Translating precision medicine for autism spectrum disorder: A pressing need

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Autism spectrum disorder (ASD) is a heterogenous group of neurodevelopmental disorders (NDDs) with a high unmet medical need. Currently, ASD is diagnosed according to behavior-based criteria that overlook clinical and genomic heterogeneity, thus repeatedly resulting in failed clinical trials. Here, we summarize the scientific evidence pointing to the pressing need to create a precision medicine framework for ASD and other NDDs. We discuss the role of omics and systems biology to characterize more homogeneous disease subtypes with different underlying pathophysiological mechanisms and to determine corresponding tailored treatments. Finally, we provide recent initiatives towards tackling the complexity in NDDs for precision medicine and cost-effective drug discovery.

Keywords: Autism spectrum disorder; neurodevelopmental disorders; omics-based data analysis; precision medicine; systems biology

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Abbreviations: ASD, Autism spectrum disorder; NDD, Neurodevelopmental disorder; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; ADHD, Attention-deficit/hyperactivity disorder; LoF, Loss of function; CNV, Copy number variation; CNS, Central nervous system; FDR, False discovery rate; GABA, Gamma-aminobutyric acid; TD, Typically developing; CAMP, Children's Autism Metabolome Project; EHR, Electronic health record; DEPI, Database endophenotyping patient identification; FDA, Food and Drug Administration; ML, Machine learning; SSRIs, Selective serotonin reuptake inhibitors; CARS, Childhood Autism

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Autism spectrum disorder (ASD) is a highly heterogenous and lifelong neurodevelopmental disorder (NDD) that affects 1/54 (18.5/1,000) and 1/89 (11.2/1,000) children aged around 8 years in the United States and Europe, respectively, with a male to female ratio of 4:1 [1,2]. ASD is defined behaviorally according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria [3] based on early onset of two core symptoms during the development age: impaired social interaction and repetitive and restrictive behaviors. Although this broad and behaviorally-based definition is useful to stratify patients with ASD based on various levels of functioning it fails to acknowledge the diversity of genetic, neurophysiological, and clinical manifestations. Moreover, it does not offer sufficient insight into the underlying genetic or molecular causes of the disease, which has become a major bottleneck for the discovery of consistent biomarkers and the development of efficient drug treatments.

Currently, the disorder remains an area of high unmet medical need, with no treatments available to address the core symptoms. To date, the only approved drugs with an ASD indication (irritability associated with ASD) are aripiprazole (Abilify®) and risperidone (Risperidal®). However, both drugs do not address the core symptoms. Considering the high heterogeneity associated with ASD and multiple biological mechanisms that could potentially explain the pathophysiology of the disorder, it is unlikely that any single treatment will benefit the ASD population as a whole. This is indeed reflected by a considerable variability and transiency in the medications prescribed to manage the symptoms and comorbidities in patients with ASD, with specific medications prescribed depending on coexisting comorbidities. Therefore, precision medicine could be a promising approach for the treatment of ASD, as it offers specific drugs tailored for specific patient subgroups to tackle different molecular backgrounds.

ASD is predominantly idiopathic or of unknown cause with only 20% of the ASD population (i.e., secondary ASD) having a specific defined cause. Remarkably, heterogeneity also applies to secondary ASD including well-defined syndromic forms of ASD such as Fragile X syndrome [4]. Beyond the heterogeneity observed within patients diagnosed with ASD, the current nosological classification also brings a blurry definition of the boundaries between ASD and other psychiatric disorders. This is exemplified by the co-occurrence of ASD and other psychiatric disorders at rates higher than would be expected by chance. The significant degree of shared symptoms, neuropathology, and genetic etiologies suggest the existence of common clusters of targetable biological mechanisms including different DSM-5-defined NDDs. Taken together, due to the high heterogeneity and lack of specificity, the current behavior-based disease definition cannot serve as a starting point for drug development.

During the past decade, different fields of medicine have increasingly translated omics-based findings in patients into clinical practice. In contrast, ASD, alongside most NDDs and other neuropsychiatric conditions, is still characterized behaviorally, mostly overlooking disease etiology. Failure to acknowledge patient's biological heterogeneity in idiopathic ASD has repeatedly resulted in failed clinical trials and hindered the advancement of targeted drug development to address the core symptoms in ASD. In this review, we summarize evidence from existing research pointing to the clinical, genetic, molecular, and therapeutic response heterogeneity in ASD, as well as present recent advances in precision medicine and discuss their potential to enable the development of efficient drug treatments.

Clinical, genetic and molecular heterogeneity in ASD

Clinical manifestation

ASD is a heterogeneous entity at several levels starting from the clinical point of view. It is well documented that ASD co-occurs frequently with other psychiatric conditions, such as attention-deficit/hyperactivity disorder (ADHD, ~28–31%), anxiety (~20–56%), depression (~11–70%), obsessive–compulsive disorder (~7–24%), oppositional defiant disorder (~25–28%), bipolar disorder (~5–7%), and schizophrenia (~2–13%) [5–11]. These psychiatric conditions co-occur in up to 70% of patients with ASD, and have a considerable impact on the quality of life, morbidity, and mortality [12]. Furthermore, intellectual disability is a frequent comorbidity albeit the prevalence remains controversial; while previous epidemiological reports suggested that ~70% of patients with ASD had co-occurring intellectual disability, due to the lack of standardized definitions and increased awareness and diagnoses, this number has decreased to ~30% in recent reports [13]. Epilepsy also co-occurs frequently (~10–26% of patients with ASD) with a higher prevalence associated with older age, lower cognitive ability, poorer adaptive and language functioning, a history of developmental regression and more severe ASD symptoms; however, no specific type of seizure shows a significantly higher co-occurrence with ASD [14–16]. Additional neurological features occurring more frequently in patients with ASD include macrocephaly, hydrocephalus, cerebral palsy, migraine/headaches, and congenital abnormalities. In addition, 80% of patients suffer from sleep disorders and 46–84% have gastrointestinal disorders [17]. Finally, electronic health records (EHRs) have shown that adults with ASD are more likely to be diagnosed with additional physical health conditions such as immune conditions, obesity or stroke, compared with adults in the general population [18]. This heterogeneity associated with both the core and co-morbid conditions reflects a heterogeneous pattern of neuropathology in ASD [19].

Genetics

In line with clinical heterogeneity observed among patients diagnosed with ASD, the genetic architecture of ASD is also highly complex and variable. It has been estimated that over 1,000 genes might contribute to the risk of ASD [20]. This includes genes with high penetrance for ASD rarely disrupted in the general population and typically resulting in monogenic ASD-related conditions, as well as those contributing to more polygenic forms of ASD in which additive effects derived from common variants can be determinant [21]. Over the last decades, substantial progress has been made towards the identification of ASD risk genes, with most gene discovery still derived from the identification of recurrent *de novo* loss of function (LoF) mutations in highly constrained genes, and some emerging insights from the analysis of rare inherited LoF variants [22]. To date, around 100 genes are confidently associated (false discovery rate [FDR] < 0.05) with ASD risk (Supplementary Table 1) and

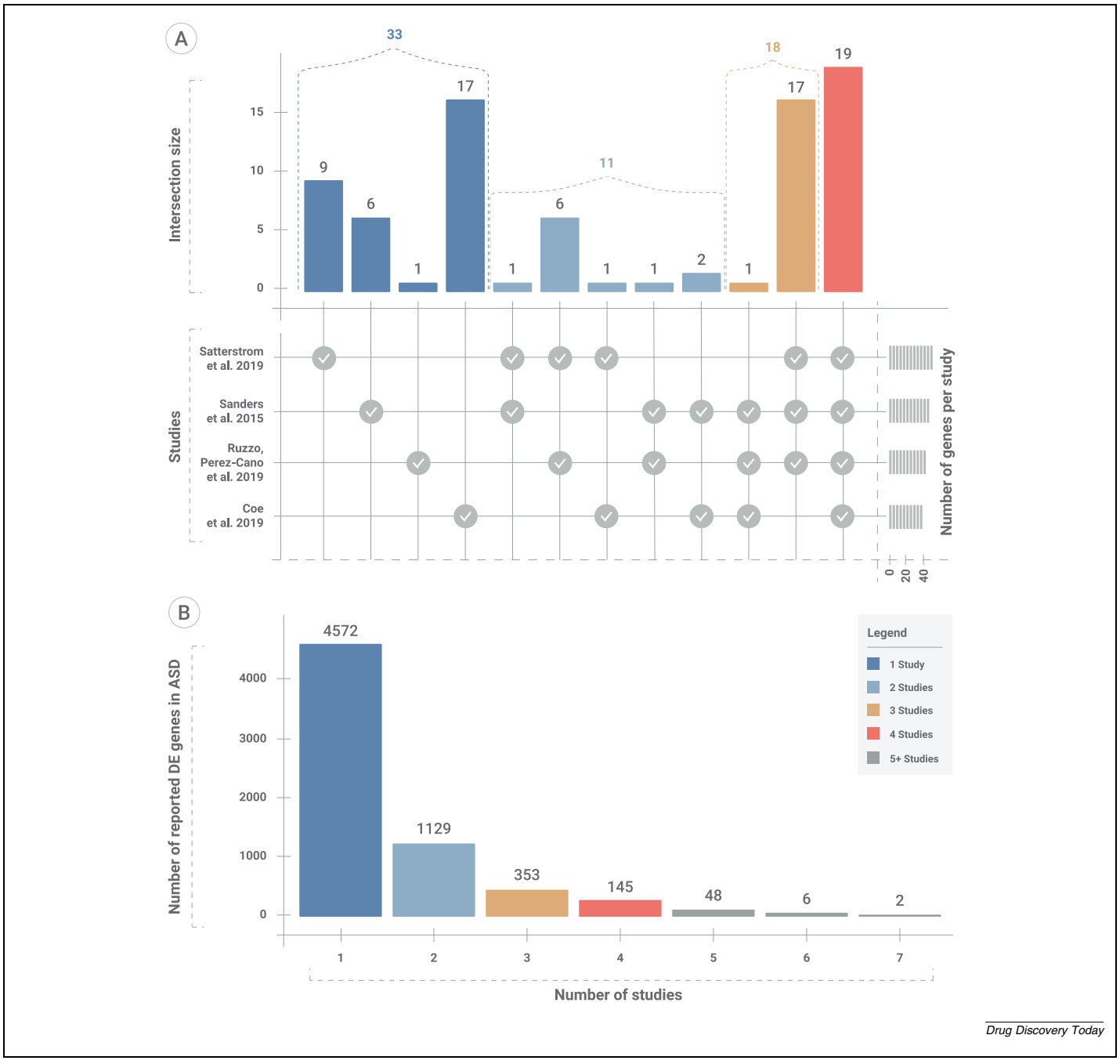

FIGURE 1

Cross-study inconsistencies across large-scale sequencing and gene expression analysis studies in ASD (A) Number of genes reported to be significantly associated with ASD (FDR < 0.05) across one or more large-scale sequencing studies in ASD [22,92–94]. The y-axis shows the number of ASD risk genes consistently reported in the studies listed below, with the colors of the bars representing the number of publications that have indicated those genes as implicated in ASD risk. It can be observed from the red bar at the right of the plot, that the same 19 genes have been reported in all studies as significantly associated with ASD, while a total of 33 genes have been identified as ASD risk genes by only one of the studies evaluated **(B)** Number of genes reported as ASD differentially expressed across the studies analyzed (see Supplementary Information). Only two genes were found to be DE in all 7 studies reviewed, whereas a total of 4572 genes were reported as DE in ASD by only one study, without being replicated in any of the 7 represented in this graph. ASD, autism spectrum disorder; DE, differentially expressed; FDR, False Discovery Rate.

>1,000 genes (also including the former ones) show suggestive evidence from genetic and functional data (https://www.sfari.com). Nevertheless, individual genes and variants associated with elevated risk of developing ASD typically account for a very small proportion of the cases [23]. Remarkably, genes significantly associated with ASD (FDR < 0.05) are not always replicated across large-scale sequencing studies in ASD (Figure 1A, Supplementary Table 1). While type I errors and the lack of statistical power might explain some of these discrepancies, this is also influenced by the underlying genetic heterogeneity in patients diagnosed with ASD and the small proportion of the cases explained by individual genetic risk factors. Consequently, even

genes that are widely accepted by the scientific community to be associated with the risk of ASD (such as CUL3), do not always reach significance in large-scale association studies.

Functional genomic analyses have shown the convergence of clusters of ASD- and NDD-related risk genes into broad molecular pathways which has facilitated the development of mechanistic hypothesis. Among those, a unifying theory proposing an increased ratio of excitation versus inhibition in the brain of patients with ASD was first described in 2003. This hypothesis was supported by investigations reporting the presence of seizures, unusually frequent spikes of activity, and low levels of gamma-aminobutyric acid (GABA) in the brain of patients diagnosed with ASD. However, other studies have provided contradictory evidence that demonstrated an increased density of inhibitory interneurons in post-mortem brain tissue from patients with ASD, as well as hypoexcitability in Purkinje cells derived from patients with tuberous sclerosis complex and ASD. These contradictory results suggest that different subgroups of ASD patients with an opposite pathophysiology co-exist. In fact, genes tightly correlated at a molecular pathway level can result in similar phenotypical profiles predisposing to ASD, while mutations in distantly related ASD risk genes can result in different or even opposite clinical manifestations (see Supplementary Information and Supplementary Tables 2 and 3). For instance, copy number variants (CNVs) are known to contribute to the heterogeneity in ASD though mirror effects that can be triggered by altered gene dosage, such as in 7q11.23, 22q11.21, 1q44, 1q21.1 and 16p11.2. Overall, accumulating evidence suggests the existence of subgroups of ASD patients with different or even opposite pathophysiology, underlining the need to account for the type and directionality of the perturbations across patients for the development of efficient and safe drug treatments in ASD.

Metabolomics

Metabolomics, the profiling of small-molecule metabolites, has emerged as a major contributor in the study of ASD pathogenesis. Indeed, the metabolome reflects the previously discussed interaction between genetic [24] and environmental influences [25] in ASD, owing to its sensitivity to capture interactions between the genome, gut microbiome, diet, and environmental factors, and therefore can provide information to bridge the gap between genotype and phenotype [26]. In this context, promising ASD diagnostic biomarkers (such as metabolites) could play a critical role in earlier disease diagnosis during infancy, enabling the initiation of early intervention which has been shown to promote improvements in the behavioral symptomatology of children with ASD [27].

Recently, the study of differential metabolites between patients with NDDs and typically developing (TD) individuals has attracted growing attention. This strategy has been widely used in the analysis of different samples (such as blood, urine, and stools) from patients with ASD and healthy subjects [28]. However, the findings across various studies analyzing changes in blood metabolites in children with ASD have been rarely consistent (see Supplementary Information). This lack of reproducibility across studies is in part due to the high heterogeneity among patients diagnosed with ASD.

Transcriptomics

Over the past few years, extensive efforts have been also dedicated to the transcriptomics profiling of patients with ASD with the objective of detecting key dysregulated genes in both brain and peripheral tissues. Recent studies focused on understanding the expression alterations involved in the pathophysiology of neuronal cells as well as studies measuring transcriptomic changes across more easily accessible tissues such as peripheral blood for the diagnosis of ASD and the development of treatment options. As observed in genetic and metabolomic investigations, genes reported to be significantly dysregulated in ASD (FDR < 0.05) have barely been replicated across studies (Figure 1B).

Large cohort studies that aimed at detecting a common signature by comparing all ASD patients as a single group against a control population pointed to several dysregulated pathways linked to the nervous system development and function, the immune system, and those related to oxidative phosphorylation and ATP synthesis [29–33]. However, similar alterations have been associated with other diseases, notably in patients with other psychiatric conditions and cancer. Along these lines, in two recent studies [34,35], a strong common signal was identified among ASD and other neurological diseases such as schizophrenia and depression, linking transcriptomics changes with a shared central neuron system genetic architecture. These results show the potential to detect dysregulated gene expression in patients with ASD alongside the existing convergences with gene expression dysregulations reported in other neurological- and immune-related diseases. They also arguably point to the lack of disease-specific information to drive precise treatment development in ASD.

Need for patient stratification and clinical endpoint refinement for drug discovery

The complexity at the clinical, genetic, and molecular level underlying ASD poses a significant challenge for drug discovery. This is reflected by the lack of success in identifying treatments that address the core symptoms of ASD (Supplementary Table 4, adapted from Siafis *et al.* [36]).

One of the main difficulties in ASD and NDD drug development is the complexity of genetic and environmental factors inducing early developmental alterations in the brain which can involve dysregulation and imbalance of several central nervous system (CNS) neurotransmitters such as serotonin, acetylcholine, histamine, dopamine, GABA, and glutamate [37]. Abnormalities in prefrontal cortex and mesolimbic circuit involving dopamine as a key mediator have an impact on behavior and emotional regulation, while alterations in other relevant neurotransmission systems can result in reduced GABAergic gene expression and/or an increase in glutaminergic signaling. The unpaired modulation of these different brain circuits in ASD leads to the development of core symptoms and a spectrum of several associated comorbidities like depression, schizophrenia, seizures, insomnia, impaired behavioral activity, attention deficit and self-injury. Pharmacological targeting of the ASD markers is currently applied in several clinical studies with potential therapeutic strategies [37–39]. Although approved treatments can

help ameliorate related symptoms and comorbidities, these treatments do not address the core symptoms and may cause several off-target and side effects. In this regard, risperidone (Risperdal®) and aripiprazole (Abilify®) are the only drugs approved by the Food and Drug Administration (FDA) for patients with ASD. These medicines can be prescribed to mitigate irritability that can be associated with ASD. Other commonly prescribed medicines include selective serotonin reuptake inhibitors (SSRIs), anti-anxiety medications or stimulants, although none have received FDA approval for the treatment of ASD.

Recent studies suggest that only a subset of the patients will respond to any given treatment and the chance that one or few therapeutic treatments will be effective on the entire ASD population is very narrow [40]. Thus, the paradigm in ASD should move from a "one-size-fits-all" approach towards a more personalized medicine approach, in which the identification of multiple "biologies" underlying ASD will be key to identify molecular targets involved in the pathophysiology of specific patient subgroups with the potential to respond to a particular treatment based on a shared biology. In addition, data related to the failure of clinical trials in ASD can be associated with sub-optimal study design and outcome measures derived from broad diagnostic criteria rather than clinically meaningful or patient centric criteria. Historically, clinical trials testing for drug candidates in ASD have included broad groups of participants resulting in failure to demonstrate clinical benefit among all study participants [40,41]. Similarly, existing clinical studies in ASD typically focus on symptoms observed in ASD patients such as irritability, hyperactivity, and anxiety as clinical endpoints, rather than molecular mechanisms underlying these symptoms [42]. More recently, the core symptoms of ASD such as social withdrawal and social communication are increasingly being considered [42]. However, despite these recent attempts to target the core deficits, there is still no substantial evidence supporting the efficacy of drug treatments addressing these core symptoms at the population-level.

In this context, in clinical studies evaluating D-cycloserine [43] and memantine [44] did report clinically meaningful effects in reducing core symptoms in some adolescents and adults with ASD. SSRIs are also often prescribed to patients with Fragile X syndrome to relieve anxiety, irritability, improve mood, and social deficits. Polymorphism of five genes involved in the serotonin pathway and its metabolism have been studied and significantly correlated with improved cognitive and behavioral scores in young children with Fragile X syndrome treated with sertraline (compared with placebo) were associated with specific BNDF genotypes [45]. Overall, the variation observed among patients in their behavioral response to treatments points to the necessity to fully consider the heterogeneity in the etiology, pathogenesis, and symptomatology of ASD patients to successfully advance therapeutics [46].

Heterogeneity in terms of treatment response was also underlined by drug treatments targeting the proposed excitation versus inhibition imbalance in ASD, as reported in the arbaclofen and bumetanide trials [47–49]. Arbaclofen, a GABA-B receptor agonist, was tested for the treatment of Fragile X syndrome based on previous evidence of behavior improvement in genetic studies in mouse models [43]. In 2017, VanderWeele *et al*. reported inconclusive results of a randomized, double-blind, placebo-controlled, phase-2 clinical study of arbaclofen administrated to patients with ASD who had a history of seizure disorder [47]. The authors highlighted the importance of narrowing the target population to detect the improvements only expected in a portion of the heterogeneous autism spectrum. *Post hoc* analysis on socialization score focusing only on consistent raters of Vineland Adaptive Behavior Scale-II scores demonstrated significant improvement under arbaclofen administration after 12 weeks. A similar attempt was made by repurposing a drug used for the treatment of hypertension known as bumetanide, a brain Na-K-Cl cotransporter (NKCC1) and renal NKCC2 chloride-importers inhibitor that reduces intracellular chloride concentration and promotes GABAergic inhibition. The efficacy of bumetanide was assessed in respective clinical studies involving children with ASD or Asperger syndrome. These studies demonstrated significant improvement in Childhood Autism Rating Scale (CARS), Clinical Global Impression (CGI) improvement and Social Responsiveness Scale (SRS) scores compared with placebo [49]. However, response based on comorbidities could not be analyzed due to the rather small sample set ($n < 100$) [49]. More recently, the largest European phase 3 clinical trial of bumetanide for the treatment of core symptoms of ASD in pediatric population was terminated due to lack of efficacy. Servier, the sponsor of the study, reported in a press release [50] that the results did not show efficacy of bumetanide compared with placebo for the treatment of the general ASD population. The main criteria for assessing core symptoms of ASD were alterations in communication and social interactions with the presence of stereotypical and repetitive behaviors. Prof. Ben-Ari, who is the president of Neurochlore, the company that originally partnered with Servier on the bumetanide clinical trial, stated that "the heterogeneity of ASDs probably makes it impossible to offer a sole treatment for all autistic children".

Taken together, these studies suggest the need for novel data-driven approaches for the identification of sub-populations of patients diagnosed with ASD and characterization of more robust endpoints that account for the molecular alterations underlying these sub-populations. Accordingly, we expect to see an increasing role for artificial intelligence based analyzes of social functioning, repetitive/restricted behavior, and cognitive impairment scores combined with multi-omics data. In parallel, clinical studies will need to employ more objective and measurable endpoints to counteract the high placebo effect. Endpoints derived from quantitative measurements such as EEG signals, levels of biomolecules in whole blood and cererbospinal fluid, and from longitudinal measures assessing cognitive, emotional, motor and sensory functions screened by the NIH toolbox platform (https://neuroscienceblueprint.nih.gov/resources-tools/blueprint-resources-tools-library/nih-toolbox-assessment-neurological-and) will play a critical role in informing on the biological mechanisms underlying specific behavioral patterns.

Computational models for characterizing patients with ASD and developing therapeutic interventions

Computational methods can help improve the characterization and classification of patients diagnosed with ASD. Stratification

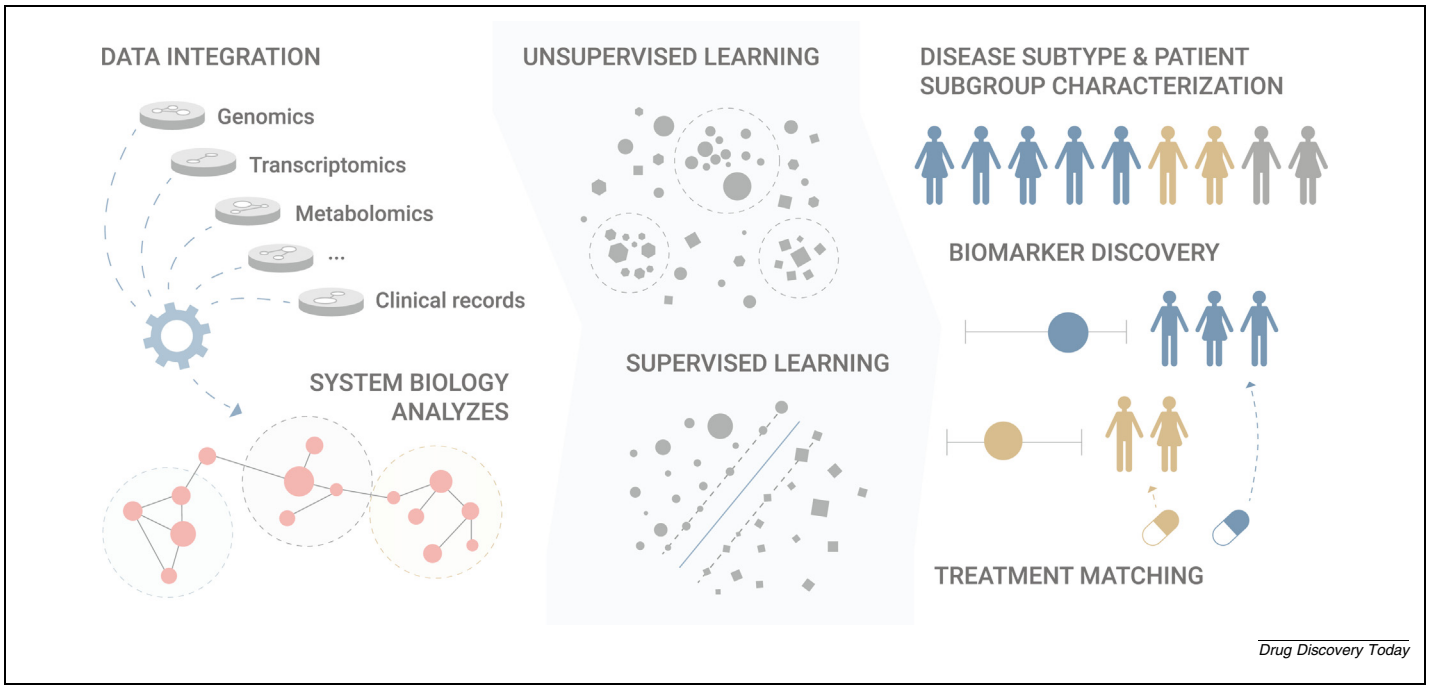

FIGURE 2

Schematic representation of precision medicine in NDDs using systems biology and computational data analysis. Multi-omics and clinical data are integrated and enriched using systems biology based multimodal data analysis. Machine learning models including both unsupervised & supervised models are used to identify the genetic and clinical characteristics underlying specific patient subgroups leading to patient stratification, biomarker, and drug discovery. NDD, neurodevelopmental disorders.

of patients into subgroups of interest offers a promising strategy to tackle the inherent heterogeneity in ASD and to establish more homogeneous disease subtypes; nevertheless, the most frequently used variables for subtyping analyses conducted so far are based on behavioral assessments [51]. In this regard, discovery of the patient subgroups with common molecular backgrounds would be key to develop targeted and more effective therapies. Over the past decade, multiple approaches have been proposed, which include both supervised learning approaches that involve training models for the classification of patients with ASD into certain categories and unsupervised learning approaches that cluster patients with ASD into more homogeneously defined subgroups (Figure 2). This has given rise to the emergence of computational psychiatry, aiming to improve diagnosis through computational analysis of large-scale behavioral and biological data sets (see a comprehensive review by Jacob and colleagues) [52]. The main limitation with these approaches, however, is that the computational models rely on small sample sizes across patient datasets, particularly given the biological and clinical heterogeneity.

Given the challenges associated with the diagnosis of ASD using behavioral and observational assessment, the clinical diagnostic data for ASD relies on various standardized psychological assessment tools such as the Autism Diagnostic Interview-Revised, the Autism Diagnostic Observation Schedule (ADOS), SRS and CARS. Children with a suspected diagnosis of ASD are usually evaluated within a developmental framework, including multiple informants from diverse contexts (e.g., parents and teachers whenever applicable). These evaluations consider a child's current behavior and developmental history to measure social and communication abilities. The data collected through such behavioral and observational assessments in the form of questionnaires or list of clinical criteria is often categorical in nature. Moreover, the collected information does not offer insight into the genetic and molecular disruptions underlying the behavioral and observational characteristics, failing to assess the quantitative features relevant to the disease condition appropriately.

In this context, one of the first studies using a machine learning (ML) classifier on ADOS generic data concluded that 8 of the 29 items of the questionnaire were sufficient to classify autism patients with high sensitivity and specificity values, which can help shorten the time for diagnosis [53] and reduce the time for clinical assessment. A recent literature review of ML approaches for ASD classification using behavioral data (22 studies assessed) highlighted that even if very good metrics were achieved by ML models, no evidence supports their readiness for clinical use [54]. Overall, the validity and generalizability of these models based on behavioral data from small cohorts warrant further research. On the other hand, a study suggested that computational methods based on more quantitative and biologically informative features such as pregnancy follow-up ultrasound and biological measurements might provide an early prognosis of ASD to enable early behavioral interventions that can efficiently attenuate ASD developmental sequels [55]. Nevertheless, problems such as data repository discrepancies, limita-

tions inherent to clinical assessment instruments that treat ASD as a general diagnostic category, and overfitting to the training data remain as potential challenges to improve these models.

The clinical assessment tools used for ASD categorization in ML models have also been used in neuroimaging data analysis, given accumulating evidence that suggests the potential utility of neuroimaging in capturing atypical brain organization in ASD. These variations can occur either at the structural level such as variations in cortical thickness, surface area or brain volume in patients with CNV in certain ASD risk regions including 7q11.23, 22q11.21, 1q44, 1q21.1 and 16p11.2 [56–58] or in pattern connectivity abnormalities revealed by functional neuroimaging techniques. A recent study using functional magnetic resonance imaging (MRI) on children aged 6 months with a high familial risk for ASD, was able to predict diagnostic outcomes at age 2 (specificity: 100%, sensitivity: 81.8%) [59]. In a parallel study, a deep-learning algorithm on MRI data from high-risk children aged 6–12 months was able to predict autism onset at 24 months (specificity: 95%, sensitivity: 88%) [60]. Provided that several difficulties such as reduced sample sizes, standardization of imaging methods or cross-center validation issues are solved, further replication of these studies in the future could result in the development of reliable imaging biomarkers. Interestingly, two studies have tried to use neuroimaging data to discern ADHD from ASD owing to the clinical cooccurrence and genetic overlap between these 2 conditions [12,61,62]. This has motivated the emergence of studies aimed to identify homogeneous ASD subgroups based on neuroimaging features, also known as neurosubtyping. Results from initial efforts do support the feasibility and potential utility of neurosubtyping, even though there are still some technical limitations [63,64].

Heterogeneity remains a fundamental issue for the characterization of patients with ASD as some biological markers may only be found within a subset of patients, even though there are clinical-level commonalities manifesting across patient subgroups. Accordingly, several reports document stratification of patients into subgroups using computation techniques applied on the clinical data. For example, Feczko *et al.* [65] attempted to identify and characterize cognitive subtypes within the ASD population using a Functional Random Forest ML classification model, which revealed 3 ASD and 4 TD putative subgroups with distinct behavioral profiles in a cohort of 47 children diagnosed with ASD and 58 TD children. In another study, Narita *et al.* [66] conducted cluster-based genome wide association studies on 712 probands and 354 controls and identified 65 chromosomal loci of interest, with some of these loci being located within or near previously reported candidate genes for ASD. These findings suggest that clustering may successfully identify subgroups with homogeneous disease etiologies. Furthermore, Luo *et al.* [67] were able to find a subgroup of the patients with dyslipidemia-associated autism using a multidimensional precision medicine approach, where healthcare claims, EHRs, familial whole-exome sequences, and neurodevelopmental gene expression patterns were combined. Other applications are emerging in the past few years, such as those based on deep learning [68], which can be widely used to predict personalized drug response and optimize medication selection and dosing using knowledge extracted from the large and complex molecular, epidemiological, clinical, and demographic datasets [69]. Often, however, deep learning-based methods require large sample sizes and lack mechanistic interpretation in regard to the applicability in a clinical setting. Taken together with the high heterogeneity in ASD, further research will be required to validate previously characterized ASD subgroups and the associated therapeutic implications. Nevertheless, the integration and use of different computational methods will provide valuable opportunities to extend the domain of applicability of each method and more thoroughly exploit information coming from diverse sources.

Recent omics- and endophenotyping-based methods towards precision medicine

Omics-based methods are increasingly gaining popularity to process large cohort datasets towards precision medicine, driven by the success of their application on patients with cancer, both for disease subtyping and for determining the most appropriate treatments [70–72]. More and more initiatives across a wide number of different conditions [73–76] have recently embarked upon driving the translational application of precision medicine with special emphasis on integrating omics-based data with clinical information from EHRs and deep phenotyping of patients. These studies support existence of endophenotypes (i.e., intermediate phenotypes corresponding to biological processes) linked to genetic and molecular changes underlying the disease pathology [77], beyond neuropsychiatric conditions. More importantly, the endophenotypes demonstrate how data integration and processing can detect recurrent patient subgroups, to enable better diagnosis and personalized treatment, also for NDDs.

The application of endophenotyping towards identification of better treatment alternatives has attracted interest in the past few years [73]. Using network medicine tools [78–80], numerous studies have investigated repositioning of drugs for cardiovascular, cerebrovascular, and other CNS disorders [81–83]. In fact, of all medical specialties, psychiatry has arguably the most to gain by incorporating mechanistic knowledge on endophenotypes to identify precision medicine treatments, considering substantial heritability of psychiatric phenotypes and the current reliance on syndrome-based diagnoses. While there are certainly challenges to overcome, omics-driven analyses provide an empirical framework upon which psychiatry can now progress towards better understanding of the disease mechanisms, better treatments, and to better ways of targeting treatments to the patients most likely to benefit from, thus paving the way for precision psychiatry [84]. Accordingly, efforts to integrate omics and clinical data towards precision medicine are increasingly being applied to have a detailed view on gene-phenotype relationships. For instance, Karczewski *et al.* integrated whole exome sequencing data from >200,000 individuals with deep phenotyping data to analyze a single mutation effect on >3,000 annotated phenotypes, providing the scientific community with an unprecedented view of gene-phenotype relationships [85]. These efforts to identify and quantify the relationship between genetic variants and patients' phenotypes are currently delivering promising results in rare diseases, enabling prompt diagnosis and personalized decisions about the treatment for patients. A representative

example is the study by Sweeney *et al.*, where they showed that the surgical operation could have been determined by a prompt application of a rapid diagnostic protocol based on whole genome sequencing data [86].

A deep understanding of omics-based molecular phenotypes could also provide a portfolio of biomarkers suitable for drug development and clinical trial approaches in NDDs. Omics has opened the door to a vast amount of information about function, protein and genetic interactions, gene product expression, metabolite and lipid content, and complex feedback processes that integrate these molecules into pathways and in time and space. Understanding the entire picture will allow us to design and test molecular biomarkers for response to different therapeutic strategies and may allow the development of personalized medicine strategies to contribute to the success of clinical trials in ASD.

Along these lines, metabotyping, i.e., subtyping based on shared metabolic phenotypes identified using metabolic biomarkers associated with the risk for ASD, can enable stratification of the disorder into distinct subpopulations based on a common metabolic dysregulation [87] and lead to potential therapeutic targets for individuals with a specific metabotype [88–89]. A previous study on the Children's Autism Metabolome Project (CAMP) cohort was able to define an altered metabolic phenotype consisting of imbalanced branch chain amino acids metabolism in 16.7% of the CAMP participants, with specificity and precision values of 96.3% and 93.5%, respectively [87]. Even though additional research is needed, it is not clear, for example, how stable these metabotypes are in a particular child [90]. Nonetheless, metabolomics data analysis has shown an immense potential in early diagnosis of ASD and further research can have a profound impact on patients' treatment and prognosis [91]. Latest research has also tackled the transcriptomic profiling of individuals with ASD from a precision medicine perspective. To this end, dissecting ASD into different subtypes and characterizing corresponding subtype-specific omics-based signatures to explain the phenotypic variability in these subtypes constitute a promising strategy for the diagnosis and personalized treatment.

Progress made in the omics and computational biology fields has also enabled emergence of not only several pioneering consortia projects towards characterizing genetic factors underlying ASD, but also various clinical stage biotech companies focused on the development of precision medicine-based diagnoses and

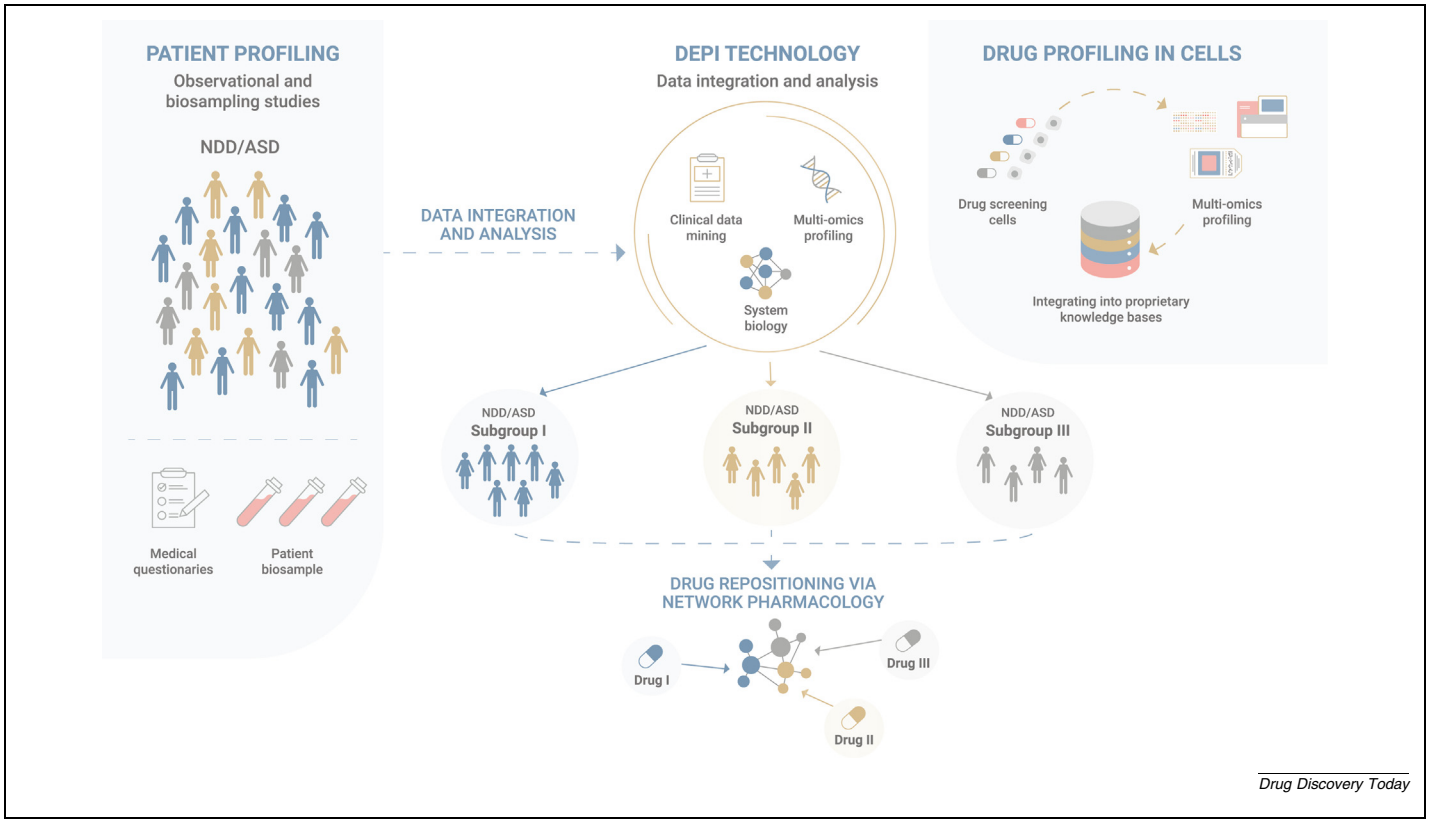

FIGURE 3

DEPI platform overview. Developed by STALICLA, Databased Endophenotyping Patient Identification (DEPI) allows the stratification and identification of subgroups of patients with NDDs, by integrating different analysis approaches such as system biology, EHR and multi-omics profiling, and using patient data such as biosamples and clinical questionnaires (patient profiling). On the other hand, drug screening in specific cell lines (drug profiling) can address the therapeutic needs of a specific group, thus generating tailored treatments for each subgroup. EHR, electronic health records; NDD, neurodevelopmental disorders.

treatment options for patients with ASD and other NDDs. For example, public initiatives such as SFARI (https://www.sfari.org), iHART (http://ihart.org) or MSSNG (https://research.mss.ng) have collected medical and genetic information from >10,000 patients with ASD. On the other hand, companies such as Stemina Biomarker Discovery (https://stemina.com/), integrates the NeuroPointDX technology, a metabolomics-based ASD diagnostic tool (NCT02548442); quadrant biosciences with Clarifi ASD®, also develops an ASD diagnostic tool based on epigenomics (https://quadrantbiosciences.com/; NCT02832557); and STALICLA (https://stalicla.com/) offers Database Endophenotyping Patient Identification (DEPI), a systems biology and multi-omics-based platform that allows stratification of NDD patients into biologically similar and clinically actionable patient subgroups (i.e., patients that share similar pathway-level alterations) and the exploration of the corresponding tailored therapeutic treatments for these patients in each subgroup (Figure 3).

One of the first applications of DEPI technology is STP1, the first precision medicine therapeutic package in the ASD space specifically developed for the treatment of patients matching the criteria for a clinically and biologically validated ASD subgroup, for which a Phase1b interventional clinical trial has recently been completed with success (NCT04644003). DEPI leverages proprietary NDD-tailored knowledge bases and combines clinical and biomedical data from large-scale NDD cohorts for endophenotyping, clustering and drug positioning (Figure 3). The endophenotypes identified by DEPI, also named actionable clinical signs and symptoms, constitute quantitative biological traits with a strong genetic component that significantly contributes to the risk of NDDs. These are based on curated and NDD-tailored catalogs that combine clinical, genetic, molecular, and pathway-level data. The clinical data include information extracted from EHRs such as medical history, where behaviorally-defined conditions are excluded using semantic-type matching based on standardized medical ontologies. Importantly, DEPI can further integrate patient-derived biomarkers with cell-based multi-omics data to prioritize drug treatments and enable the matching of the right patients with a NDD with the right drug treatments. The broad applicability of DEPI is being investigated in an ongoing observational study consisting of 250 patients (NCT04273087) for STP1 and STP2, the two precision medicine packages targeting two ASD subpopulations.

Conclusion

The clinical, genetic, and molecular heterogeneity observed in patients diagnosed with ASD and the application of a "one-size-fits-all" approach in the past clinical trials have hindered the development of efficient drugs for the treatment of patients with ASD and other NDDs. In this review, we have highlighted various studies in which patients with ASD were treated indistinctively despite clear biological and clinical differences. The poor overlap between the genetic- and molecular-level perturbations across different cohorts reflects the limitations of existing clinical studies that use ASD as a broad diagnostic category and emphasizes the urgent need to stratify the heterogenous ASD population into biologically-defined ASD subgroups to improve the clinical response success rate. The recent paradigm shifts toward "multi-omics" data integration together with systematic and systems biology based analyses of large-scale data can facilitate identification of ASD patient subgroups with a similar underlying "biology", which may allow the identification of consistent biomarkers. Such data-driven analyses can be used to translate mechanistic perturbations underlying different patient subgroups into actionable molecular targeting strategies. Therefore, to advance the identification of novel personalized treatments and overcome the current challenges and pitfalls experienced in past ASD clinical studies, it is critical to apply robust modeling strategies that account for heterogeneity by ensuring cross-cohort replication and by using interpretable models that are not prone to overfitting, especially for small cohorts. Similarly, there is a need for quantifiable biomarkers as primary endpoints to characterize mechanistically changes in core deficits upon treatment across patient subgroups. Quantifiable endpoints will allow to assess the effect of treatments more accurately and circumvent the high placebo effect observed in some past clinical trials.

Along these lines, recent studies and public and private translational initiatives open a new era in the diagnosis and treatment of ASD and other NDDs, offering solutions to fill the existing translational gap to improve the quality of life of the patients with more severe forms of these conditions.

Data availability

The references from which the data is collected for the supplementary tables are listed in the supplementary information.

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Conflict of interests

All authors are employees of STALICLA.

Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.drudis.2023.103486.

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