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DEVELOPMENT OF PHARMACOTHERAPIES FOR AUTISM SPECTRUM DISORDERS: A MOLECULAR MEDICINE FRAMEWORK FOR NEUROPSYCHIATRIC DRUG DISCOVERY

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SUMMARY

Innovative advances in human molecular genetics, animal models with tighter correlates to human disease and translational biomarkers are accelerating our understanding of brain disorders. They are also providing a therapeutic framework for diseases previously not considered tenable for drug intervention. Such is the case of autism spectrum disorders (ASDs), where clinical heterogeneity and gaps in our understanding of the underlying biology of the disorder have been impediments to investment by companies. Evidence for therapeutic rescue of deficits in mouse models of rare gene variants of major effects, combined with insights derived from technological developments in whole exome sequencing and functional neuroimaging, are beginning to inspire investment in pharmacotherapeutic approaches to ASDs.

INTRODUCTION: CORE SYMPTOMS, PREVALENCE AND SOCIOECONOMIC BURDEN

Autism spectrum disorder (ASD) is the commonly used terminology to reflect the spectrum of developmental disabilities that now define "autism" (for review see 1). Originally defined by Leo Kanner in 1943 in a small cohort of children that presented with severe aloofness and indifference to other people, autism is presently diagnosed at increasing rates and has achieved significant public attention in both the scientific and lay communities. Despite the fact that ASD patients represent a heterogeneous population that spans averbal patients with severe intellectual disability to relatively high-functioning patients, the diagnosis in all cases relies on the presence of three core behavioral deficits. Autistic disorder is diagnosed when there is qualitative impairment in social interaction and communication, restricted and stereotyped pattern of behaviors, interests and activities and impaired or delayed development of language. Diagnostic criteria for higher functioning cases of ASD, such as Asperger's disorder, are very similar, except that there is no demonstrable impairment in language development or communication. Pervasive developmental disorder, not otherwise specified (PDD-NOS), the remaining ASD, is a subthreshold diagnostic term for when a child demonstrates impairment that is not sufficient to meet full criteria.

The prevalence of ASD in North America and Europe (circa 2000-2006) is approximately 6 per 1,000 (1). In 2000, the Centers for Disease Control and Prevention initiated the Autism and Developmental Disabilities Monitoring (ADDM) Network. This network is a multisite, records-based surveillance program that relies on developmental evaluation records for determining the prevalence of ASD at age 8 years. The latest surveillance report (CDC report for 2006 cohort; 2) indicates that 1%, or approximately 1 per 110 children, are classified as having an ASD. The reason for well-publicized increases in prevalence is not clear, although a number of factors have been noted: heightened public awareness, historical broadening of diagnostic criteria and better ascertainment through increased diagnostic proficiency.

ASD is a very expensive, lifelong disorder that costs upwards of USD 35 billion in direct and indirect costs to care for individuals over their lifetime (3). The lifetime per capital incremental societal costs of ASD have been recently estimated to be USD 3.2 million (4). The largest component of these costs is lost productivity as adults and

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adult care, facts that may be underappreciated, as ASD is often depicted and discussed by lay media simply in the context of childhood disability.

CURRENT TREATMENT APPROACHES

The treatment of individuals with ASD often involves the application of various behavioral practices. The scientifically validated treatment, applied behavioral analysis (ABA), is the only standard of care recognized over the past 20 years in improving the developmental progress and intellectual performance of young children with ASD (5). The development of safe and effective drug treatments for patients diagnosed with ASD is desperately needed. Current drug treatment is often limited to an "off-label" polypharmacy with medications used for the treatment of other disorders. Only two marketed antipsychotic drugs, risperidone and aripiprazole, are presently approved for the treatment of irritability in ASD. These drugs offer some benefit, but neither treats the core deficits of the disorder. These drugs are also associated with adverse metabolic side effects and weight gain, often leading to discontinuation and challenges with patient compliance. The use of alternative therapies (e.g., nutritional and chelation therapies) that are not scientifically proven, and may even pose significant risks, speaks to the urgent need for effective treatments. Effective treatment of associated symptoms and comorbidities represents a significant unmet need. A novel therapy that mimics the actions of risperidone or aripiprazole without side effects would provide incremental clinical benefit in the near term. Additional comorbidities, such as sleep disturbances and anxiety, represent a significant burden for both patients and family members and can negatively impact the current standard of care (6, 7). Given the multitude of drugs in clinical development or marketed for the treatment of these disorders in other patient groups, a key strategy for near-term impact is to assess their efficacy in ASD patients in accordance with clinical trial methodologies accepted by regulators. Recent data demonstrating synergistic benefits of risperidone when combined with behavioral intervention (8) support novel clinical trial schemes to demonstrate added pharmacotherapy benefits in maximizing outcomes with current standard of care.

GENETICS OF ASD

ASD represents the most heritable of all neuropsychiatric disorders. Twin studies demonstrate > 95% concordance rates in monozygotic and 23% concordance rates in dizygotic twins (9, 10). Furthermore, the risk of being diagnosed on the spectrum is increased 20-fold in siblings of affected individuals (11). Emerging advances in the human molecular genetics of ASD are leading to a consensus view that variable genetic insults in ASD alter the development of specific circuits involved in social cognition and language (12). Early insights are provided by single gene disorders (e.g., fragile X syndrome, tuberous sclerosis, Joubert syndrome) that present with autistic symptoms at frequencies far greater than the typical population. Chromosomal aberrations, hallmarks of several different neurodevelopmental syndromes, are often associated with autism and multiple chromosomes have been implicated (12, 13). Cytogenetic studies of maternally inherited duplications of chromosome 15q11-13 and other rare chromosomal anomalies also represent an important etiology of autistic cases. Recent advances in array-based and gene resequencing technologies have helped identify other single genes of major effect, including *NLGN4, NRXN1* and *SHANK3*. These rare variants collectively account for no more than an estimated 15-20% of all children with an ASD, and individually only represent 1-2% of ASD cases. In addition to these rare variants of major effects, linkage studies have identified more common variants of lesser effect, although these findings are not always reproduced. Figure 1 represents a timeline of candidate genes reportedly associated with ASD, many of which have been independently replicated by different investigators using different approaches. The remainder of "idiopathic" ASD cases result from a panoply of unknown genetic and/or environmental etiologies. The most recent Autism Genome Project studies (14) strengthen the growing evidence that rare structural variants including both inherited and de novo copy number variants (CNVs) are associated with ASD. Some rare CNVs may be "large-effect" mutations. Preclinical mechanistic studies of rare variants may help in the identification of common pathophysiological elements that broadly account for ASD symptoms and anatomical features (e.g., accelerated brain growth). Such inquiry may inform a framework for pharmacotherapeutic intervention, as in the case of familial Alzheimer's disease (AD). Mutations in the presenilin genes, which accounted for only < 1% of all cases of AD, revolutionized the field with the "amyloid hypothesis". This hypothesis inspired the drug industry to focus on β-amyloid (Aß) reduction for modifying the progression of sporadic AD.

The idea that a large number of genes contribute to genetic "load" is an emerging theme in a variety of different complex conditions, including diabetes, heart disease, inflammatory diseases, obesity and schizophrenia. In Crohn's disease, for example, 30 different genes have been identified that robustly influence disease and are now implicating novel biological pathways (e.g., autophagy) involved in disease pathogenesis (15). Interestingly, several candidate genes defined to date confer susceptibility not only for ASD but also for distinct neuropsychiatric disorders. For example, *CNTNAP2* is associated with obsessive–compulsive disorder, Tourette syndrome (16), language impairment (17), intellectual disability (18), attention deficit hyperactivity disorder (19) and epilepsy (20), while disrupted in schizophrenia 1 (*DISC1*) and neuregulin (*NRG1)* are implicated in schizophrenia (21). Mutated genes can also confer subclinical impairment in siblings of patients with ASD, as they can exhibit significantly lower performance across measures of social–communicative development, cognitive functioning and ASD symptoms relative to their low-risk peers (22).

Some argue that the heterogeneity and genetic complexity of ASD render the disease too high risk for therapeutic investment. While we recognize the genetic complexity, advances in molecular technology are facilitating new discoveries at a rapid pace. Arguably, ASD represents the best opportunity to deliver on the promise of personalized molecular medicine in central nervous system (CNS) disorders. First, genetic profiling has become technologically affordable and efficient, permitting hypothesis-free genotyping of hundreds of thousands of distinct variants within a single genome. It is possible that in the foreseeable future molecular tests will be available that allow prenatal testing for a high-risk panel of genes, or "molecular signature", that predicts the likelihood for developing ASD. Indeed, a recent study reported the identification of a four-gene panel for ASD risk (23). Translation of genetics into evidence-

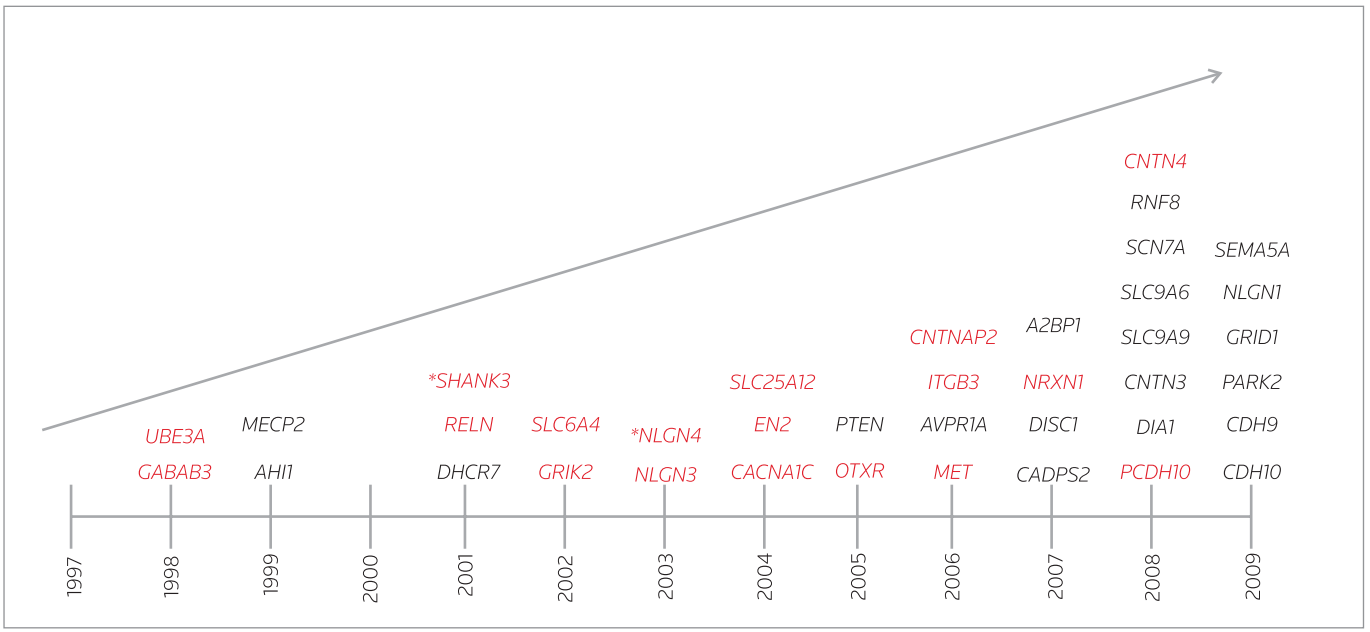

Figure 1. Timeline of genetic findings in ASDs. *> 10 mutations. Red text indicates independent replication (12, SFARI gene database: http://sfari.org/sfari-gene).

based clinical use is being advocated in oncology with a 21-gene panel (24).

Another enabling theme for ASD drug discovery is the intriguing observation that most candidate genes encode proteins of the synapse, and may contribute to an underlying and potentially reversible "synaptopathy". Understanding how candidate genes alter the number, structure and function of synapses may therefore provide a pioneering approach to developing therapeutics for ASD. The identification of the neuroligin genes that originally emerged from cytogenetic analyses has since been strengthened by the identification of additional genes encoding synaptic proteins, including *SHANK3, SHANK2, NRXN1, CNTNAP2* and *CNTN3/4* (reviewed in 25-27). Such genes encode proteins that play roles in signaling, cell adhesion and synaptogenesis. In vitro studies of mutated proteins often shows altered synaptic integrity and function (28, 29). Transgenic mice that express modified proteins, e.g., CADPS2 (30) and neuroligin 4 (31), often present "autistic-like" behavioral phenotypes. Moreover, combinatorial pathway mining of candidate genes reveals surprising convergence at the molecular level (32). For example, mTOR/PI3K signaling, originally defined as the key pathway implicated in tuberous sclerosis, may also be associated with other gene defects, i.e., *FMR1* (33) and *MET* (34). Mutations in *MECP2,* previously thought to be restricted to Rett syndrome, are now emerging in larger molecular genetic studies of idiopathic autism cases (35). Collectively, these findings comprise an emerging link among genes, phenotypes, pathways and potentially "drugable" therapeutic targets for disease modification.

TARGET IDENTIFICATION AND VALIDATION FRAMEWORK FOR DRUG DISCOVERY

Drug discovery for neuropsychiatric disorders has been impeded by the lack of experimental models that recapitulate key aspects of human disease. Many scientists now surmise that classical animal models of psychiatric disease may simply be "sensors" of specific pharmacology, with limited predictive value for novel drugs from diverse pharmacological classes. This gap has contributed to an unsustainably high failure rate in human phase II trials and a dearth of innovation in the last decade. This challenge no doubt contributed to recent strategic decisions by two large pharmaceutical firms to exit psychiatric research. The humbling fact is that most currently marketed psychiatric drugs are reverse-engineered derivatives of earlier clinical serendipity rather than the result of prospective design from modern knowledge of disease biology.

As trail-blazed by oncology in the last decade, new understanding of human neuropsychiatric illness (i.e., powered by genetics, molecular mechanisms, functional imaging, qEEG) is beginning to translate from humans back to the lab to inform more elegant and hopefully predictive models for CNS drug discovery. Examples of this are already evident in monogenic forms of ASD, where brain slices and embryonic neurons isolated from monogenic transgenic mice are being used as in vitro model systems for drug testing. Another common approach to defining phenotypes of candidate genes is to study gene expression acutely modified by interfering RNA. Mechanistic studies utilizing biochemical, structural (e.g., changes in synapse density, spine numbers or morphology) and electrophysiological (e.g., acute and plasticity changes) endpoints can illuminate gene-specific synaptic impairment and provide a platform for testing of mitigating treatments. Translational validity of these models can then be tested against tissues from genetically modified animals (36-38), and even in pluripotent stem cells isolated from patients (39). Circuit-level analysis in situ can be facilitated by tissue-specific changes in transgene expression by lentiviral vectors, or circuit-specific gene rescue of transgenic "knockout" (KO) mice (e.g., 40). Optogenetics is an innovative tool that can advance a more spatial-

ly refined understanding of candidate gene expression and activity of neural circuits and behavior (41).

Mouse models of monogenic syndromes of ASD such as fragile X syndrome (*Fmr1*), Rett syndrome (*MeCP2)* and tuberous sclerosis (*TSC1/2*) recapitulate behavioral deficits and altered neuropathology as near phenocopies of the human syndromic diseases. Examples include macrocephaly in *Pten* conditional knockout mice (42), synaptic plasticity deficits and macroorchidism in *Fmr1* mice (36), and respiratory disturbances and dendritic spine abnormalities in *MeCP2* mice (43). A new mouse model of human 15q11-13 duplication (44, 45) has generated great enthusiasm, as it models deficits of a key recurrent cytogenetic aberration associated with 2-3% of ASDs (46, 47).

Both genetic rescue and pharmacologic strategies have been shown to reverse functional and behavioral deficits in mouse models of monogenic forms of autism. Table I shows a list of ASD syndromic mouse models and interventions that rescue specific deficits in genetic mouse models. It is clear that much of the optimism for therapeutic intervention is being fueled by the metabotropic glutamate $mGlu_5$ receptor theory of fragile X syndrome in which genetic reduction of the $mGlu_5$ receptor in *Fmr1* knockout (KO) mice rescues six of seven hallmark phenotypes (36). The $mGlu_5$ receptor theory of fragile X proposes that various neural symptoms of the disorder result from the loss of translational regulation by fragile X mental retardation 1 protein on mGlu receptor signaling, most notably enhanced AMPA receptor internalization and long-term depression (48). An attractive feature of the $mGlu_5$ theory is that multiple deficits could be rescued concurrently with a single mechanism. Another seminal discovery is that rescue of the deficits can be observed after the brain has undergone early stages of development (49). For example, dominant-negative expression of PAK in *Fmr1* KO mice was found to rescue spine deficits when PAK expression was conditionally abrogated in the postnatal period (37). Acute treatment with an $mGlu_5$ receptor negative allosteric modulator (NAM) reversed presynaptic deficits in the lateral amygdala of *Fmr1* KO mice (38). Several pharmaceutical companies have advanced $mGlu_5$ receptor NAMs (e.g., RO-4917523, AFQ-056, NPL-2009; clinicaltrials.gov) in phase I/II clinical testing in adult fragile X patients. Novartis has publicly cited encouraging preliminary findings in early clinical trials (50).

We appreciate the current debate as to whether rare monogenic variants (e.g., correcting FMRP or SHANK3-mediated disability) is the appropriate focus for drug discovery in ASD, as there is uncertainty as to whether these clinical entities would inform treatments for idiopathic disease. For those of us in the risky business of commercial drug discovery that need to deliver value to capital providers, one should reframe the question this way: Should you first start to treat 20% of patients with clearer mechanistic etiologies, that offer more translatable preclinical models of human disease and a focused and more economical clinical trial design, or do you invest in the elusive "magic bullet" therapeutic for a heterogeneous group of patients of unclear, diverse etiologies? The latter strategy has not been particularly successful for other CNS disorders. Additionally, we posit that in-depth study of these models should elucidate important and transferable insights of disease. Recent data from genetic mouse models are elucidating potential connections between diverse autism candidate genes. For example, mTOR signaling (33) and gene expression of *MeCP2* and *Wnt2* (51) have been shown to be altered in *Fmr1* KO mice, and *MeCP2* deficiency causes reductions in *GABAB3* and *Ube3a* expression (52). Finally, synergistic effects on expressed autism-relevant phenotypes have been observed when crossing genetic mouse models derived from distinct candidate genes (53, 54). These results lend mechanistic support to the hypothesis that there may be canonical mechanisms among distinctive autism candidate gene pathways that could ultimately inform some common therapeutic approaches. For example, molecular defects in mechanisms controlling synaptic protein synthesis may underlie cognitive impairment or even examples of savant abilities in ASD (55).

Forward genetic strategies are also enabling animal models that recapitulate core deficits in ASD, such as impaired social interaction

Table I. *Mouse models of monogenic disorders with a high prevalence of autism spectrum disorder (ASD).*

Human disease	Gene targeted in genetically modified mouse	Key phenotypes	Pharmacologic or genetic rescue	Refs.
Tuberous sclerosis	*Tsc1/2*	Multiorgan tumors; learning and memory deficits	Rapamycin	73
PTEN hamartoma syndrome	*Pten*	Macrocephaly; brain hypertrophy	Rapamycin	74
Fragile X syndrome	*Fmr1*	Macro-orchidism; dendritic spine alterations; enhanced seizures; impaired synaptic plasticity	$mGlu_5$ receptor negative allosteric modulators, minocycline, brain-derived neurotrophic factor, PAK inhibitors	36, 37, 75, 76
Rett syndrome	*Mecp2*	Reduced life span; respiratory dysfunction; dendritic spine alterations; synaptic deficits	IgF_1, brain-derived neurotrophic factor, desipramine, AMPA positive allosteric modulators	77-80
Angleman syndrome	*Ube3a*	Impaired visual plasticity; impaired learning and memory; motor dysfunction; enhanced seizures	Phospho-CaMK-II inhibition	81
Phelan-McDermid syndrome	*Shank3*	Synaptic plasticity deficits; dendritic spine alterations; behavioral abnormalities	IgF_1	82

and vocalization, seizures and restricted repetitive behaviors (56). Profiling of behavioral phenotypes across multiple inbred mouse strains has led to the identification of the BTBR mouse strain that manifests social impairment and repetitive behaviors of ASD (57, 58). Reversal of behavioral deficits with pharmacologic and behavioral intervention approaches suggests construct validity for this model (59, 60). Molecular profiling studies are under way to define the genes that underlie this phenotype (61).

PERSONALIZED MEDICINE APPROACH IN CNS DISEASES

The pharmaceutical industry faces significant challenges in innovation while being faced with an increasingly restrictive regulatory environment. We are nearing the end of the blockbuster era and entering a more specialized era for drug discovery (62, 63). In oncology, pharmacotherapy is advancing closer to the promise of personalized medicine and it may become a standard of care in the near future (64). Examples of success include herceptin in *HER2* gene-positive breast cancer therapy and *KRAS* screening in epidermal growth factor receptor (EGFR) inhibitor therapies. The concept of defining patients and corresponding therapy based on their underlying molecular signature is at a stage of infancy in neuroscience. Patient diagnosis, trial design and recruitment, and regulatory paths are all defined by rather blunt diagnostic and treatment outcome instruments that belie the complex and heterogeneous etiology and manifestation of disease. Like "autisms", there are "schizophrenias" and "depressions" –individual practitioner and clinical trial experience have pointed to that fact all along. Marketing analysis without the ability to properly segment patients is inherently flawed. Commercial analysis that collapses diverse patient subgroups of disease and maximizes pro forma revenue projections is no recipe for clinical trial or commercial success. Defining the correct patient population to treat under the concept of "right drug, right patient" is a particular necessity for CNS research where challenges for success are greater than any other disease area (65, 66).

ASD represents a unique CNS disorder in which drug target identification and validation can be conducted using animal models that at least partially phenocopy underlying human disease pathways. This approach affords a cost-effective testing of specific mechanistic hypotheses with therapeutics in discrete, genetically defined patient populations. The prospect that multiple patient subgroups could converge at the signaling pathway or circuit level can be confirmed preclinically and inform subject recruitment efforts for a clinical program. An example might be the use of an mTOR pathway inhibitor for patients with tuberous sclerosis, as well as patients with *PTEN* mutations with prominent macrocephaly based on their association mechanistically with the PI3 kinase pathway.

OPPORTUNITIES AND CHALLENGES IN CLINICAL SCIENCES

Aside from the genetic "anchors", imaging techniques are elucidating key dysfunctional neuroanatomical circuits in ASD. There is a need to integrate detailed molecular knowledge of ASD candidate genes with the neurocircuitry and functional endophenotypes in humans. Biomarkers such as qEEG, event-related potentials (ERPs), magnetoencephalography (MEG), functional magnetic resonance imaging (fMRI) and eye tracking, developed first for diagnostic value, are poised to measure changes in circuitry linked to social cognition and response to drug treatment. For example, hypoactivation of the fusiform gyrus by fMRI (67), N170 ERP abnormalities (68) and eye tracking deficits (69) all represent methods for assessing key deficits in face recognition, some of which have been correlated with the level of social impairment and disability (69, 70). These noninvasive and quantitative biomarkers should provide great utility in driving investment decisions for clinical development.

One of the largest gaps in ASD drug discovery is clinical trial doability. A paucity of well-designed, placebo-controlled, multicenter trials has been carried out to date in ASD patients. Clinical trial outcome measures in ASD rely on subjective rating scales, often by parents, and can be inherently associated with significant limitations (71). Adapting clinical rating scales originally defined for a different patient population (e.g., obsessive–compulsive disorder; 72) can be problematic and risky. Joint efforts by industry, academia and government are needed to help define reliable outcomes that gauge effective treatment effects and provide a suitable regulatory path to approval and commercialization. The emergence of precompetitive consortia, involving joint partnership between industry, academia, regulators and nonprofit agencies, represents an ideal framework to enable clinical trial doability for ASDs.

Given the increased funding, emerging breakthroughs in the underlying neurobiology and genetics, as well as translational animal models, we propose that the time for the pharmaceutical industry to invest in and impact this disease of high unmet medical need is overdue.

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