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Animal Models of Neurodegenerative Diseases

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Abstract

Animal models of adult-onset neurodegenerative diseases have enhanced the understanding of the molecular pathogenesis of Alzheimer's disease (AD), Parkinson's disease (PD), frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). Nevertheless, our understanding of these disorders and the development of mechanistically designed therapeutics can still benefit from more rigorous use of the models and from the generation of animals that more faithfully recapitulate human disease. Here we review the current state of rodent models for AD, PD, FTD and ALS. We discuss limitations and utility of current models, issues regarding translatability, and future directions for developing animal models of these human disorders.

Introduction:

Alzheimer's disease (AD), Parkinson's disease (PD), frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are devastating neurodegenerative disorders that inexorably progress to severe disability and death. Though in many individuals these neurodegenerative disorders have no clear genetic causes, the field has been guided by the discovery of mutated genes that deterministically drive these disorders as well as genetic

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variants that alter risk. These genetic guideposts, along with the biochemical identification of proteins defining the pathological hallmarks of these diseases such as amyloid-β (Aβ), α-synuclein, tau and TDP-43, have provided essential insights into the pathophysiology of neurodegenerative disorders, and provided the opportunity to create animal models for these diseases. Genetic forms of the human disorders do not always perfectly phenocopy sporadic disease, but in many cases are excellent surrogates, providing intrinsic validity to genetic-based models of neurodegenerative disorders. Indeed, models based on genetic forms of these disorders have provided both insight into molecular mechanisms and the temporality of changes of the human disease and helped to identify candidate, potentially disease modifying, therapies.

Arguably, no animal model of AD, PD, FTD or ALS fully phenocopies human disease. Many models recapitulate the initial proteinopathy or other pathological features linked to the human disorder. Some models also develop a more complete neurodegenerative cascade, but it remains uncertain as to whether the entire sequence of pathophysiologic events that occur in the human disease are fully captured. Nevertheless, discoveries in animal models have led to a greater understanding of the molecular and cellular mechanisms leading to brain cell dysfunction and degeneration. Animal models have enabled the field to develop, test and refine targeted therapies, but, for reasons elaborated on below, studies conducted in mouse models have had poor predictive power for drug efficacy in human neurodegenerative diseases. However, this failure to translate is not always attributable to shortcomings of the animal model per se. Despite the ever expanding repertoire of human cellular models of neurodegenerative diseases[1], these models are limited in terms of maturation and complexity including the lack of complex neuronal circuits, lack of a full complement of glial complexity as well as the absence of vascular and immunologic components. Thus, there remains, for the foreseeable future, a need for engineered animal models that recapitulate the complexity of an intact nervous system. Here, we i) review the current state of rodent models for AD, PD, FTD and ALS – based largely on genetic forms of these disorders, ii) highlight common challenges inherent to the modeling of neurodegenerative diseases in rodents (Box 1), iii) discuss the potential utility of non-rodent models, iv) explore the challenges of using current models to inform therapeutic development, and v) provide suggestions both for how to utilize current models and develop new models that may be more suitable for preclinical therapeutic development

Animal Models of Alzheimer Disease (AD) and related disorders

What are we modeling?

AD, the leading cause of dementia, is typically characterized by early progressive memory loss followed by impairments in executive functions and other behavioral disturbances including agitation and paranoia. AD is characterized by three hallmark pathologies: senile plaques, neurofibrillary tangles (NFT), and hippocampal and cortical neurodegeneration[2, 3]. Senile plaques, whose major insoluble component are fibrillar aggregates of Aβ, are specific to AD, whereas NFT, whose principle component are hyperphosphorylated, fibrillar forms of the tau proteins, are found in numerous neurodegenerative conditions besides AD including FTD linked to Chromosome 17 (FTD-MAPT)[4]. Mutations in the amyloid

precursor protein (*APP*), presenilin 1 (*PSEN1*) and presenilin 2 (*PSEN2*) are the main causes of autosomal dominant early-onset AD, while the *APOEε4* allele is a major risk factor for late-onset AD[5]. There are at least 25 other loci and rare coding variants associated with disease risk[6] and a number of these genetic associations implicate the immune system as playing an important role in AD[7].

Genetic-Based Models of Amyloid Pathology.

Transgenic rodents that drive Aβ aggregate accumulation, model amyloid deposition in senile plaques and in some cases cerebrovascular amyloid. AD-linked human mutations in *APP, PSEN1* and *PSEN2*, function in the mouse model much as they do in humans[8–11]. *APP* mutants either increase total Aβ or more commonly, increase the relative production of the more aggregation prone Aβ42[12]. *PSEN½* mutants alter endogenous processing of mouse APP, but do not lead to amyloid deposition. Co-expression of *PSEN½* mutants with an *APP* transgene dramatically accelerates the amyloid phenotype by increasing the relative production of Aβ42, or in a few cases Aβ43[13]. Abnormal dystrophic neurites, which appear to be axonal swellings filled with various organelles that are induced by Aβ aggregates, mimic what is seen in the AD brain quite well except for the lack of prominent tau accumulation, though tau-containing neurites can be observed following seeding with AD tau[14]. Astrocytosis, microgliosis and the molecular signatures of alterations in innate immune activation are reasonably well modeled[8–10].

Behavioral alterations in most APP rodent models correlate relatively poorly with accumulation of visible Aβ aggregates and, typically, the behavioral deficits do not progress[8, 9]. Mouse models that drive Aβ production and deposition in the absence of *APP* overexpression, including knockin models, either do not show behavioral abnormalities or show more subtle behavioral abnormalities that coincide better with the development of amyloid pathology[15–17]. Though these models may mimic some aspects of preclinical asymptomatic AD, given the *lack of* tau pathology, robust neurodegeneration and neurotransmitter abnormalities that accompany the symptomatic phases of human AD, it is clear that mice engineered to accumulate Aβ are not AD models, and especially not models of AD cognitive and behavioral dysfunction, but models of Aβ aggregate pathology.

Genetic-Based Models of tau pathology.

Transgenic mice that develop robust neuronal tau inclusion pathology (tauopathy) are largely based on transgenic overexpression of mutations that cause FTD with Parkinsonism linked to chromosome 17 (FTD-*MAPT*)[9, 18,19]. Unlike models of Aβ, these animals exhibit overt neurodegenerative changes. A reasonable concern, however, is whether these models of FTD-MAPT tauopathy are relevant to the tauopathy in AD. Indeed, the inclusions in mice often bear more resemblance to Pick bodies than the classic AD neurofibrillary tangle (NFT) pathology. Further, widely used tau transgenic mice are based on either the FTD-linked P301L or P301S *MAPT* mutations while it is not even clear that these mutations are representative of tau dysfunction in all forms of FTD-*MAPT*[20]. The human BAC hTau mouse that expresses all human tau isoforms (wild-type) in the context of a mouse *MAPT* knockout background exhibits limited tau pathology and subtle age-dependent neurodegenerative changes that may be more relevant to AD tauopathy[21].

High-level expression of the mutant tau transgene in the spinal cord leads to a motor phenotype[22], while more directed expression in the forebrain, hippocampus, or entorhinal cortex, results in tau pathology and neurodegeneration in those regions[23–25]. Thus, mutations in tau that drive tau aggregation can produce neurodegeneration in many neuronal cell types. Although human tau is widely expressed throughout the CNS, a gap in knowledge remains as to why different neuronal populations succumb in AD and FTD. Despite a robust neurodegenerative phenotype, the precise mechanisms of tau-induced neurodegeneration remain enigmatic. The field has yet to reach a consensus or understanding of how tau dysfunction and aggregation drive neurodegeneration[18].

Co-expression of both mutant human tau protein and mutant APP accelerates tau pathology, as has injection of aggregated Aβ into a tau transgenic model[26–29]. Other reports fail to find such synergistic interaction between Aβ/APP and tau pathology[30]. Though conceptually attractive, the utility of animals containing both human mutant tau, mutant APP and in some cases mutant PSEN½, remains uncertain, as there is ongoing controversy as to whether these models show synergistic interactions between the two pathologies. Ongoing more stringently controlled studies such as those supported by MODEL-AD may help to settle these and other controversies, as well as refining our current models.

Animal Models of Parkinson's Disease and Parkinsonism

What are we trying to Model?

PD is characterized by the progressive loss of dopamine (DA) neurons in the substantia nigra pars compacta (SNpc) and the presence of misfolded α-synuclein in Lewy bodies and neurites throughout the nervous system[31, 32]. DA neuron loss leads to motor symptoms that include a rest tremor, bradykinesia and rigidity. There are many different causes of PD both genetic and environmental. Mutations in α-synuclein and leucine repeat kinase 2 (LRRK2) cause autosomal dominant PD, while mutations in parkin, PTEN-induced putative kinase 1 (PINK1) and DJ-1 cause autosomal recessive PD[33–35]. Other genes have been linked to syndromes with PD-like features including mutations in ATP13A2, FBX07, DNAJC1, SYNJ1 and PLA2G6[35] and genes such as eiF4G1, VPS35 and CHCHD2 seem to cause very rare forms of PD with varying degrees of penetrance. Also, genome-wide association studies have putatively identified at least 41 PD risk loci[36].

In sporadic PD, neuropathologic assessment of the distribution of misfolded α-synuclein indicates that it is a global nervous system disorder with DA neurons becoming affected during mid-stage in the course of the disease[37]. α-Synuclein pathology is also found as a prominent feature in diffuse Lewy Body disease (LBD), (characterized by features of PD, along with fluctuations in cognition, REM sleep behavior disorder, and visual hallucinations) and Multiple System Atrophy (MSA) (characterized by parkinsonian features, autonomic and cerebellar ataxia). In contrast to PD where Lewy body pathology predominates in the SN, α-synuclein pathology in LBD is much more widespread throughout the limbic and neocortex and in MSA is found in glial cytoplasmic inclusions in white matter of the midbrain and cerebellum. Because of the etiologic heterogeneity of PD, modeling efforts have focused on i) pharmacologic and toxin models that recapitulate

midbrain dopaminergic signaling dysfunction, ii) modeling of α-synuclein pathology to try to recapitulate features of PD, DLB and MSA and iii) modeling of genetic forms of PD.

Pharmacologic based models of PD.

Historically drug-induced models were used to advance successful symptomatic therapies for PD where they ultimately led to the mainstay of symptomatic treatment for PD, namely L-dopa in combination with carbidopa, a noncompetitive aromatic L-amino acid decarboxylase inhibitor[38, 39]. Extensive use of the 6-hydroxydopamine (6-OHDA) and the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models, have refined therapies that are used to treat the symptoms of PD[40, 41]. Despite their utility as effective models for symptomatic therapies for the motor symptoms in PD, therapeutic approaches tested in drug-induced models to date have failed in numerous clinical trials to demonstrate any utility in the identification of disease modifying therapies[42, 43].

Genetic-Based models of α-synuclein pathology.

Mutations in α-synuclein cause autosomal dominant PD with characteristic Lewy pathology. Numerous models of α-synuclein induced degeneration using a variety of different promoters that constitutively overexpress different familial-PD associated mutant forms of α-synuclein (A53T, A30P and E46K) exhibit varying degrees of neurodegeneration and develop many clinical and biochemical features of PD, LBD and MSA including the formation of oligomeric and fibrillar α-synuclein. However, the degeneration typically occurs in the absence of any measurable loss of DA neurons[44–47]. These α-synuclein transgenic models exhibit robust non-dopaminergic deficits including anxiety, gastrointestinal dysfunction, non-DA related motoric dysfunction among others[48, 49]. Although many mutant α-synuclein transgenic models exhibit substantial neurodegeneration, the absence of a loss of DA neurons is viewed by some as a major shortcoming in these models. In contrast, conditional, temporal and/or cell type specific overexpression of mutant α-synuclein leads to degeneration of DA neurons [50, 51]. Viral overexpression of both mutant and wild type α-synuclein, following targeted delivery to the SN also leads to robust degeneration of DA neurons in rats, mice and non-human primates[52, 53]. Transgenic mice expressing a more toxic truncated c-terminal form of α-synuclein leads to loss of DA neurons[54].

Models of other genetic forms of PD.

Germline LRRK2 transgenic models based on overexpression of the G2019S or R1441C/G mutation have varying degrees of DA abnormalities, but lack convincing age-dependent degeneration of DA neurons[52, 55–57]. In a similar manner, knockin mutations of G2019S or R1441C of LRRK2 failed to lead to neurodegeneration[55]. Like α-synuclein models conditional, temporal and/or cell type specific overexpression or viral mediated transduction of mutant LRRK2 lead to degeneration of DA neurons[58–60]. Knockout of both LRRK2 and LRRKK1 lead to age-dependent neurodegeneration of DA neurons, suggesting that loss of LRRK function may contribute to the degenerative process of PD[61]. Both cell autonomous and non-cell autonomous mechanisms contribute to neurodegeneration of DA neuron in LRRK2 transgenic models as well as α-synuclein models [62, 63].

In the case of parkin and PINK1 there is robust degeneration of DA neurons when these genes are deleted in adult mice[64, 65], while germline knockout of parkin, PINK1, DJ-1 and even germline knockout of all three genes in mice failed to induce neurodegeneration[48, 57, 66]. On the other hand germline knockout of PINK1 and DJ-1 in rats leads to loss of DA neurons[67]. Overexpression of c-terminal truncated human mutant parkin (Parkin-Q311X) also lead to progressive loss of DA neurons in mice as well as overexpression of some parkin substrates[64, 65, 68, 69]. Developmental compensation and plasticity of the rodent nigrostriatal pathway may account for why conditional overexpression or knockout in adult mice lead to loss of DA neurons[48].

Taken together, there are experimental model systems to study the molecular mechanisms of neurodegeneration in PD due to mutations in α-synuclein or LRRK2 as well as loss of function models of parkin, PINK1 or DJ-1. As new genes are identified, conditional, temporal and/or cell type specific overexpression or deletion will likely lead to additional systems to study the role of these mutations in the degeneration of DA neurons. Since the majority of PD patients do not have inherited disease, it might be relevant to combine toxin/environment insults with genetic risk factors (e.g. LRRK2 or GBA mutations).

Animal Models of Amyotrophic Lateral Sclerosis and Frontotemporal Dementia.

What are we trying to model?

ALS is characterized by premature loss of upper and lower motor neurons leading to fatal paralysis with respiratory failure typically within 1–5 years[70]. FTD is characterized by the progressive degeneration of the frontal and temporal lobes. Most ALS and FTD cases are sporadic, but mutations in specific genes can cause either ALS, such as in the copper/zinc superoxide dismutase 1 (*SOD1)* gene, or FTD for mutations in the *progranulin* and *MAPT* genes. While ALS and FTD are clinically distinct, motor neuron degeneration and cognitive deficits can be concomitant in patients or families. Indeed, many forms of ALS and FTD have clinical, genetic and pathological overlap. The discovery of TDP-43 inclusions as a common pathological hallmark in ALS and FTD[71] and the finding of causal mutations in TDP-43 initiated a paradigm shift in which RNA metabolism plays a crucial role in these neurodegenerative disorders[72]. Recognition of mutations and/or mislocalization other RNA binding proteins including FUS/TLS, TAF15, EWSR1, hnRNPA2B1 and A1, ataxin 2, MATR3 and Tia1 in ALS and/or FTD further implicated RNA metabolsism[73]. Mutations in ubiquilin-2, VCP, TBK1, PFN1, or TUBA4A incriminated other pathways including altered protein homeostasis, cytoskeletal function and axonal transport[73]. Finally, the strongest genetic link between ALS and FTD is a G_4C_2 hexanucleotide repeat expansions on the order of hundreds to thousands in the first intron of the *C9orf72* gene[74, 75].

Mutations in *SOD1* to model ALS or the *MAPT* gene to model FTD[76] have been invaluable tools to dissect disease mechanisms and served as preclinical models to test therapeutic approaches. However, mutations in SOD1 and Tau represent only a fraction of the human disease spectrum[77] and the identification of more than 20 genes associated with ALS and FTD provides additional opportunities to develop new animal models.

Models of SOD1-related ALS.

Transgenic mice and rats overexpressing various SOD1 mutations develop significant denervation of the neuromuscular junctions, cortical and spinal motor neuron loss, glial activation, accumulation of misfolded SOD1 protein, and progressive paralysis with reduced lifespan[78, 79]. The level of transgene expression determines the severity of disease, while SOD1 gene deletion does not lead to motor neuron disease[80, 81]. A combination of elegant approaches using mice allowed researchers to dissect *in vivo* the relative contribution of different cell types (motor neurons, microglia, astrocytes, muscle, endothelial cells, oligodendroglia or Schwann cells) to the onset and progression of SOD1-related disease[82]. However, a major caveat of SOD1 models is that they do not recapitulate TDP-43 pathology which is present in the vast majority of ALS patients.

Models of TDP-43 pathology.

The discovery of TDP-43 as the major component of cytoplasmic inclusions in sporadic ALS and FTD patients provided an opportunity to investigate sporadic disease[71, 72]. TDP-43 is an essential protein that influences the processing of hundreds of RNA targets and its ubiquitous deletion in mice is embryonically lethal[83–85]. An important caveat for animal modeling is that the repertoire of RNAs bound by TDP-43 differs between species and RNA processing alterations elicited by TDP-43 dysfunction are largely distinct between mice and humans. Numerous transgenic rodents expressing human TDP-43 with or without disease-causing mutations and under various promoters have been generated[79, 86]. Overexpression of TDP-43 induces a severe lethal phenotype independent of the presence of mutation[79, 86]. However, mice expressing levels close to endogenous TDP-43 develop mutant and age-dependent neurological phenotypes including motor and cognitive deficits, motor neuron degeneration and neuromuscular denervation but without paralysis or reduced lifespan[87, 88]. TDP-43^{Q331K} knockin animals develop mild cognitive dysfunction without spinal motor neuron degeneration[89]. Transgenic mice expressing low levels of TDP-43 as well as knockin animals develop mild phenotypes despite the absence of TDP-43 large cytoplasmic aggregates and nuclear clearance that are pathological hallmarks in ALS/FTD patients. Conditional overexpression of human TDP-43 with a mutation in the nuclear localization signal (NLS) leads to biochemical and pathological features characteristic of TDP-43 proteinopathies[90]. Suppression of transgene expression after disease onset using a Tet ON/OFF system was followed by microglia-dependent clearance of pathological TDP-43 and partial functional recovery indicating that abnormal TDP-43 may be dynamically cleared and disease partially rescued[90, 91].

Models of other forms of ALS and FTD.

Overexpression of wild-type or mutant FUS protein is toxic with reduced lifespan in rodents[92]. However, FUS mutant knockin mice develop progressive motor neuron degeneration, albeit without lethal paralysis[93, 94]. Notably, paralytic phenotypes are observed transgenic animals with UBQLN2[95] or PFN1[96, 97] mutations.

Intense efforts towards understanding disease mechanisms linked to *C9orf72* expansions have reshaped ALS/FTD research since its discovery. Although the relative contribution of different proposed mechanisms to neuronal death is not yet established[98], there is mounting

evidence for a gain of toxic function either from accumulation of expansion-containing transcripts into RNA foci or from the accumulation of dipeptide repeat proteins (DPRs) translated from the *C9orf72* expansion. Importantly, the generation and characterization of C9orf72 mice models indicate that the reduced level of C9orf72 observed in patients may contribute, but is not sufficient to trigger neurodegeneration. C9orf72 reduction sensitizes human cultured motor neurons to glutamate and DPR toxicity[99] while C9orf72 knockout mice don't exhibit neurodegeneration but instead develop a peripheral phenotype consistent with a role for C9orf72 in immune cells[100–103]. Models expressing expanded repeats either through AAV-mediated somatic transgenesis[104] or under the human C9orf72 promoter in BAC transgenic animals[103, 105–107] develop pathological features of disease including accumulation of RNA foci, DPRs and phospho-TDP-43. Varying degrees of cortical and/or hippocampal neurodegeneration associated with cognitive abnormalities as well as premature death and massive neurodegeneration in the brain and spinal cord with incomplete penetrance have been reported[103, 104, 107]. Five DPRs translated from all frames of sense and antisense expanded RNAs are the major components of cytoplasmic p62-positive, TDP-43-negative aggregates that represent a unique pathological hallmark in C9orf72 ALS/FTD patients. AAV-mediated expression of GFP-$(GR)_{100}$ in mouse brain induce age-dependent neurodegeneration with motor and memory deficits associated with impairment of protein and stress granule dynamics[108]. While toxicity of poly-GR and poly-PR appears dominant in overexpression studies, evidence for neuronal toxicity of poly-GA occurs in cells and mice expressing various lengths of codon-modified poly-GA. These animals develop varying degrees of neuropathology, motoric and neurobehavioral deficits depending on the approach[109, 110]. Together, these models support a toxic property of poly-GR and poly-GA, the most abundant DPR in C9orf72 ALS/FTD patients, and may be useful for testing therapeutic approaches directly targeting DPRs.

"Seeded" Models

Like models of prion disease based on inoculum of mice with pathological prion protein conformers[111] intracerebral injection studies of purified recombinant proteins, protein aggregates from cell culture models, human disease lysates or mouse brain lysates are being used as a way to "seed" Aβ, tau, α-synuclein and other FTD/ALS associated inclusion pathology in the brain of both transgenic and in some instances wild-type rodents[112–115] (Box 2). A full discussion of these models is in an accompanying article in this issue.

Non-rodent Models

Rodents are not the only organisms used to model these disorders. *Drosophila, C. Elegans, Danio rerio*, yeast models and other organisms have been used to gain insights into how proteins implicated in these neurodegenerative disorders cause cellular and organism pathology toxicity[116–121] . There are limited number of naturally occurring non-rodent animal models, such as SOD1 mutations that cause ALS in canines. Aged, non-human primates have also been extensively studied as potential models of AD, as they do accumulate Aβ in plaque-like structures. However, even seeding of AD brain lysate in these models has not induced tau pathology and neurodegeneration. Efforts to develop non-rodent models are ongoing, especially in non-human primates. However, given that no "next-

generation", non-rodent model currently exists, and will likely not exist for many years, the field cannot wait for such advances and instead we need to focus on using rodent models in ways that are likely to be more translatable.

Improving Translatability

One of the primary goals of animal model development is to identify key points in the process of neurodegeneration that represent therapeutic targets. To date, clinical trials in AD, ALS, FTD and PD, based on therapeutics that showed success in an animal model have largely failed. This lack of success is especially true for disease-modifying therapies targeting the underlying pathologies or attempting to slow neurodegenerative changes. Failures in the translation may be attributed to numerous factors in both preclinical and clinical studies including inappropriate preclinical study designs, imperfect animal models, overly optimistic interpretations of the preclinical data, lack of target engagement in humans, and the limitations of clinical trials conducted in absence of informative biomarkers[122].

One major issue that can be addressed more consistently is the lack of alignment between clinical and preclinical studies[12]. In the design and interpretation of preclinical studies, it is important to consider the timing of treatment initiation and whether onset or progression of the disease is altered. Therapeutic approaches found to impact disease onset rather than progression are unlikely to have a beneficial effect in already affected patients.

In preclinical studies, a novel AD therapeutic has typically been tested at a time when the models have only modest Aβ or tau pathology. In this setting, the therapeutic shows efficacy. In most cases, the therapeutic modality is not tested in models with AD-like pathology loads (or that study is never reported). However, all disease-modifying therapies that have completed human studies have failed when tested in humans with symptomatic AD and long-standing Aβ and tau pathologies. Further, many therapies are only tested in an Aβ model or a tau model, but not both. Especially as immune therapies are considered, it will be paramount to test such therapies in both models, as the therapy could improve the phenotype in one and accelerate the phenotype in the other. In all cases, the field should insure that there is alignment between the timing in which the therapeutic effect observed in the model and the stage of pathology in the intent to treat population in the clinical trial. Indeed, anti-amyloid agents are now being tested in secondary prevention studies (i.e., initiating treatment in individuals with amyloid pathology who are asymptomatic), and may someday be tested in true primary prevention studies[123–125].

In ALS, SOD1 transgenic mice have been extensively used as preclinical models to test therapeutic strategies. However, numerous studies have not been replicated in part due to poor preclinical design with small cohort sizes[126] motivating the publication of guidelines for optimal use of $SOD1^{G93A}$ animals in therapeutic development[126, 127]. Notably, Riluzole was shown to increase survival by slowing progression of disease rather than delaying its onset[128]. Edaravone was administered after disease onset and shown to improve motor performance and reduce accumulation of mutant SOD1 in mice[129]. While studies conducted in SOD1 rodent models are at the stem of several clinical trials that failed to show efficacy, it

is noteworthy that these trials were mostly performed in sporadic patients. Therapeutic approaches may have a different impact on various forms of the disease and enrolment of patients with specific genotypes[130] or stratification of the analysis by gene mutation may improve translatability[131].

In PD, disease-modifying therapies based on current genetic models of PD are beginning to enter the clinic, but the PD community should take lessons from the clinical failures in AD and ALS. Advancing therapeutics tested in a single model is a risky choice and strong consideration should be given to testing in multiple models. Further, readouts for disease modification in human PD are not yet well-established. “Seeded” α-synuclein models may provide more efficient and reliable preclinical studies, but the intrinsic validity of such seeded models will need to be further established (Box 2).

Finally, for all of these disorders more emphasis is needed with respect to effect size and significance in the preclinical studies. Too often small but significant effect sizes in the preclinical model are used to help justify a human trial, that is unlikely to find evidence for a similar subtle disease modifying effects. Alternatively, if the clinical study is designed to find such small effect sizes, the study will likely require a huge number of participants and therefore be very expensive with a firm readout only upon completion of a very large phase 3 study. Strategies to enhance success in a clinical trial are highlighted in Box 3.

Future Directions: New Models?

What might be the next phase of modeling AD, PD, ALS and FTD in animals? The first step might be to reach consensus on what aspects of the models might be predictive of efficacy in humans. Unfortunately, this step is somewhat predicated on some drug developed in a given model showing efficacy in humans with that disease. One might also debate whether one should try to develop consensus with respect to selecting “preferred” models. However, given the vast number of models already developed and likely to be developed in the future, in the absence of additional large-scale data developed collaboratively, such a debate would likely not be fruitful. There could be utility in trying to develop more consensus around reproducibility and rigor using data driven approaches that might address the questions of “How might we know what the best model is?” and “How would we know if we made a better model?” (Box 4). Further, key issues around development of new models for these disorders are highlighted in Box 5.

APP and tau animals are useful models of the two major proteinopathies in human AD, but they do not fully phenocopy human AD. APP models are excellent to test factors that regulate Aβ deposition and likewise tau models are extremely useful to evaluate factors that regulate tau aggregation and possibly tau-induced neurodegeneration. They also might be reasonable models to explore some aspects of downstream events, but efforts are needed to ensure that the downstream events actually reflect a process occurring in humans. We need to understand what features of the human disease they recapitulate well and what they do not. We should also be cognizant not to impose artificial barriers regarding validity of the models, as a prerequisite for publication or further study. For example, a model of α-synuclein induced neurodegeneration may be perfectly suited to test a therapy targeting α-

synuclein aggregation even in the absence of DA neuron loss that induces the parkinsonian phenotype.

In PD, the drug-induced models are highly predictive for testing symptomatic therapies that are due to the loss of DA. As we learn more about the circuit dysfunction in PD and the non-motor features of PD such as anxiety, depression, cognitive impairment, sleep disturbances, autonomic dysfunction and gastrointestinal dysfunction among others, might we need new experimental systems to develop symptomatic therapies for these non-motor systems? Given the prior utility of the drug-induced PD models in identifying effective symptomatic therapies, might we not also want to develop models of the complex network dysfunction characteristic of the non-motor symptoms of PD, as well as the behavioral dysfunctions in AD, FTD and ALS to develop targeted symptomatic therapies? These may be unrelated to the triggering mutant genes or proteinopathies but should have validity in that they model to the degree possible the behavioral or functional deficits observed in humans. We should collectively welcome new modeling approaches that are designed to help uncover targets that provide symptomatic relief, even if transient.

For disease modifying therapies (i.e. those that prevent degeneration), we need models that consistently and robustly reproduce progressive cellular demise, not just of neurons but of all the cell-types altered by these disorders. A better description of the cell-type changes that occur over time in both our models and in humans is also required. There is growing evidence that neurodegenerative disorders involve many secondary pathological events that may become independent of the disease trigger. Indeed, therapies ultimately may need to target multiple pathways, perhaps using a combinatorial approach, to show efficacy in humans. Further development of such therapies must consider the widespread cellular dysfunction that is not just limited to neurons.

Given the advances in genome editing and increasing power of viral mediated somatic transgenesis there is an opportunity to test the effect of mutations in mice with various genetic backgrounds. In particular for ALS and FTD, factors influencing the development of a specific phenotype in an individual are not understood but likely to be influenced by genetic modifiers that are not recapitulated in congenic mice. Future studies will be necessary to determine whether different mouse genetic backgrounds result in distinct neuronal vulnerabilities when expressing mutations implicated in both ALS and FTD. There may also be some merit in new modeling initiatives in mammals other than mice. For example developing rat models to study various forms of parkinsonism might be worthwhile as there is evidence that rat SNpc DA neurons are more vulnerable to genetic alterations linked to human PD[67]. Rats can also provide better models with respect to various behavioral tasks, and if that is the goal then additional efforts to model behavioral phenotypes in rats are warranted. These technology advances will almost certainly underlie future attempts to develop non-human primate models of these disorders. However, given the resources needed to create such models and questions about how widely they might be available once developed, such efforts should be carefully considered.

In all cases, characterization of any new model is crucial and remains a bottleneck. Efforts to more symmetrically and comprehensively catalogue the phenotypes associated with each

model are needed, including more routine multi-omic studies. We often do not even know the transgene insertion site, which can create confounds in individual models. Pathological analyses should also be more systematic including effects outside the CNS. Novel sophisticated technologies such as *in vivo* imaging or touch-screen based behavioral testing for cognitive assessment should be more broadly used to characterize the different models. Unless there is compelling evidence, many in the field will be hesitant to switch from use of their own favorite models. This hesitance is well-founded, as there is little substitute for long-standing experience with a given model.

For human neurodegenerative proteinopathies, which are not completely phenocopied in an animal model, it is worthwhile to reflect on the reasons why the complete disease process might not be apparent. One factor may simply be time, since even in humans the proteinopathy does not drive degeneration for years if not decades. Even though rodent models age, perhaps there is simply not enough time to elicit the full cascade of secondary events? The flip-side of this is that the major demographic risk factor for many neurodegenerative disorders is age. Might physiologic changes associated with aging in humans not be replicated fully in mice? To extend this concept there may be numerous inherent differences in the physiological response to the proteinopathy. We really do not know yet if the integrated response to the pathology in mice is the same as in humans. We also need to consider more carefully that mice are housed in relatively deprived environments with limited exposure to pathogens. Though useful for standardization, the lack of environmental or microbiome exposures in mice is a major difference from what occurs in humans. This is especially important given the increasing evidence for a crucial role of the immune systems and immune–microbiome interactions in various neurodegenerative disorders.

Ultimately, to fully understand how useful a model is, it must constantly be compared to the human disorder. As our understanding of human disease evolves our appreciation of both the utility and limitations of our animal models must evolve as well. Much of our past work has focused on overt phenotypes in models, or specific signaling pathways. A broader system level approach including omics studies might be warranted and useful where widespread molecular markers of pathology in both human disease and mouse models are compared[132]. Such a comparison might provide clues as to why a model is not a perfect phenocopy of the disease, or why a mouse brain or spinal cord is resistant to further pathology. Defining a broader molecular signature developed in each mouse model would provide a less biased approach to understanding how fully a given intervention modified the phenotype and how it models the human disease.

Concluding Thoughts

Overall to date, there have been limited success in translating insights gained from mouse models of these human neurodegenerative diseases into targeted therapies. Although some in the field might suggest that both studying models that incompletely phenotype the human disease and using such models to develop therapies is futile, we disagree. When the models are appropriately used it is clear that they inform on fundamental biology that is relevant to the human condition and they can inform therapeutic development. Perhaps the lesson we

have learned is that we need to be more conservative about findings based in one model at one time point. Instead we should perform a much more thorough preclinical study to understand both the potential and limitations of a given intervention, and depending on the target test that preclinical intervention even in multiple models. Indeed, it is critical to substantially invest more in the preclinical phase before embarking on much more expensive human studies.

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Box 1. Common challenges in modeling neurodegenerative disorders in rodents

- Limited relevance of models based on expression of rare genetic variants for understanding and treating sporadic disease. Such models are invaluable to understand disease mechanisms and develop gene-specific therapy and silencing approaches but may be inappropriate when molecular and cellular events leading to neurodegeneration differ from other forms of the disease.
- Inherent caveats related to the methods used to generate animal models. The artificial overexpression of proteins in transgenic and AAV-mediated models can be overcome by the generation of knock-in animals. However, such models often demonstrate a mild phenotype, if any, requiring deep and time-consuming phenotyping. The use of sensitive methods including automated behavioral analysis, *in vivo* imaging, electrophysiology, -omics approaches, or detailed evaluation of specific molecular and cellular defects has the potential to identify valuable readouts that may be more relevant than blatant behavioral deficits or reduced survival.
- The short life-span of rodents may contribute to the incomplete development of pathological hallmarks and/or neurodegeneration representing major limitations in the modeling of aging-related neurodegenerative diseases.
- Inherent differences in the development and function of rodent and human brains. In particular, caution should be employed when interpreting behavioral deficits especially when evaluating cognitive, emotional and language deficits characterizing human disease[133].
- Genomic differences between rodent and human may have profound implications when modeling neurodegenerative diseases. For example, binding sites of RNA binding proteins associated with neurodegeneration are not well conserved and RNA processing alterations are not fully recapitulated in mouse models. Another example is the different impact of amino acid changes in various species (the normal endogenous murine *Snca* actually corresponds to the human disease-causing A53T mutation).
- Limitations related to the use of inbred animals that do not reflect the genetic diversity of a population. Notably, new resources have been developed to address this issue including the Collaborative Cross (CC) and Diversity Outbred (DO) mouse sets[134, 135]. In addition, the emergence of CRISPR/Cas9 and viral-mediated expression of genes opens the possibility to generate mice expressing mutations on different genetic backgrounds[134, 136].

Box 2. Seeded" Models

- Intracerebral injection studies of purified recombinant proteins, protein aggregates from cell culture models, human disease lysates or mouse brain lysates are used as a way to "seed" Aβ, tau, α-synuclein and other FTD/ALS associated inclusion pathology in the brain of both transgenic and in some instances wild-type rodents[90, 112–114, 137]. These studies demonstrate there may be different prion-like "strains" of these aggregates that template aggregation of either the endogenous protein or the human protein expressed from a transgene to produce different types of inclusion pathologies[137–140]. Though sometimes claimed to produce a better model and more closely mimic AD inclusion pathology, to date they have failed to produce animals that mimic the entire human pathological cascade. Even aged non-human primates inoculated with extracts from AD brain that can accelerate amyloid deposition do not have much evidence for additional pathologies[141].
- Seeding with preformed aggregates of α-synuclein can induce and synchronize α-synuclein pathology in transgenic α-synuclein overexpressing mice[142–144]. Moreover, seeding with preformed aggregates of α-synuclein into the striatum of wild type mice has been reported to drive degeneration of DA neurons with accompanying behavioral and pathologic features of PD[145]. Though additional studies will be needed to demonstrate their overall utility, these seeding models enable more robust well-powered preclinical studies of α-synuclein pathology and neurodegenerative phenotypes paradigms that have been challenging due to the intrinsic variability of other PD models.
- Notably, except for a limited number of cases of prion disease there is very limited and somewhat controversial evidence that these human proteinopathies are truly transmissible[146, 147]. Other mechanisms could account for, or contribute to spread of pathology in humans, including intrinsic disruption of proteostasis or generation of secondary "toxic" signals that drive the spread of pathology[148]. Indeed, there is at least one example where injection of brain lysates lacking α-synuclein triggers the full-blown proteinopathy[149], suggesting that other mechanisms should be considered. Further, even within a single brain there is often evidence for multiple conformations of the inclusion pathology[113]. Though there are reports linking a specific conformer, or "strain", of aggregated protein to the formation of different inclusion pathologies, more work needs to be done to fully understand the role of different strains and the contribution of cell-to-cell transmission in the pathogenesis of these disorders.

Box 3. Strategies to enhance success in translating preclinical results in animal models of AD, PD, ALS and FTD to the clinic

- Increase rigor of preclinical studies, ensure adequate replication and group sizes, assess sex effects, and if appropriate test in multiple disease relevant models. The later point is important if one is testing a neuroprotective therapy that may be independent of the presumptive trigger of the disease. Evaluating whether the agent has neuroprotective effects in multiple models that exhibit a degenerative phenotype would increase confidence that it could be translatable.
- Understand the effect size of the intervention in context. For example, a highly significant 50%, and apparently impressive reduction in pathology may only translate into a 2-week delay in pathology in the model and might not show any evidence for modification of other phenotypes. Understand that even a study that is significant at a p of 0.05 with normal variance will likely replicate less than 50% of the time if powered similarly as the initial study[150].
- Insure that there is alignment between the timing of the treatment with respect to therapeutic effect observed in the model and the stage of disease in the intent to treat population in the clinical trial design. If possible, test the efficacy in both prevention or early intervention studies and a setting more likely to be observed in humans enrolled in initial trials (i.e., a more therapeutic study).
- For preclinical studies of small molecules or biologic therapies, insure that there is adequate PK and PD assessment as well as evidence for target engagement in the CNS (if that is the proposed mode of action).
- If possible, identify translatable biomarkers that track with disease progression in both humans and the animal model.

Box 4. What are the best models, and how would we know if we made a better model?

- The concept of a “best model” is probably misguided. Utility of a given model is best viewed in the context of the question being asked. For example, if one is simply trying to understand factors that regulate inclusion formation, then as long as that model reliably develops the inclusion pathology, it is probably appropriate. In such a case, choice of a given model will likely be dictated by pragmatic issues and should not necessarily be dictated by intrinsically biased notions of what is “best”. Pragmatic issues might include inherent variance, reproducibility of the phenotype over-time, timing of disease onset, and investigator experience with a given model.
- Given the imprecise correlations between various models and the extent to which they phenocopy the human disease, it is important that claims about what the models show remain conservative and consider previous efforts in the field. For instance, some APP rodent models show evidence for neurodegeneration, albeit limited, but most do not. Thus, although neurodegeneration can be studied in the APP models that show such a phenotype, it is not clear whether this is relevant to the human disease. Unless a mechanistic basis that caries construct validity is found for the discrepancy in phenotype between the various models, then testing for factors that modify neurodegeneration in such models is likely to be of limited utility. There is inherent utility in deciphering which aspects of the models do not recapitulate features of human disease. Such data will undoubtedly guide better model development, and also could provide insights into new therapeutic approaches.
- Many publications purport the development of a superior model. These claims are typically based on phenotyping that is inherently limited and biased in nature. More complete phenotyping and comparison to parallel multi-omics data from humans might be used to better understand at a systems level how well the model phenocopies the human disease. In this regard, a systematic effort to collect such multi-omic data from longitudinal cohorts of widely used models would have general utility for the field. Indeed, once generated such datasets could serve as readouts for therapeutic intervention. Efforts such as AMP-AD are beginning to collate such data primarily for AD-relevant models and make it publicly available.
- Better models in some cases simply might mean ones that are less-costly to use, or models that better enable testing of a specific hypothesis.
- There is an inherent resource barrier to adoption of a novel model, even when the initial reports suggest it might have increased utility over current animals. We should collectively try to develop systems that would enable more rapid dissemination of novel models.

Box 5. Strategies for the development of new models for these disorders.

- Efforts to better model various non-genetic factors and co-morbid diseases that commonly occur in humans suffering from various neurodegenerative disorders might be warranted. Efforts to standardize housing conditions to eliminate various experimental confounds, may have introduced their own confounds. We should recognize that the relatively sterile environments, in terms of behavioral enrichment, microbiome, and environmental exposures, do not represent the exposure history of humans who develop neurodegenerative conditions.
- More studies should be conducted in the setting of old models that better mimic the physiology of aging typically present in humans with these diseases. Given the expense associated with such studies, like the aging rat colonies developed by the NIA, the field may consider a standard resource that generates such aged models to enable more standard use.
- We should try to understand why select phenotypes are not fully recapitulated in mouse models, in order to create hypotheses that might enable us to engineer better models. For example, efforts to understand the relative resistance of DA neurons to α-synuclein pathology in transgenic mice might provide insights that could not only enable the generation of a model developing a more complete phenotype but would also have obvious therapeutic implications.
- We should make a more concerted effort to understand and model selective CNS-cell vulnerability. Though often thought of in terms of neuronal vulnerability, a major gap in knowledge relevant to these human disorders is that despite widespread expression of a given mutant protein, not all areas of the brain degenerate at equivalent rates. Such selective regional or cellular vulnerability is often not carefully considered in most of our current models.
- Use of novel tools and techniques (e.g., gene editing) to generate models in mammals other than mice should be considered, but only when an appropriate underlying hypothesis frames the study. Development of such models will inherently be expensive and likely challenging to widely disseminate. To avoid huge investment of resources, development might be staged. For example, by first using viral-based modeling methodologies or inoculation with “high-titer” seeds with subsequent investment in more stable genetically modified models.