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Identification and validation of biomarkers for autism spectrum disorders

Eva Loth et al.

Autism spectrum disorder (ASD) is one of the most common neurodevelopmental disorders, but effective medical treatments for the core symptoms of the disorder are still lacking. According to the *Diagnostic and Statistical Manual of Mental Disorders*, fifth edition (DSM-5), the core symptoms of ASD comprise deficits in social communication and interaction, and repetitive and restricted behaviours, which include sensory abnormalities. Novel genetic and preclinical approaches now provide unprecedented opportunities to identify the underpinning pathophysiological mechanisms and aetiology-based treatment targets, as discussed in a Review article by Ghosh *et al.* (Drug discovery for autism spectrum disorder: challenges and opportunities. *Nat. Rev. Drug Discov.* **12**, 777–790 (2013))[1]. This has led to more interest from the pharmaceutical industry in an area in which the overall risk of failure is seen as very high because key parameters of drug efficiency are not yet established and the regulatory environment is uncertain. For example, industry has recently invested in several pre-competitive projects, such as the European Union (EU) Innovative Medicines Initiative (IMI)-brokered public–private partnership EU-AIMS (European Autism Interventions — A Multicentre Study for Developing New Medications)[2].

However, even when new compounds that show preclinical promise for ASD are found, there are still considerable challenges in testing them in clinical trials. For instance, the current practice of testing treatments in clinically and biologically heterogeneous patient groups hampers the ability of investigators to detect potentially significant efficacy signals in specific subgroups who 'respond'. Therefore, we need biomarkers that stratify patient populations according to distinct biological subtypes. So far, the identification and validation of biomarkers has been limited by studies with small sample sizes that have insufficient power and/or because studies use different (and often not standardized) measures. We also need quantifiable, reproducible outcome measures — including surrogate end points — that are sensitive to change, in order to assess treatment efficacy.

Currently, the EU-AIMS Longitudinal European Autism Project (LEAP) is the worldwide largest multicentre, multidisciplinary study to identify stratification biomarkers for ASD and biomarkers that may serve as surrogate end points. In total, the study will include approximately 450 individuals with ASD between the ages of 6 and 30 years, and 350 control participants with typical development or mild intellectual disabilities. All participants are comprehensively characterized in terms of their clinical symptom profile, comorbidities, quality of life, level of adaptive function, neurocognitive profile, brain structure and function (assessed using structural magnetic resonance imaging (sMRI), functional MRI (fMRI) and electroencephalogram (EEG)), biochemical biomarkers, prenatal environmental risk factors and genomics (see Supplementary information S1 (table)).

To understand whether data generated in this study would be accepted in regulatory decisions for future clinical trials, the LEAP Group obtained scientific qualification advice from the European Medicines Agency (EMA) on the population selection criteria, clinical end points and biomarker methodologies to be used. The EMA's Committee for Medicinal Products for Human Use (CHMP) offers tailored advice to support the qualification of innovative methods that have been developed for a specific intended use in the context of research into and development of pharmaceuticals. The goal of using qualified methods is to enable a more robust assessment of risks versus benefits in clinical trials. Another advantage of the procedure of qualifying these methods is that, once qualified, these clinical study instruments may be applied by any investigator in subsequent clinical research, thus ensuring greater scientific rigour.

Population selection criteria

The CHMP agreed to following the DSM-5 criteria for a diagnosis of ASD and stressed the use of the 'clinical specifiers', such as cognitive ability, symptom severity, association with a known medical or genetic condition or environmental factors. Reaching ASD cut-offs on 'gold-standard' clinical instruments, such as the Autism Diagnostic Interview-Revised and the Autism Diagnostic Observation Schedule, was not considered necessary for participant inclusion. Instead, comparison between participants who do versus those who do not reach the cut-off on these measures may help to ascertain whether a particular biomarker extends to the 'broader' autism spectrum. Inclusion of nearly all psychiatric comorbidities was agreed on, as up to 70% of people with ASD have one or more comorbid conditions, such as attention-deficit hyperactivity disorder (ADHD), anxiety or depression[3]. Two exceptions are psychosis and bipolar disorder, as they represent severe psychiatric illnesses that typically require careful medical management, which may interfere with participation in research studies.

The CHMP also supported the inclusion of people with ASD and mild intellectual disabilities (as defined by an IQ below 70 ± 5 and low adaptive behaviour). So far, the vast majority of biomarker studies has focused on high-functioning individuals with ASD, even though approximately 55% of individuals with ASD have mild-to-severe intellectual disabilities[4]. Therefore, relatively little is known about cognitive or neurobiological biomarkers in the patient group that tends to have the poorest outcome and for whom medical treatments are arguably particularly urgent. Likewise, the inclusion of participants on stable medication (that is, lasting more than 10 weeks) was also accepted. The reality is that 30–70% of patients with ASD are prescribed at least one medication to treat associated symptoms. Finally, as a reasonably stable ASD diagnosis can be made from the age of 2–3 years onwards, and as a number of assessment tools used in the LEAP have been validated for use in children from the age of 4 years, the CHMP stressed the importance of also identifying and validating biomarkers across the preschool age range (that is, below 6 years of age).

Clinical outcomes

All of the proposed clinical scales (see Supplementary information S1 (table)) were accepted as clinical outcomes. These scales are not to be used to validate diagnosis, however, as the specificity of many is limited. Indeed, several recent frameworks, including the US National Institute of Mental Health Research Domain Criteria, now recognize that abnormalities in many fundamental behavioural dimensions probably cut across distinct, categorically defined psychiatric disorders[5]. This implies that instruments probing for those behaviours will inevitably have less than 100% specificity. It is also possible that future treatments will be

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aimed at symptoms or cognitive or neural system domains that may be shared between diagnostic categories.

A major priority for the LEAP was the need to define cut-offs for each clinical instrument that represent clinically meaningful changes. Cut-offs can be determined by testing whether changes in symptom severity from baseline to follow-up predict functional changes in quality of life or adaptive behaviour. For instance, changes in functional scores that are smaller than 0.25 standard deviations may be considered too small to have clinical relevance, whereas an improvement by a full standard deviation may be considered 'clinically significant' (REF. 6).

Biomarker stratification approaches

The CHMP agreed to the testing of all the proposed methodologies (including cognitive, eye-tracking, EEG, brain-imaging and biochemical markers) as potentially enriching biomarkers. However, the goal of identifying ASD subgroups that are more biologically homogeneous requires novel stratification approaches that go beyond the predominant focus on case–control differences (FIG. 1).

First, individuals are stratified by population criteria, such as comorbidities or sex. For example, sex differences both in typical development and in ASD have been reported at multiple levels, including in serum biomarkers, in brain structure and function, and in several aspects of cognition[7]. Given the strong sex bias towards males in ASD, we are selectively over-recruiting females to identify potentially sex-specific biomarkers.

Second, based on an accelerated longitudinal design, we aim to establish whether some ASD biomarkers may only be detectable at certain developmental stages. This involves first constructing cross-sectional developmental trajectories for each measure (for example, performance on a cognitive task or brain anatomical indices) in the typically developing (TD) group. Confidence intervals around the TD trajectory will then be used to assess, for each individual with ASD, whether they fall outside the range of performance expected for their age group. On this basis, cut-offs for stratification biomarkers can be established for a particular developmental level. To test the stability of and changes in a biomarker over time, the cross-sectional trajectories will be validated by the longitudinal follow-up data.

Third, we will use multivariate and multimodal approaches to divide individuals into groups according to differences in brain development and function that underpin key cognitive systems. fMRI studies have delineated regional functional activation and connectivity differences in individuals with ASD when performing certain cognitive tasks or during resting state. Substantial variability (for example, in hypoconnectivity versus hyperconnectivity patterns in individuals with ASD), both among studies and among individuals within studies, may indicate substantial heterogeneity among individuals with ASD. The large cohort of the LEAP will allow us to delineate ASD subgroups using multivariate pattern-classification approaches based on profiles

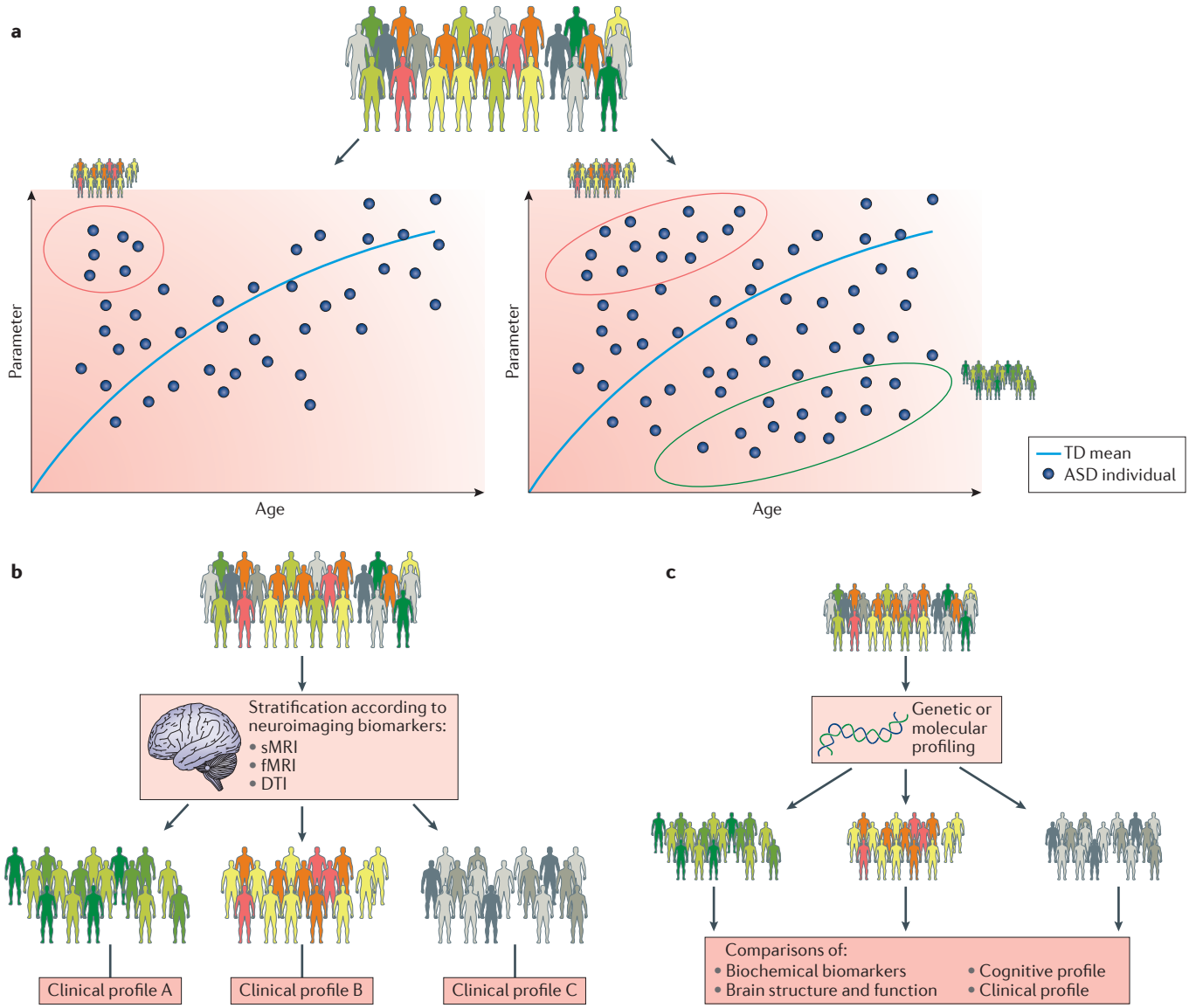

Figure 1 | **Examples of biomarker stratification approaches. a** | For each cognitive, neuroimaging and biochemical measure, the abnormalities of each participant with autism spectrum disorder (ASD) are estimated based on deviations from the typical development (TD) at a particular age. In the left panel, the abnormality is only seen in a subgroup of people with ASD at a particular developmental stage (for example, during middle childhood). In the right panel, subgroups with opposing abnormalities (for example, functional hyperconnectivity versus functional hypoconnectivity in particular networks) are identified that persist across different ages. Both scenarios are hypothetical only. Ovals represent the establishment of different (distinct) subgroups. **b** | Patient stratification according to neuroimaging biomarkers that combine different indices of, for example, brain structure and connectivity, as derived from structural magnetic resonance imaging (sMRI), functional MRI (fMRI) and diffusion tensor imaging (DTI). Different subgroups are then mapped on to potentially shared versus potentially distinct clinical profiles. For example, clinical profile A may be characterized by particular sensory abnormalities, whereas clinical profile B may be characterized by a pattern of particular social-communicative deficits. **c** | Subgroups can be identified according to differences in their genetic–molecular profile. These stratified groups can then be compared with one another in terms of biochemical biomarkers, brain structure and function, cognition and clinical profile.

of brain structure[8] and function[9] and relate them to clinical outcome. Neuroimaging techniques also help us to identify the mechanisms through which interventions improve functioning. Recent event-related potential and pharmacological fMRI[10] studies have been used to ascertain whether treatment effects are reached because of the normalization of atypical neural processes, or owing to the development of compensatory mechanisms.

Last, molecular biomarkers will be crucial in predicting treatment response. Network-based stratification approaches have recently been successfully used in cancer research to identify tumour subtypes. We will use similar approaches to identify molecular ASD subgroups on the basis of the entire genetic mutation profile. We then aim to map these molecular groups, from the 'bottom up', to neurobiological biomarkers and clinical symptom profiles.

Conclusions

The CHMP's key recommendations on the LEAP study design and biomarker approaches included the need to establish sensitivity and specificity for all candidate biomarkers and to define cut-offs for quantitative stratification markers. The CHMP also highlighted the need to establish, for each candidate biomarker, how abnormalities map on to differences in prognosis, and to define what would be considered

as clinically relevant differences. As this is an exploratory observational study, the large number of end points tested was recognized. To achieve a balance between the risk of false positives and false negatives (if one were to correct for the multiplicity of tests), replication in an independent data set will be required. This will be particularly necessary for the validation of any candidate biomarker as a surrogate end point. To facilitate data pooling and replication, we are sharing our protocols and standard operating procedures with other research groups (for example, the Australian Cooperative Research Centres (CRCs), the French Fondation FondaMental, the Chinese Key 973 programme, the Foundation for the US National Institutes of Health (FNIH) and the Canadian Province of Ontario Neurodevelopmental Disorders (POND) network).

The outcome of this qualification advice process is an important step towards a shared understanding of biomarker criteria for ASD between academia, industry and regulators. Basic science is now at the brink of being able to identify molecular mechanisms and translate them into effective therapeutic targets for the treatment of individuals with ASD. The validation and qualification of ASD biomarkers will be key to: help to give industry the confidence to carry out the costly large-scale clinical trials that are needed to assess the efficacy and mechanism of therapeutic interventions; delineate the patient populations that will benefit from such interventions; and facilitate the regulatory approval of new therapies.

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Competing interests statement
The authors declare competing interests: see Web version for details.

FURTHER INFORMATION

***Diagnostic and Statistical Manual of Mental Disorders*, fifth edition:** http://dsm.psychiatryonline.org/doi/book/10.1176/appi.books.9780890425596

SUPPLEMENTARY INFORMATION

See online article: S1 (table) | S2 (box)

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