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Neurodevelopmental Disorders

What Is to Be Done?

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Abstract

Autism and schizophrenia are associated with abnormalities in brain development, the genetic and environmental risk factors of which are just now being discovered. How can these advances be translated into treatment? This chapter reviews the psychiatric classification of autism and schizophrenia, and discusses how this has impeded progress in understanding disease mechanisms. It recommends that future research not be constrained by current nosology and explores alternative diagnostic approaches. It discusses recent studies of prevention in the early stages of illness and discovery of genetic and environmental risk factors. Both sets of observations should be used to guide neuroscientific exploration of neurodevelopmental disorders unimpeded by current psychiatric nosology.

The Status Quo of Psychiatric Diagnosis

To appreciate the limitations of current psychiatric nosology, we need to review (a) the diagnostic encounter, during which a clinician collects data from a patient and assigns the patient to a diagnostic group; (b) the diagnostic rules that guide the clinician in the selection of clinical features and the interpretation of additional data (e.g., historical information and laboratory tests); and (c) the diagnostic paradigm, which asserts that diagnosis can be validated by clinical presentation, outcome, and disease mechanisms.

Typically, the diagnostic encounter is a cross-sectional assessment of psychopathology. The major focus has been on reliability: How can we make sure that a patient (seen by the same clinician or by several clinicians at various

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time points) receives the same diagnosis? The type of information collected in the psychiatric encounter is often constrained by schools of psychopathology and the training of the clinician. While it is well known that the reliability of the diagnostic assessment can be improved by collateral information (Ho et al. 2004), such information is often not collected. The psychiatric encounter occurs in a clinical setting and is thus unable to examine the person in the real world. Recent studies offer compelling examples that experience sampling can provide much richer phenotypic data (van Os et al. 2013). In addition, the social context of the person needs to be taken into consideration to understand the complexity and unique features of the individual struggling with a mental illness.

Diagnostic rules have been shaped primarily by the extensive literature on clinical psychopathology. For example, the current concept of schizophrenia is built upon the diagnostic concepts of Kraepelin, Bleuler, and Schneider. The International Pilot Study of Schizophrenia (Wing and Nixon 1975) and the U.S./U.K. study (Kendell et al. 1971) revealed wide discrepancies between the European and the American concepts of schizophrenia. This led to a much narrower definition of schizophrenia in the third edition of the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM-III) (Andreasen 1989). Since then, only five domains (delusion, hallucination, thought disorder, catatonic behavior, and negative symptoms) have been included in the diagnostic criterion set for schizophrenia; others have been excluded (e.g., cognitive impairment, depression, mania). In addition, gatekeepers have been added to exclude (a) less severe forms of psychosis, (b) patients with prominent mood symptoms, and (c) patients with psychosis due to substance use or medical and neurological disorders. While these diagnostic criteria have increased the reliability of the schizophrenia diagnosis (intraclass kappa of 0.5 for DSM-5 diagnosis of schizophrenia) (Regier et al. 2013), they have threatened the validity of the concept (Andreasen 2007).

The diagnostic paradigm can be traced back to Kraepelin's concept of the natural disease unit (Hoff 1985). Kraepelin proposed that a psychiatric diagnosis can be validated through (a) clinical presentation, (b) disease course and outcome, and (c) the cause and mechanism of the illness. Implicit in this paradigm is the strong hypothesis that all validators converge on the diagnosis and reflect an underlying biology, for instance, the natural disease unit. Although there is little doubt that the validators' clinical presentation and course/outcome have much to offer in clinical practice, it is now clear that they often do not converge with the cause and mechanism of illness. In short, it is likely that several mechanisms and causes will be identified for one psychiatric disorder, and that shared mechanisms and causes will be identified for clinical features that traverse diagnostic categories (Heckers 2008). This challenges the current design of psychiatric research, for instance finding the etiology and pathophysiology of a diagnostic category defined by clinical presentation.

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Where do we go from here? Do we need a paradigm change now or should we continue the current research strategy, with better methods and greater resources? For example, investigators could study patients with several diagnoses in one or multiple chapters of the DSM (e.g., psychotic disorders, mood disorders, anxiety disorders). In addition, the major domains of psychopathology could be redefined along dimensions (normal, mild, moderate, severe) rather than categories (Barch et al. 2013). We will review one proposal for a change in psychiatric nosology.

The Research Domain Criteria

The genomic and biomedical revolution of recent years has superseded the classical model of diagnosis based on symptoms, relatively gross measures of pathology, and standard clinical tests. In oncology, for instance, cancers are increasingly diagnosed by the genetic composition of the neoplasm rather than tissue of origin; although much treatment still relies on surgery and broad-spectrum chemotherapies, new approaches seek to determine optimal matches between the genetic signature and the particular chemotherapeutic agent. This has led to the development of large-scale adaptive trials that incorporate simultaneously a number of genetic factors; in breast cancer, for example, iSPY2. Even monogenic disorders are now known to comprise a number of different "diseases" at the genetic level. For instance, ivacaftor (Kalydeco™) is highly effective in treating patients with the clinical phenotype of cystic fibrosis who have the R117H mutation of the CFTR gene. R117H, seen in only 4% of cystic fibrosis patients, is one of over 1,000 different mutations that can produce the clinical syndrome, and subsequent clinical trials are going forward one mutation at a time.

While diagnostics in other areas of medicine have moved to increasingly sophisticated clinical and genetic tests, psychiatric diagnosis remains stuck in the practices of the past. The current DSM and ICD categories rely entirely upon presenting signs and symptoms to confer diagnoses. Further, they utilize an infectious disease model in which disorders are considered to be either present or absent, notwithstanding accumulating evidence that most symptoms can be arrayed along some dimension of severity and/or impairment.

This system largely suffices for current treatment practice, since both drug and behavioral modalities tend to be moderately effective across a range of mood/internalizing or psychotic spectrum disorders. However, modern genomics and neuroscience research increasingly indicates that the categories are a poor reflection of nature. Genetic risks appear across multiple disorders, such as unipolar and bipolar depression or bipolar disorder and schizophrenia, with a recent paper reporting commonalities across five different disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium and Genetic Risk Outcome of Psychosis Consortium 2013). Further, neuroimaging and other

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studies indicate that common mechanisms are present across multiple disorders (e.g., emotion regulation or working memory disruptions), while almost all disorders are highly heterogeneous both in terms of symptom combinations and mechanisms of pathophysiology.

Unfortunately, the current symptom-based scheme has become the *de facto* standard for virtually all research into mental disorders, and it similarly constrains clinical trials, since the categories serve as the indications for new drug approvals by regulatory agencies. This situation severely hampers attempts to understand the fundamental genetics and pathophysiology of mental disorders and consequent attempts at mechanistically targeted treatment development. The paradox is thus that psychiatric research urgently needs studies which cut across diagnostic boundaries in order to provide the basis for developing an improved nosology that can usher the field into an era of precision medicine. Such a database cannot be developed, however, as long as research grants are funded solely in terms of DSM/ICD categories that obscure the relationships of genes, pathophysiology, and specific functional impairments.

This dilemma led the U.S. National Institutes of Mental Health (NIMH) to develop the Research Domain Criteria (RDoC) project, based on a goal in its 2008 Strategic Plan: "to develop, for research purposes, new ways of classifying mental disorders based on dimensions of observable behavior and neurobiological measures." RDoC is not a fully developed alternative system; it is a framework for research that is intended to provide a research literature that can support future revisions to the DSM and ICD that incorporate genetics and other areas of neuroscience. RDoC represents an attempt to free investigators from the shackles of research constrained to single DSM/ICD categories in several ways:

1. The system calls for research in fundamental functional dimensions (e.g., fear, reward, or working memory, and are formally termed "constructs") as studied along a full range of performance, from normal to abnormal. (It should be noted, however, that enriched sampling of subjects at the extremes of a distribution—that would correspond to DSM-level psychopathology—is envisioned for most studies, to maximize translation to clinical issues.)
2. Investigators are strongly encouraged to study functional dimensions in terms of multiple levels of measurement, including genes, circuit activity, behavioral measurements, and self-reports (at the extremes of a distribution, the latter measures would comprise symptoms).
3. The framework does not specify cutoffs for the presence of disorders, so as to focus attention on the dimensional relationships among the various measurements.
4. Researchers are free to devise sampling frames that may include patients drawn from multiple DSM categories (as well as a range of control subjects with subsyndromal pathology or normal functioning).

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5. Studies of neurodevelopmental trajectories and environmental influences are encouraged for all areas of psychopathology to develop new ideas regarding pathogenesis.

Particularly in the early phases of the RDoC project, it is anticipated that research projects will provide maximum yield by including, in the sampling frame, a group of subjects drawn from related diagnostic groups (and milder forms of pathology along the same putative clinical spectrum; e.g., all of the anxiety disorders, unipolar or bipolar depression, or autism spectrum). In many cases, it may be advantageous to cover a range of clinical severity and include in the sampling frame all patients from a given chapter of the new DSM/ICD metastructure; for example, the new "Schizophrenia Spectrum" chapter contains not only schizophrenia but also schizophreniform disorder, acute and transient psychotic disorder, schizotypal disorder, etc.

Once a sampling frame is specified, the investigator is at liberty to implement a research design with independent and dependent variables appropriate to the research question. For instance, in a study with autism spectrum children, the independent variable could include genetic combinations, as identified by a computer algorithm, and dependent variables of social cognition and/or language facility. In a study with patients from multiple anxiety disorders (and controls), a hypothesis could be based on recent reports that patients with blunted fear-potentiated startle in an emotional imagery challenge (independent of anxiety disorder category) report higher symptomatic levels of overall distress and diffuse anxiety compared to patients with high levels of fear-potentiated startle (McTeague and Lang 2012); thus, the independent variable could be a magnitude of fear-potentiated startle, while the dependent variables would be scores on various measures of distress and nonspecific anxiety.

The goal of RDoC is to support research that contributes to the validation of intermediate phenotypes based on particular behavioral functions and the neural circuits that implement them. It is hoped that such constructs will provide an improved data set for relating findings from genomics and neuroscience to important clinical symptoms, such as excessive fear and anxiety, disrupted reward behavior (as in anhedonia or, at the opposite extreme, mania), or impaired cognitive functioning. RDoC is clearly intended to be a long-term effort, one that will take ten years or more to mature. However, it is also hoped that initial studies will yield near-term benefits, to inform the development of new ideas about psychopathology and new approaches to clinical trials. RDoC is frankly an experiment on a large scale, and its precise ramifications remain to be seen. We anticipate that it will inform necessary long-term efforts to incorporate genetics and pathophysiology research into new avenues for precision medicine in psychiatry.

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The Early Stage of Neurodevelopmental Disorders: Detection, Intervention, and Prevention

The ultimate goal of our current research efforts is the early detection and prevention of neurodevelopmental disorders. How can we get there? One strategy involves the identification of genetic and environmental risk factors. If we can detect such factors in individuals before the disease phenotype becomes evident, this may provide an avenue for intervention and prevention. This strategy, however, creates ethical issues. For example, how do we weigh the benefits of diagnosis and treatment if we are unable to provide any treatment, or if there is the risk of adverse effects? Here we review some recent efforts to detect risk for neurodevelopmental disorders and to intervene before the full phenotype has developed.

It has been well established that untreated psychosis leads to poor outcome (Perkins 2006). This has led to intensive research of the early stages of psychosis, with the hope of being able to intervene early (Marshall and Rathbone 2011). More recently, researchers have moved further upstream in the disease process to study the psychosis high-risk state (also known as "ultra-high risk," "at-risk mental state," or "prodrome") (Fusar-Poli et al. 2013). The psychosis high-risk state can be defined as a genetic at-risk state (e.g., history of psychosis in parents) or as a subsyndromal clinical presentation, also known as attenuated psychotic symptoms or brief limited intermittent psychotic symptoms (BLIPS).

The North American Prodrome Longitudinal Study (NAPLS) reported five clinical variables that predicted later conversion to psychosis: genetic risk with functional decline, low social functioning, unusual thought content, paranoia, and history of substance abuse (Cannon et al. 2008). Additional studies provide preliminary evidence that the prediction of psychosis can be improved further by combining these clinical variables with family history of psychosis, neurocognitive measures, and advanced imaging studies (Fusar-Poli et al. 2013).

As cognitive deficits are core to schizophrenia syndrome, measures of cognition have been applied in the investigation of help-seeking people with prodromal features. Lacking are normative "growth charts" in well-phenotyped populations; these would enable integration of neurocognitive measures and psychosis features across development (Cannon 2014).

The Philadelphia Neurodevelopmental Cohort stratified youths (age 8–21 years) by degree of reported psychosis features assessed in a structured interview. All participants were administered a computerized neurocognitive battery, based on tasks acquired in functional neuroimaging studies. To compare performance-based growth charts, neurocognitive age was related to chronological age, established in typically developing participants (~1600). Across ages, the psychosis spectrum group (~1400) had a greater developmental lag than the group reporting mild psychotic symptoms (~900). Furthermore, neurocognitive delay was more prominent in youths endorsing psychotic symptoms

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than participants reporting nonpsychotic symptoms (~900). Combined clinical and neurocognitive assessment can facilitate early detection of individuals at risk for psychosis (Gur et al. 2014).

Early intervention of psychosis is a rapidly developing area of research (Marshall and Rathbone 2011). A small number of studies has found antipsychotic medication to be of little benefit in the prevention of psychosis. There is, however, compelling evidence that cognitive behavioral therapy (alone or in combination with antipsychotic medication) is helpful, and there is one intriguing study of omega-3 fatty acids reducing the conversion to psychosis (Amminger et al. 2010). These early studies provide hope that early intervention of psychosis is possible.

Not surprisingly, there is also considerable interest in both early detection and intervention in the service of secondary prevention of autism spectrum disorders (ASDs). There is currently a good deal of optimism regarding the value of reducing the age at diagnosis, which now is often several years after the initial signs are noted by parents, physicians, or caregivers, and in intervening as early as possible to alter developmental trajectories. Although some data supports this view, there remains a need for a substantial investment in prospective well-controlled longitudinal studies to address these issues definitively, including identifying the most efficient means of early detection and defining an evidence-based armamentarium for early behavioral and educational treatments (Kaale et al. 2014; Daniels et al. 2014).

At this stage, given the limitations of the available somatic treatments for ASD and the absence of therapeutics that target pathophysiological mechanisms, there is only a very small body of literature addressing early (as young as two years of age) pharmacological treatment (DeLong et al. 1998, 2002). However, as novel rational treatments are developed, and in light of the increasing ability to detect risk mutations at birth or before, the opportunities to test pharmacological therapies in the first weeks to months of life are likely to become conceivable, thus raising important clinical as well as ethical concerns.

From Genes to Treatment Targets in Neurodevelopmental Disorders

Recent successes in gene discovery in autism and schizophrenia are prompting a reassessment of established strategies for "bottom-up" translational neuroscience. The traditional approach of identifying a single gene of interest, creating one or more animal knockouts or knockins, and then elaborating molecular, cellular, and circuit level mechanisms has been tremendously fruitful in advancing basic understandings of the central nervous system and of rare monogenic brain disorders (e.g., FMRP, MECP2, and NF-1). The traditional approach has led to the identification of putative treatment targets and strategies. However, recent progress in the genome-scale genetics of neuropsychiatric disorders has

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revealed that, in general, risk of these disorders is polygenic. The scientific approaches that proved useful for the analysis of highly penetrant mutations that cause monogenic disorders are not likely to prove fruitful for the analysis of less penetrant genetic variants that act in diverse combinations to produce risk.

While the yield of recent gene discovery has been impressive, it has also revealed that both autism and schizophrenia (and other related neurodevelopmental disorders) reflect tremendous genetic heterogeneity. The genetic variants (alleles) involved tend to be biologically pleiotropic, and there is a surprising degree of phenotypic variability emerging from the same allele.

Given the early state of the research, the emerging picture of the genetic architecture of each of these syndromes remains unsettled. A dozen or more rare copy number variants (CNVs) have been found to increase risk substantially for both autism and schizophrenia.

Thus far there has been relatively greater success in schizophrenia in the search for common variation (polymorphisms) and somewhat greater success with regard to ASD in the discovery of rare *de novo* point mutations. It is not yet clear to what extent this reflects differences in the underlying genetic architecture that might be expected from the differences in severity and effects on fecundity versus differences in the predominance of different research strategies applied to the two disorders. However, this distribution of identified risk alleles has relevance for the design of translational strategies: common variation facilitates the identification of cohorts carrying alleles of interest, but individual alleles may be difficult to interpret *prima face* as they are often found in noncoding segments of the genome, and they typically carry small effects. In contrast, rare point mutations in ASD have been discovered through the analysis of *de novo* coding mutations. These are easier to interpret with regard to impact on protein function and, in some cases, may carry very large effect sizes; however, the population frequency of these alleles is low, with even the most common genes implicated by multiple independent mutations occurring in less than 1% of affected individuals. Moreover, none of the mutations identified to date are 100% penetrant and, for some, the range of possible outcomes has already been shown to involve a wide range of neurodevelopmental syndromes.

Together, these complexities make several aspects of the standard translational model more difficult:

- The selection of genes to study is complicated by their sheer numbers, and this will increase substantially with further genetic studies.
- Few of the identified mutations can be faithfully modeled by full knockouts, either for common or rare variations.
- Most disease-related variants identified so far are subtle variations that are present in either a predisposing or protective genetic background, which is a challenge for modeling the effects in inbred mouse strains.
- The means to model, in any system, multiple alleles of very small effect (either together or in parallel) are only just emerging and are currently

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limited to a handful of risk alleles rather than the many hundreds likely to be implicated in neurodevelopmental disorders.
- The manipulation of biologically pleiotropic genes with alleles of varying effect sizes suggests that multiple functional (and potentially subtle) phenotypes are likely to emerge across development. To date there is little information regarding precisely where and when to look for a human specific pathology.

Despite the very recent nature of systematic and reliable gene discovery in complex neurodevelopmental disorders, multiple conceptual strategies have already started to emerge to begin to address these problems. Roughly, these can be characterized as follows:

- Continuation of the current traditional approaches: knocking out genes in animal models, which focus on recent findings, point to the most highly penetrant risk genes.
- Use of a systems biology approach to organize disparate risk alleles of varying effect sizes into molecular pathways as a first step to organize both the choice of which genes to model further and the functional phenotype on which to focus.
- Use of a systems biology approach to organize risk alleles into networks based on spatiotemporal expression properties to capture developmental as well as molecular contexts.
- Leveraging high throughput technologies to engineer mutations in *in vitro* systems, looking for convergence of functional phenotypes *post hoc* via high throughput, unbiased phenotyping approaches.
- Human genetics that searches for protective alleles as markers of treatment target development via large-scale population studies of at-risk cohorts.

While the traditional approach of deeply modeling a single gene knockout may not *a priori* address all of the complexity described above, the focus on highly penetrant alleles has already demonstrated an ability to elaborate critical biological and pathophysiological processes and to help identify questions that will need to be addressed in broader systems biological approaches. Next we review synaptic changes in autism as an example of the progress of this approach.

The Synapse in Neurodevelopmental Disorders

Human genetics, animal models, and iPSC studies have yielded compelling evidence that synaptic proteins are affected in neurodevelopmental disorders. The first evidence came with the identification of rare X-linked mutations affecting the cell adhesion molecules neuroligins (NLGN3-4X) in patients with

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autism or Asperger syndrome (Jamain et al. 2003; Laumonnier et al. 2004). Following these results, further support came from heterozygous *de novo* mutations in the scaffolding protein SHANK3 and the presynaptic cell adhesion molecule NRXN1, lending credence to the hypothesis of a gene dosage-sensitive synaptic pathway in ASD (Autism Genome Project Consortium et al. 2007; Durand et al. 2007). Since then, mutations in more than 50 genes related to synaptic functions (receptors, cell adhesion molecules, scaffolding proteins, ion channels) or regulating synaptic gene levels (chromatin modeling factors, transcription and translation factors, signaling pathways) have been reported in patients with ASD (Huguet et al. 2013). Unbiased pathway analyses also confirmed a significant enrichment of mutations in genes related to synapses and/or to the fragile X pathway (Steinberg and Webber 2013).

Remarkably, variation in synaptic genes such as NLGNs, NRXNs and SHANKs are not restricted to ASD, but have also been detected in patients with intellectual disability (ID), schizophrenia, attention-deficit/hyperactivity disorder (ADHD), and bipolar disorders (for a review, see Guilmatre et al. 2014). Among these synaptic genes, *SHANK1–3* genes code for large synaptic scaffold proteins of postsynaptic density (Grabrucker et al. 2011). Interestingly, deletions, duplications, and coding mutations in *SHANK* genes have been detected in the whole spectrum of autism, but with a gradient of severity in cognitive impairment. Mutations in *SHANK1* are present in males with normal IQ and autism; mutations in *SHANK2* are present in patients with ASD and mild intellectual disability; mutations in *SHANK3* are present in patients with ASD with moderate to profound ID (Berkel et al. 2010; Leblond et al. 2012; Sato et al. 2012).

SHANK3 is the most studied gene from the SHANK family since its haploinsufficiency has been identified in more than 900 patients affected with chromosome 22q13 deletion syndrome, known as Phelan-McDermid syndrome (Bonaglia et al. 2011). The genomic rearrangements observed in these patients are diverse, ranging from simple 22q13 deletions (72%), ring chromosomes (14%), and unbalanced translocations (7%) to interstitial deletions (9%), all resulting in haploinsufficiency of the *SHANK3* gene (Bonaglia et al. 2011). In more than 80% of the cases, autism or autistic-like behavior is present (Betancur and Buxbaum 2013).

Mice lacking any of the SHANK proteins display phenotypes relevant to ASD (Jiang and Ehlers 2013). *SHANK1* knockout mice show increased anxiety, decreased vocal communication, decreased locomotion and, remarkably, enhanced working memory but decreased long-term memory (Hung et al. 2008; Wohr et al. 2011). *SHANK2* knockout mice show hyperactivity, increased anxiety, repetitive grooming, as well as abnormalities in vocal and social behaviors (Schmeisser et al. 2012; Won et al. 2012). *SHANK3* knockout mice show self-injurious repetitive grooming and deficits in social interaction and communication (Peca et al. 2011; Yang et al. 2012b; Wang et al. 2011). Consistent with gene dosage sensitivity, a Shank3 transgenic mouse modeling

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a human *SHANK3* duplication exhibits manic-like behavior and seizures (Han et al. 2013a). Interestingly, the mood-stabilizing drug valproate, but not lithium, rescues the manic-like behavior of Shank3 transgenic mice.

Cortical neurons derived from induced pluripotent stem cells (iPSCs) have been generated from patients with *SHANK3* mutations (Shcheglovitov et al. 2013; Boissart et al. 2013). In accordance with the haploinsufficiency, neurons display lower *SHANK3* mRNA and protein levels. They also present defects in excitatory, but not inhibitory, synaptic transmission that could be restored by overexpression of SHANK3 or IGF1 treatment (Shcheglovitov et al. 2013).

The Glutamatergic Synapse in Schizophrenia

Pathway and network approaches offer a viable avenue to organize disparate genes and address the observed genetic heterogeneity. At present, methods development is a key issue and, as discussed below, the resources necessary to carry out rigorous unbiased systems biological pathway or network analyses are limited, with resulting limitations on the reliability, biological depth, and specificity of the output.

It is highly encouraging that, despite the complexity of genetic findings in schizophrenia and the realization that we are still at the beginning of a longer process of gene discovery, there is already evidence for some convergence onto specific and highly plausible biological processes. Results from genome-wide association studies (GWAS) (Ripke et al. 2013), CNVs (Kirov et al. 2012), and sequencing studies (Purcell et al. 2014; Fromer et al. 2014) point to the involvement of functionally related sets of postsynaptic proteins in synaptic plasticity, learning, and memory. These include L-type calcium channels, postsynaptic scaffolding proteins involved in NMDA signal transduction, and proteins that interact with activity-regulated cytoskeleton-associated (ARC) protein, referred to as the ARC complex (Kirov et al. 2012), and brain-expressed genes that are repressed by fragile X mental retardation protein (FMRP).

These findings are notable not only for their consistency across several studies, but also for their convergence into a coherent set of biological processes involved in the regulation of plasticity, particularly at glutamatergic synapses. These synaptic processes have been implicated in cognition (Grant et al. 2005) as well as in a range of neuropsychiatric conditions, including ASDs and ID (Fromer et al. 2014). While it is highly unlikely that this is the only set of biological processes implicated in the disorder, the identification of at least one system involved in risk for schizophrenia and related disorders paves the way for more detailed mechanistic studies and potentially to stratified and novel therapeutic approaches.

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What Do We Need Now?

One strategy that has been suggested by many, albeit with clear downsides, is to differentiate fibroblasts, iPS cells, or hESC cells into desired neural types, and to purify them with a reporter gene that could be engineered into them. This would provide enough material for transcriptomics, proteomics, and epigenomics on a population of uniform cells. The downside, of course, is that cells *in vitro* are expected to be quite different from cells embedded in a circuit in a brain. Thus, to produce the best possible databases, it will be critical to iterate between postmortem human brain tissue (with all its problems) and *in vitro* cellular models.

High throughput cell-based assays, which leverage the recent development of iPSCs and genome editing, represent an exciting emerging possibility. The iPSC approach, like pathway and network analysis, seeks to leverage heterogeneity and phenotypic convergence, but does so based on the output of genetic manipulations. To achieve this goal, we need to develop assays that are reliable and replicable, address whether to model patient mutation in a natural genomic context or engineer known risk alleles into an isogenic background or both, and limit cell types that can be modeled and a set of phenotypes that might be reliably assayed using this approach.

Human genetics strategies that focus on protective alleles have recently been shown to be a productive avenue in identifying treatment targets subsequent to the identification of risk alleles (Flannick et al. 2014). These strategies would seem to be ideally suited at present to schizophrenia, given the highly productive efforts at common variant identification. In this case, a study would focus on a population sample, looking for individuals at high polygenic risk who do not have evidence of the phenotype. A similar strategy could be applied to rare loss-of-function (LoF) variants in ASD-associated genes, such as CHD8 in which LoF alleles have not yet been seen in large samples of unaffected individuals. Very large-scale research sequencing, or in-time, routine clinical sequencing, might conceivably capture a sufficiently large population to find first individuals with disruptive CHD8 mutations without ASD and then identify genetic moderators of that phenotype. Finally, we should not overlook the potential of high-throughput whole exome and genome sequencing in large samples to identify protective alleles at the population level by identifying rare alleles that are overrepresented in controls relative to cases. We note, however, that this will require the sequencing of large control populations.

These strategies all have considerable strengths as well as some limitations. In the final analysis, they represent important short-term options to begin to move forward with translational efforts in a broader context than is often employed at present. Of course, it is likely that complementarity among these approaches and very likely critical interactions will drive progress.

A key issue, however, is the lack of the foundational resources that are required if we are to exploit genetic discoveries through systems biology.

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Understanding the functional effects of risk alleles requires an investigation of the biochemical pathways or protein networks in which they act. Genetic variation can impact directly on protein structure and function, but many of the small effect risk alleles identified in GWAS lie outside the protein coding sequence and presumably impact on regulatory regions. Put simply, the underlying idea is that the alleles contributing to polygenic disorders act by making small nudges in a protein network which either summate or reach a tipping point to alter its function. Thus, the identification of such networks provides a point of biological convergence to study pathogenesis. Moreover, the most promising drug target may be a protein within the network that is not itself implicated genetically.

If we are to move rapidly and efficiently from the implication of multiple disease risk alleles to mechanistic insights, we need access to large-scale data bases which can be interrogated to identify sets of functionally related risk alleles. For example, we wish to know whether specific gene expression networks or functionally related groups of proteins are enriched among the genes implicated in a specific disorder. The annotation of the genome and proteome is relatively advanced in some tissues, but there is a dearth of such data for the brain. This so-called annotation gap will limit progress as we seek to translate genetic findings into mechanistic insights and new models of disease pathogenesis. The field needs shared resources that will provide information about transcriptomes, proteomes, and epigenomes at different stages of development and about protein interactions. Initially a realistic short-term target is to obtain such data from several hundred brains in multiple brain regions over several different developmental periods. However, with time, the goal should be to have such data available for individual cell populations and types. The ability to isolate individual cells from the brain and to have enough material from which to perform single cell RNA sequencing or proteomics are still a challenge. For the former, there are encouraging experiments that single cell RNA sequence in neurons will be possible in the short term. However, we are a long way off from being able to perform proteomic studies in single cells.

In addition to these resources for genetic research, there are several other methodological advances for clinical and translational research:

- More complex phenotyping: experience sampling of behavior and mood states (van Os et al. 2013; van de Leemput et al. 2014) as well as more comprehensive documentation of clinical features not currently included in the diagnostic criteria for neurodevelopmental disorders, but which may be relevant as gene-behavior relationships are explored (e.g., sleep, circadian rhythms, gastrointestinal problems, anhedonia).
- Longitudinal studies of behavior and brain could provide growth curves for brain measures (volume, function) and behavior in healthy controls and patient cohorts: eye movement behavior in autism (Jones and Klin 2013) or cognitive function in schizophrenia (Cannon 2014).

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- Studies of cognition as a continuous, quantitative measure to compare probands with noncarrier family members rather than a qualitative, dichotomous trait (Moreno-De-Luca et al. 2013).
- Studies of environmental risk factors (e.g., childhood maltreatment) and their effect on pathophysiological processes in neurodevelopmental disorders (Mehta et al. 2013).

Looking into the Future

We are making rapid progress in our study of neurodevelopmental disorders. At the same time, we face significant challenges in realizing the full potential of the opportunities given to us. Here we recommend three actionable steps for the research community.

First, patients and relatives need to become active participants in research. Translational neuroscience needs to learn from the experiences of the biotech and stem cell communities and work proactively to ensure that investigators stay connected with the public on research advances. The risk of negative backlash on research developments is high, given this group's focus on children, development, and intellectual/academic life achievement. In addition, public reaction to advances in understanding genetic and environmental risks will vary by country, culture, and generation, so anticipating communication issues will be helpful.

Patient-led initiatives to incorporate patient/public involvement (PPI) in the research process offer an important opportunity to inform the public and mitigate negative responses to research advances. Realistic steps include providing for PPI representation in research priority setting, consortia management, protocol development, study execution, and reporting. To be effective, PPI representatives need to have more than a general knowledge of science, statistics, and the translational research process. Training efforts have been incorporated into some PPI mechanisms, for example, in conjunction with the U.S. National Institutes of Health Councils, the INVOLVE program of the U.K. National Institute for Health Research, and programs sponsored by the U.S. Food and Drug Administration and the European Medicines Agency. Access to these programs, however, is limited and not necessarily focused on neuroscience. We recommend development of an integrated and internationally harmonized public education program to train patients/caregivers in the research process. The group of trained PPI representatives could be supplemented by seeking patient/caregiver representatives from within the ranks of neuroscience researchers. The Society of Neuroscience alone consists of over 33,000 international scientists, among whom mental health problems surely occur.

With best practices for PPI still in development, it is incumbent on research leadership to make the case for this approach. In the short term, PPI representation can have a positive effect on clinical research, shortening recruitment

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times and generally increasing satisfaction with research participation for the participants and investigators (Ennis and Wykes 2013). It is hoped that patient involvement will improve outcome assessments and trial design, thereby increasing the likelihood of successful studies. In the longer term, neuroscience must recognize the need for public education and take action to inform and advance research ethics discussions.

Second, research would be greatly enhanced by greater sharing of data and more stringent falsification of hypotheses. A fast-to-fail culture, well known in drug development, would speed up the neurobiological and genetic search strategies. This requires a change in the incentives for biomedical researchers. For example, researchers who need to compete for federal funding tend to produce more positive findings (Joober et al. 2012). NIMH has adopted the fast-to-fail study design for treatment trials, including a trial of kappa receptor antagonists for anhedonia (Reardon 2014).

Finally, we need to transform the education of mental health care providers and the public at large. We need to liberate ourselves from the constraints of diagnostic systems, to get closer to the individual as well as to the neural and genetic mechanisms of the mental disorders. We also need to develop a more positive relationship with regulatory agencies, so that the development of new treatments is not constrained by current diagnostic systems.

Similarly, we need to develop a core neuroscience curriculum for psychiatry residents and articulate a more differentiated view of genetic and environmental risk factors of neurodevelopmental disorders. For primary care, greater awareness of risk factors and attention to mental health assessments will be necessary for early identification and intervention strategies.

If we succeed with the research program as outlined in this chapter, what would success look like in ten years? We would have completed a full genetic screen of all risk alleles for neurodevelopmental disorders. We would have evidence for the causal mechanisms of environmental risk factors. We would have reasonable estimates of how much these genetic and environmental risk factors contribute to the development of a neurodevelopmental disorder in an individual. Finally, we would have made reasonable progress toward drug development, including the discovery and validation of new drug targets. Reaching these goals would greatly increase the return on research (see Wooding et al. 2013) and improve the lives of persons diagnosed with neurodevelopmental disorders.

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