive comorbidities (Figure 1)<sup>20</sup>. The identification of comorbid medical conditions associated with autism may help us to develop meaningful subtypes that could result in a better understanding of causative and biological mechanisms<sup>14</sup>. Furthermore, it is quite possible that some of these medical conditions suggest the presence of biologic markers, which, if identified, could assist in diagnosis, and even be

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**Figure 1:** Autism symptoms, comorbidities and biomarkers. The core symptoms of autism are represented in the centre and represent the common features required to receive a diagnosis. All three social communication/social interaction symptoms are required to receive a diagnosis in the DSM-V draft criteria. This domain represents the ‘negative symptoms’ of autism, that is, absence of appropriate social communication. Two of the four restricted/repetitive behaviour symptoms are required to receive a diagnosis in the DSM-V draft criteria. This domain represents the ‘positive symptoms’ of autism, that is, the presence of unusual restricted, repetitive or sensory behaviours. Around the periphery of the figure are symptoms or biomarkers that are not required for an autism diagnosis but are more common in autism than in the general populations. As might be expected from the range of comorbid symptoms, biomarkers and genetic findings also reveal significant heterogeneity across individuals with autism. Reprinted by permission from Macmillan Publishers Ltd: *Neuropsychopharmacology*<sup>20</sup>, copyright 2012.

used to develop targeted treatments. The aim of this review was to discuss the scientific challenges in developing biological markers for autism.

## Discussion

### Applications of biomarkers

There are several potential applications of a biomarker for autism. Biomarkers could provide a reliable screening tool for infants and children to determine risk. Twin and family studies have demonstrated

that both genetic and non-genetic factors contribute to an increased susceptibility to autism<sup>21–23</sup>. Research on risk factors for autism has therefore focused on examining genetic linkage or association studies<sup>24</sup>, although more recently this has shifted to determining gene variants in autism<sup>25–27</sup>. Incomplete penetrance, significant locus heterogeneity and substantial phenotypic variability greatly complicate the identification of susceptibility gene variants<sup>28</sup>, and

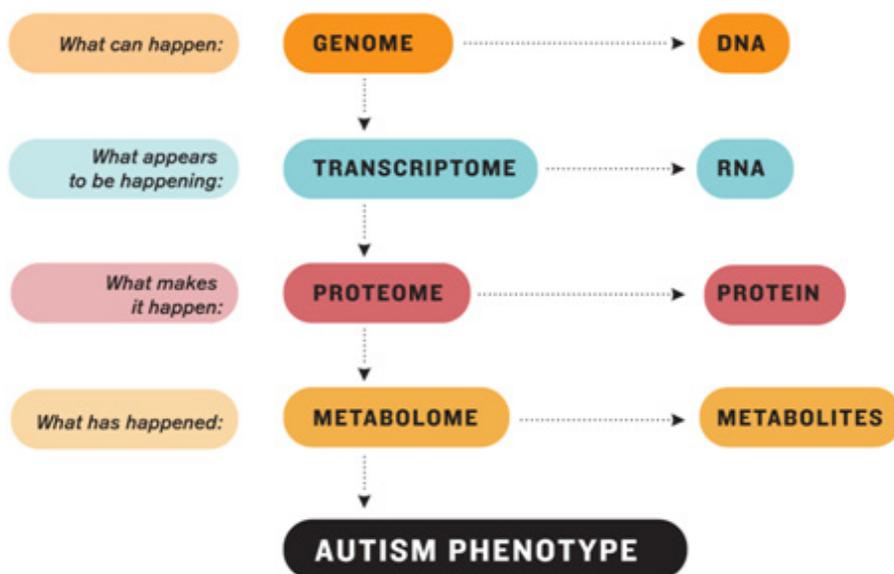
none of the putative genetic markers thus far identified have been found to be universal<sup>29–31</sup>. While compelling genetic markers of risk for autism are proving to be elusive, novel diagnostic testing, such as chromosome microarray analysis, is now commonplace and may eventually lead to routine prenatal testing for autism. The existence of such tests is likely to have significant implications for family planning and should be informed by a full discussion of the ethical issues surrounding the use of these technologies<sup>32</sup>. For a comprehensive review on this subject, see Singh and Rose (2009)<sup>33</sup> Pellicano and Stears (2011)<sup>34</sup> and Walsh et al. (2011)<sup>35</sup>.

Several putative biomarkers have been proposed for assessing autism risk (Table 1)<sup>35</sup>. These broadly fall into two categories: biological markers, which rely on the analyses of biological samples (such as blood and urine), and neuromarkers, which typically utilize imaging or assessments of the brain (such as MRI, DTI and EEG). Biological markers have been identified through the use of modern, highly innovative molecular technologies. These have helped us recognize that autism is the result of multiple systems gone awry. Targeting systems, rather than single molecules, will likely result in more measurable biomarker profiles (Figure 2). However, the often heterogeneous populations of subjects analysed, the use of siblings as controls and the disparate age ranges between controls and subjects with autism may have compromised findings as no biological marker or neuromarker has thus far indicated the presence of autism in most cases.

Biomarkers could also be useful as predictive markers while a child is presymptomatic. The behaviours that are characteristic of autism typically emerge and then evolve over the first few years of life<sup>36</sup>. In many cases though, it is difficult to distinguish the first symptoms of

Biomarker Type	Sample/Measure
Gene-expression profile	Blood samples
Proteomic profile	Serum samples
Metabolomic profile	Urine samples
Head size	Head circumference trajectory
Brain size and structure	MRI, DTI
Brain function	Functional MRI, EEG, ERPs
Eye movement	Looking measures, saccadic reaction time

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DTI, diffusion tensor imaging; EEG, electroencephalography; ERPs, event-related potentials.



**Figure 2:** The ‘-omic’ cascade in autism. The ‘-omic’ technologies have helped us recognize that autism is the result of multiple, interrelated systems gone awry. Targeting systems, rather than single molecules, may help identify abnormal patterns of expression associated with biosystems, thus leading to the discovery of more measurable biomarkers for ascertaining risk, confirming diagnosis and monitoring response to treatment.

autism from normal variations in development, or from transient delays that then resolve over time<sup>37</sup>. This is particularly important to determine in younger siblings of children with autism who are at higher risk for developing the disorder<sup>38</sup>. Thus, a clinically useful biomarker that could identify susceptible children before the onset of clear symptoms is apparent will help in

the early detection of autism enabling interventions to be initiated sooner. The effectiveness of early behavioural intervention has been recently demonstrated by Dawson and colleagues using the Early Start Denver Model (ESDM)<sup>39,40</sup>. In these studies, the ESDM applied to children as young as 12 months of age and diagnosed with autism resulted in gains in IQ, language and

adaptive behaviour<sup>40</sup>, as well as normalized patterns of brain activity as measured by EEG<sup>39</sup>. The potential of early intervention to alter the course of brain and behavioural development in young children with ASD is an exciting prospect.

Biomarkers may also be useful for providing more definitive diagnostic tools to improve the reliability of clinical diagnosis, which is currently defined on the basis of behavioural criteria including the ‘gold standard’ Autism Diagnostic Observation Scale (ADOS) and Autism Diagnostic Interview—Revised (ADI-R) assessments. While these evaluation procedures are costly to implement and limit the rate at which larger sample sizes can be achieved, they ensure accurate diagnosis and the collection of rich phenotypic data<sup>28</sup>. Ultimately though, the accuracy of any biologically driven marker can never exceed the sensitivity and specificity of these diagnostic tools<sup>32</sup>. It would therefore follow that a biomarker or biomarker profile would not be used in isolation, but rather in combination with conventional behavioural diagnostic assessments. Since autism is associated with a wide range of comorbid medical conditions, the translation of these phenomena into relevant biomarkers may prove to be clinically useful for enhancing the validity and efficiency of existing diagnostic methods<sup>35</sup>.

Finally, biomarkers may also be useful for identifying novel treatments to confirm the need for a specific treatment or to monitor treatment efficacy. The field is still far from being ready for the clinic, but holds great promise in that markers could allow for earlier treatment and a better prognosis. The likelihood of finding clinically useful biomarkers might be particularly high in autism because it can be assumed that it is ‘very biological’ in nature<sup>41</sup>. Exploring the biology of autism may also be useful for enabling autism to be sub-typed based on physiological criteria,

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creating a more personalized approach to treatment.

### Scientific challenges in biomarker discovery

There are a number of scientific challenges that pose significant issues in the development of biomarkers for autism. Autism represents a complex, multidimensional phenotype. Behavioural symptoms are typically identified through standardized assessments, semistructured play-based interviews and questionnaires, combined with expert clinical evaluations. These are the gold standard tools for making an autism diagnosis. However, an inherent limitation of this diagnostic method is that a child must be of a certain age in order to assess the absence or presence of critical diagnostic behaviours; for example, the ADOS cannot be administered until a child is at least 12 months of age and walking independently<sup>42</sup>. For a biomarker to be useful diagnostically, it would need to be present prior to the onset of behavioural symptoms.

The restructuring of the diagnostic classification for ASDs into a single category (DSM-5)<sup>43</sup> may initially pose some challenges from a research perspective, especially with regard to on-going studies in which subject recruitment spans both DSM-IV and DSM-5 criteria. However, the proposed changes aim to better reflect the relationship between DSM criteria and current research by consistently identifying the core features in social/communication and restrictive and repetitive behaviours that are specific to ASDs<sup>44,45</sup>. Typically, a clinical diagnosis of ASD must also be supported by standardized assessments and verified by expert clinical opinion in order to sufficiently document the diagnosis prior to subject participation in any research study. While this approach may result in some individuals with very mild ASD symptoms being excluded from studies<sup>46</sup>, it ensures a consistent diagnostic

categorization of ASD across studies<sup>47</sup>. Currently, most researchers use assessments such as the ADI-R to obtain early developmental history from parents, along with semistructured, standardized observations, such as the ADOS. It follows, then, that if these assessments continue to be utilized for screening subjects, there should be little change to subject inclusion in research studies.

The clinical phenotype of autism is widely heterogeneous. Despite common areas of impairment that define autism as a condition, individuals with autism show a vast clinical variability in the expression and severity of their symptoms. This heterogeneity spans the entire range of development, including a wide array of social and behavioural issues, as well as complex genetic and environmental contributions. The general consensus is that this heterogeneity leads to a single spectrum disorder<sup>48-51</sup>. However, some researchers have hypothesized that this heterogeneity represents many distinct behavioural and physical symptoms, leading to multiple causes<sup>52,53</sup>, rather than a unifying cause, for autism. Comorbid conditions further complicate this picture and must also be identified and, if necessary, analysed separately, as any putative biomarker should not only be sensitive to autism, but also specific to autism. A biomarker must allow for a differential diagnosis in cases of attention-deficit hyperactivity disorder (ADHD) or epilepsy, for example. Hormones, neurotransmitters and many other biological systems change as a child ages, demonstrating that age plays a very important role when designing studies.

Autism, like many other neurodevelopmental disorders, also displays a striking male bias in prevalence, with approximately four affected males for every one affected female. The consistency of this observation over time and across populations strongly implicates the involvement of sex-specific biological factors in

autism cause<sup>54,55</sup>. This provides an intriguing avenue of research that may speak to the aetiology of autism, and yet few studies have addressed whether autism affects males and females differently. Consequently, biomarker studies in autism may be fundamentally flawed if they fail to include samples from both males and females in their study design and analysis. The reporting bias inherent in gender-specific research creates a situation in which guidelines based on the study of one sex may be generalized and applied to both. This could be disastrous for advancing autism biomarker research since distinct sex-specific differences in phenotype<sup>56,57</sup>, genetics<sup>58,59</sup> and biology<sup>60</sup> have been reported.

The profound clinical heterogeneity in autism highlights the importance of developing large repositories of biological samples that have undergone rigorous phenotyping in order to validate the clinical significance of putative biomarkers. These biological specimens should be representative of the autism population, including age, gender, ethnicity, diagnoses and geography. They must include samples from well-characterized autism subgroups, as well as from multiple 'risk' populations, such as children at risk for ADHD, anxiety and dyslexia. These will help establish that a putative biomarker is not present in multiple disorders. Several national autism biorepositories already exist. Specifically, the Autism Genetic Resource Exchange<sup>61</sup> and the Simons Simplex Collection<sup>62</sup> have implemented protocols for collecting samples from multiplex and simplex families, respectively. While these on-going collections have amassed several thousand genetic samples, neither of these repositories include biological samples collected from typically developing controls that do not have a sibling on the autism spectrum. From a biomarker perspective, biological samples from age- and gender-matched typically developing

children, both with and without a sibling on the autism spectrum, are imperative, and their lack of current availability represents a major scientific challenge for researchers.

### Conclusion

The progression of biomarker research in autism mirrors that of other neurologic disorders in that it is still in its infancy and marked largely by discovery rather than validation. The often heterogeneous populations of subjects analysed, the use of inappropriate controls and the disparate age ranges between controls and subjects with autism cloud the interpretation of some studies. Despite these concerns, the search for biomarkers for autism will continue, given the depth and range of their potential benefits for people with autism and their families. If successful, biomarkers for autism may one day prove invaluable for ascertaining risk, assisting with diagnosis and/or identifying therapeutic interventions.

### Abbreviations list

ADHD, attention-deficit hyperactivity disorder; ADI-R, Autism Diagnostic Interview—Revised; ADOS, Autism Diagnostic Observation Scale; ASD, autism spectrum disorder; CDC, Centres for Disease Control; DSM, Diagnostic and Statistical Manual

ESDM, Early Start Denver Model; PDD-NOS, pervasive developmental disorder not otherwise specified

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**FOR CITATION PURPOSES:** Hewitson L. Scientific challenges in developing biological markers for autism. *OA Autism* 2013 Mar 10; 1(1):7.

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