Phenotyping, Etiological Factors, and Biomarkers: Toward Precision Medicine in Autism Spectrum Disorders

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ABSTRACT: Despite the progress made in understanding the biology of autism spectrum disorder (ASD), effective biological interventions for the core symptoms remain elusive. Because of the etiological heterogeneity of ASD, identification of a "one-size-fits-all" treatment approach will likely continue to be challenging. A meeting was convened at the University of Missouri and the Thompson Center to discuss strategies for stratifying patients with ASD for the purpose of moving toward precision medicine. The "white paper" presented here articulates the challenges involved and provides suggestions for future solutions.

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Significant progress has been made in understanding the biology of autism spectrum disorder (ASD) over the past decade. However, effective biological interventions for the core symptoms remain elusive. Instead of a single or even a small set of causes, a consensus has emerged that genetic and environmental causes of ASD are likely multifactorial. The genetic architecture of ASD has become increasingly clear and increasingly complex with estimates of at least 1000 genetic alterations associated with the risk for ASD.[1] These findings hint at starting points for patient stratification and precision medicine for ASD, and indeed, gene targeting has spawned efforts at clinical trials. For example, research exploring the synaptic mechanisms impacted by the fragile X gene in multiple preclinical animal models has led to trials in fragile X and ASD with negative modulators of metabotropic glutamate 5 receptors.[2] Evidence from other case series[3,4] has fostered clinical trials that aim to modulate glutamatergic and GABAergic functions. Despite the promise of targeted therapies based on a biological rationale, much heralded trials with agents such as the GABA B receptor agonist arbaclofen failed to reveal significant effects for the selected primary outcome measures in Phase II clinical trials.[5] This perceived "failure" is likely due to the etiological heterogeneity of the subjects with ASD who received the specific treatment. A review of the data for the arbaclofen study suggests a strong positive response for at least a subset of fragile X and patients with ASD. Positive responses in some individuals, but otherwise statistically nonsignificant beneficial group effects, are characteristic of most of these early pharmacological treatment trials of ASD. Thus, a critical challenge is to identify those individuals (or a subset of individuals) who may benefit from a particular treatment in a clinical trial. A meeting was convened on October 18, 2014, at the University of Missouri and the Thompson Center to foster discussions on strategies for stratifying patients with ASD for the purpose of translating this information to targeted and individualized experimental therapies, a core principle of precision medicine. Attendees agreed that the ultimate development of biomarkers would allow for patient stratification in treatment trials and could translate into safer and more effective individualized treatments. The "white paper" presented here articulates the challenges involved in developing better diagnostics and treatments based on individual biomarkers, and provides some suggestions for future solutions.

COMPLEXITIES OF AUTISM SPECTRUM DISORDER

Autism spectrum disorder (ASD) encompasses a wide range of clinical presentations.[6,7] Heterogeneity can even be observed in the former nomenclature for autism spectrum disorder, consisting of autistic disorder (impaired communication and socialization, repetitive behaviors, and onset before age 3), Asperger disorder (without delays in language or cognitive development), and pervasive developmental disorder—not otherwise specified (features of ASD but not meeting criteria for either autistic disorder or Asperger disorder).[8] As a result, multiple studies have attempted to suitably cluster

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symptoms in large populations. A number of studies have explored factor analysis to determine the structure of symptoms, focusing on "core features" of ASD, revealing a variety of sets of clusters, but overall suggesting that social/communication issues may be distinct from restricted and repetitive behaviors and interests.[9] Another recent study identified 4 phenotypic clusters and found that they varied in short-term prognosis regarding diagnostic stability.[10] To understand how ASD-related characteristics are manifested in the general population, one recent study clustered 2343 cases based on the autism spectrum quotient (AQ), revealing 2- and 3-factor solutions varying in combinations of severity of impairments in socialization, mentalizing, and orientation to detail.[11]

Autism spectrum disorder can also be associated with a range of co-occurring medical and/or psychiatric conditions, including seizures, gastrointestinal conditions, sleep disturbances, aggressive behaviors, anxiety symptoms, and attentional deficits. These conditions may or may not be associated with cognitive impairment. One recent study also incorporated co-occurring medical and biological variables in the generation of data-driven phenotypic clusters, revealing clusters for (1) circadian and sensory dysfunction, (2) immune abnormalities, (3) neurodevelopmental delay, and (4) stereotypic behaviors in one analysis of ASD-associated features.[12] Although the best course of treatment is clear for some of these conditions (i.e., treat seizures with antiepileptic drugs), it is not known how these various co-occurring phenotypic aspects might relate to potential targeted treatment of the core features.

Several studies have assembled a rich phenotypic database in cases in which genetic information is available, yielding a set of genotype–phenotype clusters.[13–15] Additionally, "complex autism," characterized by the presence of prominent dysmorphic features suggesting altered early morphogenesis, has been found to be associated with greater impairment and a markedly higher rate of chromosomal disorders or broader syndromic conditions in which ASD is a common manifestation.[16,17] Other studies have also examined how clusters derived based on diagnostic scores relate to detected genomic variations.[18,19] Distinguishable subphenotypes of ASD for transcriptomic and genetic analyses have been found based on multivariate cluster analyses of severity scores queried by the Autism Diagnostic Interview-Revised diagnostic instrument,[20,21] revealing differential gene expression (relative to nonautistic controls) by the ASD subtype[22] as well as subtype-dependent single-nucleotide polymorphisms[23] and linkage regions,[24] with class prediction analyses suggesting the potential for developing biomarker screens.[25] One group more recently subtyped ASD into 2 networks of highly connected genes,[26] while others have examined genetic factors associated with ASD in 6 genetic syndromes that increase the risk for ASD, finding that the pattern of genetic factors could be applied to detect a similar signature in idiopathic ASD.[27] Another approach looked at genetic profiles of specific symptoms, such as impaired social communication,[28] and yet others have found evidence that the core deficits are genetically heterogeneous.[29]

Clearly, subtyping of ASD according to the cause or pathobiology could be highly relevant to individualized treatment. With at least several hundred to 1000 different genes[1,30] estimated to play a role in ASD risk, development of a "one-size-fits-all" pharmacological intervention would be tremendously challenging. Some of these genes contain rare variants with high penetrance and are directly involved in the cause of ASD, whereas other genes serve as risk factors for ASD that may act in concert with other genetic or environmental risk factors.[1,31–34] Some individuals who harbor such variable penetrance variants develop ASD, whereas others harboring the same variants do not. In most cases, no genetic factor is identified to contribute to the diagnosis. Increasing recognition of environmental factors that seem to contribute to ASD may impact a cause–phenotype map. Therefore, a detailed assessment of factors in ASD that would be potentially meaningful in guiding a precision medicine approach must also explore a range of factors beyond genotype and clinical phenotype.[35] Finally, it is not yet known whether the heterogeneity of ASD in this context is represented by continuous variability on multiple dimensions or is represented by clusters, which may also have implications for treatment.

WHAT IS A "BIOMARKER"? ARE BIOMARKERS FOR A BEHAVIORAL CONDITION SUCH AS ASD DEFINED THE SAME WAY AS THOSE FOR A DISEASE SUCH AS CANCER?

Biological markers, or "biomarkers," are broadly defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.[36] For autism spectrum disorder (ASD), a broad range of candidates could be considered relevant biomarkers. Biomarkers can be markers of brain activity or anatomy (electroencephalogram [EEG], imaging), genetic, epigenetic, or proteomic, and metabolomic markers (which can range from indicators of immune function, to oxidative activity, to neurotransmitter function). Broadly speaking, clinical outcomes, that are based on direct assessment of the individual, could be considered as markers as well. Certainly, medical (seizures, sleep disturbances, gastrointestinal conditions) and psychiatric comorbidities (aggression, anxiety, attentional deficits) can direct a pharmacological treatment approach, as described in the previous section. For other markers more specific to ASD, the relationship to treatment is less clear. Specific core features such as social communication and reciprocity deficits, repetitive behaviors/hyperfocused interests, and associated features such as sensory hypersensitivity may represent relevant treatment targets.

Additionally, the developmental trajectory must be considered in any biomarker approach, as mechanisms of actions that impact the developmental trajectory of neural systems at one stage may have an entirely different relevance at a later stage.[37] Thus, a developmental systems approach is also necessary in consideration of relevant biomarkers.

The Research Domain Criteria (RDoC) initiative at the National Institute for Mental Health[38] targets specific feature domains as an approach to research across a range of mental disorders, an important consideration in light of the marked heterogeneity of ASD. It proposes the use of targeting symptom domains rather than focusing on targeting a diagnosis that represents a constellation of symptoms. In ASD, it will be important to determine how the severity of specific symptoms cosegregates with the presence of genetic or nongenetic biomarkers, including anatomical and functional indicators of alterations of neural systems, while accounting for the relevant impact of the developmental trajectory, to best facilitate symptom-specific individualized treatment approaches. Although previous work, described in the previous section, has examined the association between genetics and clinical markers,[17,19,28,29] incorporation of other molecular data (e.g., transcriptomes[39]) will also need to be considered.

In addition to protein-coding genes, noncoding RNAs may also play a role in the cause of ASD, as shown by dysregulation of microRNAs in ASD.[40–44] A recent genome-wide association study (GWAS) identified a significant association with an single nucleotide polymorphism that is not located on a coding region but rather resides in a noncoding RNA that is an antisense inhibitor of the gene for moesin, a protein that regulates neuronal architecture.[45] This discovery demonstrates the potential contribution of noncoding RNA in ASD risk. Furthermore, evidence of convergence of the molecular pathways has been reported at the alternative splicing[46,47] and transcriptome level,[48] and the importance of mRNA expression has been increasingly emphasized in recent years.[32,33,49] Other epigenetic markers have also been identified in association with ASD.[50,51]

In general, the roles of DNA methylation, histone acetylation, and microRNA markers in ASD are less well understood at this time. However, these issues have become increasingly important in other fields of medicine, such as in the treatment of cancer. With many different types of cancer, recent research has revealed the importance that biomarkers play in optimizing treatment approaches. DNA methylation patterns have predicted who is most likely to respond to certain glioblastoma therapies,[52] and it is widely known that hormonal markers predict the response to hormone therapy in breast cancer.[53] Therefore, related approaches may become increasingly important in ASD.

Particular attention should also be paid to biomarkers that have a relevant function or relationship to neural systems responsible for the expression of particular phenotype(s) during specific developmental epochs. For example, certain synapses or regional circuits may be excitatory during one phase of development but inhibitory during another epoch. This synaptic physiology may differentially affect expression of a given phenotype or biomarker during specific developmental periods. Abnormalities in the glutamatergic and GABAergic systems have been observed with some consistency in ASD in postmortem brain studies,[54–56] and in vivo with regional findings from magnetic resonance spectroscopy (MRS),[57,58] or when expressed as a ratio of GABA to glutamate with MRS.[59] There may also be the potential for peripheral measurements.[60] Mutations affecting the GABAergic system have also been associated with ASD.[61,62] As recent large clinical trials have attempted to target glutamatergic (memantine) and GABAergic (arbaclofen) systems,[5] markers representing activity in these systems, as assessed by MRS or other proxy markers such as EEG gamma band activity,[63] would seem highly relevant.

Other markers that may be relevant for treatment might include immune markers that are often atypical in ASD,[64,65] whole-blood serotonin,[66,67] genetic polymorphisms that impact the serotonergic system,[68] or serotonin ligand markers on positron emission tomography.[69–73] Oxidative reactivity,[74] and psychophysical reactivity indicative of sympathetic/parasympathetic tone,[75] may identify subjects who may be more responsive to metabolic or adrenergic treatments.

Aside from the role of MRS described above, the plethora of neuroimaging findings (especially from anatomical and functional MRI and from more recently diffusion-weighted imaging) raises the question whether any of these may reliably relate to etiological and other biomarkers discussed above, or whether some imaging findings themselves may be considered biomarkers of ASD. A serious challenge is the frequent lack of replication of functional and anatomical imaging findings, which can be in part attributed to methodological issues,[76,77] and the complexity of maturational trajectories.[78] Functional and anatomical information about the brain is being made available through the large neuroimaging initiative, the Autism Phenome Project (APP), exploring brain size and structural changes across development in a systematic manner.[79] Additionally, the NIH-funded Autism Centers for Excellence Program include some projects related to this issue, including brain imaging studies looking at neurodevelopmental patterns associated with genetic variants, studies examining predictors of social disability and language development, and studies aimed at better understanding the role of gender in ASD.[80] The grass-roots datashare consortium ABIDE[81] provides large multiscale samples of resting-state functional magnetic resonance imaging (MRI) and anatomical MRI data that may be leveraged for identification of imaging-based ASD subtypes.

Common sample size limitations highlight an additional deeper problem: the presence of expected,[82,83]

but currently unknown etiological subtypes of ASD. In typically sized samples of 20 to 50 participants with ASD, different (unknown) subtype compositions may bias imaging findings one way or the other, resulting in divergent findings across groups and studies. However, a recent study by Ellegood et al.[84] found that 26 different ASD-associated mouse models converged onto 3 clusters of brain anatomical features from MRI, which suggests that outcome neuroimaging may be a powerful tool in the detection of ASD subtypes with specific treatment response, despite genetic heterogeneity. Among the well-replicated imaging findings in ASD is anatomical overgrowth in the first postnatal years,[85–89] with some concordant evidence from diffusion-weighted imaging.[90–92] Because early overgrowth is found not only in gray but also in white matter, it may be a causal contributor to network connectivity abnormalities that have been detected in numerous functional connectivity MRI studies of older children and adults with ASD,[93] despite residual uncertainty as to the implications of underconnectivity[94] versus overconnectivity[95,96] findings. Functional connectivity has also been proposed as a biomarker with relevance to treatment.[97] These factors also need to be framed in terms of the neural systems impacted during specific developmental time windows. Because several factors may have critical temporally specific effects on neural systems, resulting in the phenotypic expression of certain behaviors, cognitive dysfunction, or other comorbidities, numerous biomarkers should be considered to best facilitate symptom-specific individualized treatment approaches, and move toward personalized medicine in ASD.

What Are Examples of Biomarkers That Have Guided Clinical Treatment?

There are a number of examples of in which biomarkers have facilitated treatment trials and individualized medicine approaches. Application of individualized medicine has been particularly helpful in the field of oncology. Although most aspects, including the pathobiology and the treatment goals, in oncology do not relate to autism spectrum disorder (ASD), lessons can be learned from this field due to the fact that it is characterized by considerable etiological heterogeneity.[98] Recent research has revealed that biomarkers in breast cancer not only provide valuable prognostic information, but can also guide therapy.[99] For example, amplification of the oncogene human epidermal growth factor receptor 2 (HER2) predicts good response to anti-HER2 targeted treatment in breast cancer.[100,101] Similarly, presence of estrogen receptors in breast cancer predicts a significantly better response to tamoxifen.[53] Other areas of cancer treatment have been impacted as well. The presence of the KRAS mutation predicts response to anti-epidermal growth factor receptor (anti-EGFR) monoclonal antibody (MoAb) therapy in the form of cetuximab or panitumumab for metastatic colorectal cancer.[102] The value of molecular subtyping has also been recognized outside the field of oncology, such as in cystic fibrosis. In studies attempting to improve lung function by increasing the activity of the cystic fibrosis transmembrane conductance regulator (CFTR) protein, the presence of at least 1 copy of the G551D-CFTR mutation enhances response to ivacaftor, a potentiator designed to increase the time that activated CFTR channels stay open.[103] Thus far, ASD has not yet benefited from this approach.

WHAT ATTEMPTS AT DEVELOPING BIOMARKERS FOR ASD HAVE BEEN PERFORMED OR ARE UNDER WAY?

Hu and Lai[25] and Pramparo et al.[104] have reported panels of differentially expressed genes that may potentially be used for diagnostic screening. To explore genetic markers for autism spectrum disorder (ASD), several large genetic collaborations have been undertaken. The Autism Genetic Resource Exchange (AGRE) is a research repository that has collected genetic information, clinical information, and biomaterials from over 2000 families, focusing on families with 2 or more children with ASD.[105] The Simons Simplex Collection (SSC) has collected genetic samples and detailed clinical information from 2600 families with 1 affected child with ASD,[106] and The Autism Simplex Collection (TASC) has over 1700 families with 1 affected child with rich phenotypical information.[107] Other much larger efforts (>10,000 participants) being developed include MSSNG (https://www.mss.ng/), a collaboration between Autism Speaks and Google to create the largest genomic database on autism, and Simons Foundation Powering Autism Research (SPARK) (https://development.sparkforautism.org/portal/page/about-spark/), funded by the Simons Foundation, which will establish a genotyped research cohort of 50,000 individuals with ASD and their families across the United States. Although the baseline phenotypic information required from all participants in SPARK will be relatively low, much effort will be directed at engaging participants, to increase their interest in deeper phenotyping efforts. Participants in this cohort will donate saliva biospecimens by mail, and further genetic analyses will deepen the field's growing knowledge of the major genetic factors that play a role in ASD. The large cohort will be open to recontacting from the research community to ultimately enable more genotype-driven clinical research in ASD, which may translate into genotype-driven therapeutics and treatment of ASD. Also, The Autism Treatment Network (ATN) has phenotypic information incorporating data with a particular emphasis on medical comorbidities on nearly 7000 patients ranging from ages 2 to 18,[108,109] and biomarker sample collection has occurred in a subset of these patients.

WHAT IS THE ROLE OF PHENOTYPING IN DISCOVERING BIOMARKERS FOR ASD?

Although biomarkers alone may provide critical information regarding an individual's underlying autism

spectrum disorder (ASD)-associated biology, phenotypic information would provide additional critical data, that is also more readily available in the clinical setting, which would allow the researcher or clinician to select the optimal individualized treatment for each patient. Additional clinical information may further interact with the relationship between a biomarker and a treatment response. Such incorporation of rich behavioral and phenotypical information alongside the biological information allows the clinician to identify characteristics that might be associated with these biomarkers for the prediction of a best treatment plan. As described with the NIMH RDoC,[38] this approach allows a more nuanced understanding of potential outcomes that a treatment might target. Previous biomarker development efforts have varied in the degree to which phenotypic information is incorporated. For subtyping patients with potential relevance for individualized treatment, greater phenotypic information will be necessary.

CURRENT STRATEGIES FOR PERFORMING PHENOTYPING

To date, strategies for conducting phenotyping have largely been either data-driven or outcome-based. Most of these previous efforts at phenotyping have been data-driven, as detailed in earlier sections, targeting symptom clustering,[11,12] gene clustering,[17,19,28,29] and genetics first approaches,[110] and most other efforts have focused on deriving phenotypical clusters within autism spectrum disorder (ASD) based on clinical aspects that cosegregate cases within this group. However, we submit that outcomes should become a key aspect of phenotyping ASD for the optimization of treatment approaches. To understand which group of patients responds best to a particular treatment, the phenotypic subtyping of characteristics will be driven by the outcomes. A large data set will ultimately be necessary for this purpose to overcome apparent variability in treatment response due to other factors or confounders (e.g., the effects of day-to-day variability on assessments and placebo effects). However, data-driven and outcome-based phenotypic groupings may have significant overlap. For example, a clinical group associated with similar markers of GABAergic function may respond similarly to administration of GABAergic drugs. Such hypothesis-driven subtyping would allow critical information to be derived from clinical interventions in a considerably smaller population. However, it is also possible that more than 1 data-driven phenotypic group may cosegregate with a similar response to treatment, thus resulting in multiple data-driven phenotypic clusters mapping to 1 outcome-based cluster. Alternatively, there may be 2 different treatment response groups, with opposite response to treatment, within 1 data-driven phenotypic group, resulting from 2 different pathophysiologies leading to 1 common clinical phenotype, but a different response to treatment. In this manner, one data-driven phenotypic cluster could map to multiple outcome-based clusters. To this point, we have very little information on how data-based and outcome-based phenotypes are interrelated. And, as mentioned in previous sections, the development of these clusters must also take into account the interactions with the developmental stage regarding the impact on the effects on the neural systems in moving toward precision medicine in ASD.

ENVIRONMENTAL EXPOSURES AS A POTENTIAL BIOLOGICALLY SALIENT STRATIFICATION VARIABLE: THE ASD "ENVIROME"

Although earlier reports based on twin studies suggested that autism spectrum disorder (ASD) has a heritability as high as 0.9,[111–115] recent evidence has suggested that the purely genetic component in the cause of ASD is somewhat less than previously believed.[116–118] Although our understanding of environmental causes is far less than that of genetic causes, their impact on the underlying neural systems associated with the expression of ASD must be considered as well. This area of research has increased in recent years, with several environmental factors gaining prominence. These lines of investigations suggest the hypothesis that the ASD "envirome" interacts with specific underlying neural systems (genetically determined) in the developing human brain to contribute to the expression of ASD.

One such nongenetic contributing factor for ASD is immune system dysregulation, which has been frequently described in individuals with ASD and their family members.[65] Most notably, mothers of children with ASD have been reported to harbor antibodies reactive to fetal brain proteins, which are absent in mothers of children who are typically developing or of children with non-ASD developmental delays.[119–121] The protein target antigens of these ASD-specific maternal antibodies were recently identified; it is the recognition of various combinations of these proteins by maternal antibodies that confers the specificity of maternal antibody-related (MAR) ASD.[122] Antibody reactivity to these proteins was noted in 23% of mothers of children with ASD, versus less than 1% in women with typically developing children, which represents a much higher proportion of ASD than any single gene. The etiological relevance of these antibodies is further supported by numerous rodent and nonhuman primate studies in which injection of these ASD-specific maternal antibodies into pregnant animals resulted in MAR autism-relevant behaviors in the offspring.[123–127]

Exposure to psychosocial stressors[128] or tropical storms[129] in the late second to early third trimester is also associated with an increased incidence of ASD. Increased risk has also been found with various other stress exposures in epidemiology studies from the Danish and Swedish cohorts.[130,131] In one study, this association was specifically present when maternal psychiatric history was incorporated in the data analysis.[132] The risk of ASD

associated with prenatal psychosocial stress seems to be linked to maternal genetic susceptibility to greater stress reactivity.[133] Furthermore, in a rodent model, prenatal stress exposure in offspring of genetically stress susceptible mothers has been shown to result in aberrant social behavior,[134] which was also associated with delayed migration of GABAergic neurons during development.[135] Maternal exposure to stress before pregnancy and even early life stress is associated with increased risk for development of ASD in subsequent pregnancies according to recent findings in data from the Nurses' Health Study.[136,137]

Data are also mounting regarding maternal exposure to pollutants resulting in increased risk of ASD. In particular, there is a growing body of literature implicating air pollutants,[138–147] with evidence of an interaction with polymorphisms of the MET receptor tyrosine kinase gene.[144] There also seems to be a modestly increased risk of ASD with exposure to drugs, including certain serotonin selective reuptake inhibitors (SSRIs) and valproic acid.[148,149] Earlier research suggested that the risk of ASD in association with exposure to β2-adrenergic agonists, commonly used to arrest premature labor, is affected by maternal polymorphisms in the β2-adrenergic receptor.[150] More such targeted approaches to gene/environment interactions of this type may be helpful in the future, exploring a targeted set of genes most likely to interact with the environmental factor under investigation, in addition to big data approaches to search for the interactions that would not be predicted in this manner. Other factors are also being explored and identified, including pesticides, endocrine-disrupting chemicals, and a host of maternal dietary factors, including a lack of folate supplementation during early pregnancy,[151–153] or even excessive supplementation of folate during pregnancy.[154] Aside from nutritional factors, the intrinsic hormonal status of individuals (especially elevated fetal testosterone levels) may also increase the risk for ASD, as suggested by the "extreme male brain hypothesis."[155] The nuclear hormone receptor RORA, a regulator of transcription of genes linked to ASD,[156] may contribute to elevated testosterone levels by reducing the expression of aromatase.[157] Increased parental age and short intervals between pregnancies have also been observed as risk factors.[158,159] Other factors have been explored that have not revealed an association with ASD, such as heavy metals.[160]

These factors are not really biomarkers in and of themselves, but rather potential risk (or protective) factors for ASD on their own or possibly in conjunction with certain genetic profiles. More typical biomarkers are being explored that could be used to indicate past exposures to these environmental factors, such as blood DNA methylation patterns that have emerged as an indicator of smoking history.[161] However, this exemplar may not necessarily be salient as a biomarker for ASD. Instead, as this literature evolves, it will be important to include potential environmental causes in the ASD biomarker development process—that is, include environmental factors in outcome-based clustering of patient populations or analyses of genetic contributors. Inclusion of environmental factors has proven valuable in other conditions. For example, genetic effect sizes have been found to vary and even new diabetes-associated specific loci have been identified when body mass index (BMI) and possible interactions with BMI are considered in the genetic analyses.[162–165] Thus, the identification of biomarkers, and ultimately perhaps the effectiveness of a treatment for any individual, may be improved by incorporating important ASD "envirome" elements in the process. Therefore, the ASD "envirome" as a risk factor must be considered in future biomarker research as we expand our understanding of these aspects and move toward precision medicine in ASD.

PHENOTYPING, BIOMARKERS, AND TREATMENT TRIALS

A. Biomarker-Rich Setting for Early Stage Trials to Inform Larger Trials

The incorporation of data on a rich set of these aforementioned factors into large clinical trials would provide important answers regarding which patients are most likely to respond to a given treatment, and which clinical aspect is most responsive to that treatment. However, clearly, this approach is neither practical nor cost efficient during the earlier stages of drug development. A strategy therefore must exist earlier in the stage of identification of distinct compounds, for identifying which biomarkers are worthy of exploration. Major investments in effort should be limited to subsequent larger trials. In light of both the failures of recent large autism spectrum disorder (ASD) trials,[5] and the current research funding climate, novel paradigms must be first developed to explore newer agents in future pilot trials. Smaller pilot clinical trials that assess these newer agents should be conducted looking for effects in the overall ASD population. However, each pilot trial should also investigate selective effects in a hypothesis-driven subset of patients that is based on biomarkers expected to be closely related to the response to that particular treatment. Exploratory analyses could also assess whether other potential markers might also be related to treatment response. For example, biomarkers of GABAergic activity might reasonably be expected to predict response to GABAergic agents in ASD, and could therefore serve as hypothesis-driven biomarkers for treatment response in this case. Results from studies at this level could then be incorporated in the planning of larger trials.

This process would exemplify a reasonable model in which neural systems are being targeted, while sensitive to the developmental timing involved. One critical question is whether earlier intervention could lead to improvement not only in symptoms at the time of the trial but also an improved developmental trajectory.

Also, it is possible that treatments that seem to benefit early, such as decreased behavior in the setting of administration of medications causing sedation, may not have optimal long-term outcomes. Thus, age of participation and long-term monitoring may be other crucial components to consider for incorporation in future clinical trials.

These aspects could also be considered to mine existing data in the recent "failed" larger trials to systematically identify subgroups that are or are not most likely to be best responders, or to identify subgroups in studies of other agents still currently under investigation, such as glutamatergic markers for memantine,[166,167] measures of oxytocin activity for treatment with oxytocin,[168–171] or serotonergic markers for buspirone,[172] among many possible examples. The response of biomarkers to treatment would also provide critical information that allows for both better understanding of the mechanism of action, and for future research to further refine treatments, through biomarker-targeted trials. Furthermore, conversations would need to occur with the Food and Drug Administration to provide guidance and establish pathways for approval with new drugs discovered in this manner. In recent years, novel drug development has focused heavily on compounds derived from exploration of the mechanism of action of ASD-associated mutations.[173] However, the current arsenal of psychopharmacological agents in clinical use today contains no significant direct contribution from drugs from this pipeline.[174] Although we believe that further exploration using this mechanistic-driven approach is vitally important in the long term, we acknowledge that an exclusive mechanism-focused approach will very likely miss opportunities for the development of new and impactful treatment options in the near future. Agents showing promise for ASD should thus be explored regardless of whether or not they were derived from a molecular mechanistic approach (Figure 1).

B. Cause/Biomarker Mapping, Allowing Development of Novel Compounds with Animal Models, Translating to the Clinical Setting in a Targeted Manner

The information derived from research in a biomarker-rich environment would inform the development and assessment of novel compounds in animal models. To optimize this approach, a cause/biomarker map should be established from large clinical populations. The knowledge from ASD envirome studies as well as developmental and cognitive neuroscience should modify the cause/biomarker map at appropriate nodes. Genes associated with ASD have been used extensively in the development of preclinical animal models, including mice, rats, drosophila, and zebrafish. These models have subsequently been used for the assessment of ASD risk genes and responses to novel treatments, with the advantage that ASD-relevant behavioral outcomes can be explored.[175] Additionally, animal models allow for the assessment of resulting behavioral and physiological effects produced by the genetic mutations and/or experimental treatments that is not possible in humans.

Although therapeutic treatments based on animal studies that use genetically modified animals in this manner can subsequently be explored in a clinical population, one can only be confident of a response in patients with ASD caused by this particular mutation (representing only a small fraction of the overall population), and potentially others with a very similar mechanism. For this reason, the Preclinical Autism Consortium for Therapeutics (PACT) was developed. The PACT aims to use a selection of genetically modified rodent models in a standardized parallel method to assess the effects of potential new pharmacological interventions.[176]

With an increased understanding of cause/biomarker maps in ASD, the investigator can make even broader predictions regarding who is most likely to respond to a drug beyond specific genetic mutations. By recognizing how different etiological factors modify developing neural systems related to biomarkers and the ASD "envirome," one can identify clusters of biomarkers that may cosegregate with specific causes. With this information, patients who do not have ASD resulting from a defined cause studied in a mouse model, but rather have similar biomarkers to the particular cause studied, might be expected to respond similarly to the drug tested. For example, patients who do not have a known ASD-associated GABAergic mutation but who have a similar level of GABAergic activity, as potentially assessed by magnetic resonance spectroscopy or electroencephalography markers,[57,63] as patients with these mutations might also be expected to respond similarly to drugs developed based on the GABAergic mutation-based ASD mouse model. Furthermore, animal models have also been developed for environmental factors, including prenatal stress models,[134] maternal immune models,[125,126,177] and drug effect models.[148] Therefore, these principles can also be extended to incorporate environmental cause/risk factors onto the cause/biomarker map of developing neural systems. Exploration of the effects of treatment response is now beginning to be explored in the environmental animal model setting as well.[178] With this approach, an expanded cause/biomarker map can result in much broader and richer translational impact with new drug development efforts through animal models. Additionally, this approach would also be valuable for refining the understanding of which individuals are most likely to be a best responder to drugs that are not derived from the new drug development pipeline, and may help reveal the mechanism of action for future, more targeted treatment (Figure 1).

C. How Can Induced Pluripotent Stem Cells Support Biomarker Development?

Another important tool in new drug development is induced pluripotent stem cells (iPSCs) in which, for

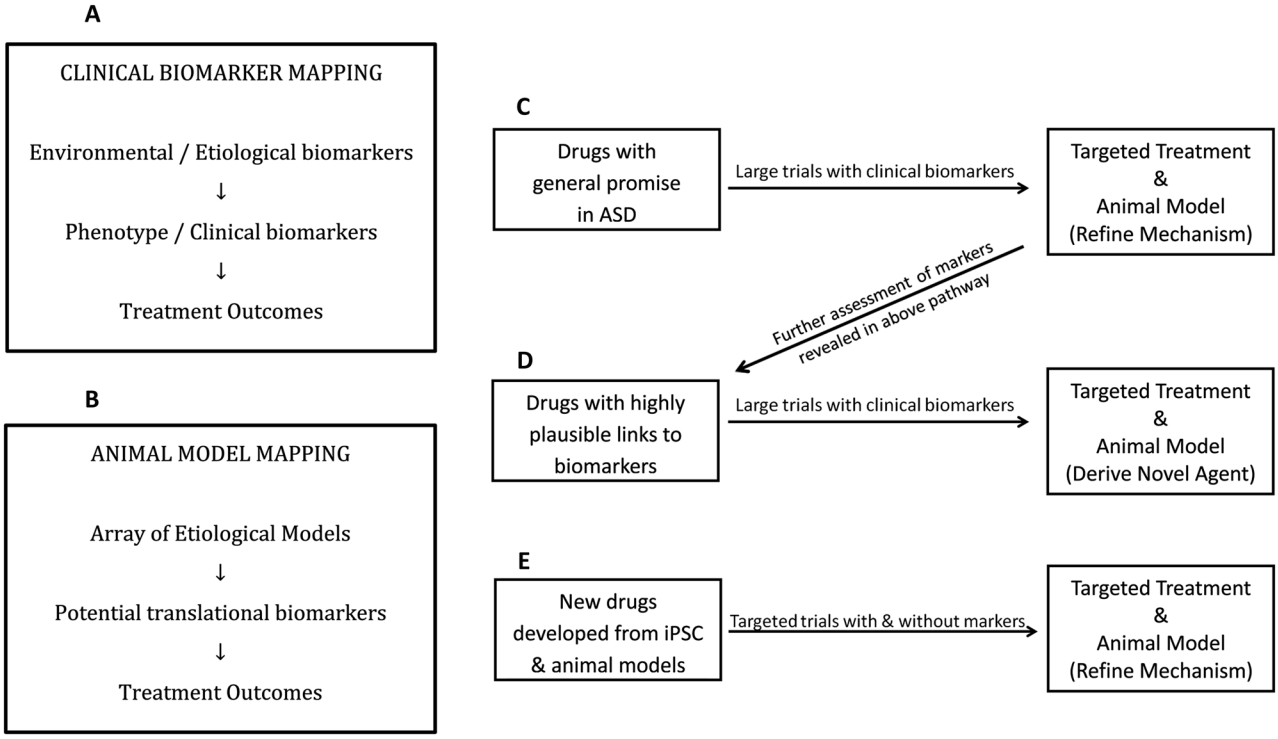

Figure 1. Outline of suggestions for research progress toward precision medicine. **A**, One critical initial step is generation of establishment of a map of how etiological factors relate to phenotypes and clinical biomarkers in the clinical setting. With this established, one can identify the biomarkers that are associated with specific causes. Furthermore, one can identify a set of other patients with no known cause that may have common biomarkers with a group with a specific cause, which then allows the possibility of determining whether they have a common pattern of treatment response in subsequent trials, either for domain-specific responses or for more global responses. Numerous efforts at exploring biomarkers are underway across a variety of selected settings. **B**, To allow animal model translation to the clinical setting, these markers should also be explored across a range of animal models. This will allow future testing of new agents across causes and biomarkers. Ellegood et al.[84] have done this for brain imaging markers across animal models. **C**, For drugs that have shown promise in a broad range of patients with autism spectrum disorder (ASD), whether derived from a molecular drug development approach or not, large trials to further explore efficacy should be taken as an opportunity for biomarker discovery, an exploratory examination for particular causes or biomarkers as they relate to treatment response. This will allow future targeted trails to confirm these associations. The effects of treatment on the biomarkers will help with understanding of the mechanism, and will also contribute to subsequent animal model studies to further refine the understanding of the mechanism and develop more targeted therapeutics. **D**, For drugs with a highly plausible link to particular causes or biomarkers (e.g., GABAergic-related biomarkers for drugs targeting the GABAergic system), or drugs with causes or biomarkers associated with treatment response discovered in the above pathway (in **C**), targeted treatment trails can be explored, with targeted biomarkers, for which the study should have sufficient power to determine salience of the biomarker for the trial outcome. With monitoring of the effect of treatment on biomarkers, this will also allow subsequent animal models to further refine the understanding of the mechanism and develop more targeted therapeutics. **E**, Similarly, new drugs derived from induced pluripotent stem cells and genetic animal models would be assessed with targeted biomarkers in the trial setting. If the patients without the predicted markers also show a positive treatment response, though, the drug could then be reexplored in the exploratory biomarker setting, described above (in **C**), and in other etiological animal models, to identify other biomarkers salient to treatment response and to move toward a better understanding of its impact.

example, skin or hair cell cultures can be generated by inducing adult cells to behave as stem cells, which are then differentiated into neurons or other central nervous system cell types. Studies of iPSC-derived neurons in other diseases show some potential for identifying disease-specific, cellular phenotypes that can lead to potential therapeutic candidates. For example, studies on amyotrophic lateral sclerosis patient-derived motor neurons found that such neurons are hyperexcitable when compared with control neurons and that gene targeting of the disease mutation corrected the hyperexcitable phenotype.[179] Furthermore, the drug retigabine was also shown to correct the hyperexcitable phenotype and improves the in vitro survival of patient-derived motor neurons. A clinical trial is now underway to evaluate the effect of retigabine in motor neuron activity in patients affected with amyotrophic lateral scleroisis (ClinicalTrials.gov Identifier: NCT02450552). Culturing these cells from individuals with ASD is a direct path to assessing the effects of mutations that are known to cause ASD, using patient-derived iPS cells, for their impact on a broad range of aspects of cell physiology. This allows the efficient assessment of the nonbehavioral impacts of a broad range of gene mutations that would require a large number of animals and a significantly longer amount of time if attempted in vivo. Although there is tremendous potential in this approach, success depends on studying specific cellular phenotypes in specific neural subtypes that are relevant to ASD pathophysiology.

A recent study using 3-D neural cultures (organoids) derived from patient-derived iPSCs with idiopathic ASD found evidence of overproduction of inhibitory neurons,

influenced by FOXG1 overexpression.[180] Knockdown of FOXG1 by RNAi in ASD-derived organoids restored the balance between inhibitory/excitatory production of neurons, identifying FOXG1 as a potential drug target. Future translational work is needed to test the therapeutic impact of targeting FOXG1 in idiopathic ASD.

Induced pluripotent stem cells offer the advantage of scalability over animal models. Effects of novel therapeutics can be assessed in a very efficient manner with iPSC models.[39,181–183] Furthermore, the fact that this is based on human mutations in human tissue provides further advantages in potential translational impact as compared with animal models. However, iPSCs are limited in their ability to explore both nongenetic causes and in cases in which ASD pathology results from effects requiring the interactions of multiple neural systems during specific developmental epochs. There are also significant technical challenges inherent in iPSC research, including challenges in the specification and maintenance of cerebral cortex neuronal cell types. Initial costs and access to technology limit the groups that can undertake large-scale studies across multiple genetic causes of ASD at this point for iPSCs. This limitation may become less problematic as these technologies become less expensive and more widely available.

THE FUTURE. WHAT WILL BE NEEDED TO DISCOVER BIOMARKERS OF ASD?

Given the complexity of the disorder and the heterogeneity of the etiologic and interacting factors, this will be a daunting task. We propose a multipronged approach, which will gather data from multiple sources that can be integrated to tackle the problem. Large studies in clinical populations at specific developmental stages will need to be performed to generate a clearer understanding of cause/biomarker maps of autism spectrum disorder (ASD) (Figure 1). Genetic, epigenetic, environmental, and other aforementioned factors would all need to contribute. A large consortium of ASD clinics/centers will be required to generate this rich data set, acquired according to identical protocols, possibly enhanced by incorporating Web-based or remote data and media capturing. Current clinical databases may have collected similar information regarding ASD features, but are each in different formats, creating a bioinformatics challenge for consolidation into 1 large data set to address issues such as subtyping. The new larger initiatives such as SPARK, described above, may help with efforts to collect such large data sets. With the ability to recontact participants, large size of this cohort will lend itself to genotype-driven clinical research in ASD and studies of environmental risk factors as well.

Valuable information has been gained from larger epidemiology studies regarding environmental risk factors for ASD, such as the Nurses Health Study and the Danish and Swedish cohorts discussed in earlier sections. The Childhood Autism Risks from Genetics and Environment (CHARGE) study is tracking large samples of ASD and unaffected children in a case–control manner to determine etiological contributions from environmental exposures, inflammatory markers, and genetic factors, and their interactions in ASD (http://beincharge.ucdavis.edu/), which has already revealed a number of the potential environmental contributors described in earlier sections. Furthermore, studies such as the Markers of Autism Risk in Babies-Learning Early Signs (MARBLES) (http://marbles.ucdavis.edu/) and Early Autism Risk Longitudinal Investigation (EARLI) (http://www.earlistudy.org/) will provide valuable information on a range of factors by closely tracking the subsequent pregnancies of families with children with autism.

With all of this information, the genetic and environmental causes of ASD can then be explored in specific neural systems during distinct developmental epochs in animal models to assess the impact of novel treatments with particular unique etiologies. Subsequent clinical trials can be performed to examine the effect of the novel treatment in the general ASD population, and the treatment's impact on a relevant subset of individuals at specific ages predicted by the animal model findings and the cause/biomarker map. By monitoring the impact of potential treatments on ASD biomarkers, this will facilitate the understanding of the ASD-specific mechanisms. Subsequent animal models will further refine the understanding of the mechanisms and lead to the development of more targeted therapeutics. Such approaches have been initiated by large collaborations, such as the European Autism Interventions Multicenter Study (EU-AIMS),[184–186] funded by the Innovative Medicines Initiative, a public–private partnership between the European Union and the European Federation of Pharmaceutical Industries and Associations, with an investment totaling €2 billion. Another such collaboration is the Province of Ontario Neurodevelopmental Disorders (POND) Network,[187] funded by the Ontario Brain Institute, with support from the Government of Ontario, with an investment totaling $40 million (Canadian) across the neurodevelopmental disorders. In the United States, in 2015, $28 million was awarded for an NIH initiative to explore biomarkers for social and communicative function in ASD in a 5-site study led by investigators based at Yale University.[188]

It is hoped that there will be support for the United States to contribute in a more significant manner in this coordinated effort. For treatments currently under exploration in the clinical setting, investigation of the relationships between response and causes/biomarkers should be supported more broadly to better understand individualized effects for development of subsequent larger trials. The resulting goal would be to optimize targeting treatments within the ASD population. Furthermore, the existing collaborative approaches of this type have not yet targeted nongenetic etiologic risk

factors. As our understanding of environmental factors expands, it will be critical to incorporate these factors into future research of this nature. Expanding beyond exclusive exploration of mechanism-driven treatment options will further enhance the impact of this effort, particularly in the near future when the benefits of existing agents can be examined. The heterogeneous condition of ASD can be systematically assessed using a data-driven approach to sort ASD into empirically supported subtypes that can each be separately explored for the ultimate development of individualized treatment approaches, whereas monitoring the impact on salient biomarkers to guide future exploration. This line of investigation would likely be far more efficacious than approaches applied broadly to all patients with ASD and represents the most powerful approach moving forward in optimization of ASD treatment. Furthermore, intervention with an individualized approach at earlier ages may have a particularly profound effect on developmental trajectories. In combination with impactful behavioral therapies,[189–193] this approach should have a significant impact on the overall burden of ASD over a lifetime in the future. Additionally, optimization of the environment at home, providing family supports and continued behavioral and educational intervention beyond the end of the typical early behavioral intervention time frame will be critical to consider for optimization of impact on outcomes with or without pharmacotherapeutic intervention.

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