Overview

Neurogenetics: Advancing the “Next-Generation” of Brain Research

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There can be little doubt that genetics has transformed our understanding of mechanisms mediating brain disorders. The last two decades have brought tremendous progress in terms of accurate molecular diagnoses and knowledge of the genes and pathways that are involved in a large number of neurological and psychiatric disorders. Likewise, new methods and analytical approaches, including genome array studies and “next-generation” sequencing technologies, are bringing us deeper insights into the subtle complexities of the genetic architecture that determines our risks for these disorders. As we now seek to translate these discoveries back to clinical applications, a major challenge for the field will be in bridging the gap between genes and biology. In this Overview of Neuron’s special review issue on neurogenetics, we reflect on progress made over the last two decades and highlight the challenges as well as the exciting opportunities for the future.

2011 marks ten years since the release of the first draft of the human genome, and as often happens with anniversaries, there has been much recent discussion, within both the scientific community and the general public, about what has often been called “the genetics revolution” and its impact on science and medicine. In this essay, we will outline the gains and the challenges of neurogenetic disease-oriented research. Considering the past two decades of advances in neurogenetics, there has been a wealth of exciting discoveries, but there are also opportunities to learn some lessons from failed experiments and a chance to reflect on challenges that might have prevented or delayed the development of successful interventions for some disorders.

There is no doubt that the sequencing of the human genome has been a critical scientific milestone that has revolutionized biology and medicine. Yet, it is noteworthy that in the decade before the completion of the human genome project, neurogenetics was already on the rise. Within both the scientific community and the general public, about what has often been called “the genetics revolution” and its impact on science and medicine. In this essay, we will outline the gains and the challenges of neurogenetic disease-oriented research. Considering the past two decades of advances in neurogenetics, there has been a wealth of exciting discoveries, but there are also opportunities to learn some lessons from failed experiments and a chance to reflect on challenges that might have prevented or delayed the development of successful interventions for some disorders.

Looking back, it is clear that many exciting discoveries would not have been possible without some key collaborations between astute clinicians and technically innovative basic scientists. It is also astounding how much these gene discoveries have taught us not only about particular diseases but also about basic neurobiology. The discovery of the gene for Duchenne muscular dystrophy (DMD) (Koenig et al., 1987) in 1986–1987 highlights the critical role of clinical genetics, cytogenetics, and linkage in delineating the location of a gene (Francke et al., 1985; Lindenbaum et al., 1979; Murray et al., 1982). DMD was one of the first gene discoveries for an inherited disorder, and over the last two decades, DMD has been a model disorder for the development of new diagnostics and therapeutics for a genetic disorder. In 1983, mapping the gene of Huntington disease (HD) to the short arm of chromosome 4 by using restriction fragment length polymorphisms and linkage in a large family marked a new era, wherein a disease gene can be mapped without any prior knowledge from cytogenetic abnormalities (Gusella et al., 1983). Likewise, the discovery of dinucleotide polymorphic repeats (Gyapay et al., 1994) and the ease of genotyping such repeats with polymerase chain reaction (PCR) facilitated genetic mapping and was a key factor in uncovering duplications and deletions of the PMP22 locus as the cause for Charcot-Marie tooth disease (CMT1A) and hereditary neuropathy with liability to pressure palsies (HNPP), respectively (Chance et al., 1994; Lupski et al., 1991). These landmark discoveries opened up the field of genomic disorders in neurobiology and beyond (Lupski, 2009).

Similar combinations of advanced cytogenetics, somatic hybrid techniques, and molecular genotyping played a critical role in refining the maps of several neurodevelopmental disorders including fragile X syndrome, Miller-Dieker lissencephaly, and Prader-Willi-syndrome (Ledbetter et al., 1981; Reiner et al., 1993; Verkerk et al., 1991). The discovery of polymorphic tri- and tetrancleotide repeats (Edwards et al., 1991) was a critical advance for defining dynamic mutations as a new mutational mechanism in several neurological disorders (see below). Thanks to this discovery, clinical enigmas such as the Sherman paradox in fragile X syndrome and the clinical phenomenon of anticipation, involving an earlier onset and more severe disease in successive generations, such as seen in disorders like myotonic dystrophy, HD, and the ataxias, were resolved. The development of large insert cloning and other physical mapping techniques (Burke et al., 1987; Schwartz and Cantor, 1984), as part of the framework for sequencing the human genome, played a crucial role in facilitating the discovery of many disease genes during the nineties. Certainly, cloning the gene for Rett syndrome would not have been possible in 1999 had it not been for the
intense mapping and sequencing efforts on the X chromosome (Amir et al., 1999).

With the release of the first drafts of the human genome sequence in 2001, the landscape of gene discovery changed tremendously, so that the once tedious physical mapping and cloning experiments slowly gave way to candidate gene analysis by sequencing. Computational analysis of mapping intervals and careful selection of candidate genes for sequencing replaced months and years of bench work. Having the sequence of genomes from several other species, like mouse, Drosophila, and, more recently, nonhuman primates, has certainly advanced neurobiological disease research, permitting in vivo functional studies in a whole range of model organisms.

Without doubt, during the last ten years, thanks to the integration of phenotypic mapping and sequencing data with tremendous analytical and computational resources, the neurobiology community has witnessed disease gene discoveries at an amazing pace, sometimes on a monthly or even weekly basis. Equally inspiring and impressive has been the fact that these gene discoveries have revealed new insights into basic biological mechanisms that extend far beyond the specific disease in question. Yet in the face of such tremendous progress, it has been surprising to see that there has been a fair amount of pessimism within the popular press about whether “the genetics revolution” has fulfilled its promise. In June of this year, the title of a prominent NY Times article stated, “A decade later, genetic map yields few new cures” (Nicholas Wade, NY Times, June 12, 2010). The first sentence read, “Ten years after President Bill Clinton announced the first draft of the human genome was complete, medicine has yet to see any large part of the promised benefits.” Questions have been raised about whether the investment in genetics and genomics has delivered.

Yet, when one considers some of the transformative changes in the realm of human neurogenetic disorders, there can be little doubt that, in fact, genetic discoveries over the past two decades have already changed not only the practice of clinical medicine in neurology and psychiatry, but also the outlook for many families afflicted by these devastating disorders. A mere 20 years ago, patients with hereditary ataxia had to undergo a number of expensive and sometimes invasive investigations including brain scans, spinal taps, numerous blood tests, possibly electromyograms, nerve conduction studies, and sometimes peripheral nerve biopsies. Similarly, children with neurodegenerative disorders or cognitive disabilities had to endure a large number of tests, scans, and sometimes skin or conjunctival biopsies; worst yet, a definitive diagnosis could not be reached and the family was typically left with an uncertain 50% or 25% chance of recurrence in subsequent offspring.

Today, a large number of childhood and adult neurological disorders can be diagnosed by a simple DNA test on peripheral blood, saving patients the pain and cost of many additional tests and providing them with a definitive diagnosis. This advancement affords families a better understanding of the disorder affecting their relatives while providing them with the option of genetic counseling and allowing for preimplantation or prenatal genetic testing, which could not have been done previously. There are several hundred neurological disorders that can now be diagnosed molecularly, including hundreds of cognitive and developmental disabilities such as autism spectrum disorders, dozens of inherited ataxias, inherited neuropathies, dystonias, muscular dystrophies, epilepsies, familial degenerative disorders, and a handful of psychiatric disorders (http://www.ncbi.nlm.nih.gov/sites/GeneTests/?db=GeneTests). While arguably the pace of development of potential therapies has been relatively slow compared to the speed of disease gene discovery, we should not underestimate the great benefits to families of disease prevention through prenatal diagnosis, and the gains in fundamental neurobiology from pathogenesis studies of neurological disorders.

Humans Provide the Largest Series of Phenotyped Alleles Revealing New Mutational Mechanisms

We should also not underestimate the impact that genetics has had on our understanding of brain development and function. It is a truism that humans are the best model system. It has been said that every nucleotide change in the human genome compatible with life is present at least once in someone on the globe. Likewise, no other species on earth is scrutinized so closely at a phenotypic level. Advances in clinical medicine and diagnostic technologies have radically expanded the range of diagnostic tests that physicians can use to unravel a patient’s symptoms and history. With respect to understanding brain function, diagnostic imaging techniques such as high-resolution MRI in 2D or with 3D reconstruction allow for visual characterization of the human central nervous system at an unprecedented level, and functional MRI and MR spectroscopy are starting to bridge the gap between sheer structural phenotyping and physiological consequences with functional relevance. Moreover, electronic communication spreads the word of unusual phenotypes globally, whereas in the past, knowledge of an extraordinarily rare disorder may have been confined to one country or even village. Thus, humans possess a genome rich in variation being examined daily by physicians for phenotypic variation—a geneticist’s dream come true.

Our ability to appreciate full phenotypic variation is directly related to the concept of intraspecies examination. Human variation is so rich because we, as examiners, and humans ourselves, can discern remarkably subtle differences among each other. This precision of deducing subtle variation and the degree by which such variation is cataloged in humans has uncovered completely new mechanisms of disease. Several novel and now well-appreciated mutational mechanisms of neurological disease owe their original description to human studies. Uniparental disomy (Spence et al., 1988), which uncovered the importance of genetic imprinting in neurobiology, has its origins in human genetics. Prions, which cause various transmitted spongiform encephalopathies, were discovered in humans and led to the then startling notion of the propagation of protein misfolding by misfolded proteins (Pruisner, 2001; Mastrianni, 2010). Dynamic mutations involving trinucleotide repeat expansions, originally discovered in fragile X syndrome and X-linked spinal and bulbar muscular atrophy, now account for well over a dozen other disorders and remain a largely
human-specific mutational mechanism (Orr and Zoghbi, 2007).

Further, as we have learned more about our own human genetic make-up, we have found insights into common biologies shared with other species as well. Consider the FOXP2 gene, identified in a family segregating developmental verbal dyspraxia or the inability of sequencing muscle movements required for articulating speech (Lai et al., 2001). While a clear-cut phenotype observed by humans in humans, the subtlety of the phenotype would be unrecognizable by us in another species. Recognizing the human phenotype, however, has now allowed the demonstration that the FOXP2 transcription factor is important in neuronal circuitry in mice and songbirds (Kelley and Bass, 2010; Schulz et al., 2010).

Same Phenotype, Many Genes—One Gene, Many Phenotypes
With better diagnostics and greater levels of clinical scrutiny over the past century, we have been able to more thoroughly document the clinical and pathological features of various disorders and classify disorders into different categories. While these clinical characterizations have been extremely valuable in making some sense of various phenotypically overlapping neurological and psychiatric disorders, they are also limiting in other respects. For one, these classifications change every few years depending on the experts evaluating the patients, better documentation of signs and symptoms, and the availability of new diagnostic tests, rendering it a challenge to keep up with the changing classifications. Likewise, at times what seem to be discrepancies between clinical criteria and pathological measures can blur the lines between categories. Now with genetics, this is beginning to change and in the process, some of the apparent confusion about classifications and categories is starting to make sense. A number of inherited ataxias have clinically similar, if not identical, features, yet genetic studies proved to us that they are indeed caused by mutations in distinct genes. Similarly, many neuropathies, dystonias, myopathies, and cognitive disorders are clinically indistinguishable yet genetically distinct. In many cases where there is apparent clinical overlap, but different genes, the similarity may be reflective of a convergent biology and common pathways. Basic research has revealed that there is indeed a biological reason for the clinical confusion and that understanding the biological basis of these disorders may shed light on why they overlap clinically. The protein products of many genes that cause overlapping phenotypes do indeed interact either directly or indirectly, and several of these proteins function in coincident pathways. This is true for proteins causing ataxias, muscular dystrophy, tuberous sclerosis, autism spectrum disorders, Parkinson disease, and Alzheimer disease (Brouwers et al., 2008; Cookson and Bandmann, 2010; Ess, 2010; Lim et al., 2006; Toro et al., 2010; see also the Perspective by Hardy [2010] in this issue). Ultimately, the discovery of the causative genes for these disorders may make the clinical classifications obsolete whereby new generations of neurologists could simply focus on the causative DNA mutation to classify a disorder. Likewise, from the standpoint of basic science, given the wealth of documented phenotypes in humans and the rapidly increasing knowledge of the causative genes, revisiting clinical data and reconsidering the implications of distinct genetic causes could lead to new hypotheses about functionally related pathways just as Drosophila geneticists have beautifully elucidated the Notch signaling pathway by starting with mutants that share similar phenotypes (Fortini, 2009).

Indeed, this is a lesson learned. The brain represents a huge mutational target where many loci encode interacting proteins and a dysfunction of any one may produce a similar consequence to the entire pathway. With this in mind, perhaps it is not surprising that very few strong genome-wide association signals have been uncovered for psychiatric disorders. If we lump phenotypes into a single clinical category (i.e., schizophrenia), the degree of genetic heterogeneity present renders association studies extremely underpowered. It may be wise to consider stratification of behavioral phenotypes into very specific subcategories, as was done for the many genetic ataxias, when pursuing gene identification.

A more surprising discovery is the finding that mutations of one gene might cause clinically distinct phenotypes in different patients. Examples include the Aristotle-related homeobox gene (APRX), which causes X-linked lissencephaly, agenesis of corpus callosum with abnormal genitalia, cognitive deficits with or without seizures, or cognitive deficits, dystonia, and seizures (Partington syndrome) (Shoubridge et al., 2010), as well as the SHANK3 gene, whose mutations can cause Phelan-McDermid syndrome, Asperger syndrome, autism, and rare cases of schizophrenia (Durand et al., 2007; Gautier et al., 2010; Phelan, 2008). Similarly, mutations in NRXN1 can cause rare forms of autism and schizophrenia (Kim et al., 2008; Rujescu et al., 2009), whereas mutations in LMNA, encoding for lamin A and lamin C (Stoskopf and Horn, 1992), can cause a variety of disorders including Emery-Dreifuss muscular dystrophy Type 2 (Bonne et al., 1999), Charcot-Marie-Tooth axonal neuropathy (CMT2B1) (De Sandre-Giovanni et al., 2002), limb girdle muscular dystrophy Type 1B, Hutchinson-Gilford progeria syndrome (Eriksson et al., 2003), and various other distinct clinical phenotypes. Altogether, these findings suggest that the neuroanatomical and physiological disturbances resulting from dysfunction of the respective genes may be influenced by the genetic background and environmental experiences of the affected individuals, leading to different clinical outcomes in different patients.

Related to this, some of the most interesting discoveries pertain to the finding that the nervous system is exquisitely sensitive to the dosage of many proteins. Haploinsufficiency or loss as well as doubling of several genes seems to cause overlapping neurological phenotypes including Parkinson disease, Alzheimer disease, the case of peripheral myelin protein 22 in neuropathies, MeCP2 in Rett syndrome, and MeCP2 duplication disorders, and the example of gain-of-function and loss-of-function mutations in neuronal ion channels causing epilepsy and other neurological deficits (Amir et al., 1999; Catterall et al., 2008; Van Esch et al., 2005; Zhang et al., 2010). Although it is still not clear how either loss or gain of the same protein or proteins causes similar cognitive and social behavior phenotypes, it is conceivable that the phenotype is a manifestation...
of a failure in neuronal homeostatic response due to downstream effects of various molecular changes (Ramocki and Zoghbi, 2008; Toro et al., 2010). Understanding neuronal homeostatic responses and their modulation may facilitate development of potential therapies for a broad class of disorders, irrespective of the underlying primary genetic defect.

**CNVs and Variable Penetrance**

Gene dosage alteration has emerged as a widespread phenomenon in neuropsychiatric disease, largely in the form of copy-number variation (CNV) (Pollack et al., 1999). The delineation of the human genome sequence allowed the widespread survey of deletions, duplications, and inversions. Surprisingly, the normal human genome is littered with such variation (Redon et al., 2006; Sebat et al., 2004). Most are considered common benign polymorphisms (although it remains unclear how such normal variation might contribute to the mutational load predisposing to disease), but the rare, large, or, even more important, de novo CNVs seem to contribute substantially to several complex genetic disorders. Sebat et al. (2007) reported in 2007 a ten-fold elevation in large, de novo CNVs in autism with similar observations reported for schizophrenia, epilepsy, and idiopathic intellectual disability. Such recurrent CNVs may finally provide a definitive genomic location for genes that may predispose to these disorders.

An emerging feature of disease-associated CNVs is the variable expressivity exhibited by many of these DNA rearrangements (Girirajan and Eichler, 2010). Deletions and duplications at 16p13.11 were initially reported in children with intellectual disability (Ullmann et al., 2007), then autism (Hannes et al., 2009), then epilepsy (Heizen et al., 2010), and finally schizophrenia (Ingason et al., 2009). Similarly, 1q21.1, 15q11.2, 15q13.3, and 16p11.2 CNVs, to name just a few, have all been associated with diverse neuropsychiatric phenotypes (Lee and Scherer, 2010). Another example of this potential clinical continuum is seen at 3q29 where similar deletions have all been associated with either intellectual disability and mild dysmorphic feature (Willatt et al., 2005), autism (Quintero-Rivera et al., 2010), bipolar disorder (Clayton-Smith et al., 2010), or schizophrenia (Mulle et al., 2010). Within the 3q29 interval lie two genes, PAK2 and DGL1, both of which have paralogs elsewhere in the genome that lead to intellectual disability and are strong candidates for further study.

While disease-related CNVs must operate, at a very fundamental level, by dosage sensitivity, it remains unclear how specific CNVs result in such disparate phenotypes. There are several possibilities. First, the precise CNV breakpoints, in many cases, are not mapped to the nucleotide level, so overlapping variants could exhibit different phenotypes. A deletion may unmask a recessive variant on the second allele or there could be cis effects where the CNV influences the expression of nearby loci. Alternatively, as has been well documented in imprinting disorders, the loci within the CNV may exhibit parent-of-origin effects with the consequence of differing levels of expression of the now hemizygous genes on the second allele.

Another possibility and perhaps a general concept for rare disease-associated CNVs is that such variants lower susceptibility thresholds and based upon genomic background and/or environmental influences result in phenotypes on a clinical continuum. Eichler proposed a more specific two-hit model (Girirajan et al., 2010) where a second CNV, elsewhere in the genome, influences penetrance and expressivity of the first CNV. Evidence for this model is best illustrated by a 600 kb microdeletion at 16p12.1 that has been found in children with intellectual disability and/or autism. Unlike most disease-associated CNVs, the 16p12.1 deletion is rarely de novo, being inherited from one parent in ~95% of the cases. The carrier parent, while cognitively intact, often exhibits milder phenotypes such as depression, mild learning disability or seizures. In 25% of the cases, the child with the more severe phenotype has a second large CNV elsewhere in the genome, a 40-fold increase over the predicted rate. A corollary of this model is that rather than a second CNV, a second conventional mutation elsewhere in the genome tips the balance to a severe phenotype. Low-cost deep-sequencing technologies should uncover such gene-gene interactions. Clearly, many more genetic studies on CNVs influencing neuropsychiatric disease are required, but from our perspective, CNV research is well worth following for the neuroscientist interested in disease mechanisms.

**Going Back to the Basics**

With the excitement and rapid pace of disease gene discovery came the expectation that therapies for these disorders are also readily within reach, and a major frustration that is often expressed by patients, their families, members of Congress, and disease-based foundations is the lack of effective therapies in spite of the formidable and exciting rate of disease gene discovery. This may be in part due to the false expectations or the hype around what a gene discovery can deliver in the short term. Gene discovery is a critical but only a first step in the path to therapeutics development. A variety of intricate in-depth investigations must take place in order to reveal pathways that lend themselves to therapeutic intervention in any given disease.

For instance, if one considers the path to the discovery of the tyrosine kinase inhibitor, Gleevec, one realizes that it took approximately 40 years of dedicated and intense basic research from the time of the identification of the Philadelphia chromosome until the FDA approved the drug. Perhaps what is more important is to identify the potential obstacles that might have contributed to such a delay and the steps in the process that are most challenging, and then one can implement solutions to accelerate the path to drug discovery. One thing is certain: to increase the likelihood of success in developing targeted therapies, one needs to understand the function of the protein or RNA mediating a specific disease process.

It is becoming abundantly clear that most proteins serve many diverse functions rather than only one or two isolated functions. Yet, traditionally, investigators have focused on one aspect of a disease protein based on one or more observations reproduced in several outstanding labs. While this research strategy is productive because it is focused and will likely yield insight into some function of the culprit protein, it is often not sufficient when one is tackling a complex
neurological disorder. Perhaps the case of amyloid precursor proteins (APP) and presenilin1 (PS1) is illustrative of this point. There is no doubt that dosage of APP is critical to the generation of Aβ, and that Aβ accumulates in the aging brain and in Alzheimer disease (AD). The vast majority of scientists and pharmaceutical companies have focused on this aspect of AD for the development of therapeutics. In recent years, however, research into other potential functions of APP and PS1 is beginning to yield additional potential mechanisms by which excess APP or mutant PS1 might compromise neuronal function. For instance, the study by Nikolaev and colleagues demonstrating that APP binds death receptor 6 (DR6) and activates caspase 6, which in turn triggers axonal pruning and neuronal death, is interesting and important. The finding highlights a role for a portion of APP distinct from Aβ, (N-terminal cleaved fragment that is also generated in a β-secretase dependent manner) as a potential contributor to AD (Nikolaev et al., 2009). More recently, Lee and colleagues demonstrated that presenilin1 is critical for targeting the v-ATPase V0a1 subunit to the lysosome, and thus for the proper acidification of the autolysosome (Lee et al., 2010). Interestingly, several AD-causing mutations in PS1 compromised this function, raising the possibility that defective lysosomal proteolysis is a key contributor to AD pathogenesis. The odds are that there are many mechanisms that contribute to AD and that it may be necessary to target more than one of these mechanisms and at multiple stages of the disease (perhaps including initiation and progression, rather than just the end stage) to rescue and treat the disease effectively. Taking a fresh and unbiased look at disease-causing proteins to pursue their characterization in depth might remove some of the barriers to accelerating research. As clues about potential new functions of these proteins emerge, validating such functions in vivo and in various disease models will be critical. Cross-species studies have been crucial for revealing in vivo protein functions, but ultimately the studies have to be done in rodent or nonhuman primates in preparation for translational studies and therapeutic interventions.

On Animal Models
One of the clear lessons learned over the past two decades is the importance of animal models in delving deep into the pathophysiology of neurogenetic diseases. Without question, mice, Drosophila, zebrafish, C. elegans, and even yeast models have provided an experimentally tractable model to test mechanistic hypotheses that might be impossible to evaluate in humans. The mouse has been the mainstay in animal models for neurogenetic disorders due to robust gene manipulation techniques, similar neuroanatomical structures, and the ability to perform electrophysiology to directly measure neuronal function and circuit activity. The toolbox for the genetic manipulation of the mouse is full of clever and highly useful approaches. In addition to knockout and knockin approaches, spatial or cell-specific regulation, as well as temporal and/or reversible regulation, have been widely utilized. A spectacular example is Adrian Bird’s demonstration that activation of a silenced MeCP2 gene reverses the Rett syndrome-like phenotype in adult mice (Guy et al., 2007). This work fueled the notion that seemingly hopeless developmental disorders, such as Rett syndrome or fragile X syndrome, may be therapeutically approachable.

Less robust but still of strong utility are mouse behavioral models of neurogenetic disorders. Interlab variation has sometimes led to discordance in the literature. Some of this variation was due to less appreciated but substantial strain differences and the need for true isogenic backgrounds for behavioral studies. Ideally, neurobehavioral studies should be performed on F1 hybrids derived from two different pure isogenic backgrounds to control for strain-specific deficits that might confound the phenotype of the engineered mutation. We also learned that investigator-specific differences sometimes lead to failure in replication, often due to subtle differences in procedures or housing environment. Alternatively, we may be simply asking too much of the humble rodent (Sousa et al., 2006). For example, a number of human loci, when mutated, lead to severe intellectual disability. The same mutations in the mouse do cause learning differences, but sometimes only by phenotypically large numbers of mutant and control mice can such differences be discerned statistically (Bouwknecht and Paylor, 2008). This is not the ideal situation for preclinical testing of drugs, since the effect size in the rodent is so small.

What can be done to improve this? Better testing paradigms may be of some help but, for many cognitive tests, laboratory mice, descendents of “fancy” or pet mice at the turn of the last century, were directly or inadvertently selected for a docile manner, which perhaps dulled their abilities to perform in many paradigms of learning and memory. Use of more outbred mice, such as M. spretus (Dejager et al., 2009), is one possibility although difficult to breed and house. Another possibility is to return to the rat, for decades the animal of choice for behavior studies that fell out of favor to the mouse, which was genetically modifiable. Recent advances in making targeted mutations in the rat may mark a return of this species (Geurts et al., 2009; Tong et al., 2010).

Another model system that has proven itself very useful in neurogenetic research is Drosophila. It was somewhat surprising initially that Drosophila models have replicated many of the pathogenic processes of human neurological disorders, despite gross anatomical and genomic differences between humans and fruit flies. Of an estimated 392 human genes that lead to cognitive deficiency when mutated, over 300 have Drosophila orthologs (A. Schenk, personal communication). The power of Drosophila as a genetic model to identify genetic interactions and epistatic loci is unmatched and permits assignment of loci to established pathways given the rich gene ontology and phenotypic data in this model organism. Studies of the consequences of loss of parkin function illustrate this point most clearly. Parkin null mice did not manifest overt neurodegeneration or behavioral phenotypes. Drosophila parkin mutants on the other hand revealed the importance of parkin for mitochondrial functions. This discovery eventually opened up the field on the importance of several Parkinson disease-causing loci for mitochondrial integrity and function (Clark et al., 2006; Greene et al., 2003; Jones, 2010; Park et al., 2006; Pesah et al., 2004).
In recent years, with the ability to develop transgenic monkeys, the nonhuman primate *Rhesus macaque* has emerged as a potential model system. Chan and colleagues (Yang et al., 2008) developed a Huntington disease transgenic *Rhesus* model that displayed a number of clinical features, seen in human that are absent in the mouse model. Although currently limited to transgenic manipulation only, the monkey, even with the expense, may prove valuable for preclinical testing, particularly when the mouse phenotype lacks certain human phenotypes, such as neuronal loss in Huntington disease (HD). In a similar vein, other large animal models, such as transgenic pigs that have recently been shown to better mimic HD than the mouse model (Yang et al., 2010), may also prove to be useful. It should, however, be pointed out that while incredibly valuable, there are potential limitations to animal models and it is critical to assess these limitations for better understanding of human disease pathogenesis. For instance, a great deal has been learned using these and other animal models of HD. However, a large number of HD pathogenesis studies rely on models that do not accurately recapitulate the HD mutation. Rather, many use a truncated short peptide containing the polyQ tract for experimental convenience and because animals expressing this short peptide manifest neurodegeneration in a short time frame. Since therapeutic strategies must target pathways affected by the entire protein, such shortcuts might, in the end prove unfortunate and counterproductive. Mouse and *Drosophila* animal models for spinocerebellar ataxia type 1 and spinobulbar muscular atrophy, two polyQ disorders, have clearly demonstrated the importance of domains beyond the polyQ tract in disease pathogenesis (Duvick et al., 2010; Fernandez-Funez et al., 2000; Kratter and Finkbeiner, 2010; Nedelsky et al., 2010).

Regardless of the model system, it will be important to conduct preclinical testing over an extended period of time. Too often, such studies are short term and leave the long-term consequences unanswered. It will be important to determine during preclinical testing which phenotypic improvements are sustained and which are not. While expensive, it is infinitely less expensive and devastating than a failed human trial. With the revelation of new insights into the molecular causes of Mendelian disorders and the wide availability of chemical library screening, it is likely that lead compounds for therapeutic development will arise for numerous human genetic disorders. The challenge will be to maintain and translate the quality and comprehensiveness of the preclinical testing into model systems. Scientists must bear the responsibility of conducting and replicating preclinical trials and insuring that meaningful outcome measures are improved. Rigorous design of preclinical trials will save hundreds of millions of dollars in failed clinical trials and will liberate patients to be enrolled in clinical trials with higher promise.

**The Future**

The field of neurobiology is well positioned to take advantage of two decades of gene discoveries, excellent animal models, and lessons learned from failed experiences. Key to the success of disease-oriented research are a few but important ingredients: in depth studies to gain knowledge about the function of the culprit proteins, excellent animal models of disease, and an understanding of the physiological and pathological consequences of disease protein dysfunction. As much as possible, investigators must use models that express the human mutation in the context of the full proteins, and in the correct spatial and temporal expression patterns. Conclusions must be based on in vivo studies that use interdisciplinary approaches including cell biological, molecular, behavioral, and physiological methods. Investigators with diverse expertise must work together for better characterization of animal models and for identification of quantifiable and reliable outcome measures.

As important as good preclinical science is to the ultimate success of translating scientific discoveries to clinical application, we also desperately need appropriate infrastructure. This requires successful partnerships between academic research, governments, private institutions, and foundations and pharmaceutical industries. Academic researchers are best at performing fundamental research studies. They are motivated to do these studies irrespective of how esoteric or rare the problem they are tackling. Performing multidisciplinary in vivo and interdisciplinary pathogenesis studies is certainly best done in academia, and such studies are likely to reveal pathways that can be targeted therapeutically. On the other hand, pharmaceutical companies have unparalleled expertise in medicinal chemistry and a rich resource of drugs and compounds that can be repurposed for specific neuropsychiatric disorders based on relevant discoveries. Somehow, we need to better bridge the interests and expertise of academic science and industry. The challenges that might keep the pharmaceutical companies from sharing their compounds for repurposing must be identified and overcome. Barriers such as a fear of the U.S. Food and Drug Administration’s response to side effects that might result from a new use must be managed by educating the FDA that drug/genotype effects need not be generalized. The case of the mGluR antagonist is an example where an existing drug was put to good use. When it was discovered that the loss of FMRP in fragile X syndrome leads to apparent overstimulation of mGluR (Huber et al., 2002), it became apparent that lowering mGluR5 signaling might suppress several phenotypes in the fragile X syndrome mouse and fly models (Bear et al., 2004). Merck, having mGluR5 antagonists in its collection, licensed a compound for development as therapeutic in fragile X syndrome, despite the fact that fragile X is a relatively rare disorder and perhaps not likely to be as profitable a market as would be expected for a development of a new drug. Pharmaceutical companies often have to weigh the risks and benefits of repurposing drugs or forging collaborations to treat rare disorders, but this is the right thing to do and a risk worth taking. Most disorders are indeed rare by themselves but cumulatively account for a large number of cases of a “common” disorder. Also, predisposition to common disorders may reflect accumulation of several rare genetic variants. Thus, if we can develop effective therapies through a successful collaboration between academia and pharmaceutical companies, the odds that we can gradually tackle
rare neuropsychiatric disorders effectively will also be enhanced.

A few principles are worth considering as we ponder solutions for the growing list of neuropsychiatric disorders. We need to invest in studies aimed at a better understanding of normal brain development. While we often think of development as being critical for understanding the integrity of the brain in infants and in children, data are gradually pointing to the fact that early developmental processes might impact the integrity and health of the adult and the aging brain as well. A better understanding of both the genetic and environmental factors that affect synapse development and maintenance is likely to provide information on how such factors might be co-opted to protect the aging brain or a brain dealing with mutations causing late-onset neurodegenerative disorders. The role of epigenetics in modulating disease processes must also be investigated. The reality is that many genes will be very hard to target or manipulate to suppress a disease phenotype. However, one can epigenetically modulate the function of genes without altering their sequence or activity. The findings that environmental factors such as diet (Waterland et al., 2006; Weaver et al., 2005; Wolf et al., 1998) or experiences (McGowan et al., 2009) can alter the epigenome open up the possibility that modulating gene function through epigenomic modifications might be a productive way to treat some disorders. Of course, it is equally important to consider the flip side of the coin, that damage to the epigenome from changing environmental factors could also contribute to or aggravate disease. The growing list of genetic defects that may lead to neuropsychiatric disorders point to the many ways any molecular perturbation can lead to devastating neurological phenotypes. However, we must not forget what we can learn from individuals who have similar mutations but milder phenotypes. Exploring environmental factors and experiences that might have protected such individuals, or identifying suppressor mutations through the new deep-sequencing technologies, might reveal factors that modify a disease process. Lastly, and perhaps a most exciting note with which to conclude, is the fact that genetic research has revealed the plasticity and resilience of the developing and adult brain. Gene discovery and creative genetic engineering in mouse models have taught us that many neuropsychiatric disorders are reversible (at least genetically) (Guy et al., 2007; Santacruz et al., 2005; Yamamoto et al., 2000; Zu et al., 2004). The findings that several disorders, including some of the most devastating developmental and degenerative diseases are reversible in mouse models, provides hope that discovering ways to counteract or suppress disease processes might halt or even reverse some of the most serious neurological and psychiatric disorders.

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Overview


