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Preclinical and Clinical Understanding

Major Gaps in Our Understanding and Capabilities

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Abstract

Age-associated diseases are an inevitable, costly, and burdensome outcome to societies as life expectancy increases. All countries face this growing problem even as there are notable successes in treating some of these diseases. For example, effective control of hypertension has been accompanied by a noteworthy reduction in the incidence of cerebrovascular disease. Unfortunately, the same cannot be said for neurodegenerative diseases, such as Alzheimer, Parkinson, motor neuron, and Huntington diseases, despite the tremendous recent progress that has been made in understanding the molecular pathogenesis of this group of brain disorders. Currently there is no effective treatment that delays the onset or slows the natural progression of these diseases. Even the very effective pharmacologic and surgical therapies for Parkinson disease are directed solely at motor symptoms; neither the nonmotor symptoms nor the gradual deterioration can be effectively treated or prevented. This chapter discusses some of the difficulties faced in discovering and developing effective treatments for these diseases, from both the preclinical and clinical perspectives. Some of the gaps in our knowledge and critical questions that must be addressed in the near future are described, with an emphasis on Alzheimer disease. The challenges are many and include incomplete understanding of disease pathophysiology, deficiencies in animal models, and inefficiencies in translating new genetic, molecular, cellular, and neurobiological insights into the clinical arena. There is increasing consensus that treatments which target the potential disease triggers will likely be more effective when given before the onset of clinical symptoms. This,

however, brings additional challenges—from the need for new biomarkers to improvements in the design and execution of clinical trials—which the research community must quickly address.

Introduction

In many ways, diseases of the brain represent the “final frontier” in our quest to understand disease causation and to discover effective treatments. For those working on diseases of the nervous system, the brain is wondrously elegant and intricate in its design and function, yet ever so complex in disease. Neurodegenerative disorders constitute a group of diseases that share in common neuronal loss in characteristic brain regions, often with pathological hallmarks, which are present in both genetic and sporadic forms, the latter frequently demonstrating genetic susceptibility traits. Importantly, as a group, they are age-associated such that their incidence rises with advancing age. As such, the increasing prevalence of this group of diseases is an unwanted outcome of the rise in human longevity. Tremendous advances have occurred over the past two decades in our understanding of the pathophysiology of many human diseases, including neurodegenerative diseases. From these advances, it has become evident that misfolding and aggregation of proteins—such as amyloid β ($A\beta$) protein, tau, TAR DNA-binding protein 43 (TDP-43), superoxide dismutase-1 (SOD-1), α -synuclein, or huntingtin—underlie most of the neurodegenerative disorders. Furthermore, aggregated proteins most likely play pivotal roles in initiating the disease processes. Yet, as with many other diseases, effective treatments often lag many years behind breakthroughs in basic research. Thus, with the possible exception of multiple sclerosis, whose pathophysiology is quite different, effective treatments for neurodegenerative diseases have been frustratingly elusive. Against the backdrop of several negative pivotal clinical trials over the past several years, the community has not surprisingly begun to question not only our current approaches but also the hypotheses underlying disease pathophysiology.

This Ernst Strüngmann Forum was convened to review the present state of knowledge of neurodegenerative diseases and to generate new ideas about research directions in basic pathophysiology and new paths for preclinical as well as clinical research. This chapter discusses some of the major gaps in our understanding, as well as needs and capabilities in preclinical and clinical research, and posits where we should be looking. Illustrative examples, where used, will be taken liberally from the Alzheimer disease (AD) field, as research in this area has exploded over the past decade in comparison to other neurodegenerative diseases.

Gaps in Preclinical/Clinical Research

Are the Proposed Cellular Pathways the Correct Targets to Test in Humans?

What Are the Right Clinical Targets?

Historically, studies on the pathophysiology of neurodegenerative diseases focused on characteristic morphological changes which helped define the disorders. For example, senile or amyloid plaques and neurofibrillary tangles are diagnostic for AD, as are Lewy bodies in the case of Parkinson disease (PD). Does this mean that these structures are diagnostic and that their very presence also points to the cause of the disease? As a corollary, would approaches to reduce the formation of these pathologic changes lead correspondingly to improvements in clinical symptomatology? Early efforts in isolating the proteins that make up these structures— $A\beta$ in senile plaques, tau in neurofibrillary tangles, and α -synuclein in Lewy bodies—did not establish whether these proteins play a causative role, not until mutations were subsequently discovered in the genes that encode the respective proteins. Yet, discovering the mutant gene and protein product does not automatically point to potential therapeutic targets. For example, knowledge of the expanded polyglutamine repeats in trinucleotide diseases, the most notable representative being Huntington disease, does not lend itself readily to an obvious “druggable” target. On the other hand, in AD, identification of mutations in the amyloid precursor protein (APP) gene strongly implicated the key role played by the proteolytic product of APP, namely $A\beta$, in AD pathogenesis. The location of the APP gene on chromosome 21 also dovetailed nicely to prior knowledge that AD neuropathology invariably develops in individuals with trisomy 21, and thus provided further support to the proposed seminal role of $A\beta$ in AD. The race to inhibit $A\beta$ production began in earnest when β - and γ -secretases (the two proteases involved in cleaving APP to liberate $A\beta$ from its parent molecule) were discovered, thus providing clear targets for pharmaceutical companies to pursue. Inhibitors to β -secretase turned out to be a difficult challenge due to the structure of the enzyme, but promising compounds have now entered Phase III testing. γ -secretase inhibitors (GSI) turned out to be considerably easier to develop. The uncomfortable surprise, however, is that nonspecific inhibition of γ -secretase activity by GSIs led to unacceptable adverse effects, likely due to inhibition of the constitutive cleavage of other γ -secretase substrates, which now number more than 50, notable examples being Notch or ErbB4. This explanation likely accounts for the negative outcomes of the late phase trials of two GSIs: not only were the primary end points unreachable but adverse side effects, including worsening cognition, were noted in subjects given semagacestat chronically (Doody et al. 2013). It can be argued that these adverse outcomes could have been predicted. One lesson from the semagacestat trial is that in our attempts to fulfill the

From “Translational Neuroscience: Toward New Therapies,”

Karoly Nikolich and Steven E. Hyman, eds. 2015. Strüngmann Forum Reports, vol. 17, series ed. J. Lupp. Cambridge, MA: MIT Press. ISBN 978-0-262-02986-5.

unmet needs for effective treatments, enthusiasm must be tempered by appropriate assessment of risks and proper consideration of the biological pathways (Blennow et al. 2013). Otherwise, additional negative outcomes in pivotal trials will do more harm to the field, especially if wrong conclusions are drawn from the negative results.

When Should the Suspected Targets Be Treated?

A theme common to virtually all neurodegenerative diseases is that mutations (often missense mutations), increased gene dosage (from gene duplication or triplication), or ineffective removal in sporadic diseases magnified during aging trigger a cascade of events to bring about full pathologic manifestation of the diseases. This cascade hypothesis only predicts that if accumulation of these aggregated and misfolded proteins—such as A β in AD, tau in various tauopathies, α -synuclein in PD, or TDP-43 in amyotrophic lateral sclerosis/frontotemporal dementia (ALS-FTD) spectrum—can be aborted or prevented, then the subsequent disease development will be attenuated or blocked. Sadly, this plausible prediction has yet to be adequately tested in any one neurodegenerative disease. In the meantime, it is unclear whether any of the pathological processes can be halted or slowed once a trigger is initiated, leading to clinical improvement. For example, it is now well established that the full spectrum of pathologic changes are evident once an individual is symptomatic with AD (Bateman et al. 2012). We also now know that these changes occur gradually over one to two decades before clinical symptoms develop, reflecting perhaps the insensitivity of purely neuropsychological and cognitive measures. Thus, where these changes are protracted but the cellular trigger(s) occurred long ago, it is unlikely that targeting the trigger of the disease (e.g., A β deposition or α -synuclein aggregation) will demonstrate substantial benefit by altering disease course because significant pathology has already developed at the time of diagnosis. Similarly, in PD, it is estimated that up to 70–80% of neurons are lost in the substantia nigra before an individual is symptomatic. Thus, reducing α -synuclein aggregation in symptomatic individuals will likely have only marginal efficacy in reversing preexisting neuronal damage.

This discussion presupposes that we have a solid concept of what the precise disease triggers are. Is this supposition really correct? Knowledge of a genetic mutation does not imply an understanding of disease causation, nor do we know whether nongenetic, sporadic forms of the same disease share identical pathophysiology. Further, have the secondary or downstream changes been recognized? If the latter were better defined to prevent downstream effects and mediators could attenuate disease progression, then more targets would be available for drug discovery efforts. Some of these secondary changes, such as tau accumulation or oxidative damage in AD, may follow different rates of development, thus providing additional windows of opportunities for therapeutic interventions. Consequently, major conversations have appropriately arisen to

argue for treatments being given as early as possible to be effective, especially in the setting where the drugs target the putative disease triggers or very proximal mechanisms to the triggers of the disease cascade. Addressing this major gap in our understanding will fundamentally impact testing and delivery of effective treatments in the near future.

Finally, the idea that effective treatments fall neatly into symptomatic or disease-modifying categories, while important or critical from the pharmaceutical industry perspective, is likely too simplistic in practice. An early example was the “DATATOP” study designed to determine whether long-term treatment with deprenyl, a MAO-B inhibitor, together with vitamin E could delay the need for levodopa treatment due to progressive motor disability in PD; in other words, delay disease progression. Results from the trial initially appeared to demonstrate precisely this desired effect; that is, subjects on treatment took longer to require levodopa therapy (Parkinson Study Group 1989). However, the symptomatic benefit of deprenyl was not taken into account such that in the subsequent full analysis of the trial results, it was concluded that deprenyl did provide clinical improvements but that the evidence did not support a neuroprotective effect (Parkinson Study Group 1993). Another example can be seen in a tetracycline-regulatable model of mutant tau transgene overexpression in mice to drive neurofibrillary tangles formation in neurons. Surprisingly, in these mice, cessation of tau expression by administration of doxycycline improved cognition and halted further neuronal degeneration, but accumulation of tangle pathology persisted nonetheless (SantaCruz et al. 2005). Thus, there was symptomatic benefit even in the presence of unremitting pathology. In this context, in AD, could toxicity of oligomeric A β be attenuated and produce symptomatic benefit if A β production could be pharmacologically inhibited in spite of possibly continued disease progression? Conversely, cholinesterase inhibitors such as donepezil have shown some evidence of mild slowing in the rate of progression in individuals with mild cognitive impairment, even when the major benefit is primarily symptomatic (Petersen et al. 2005). In short, it is not always possible to draw a clear distinction between symptomatic and disease-modifying treatments. From a patient’s standpoint, this may be an academic debate, especially if a particular treatment is truly efficacious.

Why Are There Not More Prevention Trials?

Given the preceding discussion that early treatments in preclinical individuals may be necessary to see efficacious outcomes, an obvious question is: Why are there not more prevention trials? A simplified answer might include the following: lack of reliable early diagnosis, lack of well-defined patient populations, lack of good drug candidates, safety, cost and duration, appropriate end points, trial design, and subject selection (Golde et al. 2011).

First, how does one choose a treatment to test? What evidence of target engagement is necessary to embark on a prevention study? How does one

obtain the necessary evidence: is demonstration of efficacy in symptomatic individuals required or, at the minimum, target engagement in the intended treatment group, such as A β removal from brain with anti-amyloid strategies in AD subjects?

Second, safety is an obvious issue when a number of individuals given the treatment may not develop the disease, so harm must not come to those individuals. Whether individuals with incurable diseases are willing to tolerate higher risks is unclear, especially when the disease course is measured in years to decades.

Third, given the necessary duration, trials are expensive. In this regard, there is an urgent need for surrogate biomarkers that track disease progression, preferably ones that reflect improvement in actual pathophysiological measures (see below). Further, is it enough to delay onset or will prevention be required?

Lastly, trial designs are not simple. An end point that demonstrates an absolute reduction in the number of new cases will be long and require a large cohort, even with enrichment strategies. Thus, methods to shorten the duration by assessing biomarkers in hereditary cases, as in two recently started AD trials (the Dominantly Inherited Alzheimer Network Trial¹ and the Alzheimer's Prevention Initiative²), may achieve the desired goals quicker than conventional clinical outcomes in a normal elderly cohort. An alternative approach was seen recently in PD treatment, where a novel delayed start design was used to test whether rasagiline could slow disease progression (Olanow et al. 2009). Conflicting outcomes between two different drug doses, however, led to justifiable skepticism about the possible effectiveness of this treatment. Perhaps the therapeutic intervention would have been successful with a longer delay (36 weeks) or a longer follow-up period (36 weeks). Regardless, even if there was slowing of clinical progression, it does not necessarily address whether the underlying natural history of the disease has been altered, as there was no way to evaluate pathologic status definitively.

Have We "Thrown the Baby Out with the Bath Water"?

If early treatment will indeed be more effective in neurodegenerative diseases given its protracted course, one might speculate whether the negative results from many Phase III trials imply that these approaches will be equally non-efficacious if given during the prodromal stages of the diseases, when only the barest changes are present in brain. The Alzheimer Disease Cooperative Study,³ the largest academic clinical research organization in the United States,

¹ <http://www.dian-info.org/> (accessed April 14, 2014)

² <http://banneralz.org/research-plus-discovery/alzheimers-prevention-initiative.aspx> (accessed April 14, 2014)

³ <http://www.adcs.org> (accessed April 14, 2014)

was formed in 1991. Since then it has conducted 23 drug trials but only the first one, tacrine, was unequivocally positive. The others (such as estrogen, NSAIDs, statin, B vitamins) were ineffective as AD treatment in individuals who suffered mild to moderate AD. Would any of these compounds be effective if they were given in the presymptomatic setting or, better yet, as a preventive measure prior to occurrence of the disease triggers? At a time when the development of a successful new drug is in the range of one billion dollars, might a careful reevaluation of previously failed drugs, given much earlier, be a fruitful exercise to undertake? For AD, active vaccination of AD subjects with A β as an immunogen led to detectable removal of amyloid from brain but no overt clinical improvement (Holmes et al. 2008). The trial was aborted due to development of encephalitis in ~5% of the research subjects. However, if a safer vaccine were to be developed, should the testing be conducted right away as a secondary prevention (i.e., following onset of first symptoms) or as primary prevention? The U.S. National Institutes of Health, in part with industry support, is now studying whether the repurposing of drugs or drug-like compounds might yield additional uses beyond their primary indications. The last decade witnessed considerable success in drug repurposing, such as metformin and thalidomide (especially newer analogs), in the treatment of various cancers. Even in neurological diseases, there is the example of amantadine, which is now used in PD treatment but was initially developed as an antiviral drug. Would a concerted repurposing strategy in neurodegenerative diseases bear fruit? Will old drugs continue to find new uses in brain diseases?

Good and Bad News from Animal Models

Limitations of Animal Models

At present, no single animal model is able to replicate all of the key phenotypes of any one neurodegenerative disease. While this group of diseases is often age-associated, aged animals (including large animals or nonhuman primates) unfortunately do not phenocopy the human disorders, although some animal species (e.g., bears, dogs, elephants, monkeys) naturally develop amyloid deposits with age. However, the amyloid deposits seen spontaneously in aged animals are not accompanied by the full spectrum of brain changes that occur in disease. There is much recent excitement over the development of transgenic nonhuman primates (Sasaki et al. 2009; Niu et al. 2014), but it is too early to tell how useful these models will be, given the expense, resources, and time required to develop and age these monkeys. Rodent models remain our mainstay, as advances in the manipulation of rodent genome has led to many mouse and increasingly rat transgenic lines that demonstrate one or more pathological features of the desired disease. Many of these lines, however, suffer from several common deficits, whose impact is difficult to assess.

First, most lines exhibit only a subset of the changes in the brain that are actually seen in human pathology. In AD, transgenic mice did not develop neurofibrillary pathology when only APP (with or without Presenilin encoding disease-associated mutations) was expressed in brain, and they generally showed a relative paucity of neurodegeneration, a necessary key feature of the disease. Therefore, it is felt that virtually all APP transgenic mouse lines represent early preclinical stages of AD pathophysiology (Zahs and Ashe 2010). In the increasingly complicated ALS-FTD spectrum of diseases, many aspects of motor neuron degeneration have been recapitulated in SOD-1 mutant mice, but the same has not been true in mutations identified in the TDP-43 or FUS/TLS (fused in sarcoma/translocated in sarcoma) genes (Ling et al. 2013; McGoldrick et al. 2013). In the latter cases, there is a conspicuous paucity of typical TDP-43 or RNA-binding protein FUS/TLS aggregates in transgenic animals, unlike in humans.

Second, most rodent lines expressed mutant gene constructs rather than the normal gene. This may be a nonissue in diseases such as trinucleotide repeat disorders, in which the cases are always genetic in origin. But there is always a question whether sporadic or nongenetic presentation of a disease follows similar pathophysiological pathways as the genetic form.

Third, most lines depend on overexpression to demonstrate the anticipated pathology. This is due partly to our own impatience to see the desired phenotype as early as possible, which has often been addressed by driving transgene expression to abnormally high levels, but also because expressing transgenes at endogenous levels often produces little to no phenotypes. Potentially, this can lead to artifacts related to overexpression, although there are now examples of gene duplication in AD and PD, as well as in neurodevelopmental disorders. Accordingly, in this context, it is sometimes difficult to ascertain which of the many abnormally elevated proteins represent the key triggers of brain pathology. For example, in spite of more than a dozen mouse and rat transgenic lines that overexpress various APP mutations using different promoters, it remains unclear whether overexpression of APP itself, rather than elevated A β levels, was responsible for some of the brain abnormalities. Unanticipated insights have been gained, such as the findings of epileptiform activity in some APP-overexpressing mouse lines which have also been seen in human cases (Vossel et al. 2013). Again, the situation is quite different in trinucleotide diseases because “knocking in” expanded polyglutamine repeats into the rodent endogenous huntingtin gene leads to many of the expected abnormalities and, further, expression is controlled by the endogenous murine promoter (Pouladi et al. 2013).

Fourth, virtually all rodent models bring with them considerable limitations, which investigators tend to minimize if not ignore. For example, the genetic background of the rodent strains can be a significant confound. Most investigators prefer inbred mouse strains, which reduces variance but can simultaneously also amplify unwanted or unrelated phenotypes. Whether using

outbred lines is necessary or desired is unclear and may depend on a number of factors which cannot be controlled *a priori*, such as insertion site of the transgene, the particular mutant gene being expressed, toxic interactions between the transgene with certain mouse genetic loci, etc. (Krezowski et al. 2004). Even gender can be important. Many lines of transgenic mice have shown substantial gender variability, much more so than seen in human diseases when the mutant gene is not X-linked. Furthermore, the age span of rodents is vastly different from humans, and the assumption that reduced life span in these animal models reflects compressed human life span is not necessarily correct and may even be erroneous. Some cellular changes may result from disease triggers that require time to manifest themselves, and these stochastic processes simply cannot be compressed into the lifespan of rodents, canines, or even nonhuman primates. One example may be the infrequency of neurofibrillary tangles outside of aged human brains or in AD. Unlike amyloid deposits found in many aged mammals and even aged salmon (Maldonado et al. 2002), presence of tangle pathology is so infrequent that each time this is detected in a different aged animal species, it is noteworthy enough to warrant a publication (Rosen et al. 2008).

Fifth, are investigators biased in choosing which pathological change(s) should be present? Given that the rodent models rarely, if ever, phenocopy the human disease, one often “picks” one or more phenotype to select for when evaluating the animal models during their development phase. For example, are intracellular aggregates of α -synuclein, tau aggregates, or amyloid plaques the best measure of whether a particular transgenic mouse line is a faithful model of the disease? Or, for example, are abnormal animal behaviors reminiscent of a particular neurodegenerative disease an accurate representation of the human phenotype and derived from a similar cellular basis? Put another way, does an animal model that demonstrates some but not all of the expected brain pathology represent a reliable model to study disease pathogenesis and treatment response in support of the discovery and development of adequate therapies?

Lastly, while the situation is improving, many of the preclinical studies reported to date are not designed with sufficient statistical power. The standard practice taken by biostatisticians and clinical trialists to perform power calculations before commencing a study is often not undertaken or ignored in preclinical studies. One consequence of this is that results cannot be replicated by other labs because the initial result was spurious due to inadequate sample sizes or open-label in design, and this was not discovered until much later. Indeed, although a systematic study has not been done, there is evidence from other disciplines that many preclinical studies cannot be replicated, and this hinders the ability of translating candidate drugs into the clinic if the initial assumption of validity of the cellular target is erroneous (Ioannidis 2005; Prinz et al. 2011a). Even if these errors did not exist, there is always the need to be cautious when extrapolating or “jumping” from rodents to humans, given the

significant evolutionary differences that have evolved between the two species, a key example of which is in innate and adaptive immunity (Shay et al. 2013).

Misalignment of Preclinical to Clinical Studies

Another unintended bias that has been introduced in preclinical studies is the initiation of treatment before or at the onset of pathology. This, in many ways, is representative of a primary or secondary prevention study, and not a treatment study, as typically carried out in humans where the pathology is well developed at the time of diagnosis. On the other hand, treatments given to mice after the onset of pathology are generally less effective. In this setting, extrapolating results from animal studies to humans is potentially flawed because trials in humans follow a different testing paradigm and would, at first blush, be predicted to be less efficacious. Furthermore, even when the pre-clinical and clinical studies are perfectly aligned, caution is always necessary given the suboptimal nature of the preclinical models, as discussed above. In this way, one major gap in our knowledge is the problem in translating misaligned preclinical studies in imperfect animal models to the clinical setting. Despite the technological prowess in rodent gene targeting technologies, the leap from rodents to humans is still fraught with dangers, and there should be renewed search for intermediate model systems to span this gap. In the end, until one “success” is realized from a treatment that is positive in both rodents (or another animal model) and humans under comparable testing conditions, the predictability of any of the myriad of animal models to neurodegenerative diseases remains completely unknown.

Biomarkers

Biomarkers for Accurate Diagnosis

Neurodegenerative diseases with a genetic basis are reliably diagnosed by genetic testing where available. In cases without a genetic cause, the situation is considerably more difficult as the usual “gold standard” is postmortem examination of the brain. Unlike in cancer, brain biopsies are not routinely conducted, and clinical diagnoses are prone to errors. Sensitive measures of brain atrophy by volumetric magnetic resonance imaging (MRI) are not specific for any one degenerative disease, as there are many causes of loss in brain volume. Fortunately, advances in imaging have been highly informative, especially from PET scanning, such as ^{18}F -Florbetapir to detect $\text{A}\beta$ deposits or DaTSCAN (^{123}I -Ioflupane) to detect loss of dopaminergic innervation in PD. This was highlighted in the recent Phase III bapineuzumab trial where 36% of apoE4 noncarriers (compared to 6% in apoE4 carriers) diagnosed with mild to moderate AD were not positive on amyloid imaging, arguing against AD as the underlying cause of dementia (Salloway et al. 2014b). Whether routine use

of amyloid imaging is warranted remains controversial in light of the fact that approximately 30% of cognitively normal individuals over 70 years of age are amyloid positive (Johnson et al. 2013). Does this mean that these are preclinical AD subjects, or does this represent incidental findings in a subset of cases whose clinical outcome is benign? Until the time when longitudinal follow-up reveals whether the cognitive status in these individuals will deteriorate in a fashion consistent with AD, a reliable noninvasive diagnostic marker is still wanting.

The desire to begin treatment at the earliest possible disease stage puts further challenges on the requirement for accurate biomarkers. If all we need is to treat individuals before the onset of clinical symptoms (i.e., in individuals who may have already developed changes indicative of disease, such as loss of dopaminergic markers suggestive of preclinical PD or presence of tau aggregates or amyloid deposits), then current biomarkers may well suffice in this setting of secondary prevention. This is because the full manifestations of neurodegeneration may only be present at the later symptomatic stages of disease. On the other hand, if we need to begin treatment prior to the onset of characteristic pathology or initiate treatment at a time when the disease triggers are just beginning, then this sets a much higher bar for success. Currently, only primary prevention studies will address this scenario as we do not know when the disease cascade, for example, begins but the accepted pathologic hallmarks have yet to develop (Sperling et al. 2011b). On the other hand, if secondary prevention is successful, then this speculation is moot.

Biomarkers for Comorbidities

Age is one of the biggest risk factors for neurodegenerative diseases, as are other chronic systemic illnesses, many of which have manifestations in brain. These illnesses lead to symptoms suggestive of neurodegenerative diseases, to complicate matters, and they occur concomitant with the underlying neurodegenerative disorders. For example, diabetes and cardiovascular diseases are highly prevalent in the elderly, perhaps even more so than AD, and they result in microvasculature changes which compromise neural function, including dementia. These comorbidities are important because they can aggravate the underlying neurodegenerative disease, complicate diagnoses, or confound response to treatment. In the latter case, agents targeting A β would not be predicted to improve microvascular damage due to chronic diabetes. This may mask some, and perhaps all, of the beneficial effects of drugs that have achieved appropriate target engagement. Therefore, an additional current gap is the need to diagnose these comorbid conditions accurately when they affect brain function and to take them into account in trial design and in measuring treatment responses.

Biomarkers as Readouts for Treatment Response

The need for sensitive and specific outcome measures in clinical trials is obvious. As mentioned, both the AD and PD research communities have recognized the importance of starting treatment as early as possible to maximize the potential beneficial effects of therapy. However, trials in presymptomatic individuals will need large numbers of subjects and take many years to complete without strategies to enrich subject selection and the use of validated surrogate markers. In this context, multiple sclerosis (MS) offers many instructive lessons. MS is a disease with heterogeneous presentations and unpredictable clinical course such that disease activity was incompletely or even inaccurately assessed by using only clinical measurements. Prior to MRI, results from treatment trials can be difficult to interpret in the face of unpredictable clinical course. Routine use of MRI in MS has had a major impact on diagnosis and in our understanding of underlying pathophysiology, and its incorporation as one outcome measure of disease activity has significantly altered MS treatment in a positive way (McFarland et al. 2002). Furthermore, the advent of disease-modifying therapies for MS has led to the acceptance that institution of treatment early in the disease course will lower long-term disability to the extent that treatment is now recommended even after only one single demyelinating event (“clinically isolated syndrome”), as this may delay or postpone development of subsequent multifocal disease (Goodin and Bates 2009).

Similarly, the AD community has recently incorporated imaging modalities into diagnostic criteria, at least in the research setting (Dubois et al. 2010; McKhann et al. 2011). The ongoing AD neuroimaging initiative, established in 2005, was designed to evaluate brain and other biomarkers that can accurately follow disease progression and monitor treatment response. The hope was that these imaging biomarkers would qualify as outcome measures by regulatory agencies. Significant data have been generated toward the former goal, one example of which is the demonstration that use of imaging end points can, in theory, significantly lower the sample size required to detect response in drug treatment trials (Holland et al. 2012). Nonetheless, whether any surrogate measurement provides the necessary sensitivity and accuracy awaits the arrival of the first successful disease-modifying trial.

Additional Considerations in Clinical Trials

From the above discussion, it is apparent that there is growing consensus that potentially disease-modifying treatments for neurodegenerative diseases have to be tested as early as possible to exact the best outcomes, preferably in the primary or secondary prevention stages (Holtzman et al. 2011). Beyond costs and safety, this shift to earlier testing places an increasing burden on discovery and validation of surrogate markers that reflect and guide treatment response and also reduce sample sizes. However, prevention trials raise a number of

ethical issues that must be considered *a priori*. For example, an individual may not be aware or may not wish to be aware of his/her high-risk genetic status. The latter would happen if the subject is enrolled in a trial that specifically tests an individual with such a risk, e.g., apoE4 homozygotes or presenilin (*PSEN*) mutation. The Alzheimer's Prevention Initiative trial in Colombia tests an anti-A β antibody in individuals with a *PSEN* E280A mutation, implying that subjects enrolled in the trial must carry this familial AD mutation. To counter this assumption, a third arm testing placebo in control (nonmutation carrier) subjects was appropriately added so that one cannot assume that an enrollee carries the *PSEN* mutation (Reiman et al. 2011).

We must also consider whether giving placebo for long periods of time to at-risk individuals is acceptable. Early AD treatment trials were as short as six weeks. Now, it is not uncommon to see trials lasting up to two years or longer, especially in prevention trials. Since the 1950s, it has been the accepted standard practice to study response to drug treatment in randomized placebo-controlled clinical trials. Is there a place for a placebo alternative, both to reduce size and cost, but also to address the ethics of withholding potentially effective treatments that may delay disease onset in a prevention study? Can computer modeling, based on data obtained through careful monitoring of at-risk or early stage patients, provide sufficient statistical power to predict progression accurately in a way that will reduce the size of the placebo arm or even the heretical thought of eliminating placebo control in highly selected circumstances (Spiegel et al. 2011)? Can other improvements in clinical trial designs improve our current way of testing new therapies, both to maximize success and to address emerging ethical issues? Ultimately, there is perhaps little disagreement that we need to discover "new ways of doing business" as, to date, the standard practice has not been successful in developing effective treatments for neurodegenerative diseases.

Conclusions

This review has highlighted some of the challenges, gaps, and needs in current preclinical and clinical research toward finding effective treatments for various neurodegenerative diseases. It is by no means comprehensive, focusing rather on clinical targets, issues in preclinical research, and the need to find sensitive and accurate biomarkers to accompany clinical trials as they move into preclinical stages of the respective diseases. It is also admittedly biased toward AD research. But in many ways, current research has been hampered by the lack of a single truly successful treatment trial, symptomatic or disease-modifying, for any neurodegenerative disease, with the exception of motor complications of PD. The seminal success, when it comes, will hopefully provide new and critical insights as to both the right and the wrong directions taken in the past and to take into the future. Although the discussion has emphasized

disease-modifying treatments, there is an important need for symptomatic treatments, not to mention the difficulties in drawing a clear distinction between these two therapeutic approaches.

Regardless of the initial presentation, virtually all neurodegenerative diseases have cognitive and/or behavioral symptoms in the later disease stages. These can be both burdensome for caregivers and costly for the healthcare system. For example, treatment of the motor complications of PD have improved immeasurably over the past decade and, as individuals survive longer with this disease, there is increasing recognition of the nonmotor complications of PD (e.g., behavioral and cognitive problems) that need to be addressed.

Further, we should rigorously ask whether there is a role for nonpharmacological approaches. In terms of preclinical studies, renewed effort should be placed on developing more sophisticated large animal models, such as transgenic minipigs or nonhuman primates. Can induced pluripotent stem cell technology pay handsome dividends in helping to understand pathophysiology, develop alternative disease models, or aid in drug discovery?

Continuing to look at other diseases will be important as they may offer practical lessons and roadmaps to impact research in neurodegenerative disease pathophysiology and treatment. Greater attention should be paid to neuroprotection, as these strategies may be applied to more than one neurodegenerative disease. In addition, are there ways to “de-risk” the drug discovery process, including clinical trial designs, to lessen cost, minimize unrealistic expectations, and maximize the value of pivotal but expensive Phase III trials? Finally, how should we approach polypharmacy? Given the complex nature of neurodegenerative disorders, it seems naïve to assume that targeting a single cellular pathway will achieve comprehensive therapeutic success.

If we are to obtain a clear understanding of the mechanisms, sequence, and disease progression of neurodegenerative diseases, supporting science must be better grounded. Genetic mutations have shed light on some of the contributory factors to disease, but not enough progress has been made. A concerted effort is needed between genetics, genomics, proteomics, circuit understanding, connectomics, etc., if we are to gain a clearer understanding of what can and does go wrong. These are daunting challenges, but they must be faced in view of the inevitable onslaught of age-associated disorders, such as neurodegenerative diseases, that is already at our doorsteps.

Acknowledgments

This work was supported, in part, by National Institutes of Health Grants AG20206, AG32179 and NS084324. EHK is co-inventor on a patent relating to AD therapeutics and has served as a consultant for Pfizer Inc. (including Wyeth Research), GlaxoSmithKline, and Roche.