Frontotemporal Degeneration, the Next Therapeutic Frontier: Molecules and Animal Models for FTD drug development (Part 1 of 2 articles)

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Abstract

Frontotemporal Degeneration (FTD) is a common cause of dementia for which there are currently no approved therapies. Over the past decade there has been an explosion of knowledge about the biology and clinical features of FTD that has identified a number of promising therapeutic targets as well as animal models in which to develop drugs. The close association of some forms of FTD with neuropathological accumulation of tau protein or increased neuroinflammation due to progranulin protein deficiency suggests that a drug’s success in treating FTD may predict efficacy in more common diseases such as Alzheimer’s disease (AD). A variety of regulatory incentives, clinical features of FTD, such as rapid disease progression, and relatively pure molecular pathology, suggest that there are advantages to developing drugs for FTD as compared to other more common neurodegenerative diseases such as AD. In March 2011, the Frontotemporal Dementia Treatment Study Group (FTSG) sponsored a conference entitled, “FTD, the Next Therapeutic Frontier,” focused on pre-clinical aspects of FTD drug development. The goal of the meeting was to promote collaborations between academic researchers and biotechnology and pharmaceutical researchers to accelerate the development of new treatments for FTD. Here we report the key findings from the conference, including the rationale for FTD drug development, epidemiological, genetic and neuropathological features of FTD, FTD animal models and how best to use them and examples of successful drug-development collaborations in other neurodegenerative diseases.

1. Introduction

Frontotemporal degeneration (FTD), sometimes referred to as frontotemporal dementia or frontotemporal lobar degeneration (FTLD), in the case of the neuropathology associated with the clinical syndrome, is a common form of dementia in individuals who are less than 65 years old at time of diagnosis. Once thought poorly understood and rare, there has been a rapid growth of knowledge about the biology of FTD over the past decade that has identified a number of potential therapeutic targets in different forms of FTD. FTD encompasses three clinical syndromes: behavioral variant frontotemporal dementia (bvFTD), and two primary progressive aphasias (PPA), a semantic variant (svPPA) and a nonfluent variant (nvPPA)\textsuperscript{1,2}. These syndromes frequently overlap with Amyotrophic Lateral Sclerosis (ALS), corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP), such that FTD, ALS, CBD and PSP are often considered as a related spectrum of diseases. Although FTD basic science has advanced rapidly over the past decade, there are no FDA-approved treatments for these disorders, and there are few data to suggest that any medications are effective in treating the symptoms of FTD or altering the progression of disease highlighting the enormous unmet medical of FTD patients. Moreover, because of significant overlap in pathogenic processes between FTD and other neurodegenerative diseases such as Alzheimer’s disease and ALS, development of disease-modifying therapies for FTD may help to accelerate drug development for more diseases, and conversely, therapies initially developed for AD and ALS, but not pursued, might be successfully exploited to treat FTD.
With this in mind, the FTD Treatment Study Group (FTSG) was formed in 2010 to promote collaborations between academic and pharmaceutical industry researchers focused on drug development for FTD and related disorders. On March 25–26, 2011, the FTSG sponsored a meeting entitled, “FTD: the Next Therapeutic Frontier,” at the Cleveland Clinic Lou Ruvo Center for Brain Health in Las Vegas Nevada. This meeting focused on pre-clinical models for FTD drug development, examples of successful academic-industry drug development collaborations in other neurodegenerative diseases, and development of tools, such as a website, to promote drug development for FTD. One of the goals of the meeting was to produce position papers focused on the rationale for and pre-clinical aspects of FTD drug development. This manuscript summarizes the presentations and discussions that took place surrounding animal models for FTD drug development at the March, 2011 meeting. The clinical and regulatory rationale for FTD drug development is discussed in the companion manuscript.

2. Neuropathology of FTD

The neuropathology underlying the clinical syndromes of FTD is heterogeneous, however there are a number of common themes and molecules that relate FTD to other neurodegenerative diseases including AD and ALS. Autopsy usually demonstrates relatively selective degeneration of the frontal and temporal lobes and frontotemporal lobar degeneration (FTLD) has become the accepted general terminology for FTD-related pathologies. In addition to non-specific microscopic changes of chronic neurodegeneration, most cases are found to have abnormal accumulation of protein within neurons and glia (inclusion bodies). The identity of the pathological protein varies among cases. The current classification of FTLD neuropathology is based on the predominant molecular abnormality, in the belief that this most closely reflects the underlying pathogenic process (Figure 1)\(^3\).

In ~45% of FTLD, abnormal inclusion bodies contain the microtubule-associated binding protein tau (\(MAPT\)) which is ubiquitinated and hyperphosphorylated. This molecular pathology overlaps with, but is distinct from, that seen in AD. In the adult brain, there are normally six isoforms of tau: three isoforms with three microtubule-binding repeats (3R tau) and three isoforms with four microtubule-binding repeats (4R tau). Tau protein in both FTLD and AD is relatively insoluble and these insoluble species can be detected by biochemistry. In AD, all six isoforms are abnormally hyperphosphorylated and migrate as three major bands and one minor band when visualized by immunoblotting. This biochemical signature may be used to distinguish AD from the FTLD tauopathies (FTLD-tau).\(^4\) Thus, brain tissue from patients with FTLD-tau where Pick bodies are present is characterized by a predominance of pathological 3R tau, while CBD, PSP, argyrophilic grain disease (AGD), and some other rare disorders are predominantly 4R tauopathies. Neurofibrillary tangle-predominant dementia (NTD) has inclusions containing a mixture of 3R and 4R tau, similar to that seen in AD; however, unlike AD, there is no \(\beta\)-amyloid protein (A\(\beta\)) in NTD. There is also a familial form of FTLD-tau caused by \(MAPT\) mutations, discussed further below. Autosomal dominantly inherited tauopathy is biochemically heterogeneous with different mutations being associated with 3R, 4R, or 3R and 4R tau. The morphology of the inclusions, the affected cell types (neurons or glia) and the anatomical distribution aid the neuropathologist in distinguishing among the various FTLD-tau entities. While each of the FTLD-tau pathological diagnoses may be associated with a variety of FTD clinical syndromes, FTLD-tau pathology is reliably predicted in PSP and cases with known \(MAPT\) mutations. These syndromes in particular may be attractive therapeutic targets for tau-directed therapeutics.

However, the majority of FTLD cases do not have tau-based pathology. Until recently, the neuronal inclusions that characterize the majority of FTLD cases were only detectable with
ubiquitin immunohistochemistry (FTLD-U). In 2006, it was discovered that the ubiquitinated pathological protein in most cases of FTLD-U, as well as sporadic ALS, is the transactive response DNA binding protein with Mr 43 kD (TDP-43). This finding confirmed that FTD and ALS are closely related conditions and established FTLD-TDP as the most common FTD-related pathology (~50% of cases). Distinct patterns of FTLD-TDP are now recognized to correlate with specific clinical phenotypes (including semantic dementia and FTD-ALS) and genetic abnormalities, including mutations in progranulin (GRN) and valosin containing protein (VCP) genes, and familial FTD-ALS genetically linked to C9ORF72.

Although the initial reports suggested that pathological TDP-43 was specific for FTLD-U and ALS, subsequent studies have found TDP-43-positive inclusions in a significant proportion of cases with other neurodegenerative conditions, including 25–50% of AD. This concomitant TDP-43 pathology is usually restricted to limbic structures of the mesial temporal lobe, but sometimes extends into the neocortex in a distribution that closely resembles FTLD-TDP. It is currently not known if this represents a coincidental primary pathological process, which contributes to the clinical phenotype, or a secondary change of little pathogenic significance, occurring in susceptible neuronal populations. The pattern of TDP-43 pathology that occurs in AD overlaps with that associated with GRN mutations and that some studies have suggested that GRN genetic variation may be a risk factor for AD. These findings suggest that progranulin and TDP-43 may represent appropriate therapeutic targets, not just for FTD, but also for AD.

The remaining 5–10% of FTLD cases includes several uncommon disorders with uncertain molecular bases. Following the recent discovery that mutations in the fused in sarcoma (FUS) gene are a cause of familial ALS, the possible role of FUS in the tau/TDP-negative FTLD subtypes was investigated. It was found that the conditions previously known as “atypical” FTLD-U (aFTLD-U; so-called because the inclusions are negative for TDP-43), neuronal intermediate filament inclusion disease (NIFID) and basophilic inclusion body disease (BIBD) are all characterized by neuronal and glial inclusions that are immunoreactive for FUS. These cases are usually sporadic and no FUS mutations have yet been identified in FTLD-FUS.

With these recent advances, virtually all cases of FTLD can now be assigned to one of three major molecular subgroups (FTLD-tau, FTLD-TDP or FTLD-FUS) (Figure 1). The specific role of the pathologic proteins and their relationship to causal gene defects remains to be fully elucidated. None-the-less, these recent discoveries have greatly improved our ability to offer meaningful genetic counseling for FTD families and bring us much closer to developing useful diagnostic tests and rational therapies.

### 3. FTD Genetics

Up to 40% of FTD patients have a family history of dementia or related condition (Parkinsonism or ALS); however, only about 10% show a clear autosomal dominant inheritance pattern. Mutations in the microtubule associated protein tau (MAPT) and progranulin (GRN), both on chromosome 17, and the C9ORF72 gene on chromosome 9, each account for 2–10% of all cases and 10–23% of these familial cases. Importantly, each of the FTD genetic alterations is associated with a specific neuropathological diagnosis, suggesting that construction of transgenic animals based on these genetic alterations can recapitulate the key molecular phenotypes of FTD.

Although MAPT mutations are very rare in sporadic cases, about 3–5% of sporadic FTD is caused by mutations in GRN or C9ORF72. Mutations in charged multivesicular body protein 2B gene (CHMP2B) on chromosome 3 were identified in a large, Danish FTD
Mutations in the FTLD pathogenic proteins TDP-43 (TARDBP) and FUS have mostly been associated with ALS. In families with inclusion body myopathy associated with Paget’s disease and frontotemporal dementia (IBMPFD), up to 35% of affected family members develop FTD. These families have mutations in the valosin-containing protein gene (VCP). Families with histories of both ALS and FTD have been linked to chromosome 9p, and a hexanucleotide repeat expansion within the first intron of the C9ORF72 gene has recently been identified as the cause of a large proportion of familial, as well as a small proportion of sporadic, FTD, FTD-ALS and ALS cases. Together, the known FTD genes explain the disease in a large number of FTD cases, however it is possible that other causal FTD genes exist.

The MAPT gene is located on chromosome 17q21.1 and encodes the 758 amino acid long tau protein. Up to 72 variants have been reported in MAPT gene causing missense, silent and splice site mutations (www.molgen.ua.ac.be). Pathogenic variants can result in microtubule disruption and accumulations of hyperphosphorylated tau filaments within neurons and glial cells. The GRN gene is located on chromosome 17q21.32 and codes for a 593 amino acid long precursor, progranulin, that under certain conditions, is cleaved into granulins. Progranulin is a growth factor and is involved in wound healing, tumor growth, and inflammation. Currently up to 149 variants have been identified in GRN that result in nonsense, frameshift or splice-site mutations (www.molgen.ua.ac.be). GRN nonsense mutations result in aberrant mRNA transcripts which undergo non-sense mediated decay (NMD), resulting in haploinsufficiency. The FUS gene is located on chromosome 16p11.2 and codes for a 526 amino acid long protein which binds to RNA and DNA and regulates DNA cellular localization, repair, transcription, and RNA splicing. TAR-DNA Binding Protein 43 (TDP-43) colocalizes with ubiquitinated protein deposits in the brain of FTD and ALS patients. CHMP2B is located on chromosome 3p11.2. The CHMP2B protein is composed of 213 amino acids and is a component of the heteromeric ESCRT-III (Endosomal Sorting Complex Required for Transport III). CHMP2B is involved in sorting and trafficking surface receptors and proteins into intraluminal vesicles (ILVs) for lysosomal degradation and binding the Vps4 protein responsible for the dissociation of ESCRT components. C9ORF72 is a recently identified gene that encodes a protein of unknown function in which large expansions of a hexanucleotide repeat sequence (100s to 1000s) within the first intron may lead to neurodegenerative disease either through decreased expression of the C9ORF72 protein or possibly by sequestering RNA binding proteins such as TDP-43 and FUS, interfering with their proper function.

There is accumulating evidence that mutations in genes that are associated with FTD present with greater clinical than neuropathological phenotypic variability (Figure 1). Patients with apparently pathogenic MAPT, GRN and C9ORF72 mutations have presented with symptoms of bvFTD, svPPA, nfvPPA, CBD, PSP, and rarely clinical, but not neuropathological, Alzheimer’s disease. The mechanisms determining the specific clinical phenotype of autosomal dominant mutations associated with FTD remains unknown. Variations in genes associated with FTD can also affect organ systems other than the nervous system. VCP mutations are associated with myopathy and Paget’s disease. Overexpression of PGRN and FUS is associated with the development of malignancies and mice lacking PGRN are highly susceptible to systemic inflammation. As discussed below, introduction of these human genes, either as wild type or disease-associated mutations, as well as mutation of animal homologues of each of these genes has been exploited to develop animal models of FTD.
4. Targets and early lead molecules

The proteins most commonly associated with FTD neuropathology are tau and TDP-43. Tau has been a potential drug target for AD for many years given its strong association with AD clinical phenomenology.

Moreover, the strong genetic associations of tau with FTD-tau and PSP provide a rationale for believing that interventions that target tau. The mechanisms by which TDP-43 is associated with FTD clinical phenotypes are less clear, given the relative inexperience with TDP-43 mouse models and the relatively weak genetic associations between TDP-43 gene mutations and FTD. Until more is known about the biology of TDP-43, development of treatments targeting TDP-43 are likely to lag behind those targeting tau. However, within the TDP-43 spectrum of FTD phenotypes, progranulin is a particularly attractive target for treatment development because of its strong association with FTD clinical phenotypes and the haploinsufficiency mechanism by which it leads to disease. Since FTD-PGRN is caused by reduced levels of PGRN (that can be measured in the blood, CSF and brain tissue), treatments that raise PGRN protein levels either by increased production or reduced clearance may be attractive candidates for treating FTLD-TDP patients.

Table 1 lists some of molecules that could theoretically be investigated for the treatment of FTLD-tau or FTLD-PGRN. In terms of human clinical trials, tau-based therapies are clearly more advanced than PGRN therapies. A number of tau-targeted drugs have been studied in clinical trials for PSP, including the GSK3beta inhibitor, tideglusib, and the microtubule stabilizing agent davunetide. The tau aggregation inhibitor, methylene blue, was studied in a Phase 2 clinical trial in AD. In addition, anti-oxidants and other mitochondrial-targeted therapies have also been investigated in PSP and demonstrated some promise in transgenic tauopathy models. For PGRN, two recent high-throughput screening studies have identified FDA-approved drugs that can increase PGRN levels. It is likely that additional drugs exist within industry compound libraries and elsewhere that also elevate PGRN levels.

5. Laboratory and animal models of FTD

Over the past ten years, there has been an explosion of new cellular and animal models that could be used for different stages of FTD drug development, including target identification, validation, drug screening and optimization, and other IND-enabling studies. Reviewed here are some of the available models that have been used to study FTD, their strengths and limitations (see also Table 1).

5.1. Induced pluripotent stem (iPS) Cells

Among the major hurdles in drug development is the significant differences in physiology and toxicology between animal models and humans. For this reason, the use of human neurons for disease studies and drug screening is desirable. One way to generate disease-specific human neurons is to differentiate human embryonic stem (hES) cells into neurons. However, developing hES cells harboring disease-causing mutations presents significant ethical, technical, and practical challenges. Some of these challenges can be overcome through the use of novel reprogramming technology in which induced pluripotent stem (iPS) cells can be derived from human fibroblasts with or without disease mutations. These iPS cells can be differentiated into human neurons or other disease-relevant cell types. This technology relies on the expression of four genes, Oct3/4, SOX2, NANOG, and c-Myc with a retroviral system. Other reprogramming methods without retroviral integration have also been developed, including through the expression of the miR-302/367 cluster. Although reprogrammed iPS cells may not exactly reproduce the pluripotent state of human...
embryonic stem (hES) cells, they appear to be ideally suited for studying diseases and testing therapies.

Through a collaborative effort, multiple iPS cell lines have been generated from patients (and unaffected “control” family members) with progranulin or tau mutations as well as from control or sporadic FTD cases (Almeida et al., unpublished). These iPS cell lines and their derivatives such as patient-specific human neurons provide a novel assay system complimentary to existing cell and animal models for drug development. The efficacy and toxicity of compounds that raise PGRN levels can now be tested in iPS-derived human neurons containing endogenous PGRN mutations. Similarly, compounds that can lower tau levels can be screened in cultures of iPS-derived human neurons.

Similarly, compounds that can lower tau levels can be screened in cultures of human neurons. Patient-derived iPS cells and their derivatives are rapidly becoming a powerful tool in the realm of drug discovery for FTD and related diseases.

5.2. Caenorhabditis elegans

Studies in the nematode *C. elegans* have contributed greatly to our understanding of basic physiological processes such as aging, sensory processing and programmed cell death and to mechanisms underlying human diseases such as cancer and neurodegeneration. Of the genes that have been linked to familial forms of FTLD and ALS, MAPT, PGRN, VCP and TDP-43 all have homologs in *C. elegans* (only CHMP2B and FUS do not.) Thus, significant opportunities exist to utilize *C. elegans* as a model organism in order to learn about FTD pathophysiology and to model disease with the goal of discovering novel drug targets.

Mutations in TDP-43 have been linked to the development of ALS and the protein itself is found in the neuronal inclusions of FTD due to progranulin deficiency. Several groups have generated transgenic *C. elegans* expressing human TDP-43 in neurons. These complementary studies all found motor defects associated with wild-type TDP-43 expression that was worsened by expression of mutant forms of TDP-43 \(^{21,22}\). Individual groups also showed synaptic loss with abnormal nuclear accumulation of TDP-43 \(^{21}\), insoluble phosphorylated and ubiquitinated TDP-43 aggregates \(^{22}\), decreased lifespan of TDP-43 expressing animals \(^{22}\), and age-associated worsening of motor phenotypes that could be abrogated by decreased DAF-2/Insulin/IGF-1 signalling \(^{22}\). Ash and colleagues also showed that the *C. elegans* homolog of TDP-43, *tdp-1*, can substitute for human TDP-43 in an exon recognition alternative splicing assay \(^{21}\). These studies demonstrate that expression of human TDP-43 in *C. elegans* neurons can recapitulate many features of human disease, including motor defects, post-translational modification and nuclear localization of the protein, aggregate formation, synaptic and/or neuronal loss, and age-associated decline. Together, they make a strong case for modeling TDP-43 proteinopathy in *C. elegans*.

Earlier, similar studies found that expressing human tau also causes an age-dependent neurodegenerative phenotype \(^{23}\). One group utilized *C. elegans* to screen for genes that ameliorate the abnormal movement phenotype of tau-expressing worms and identified a novel target, SUT-2, a highly-conserved CCCH zinc finger protein \(^{24}\).

In addition to modeling disease, *C. elegans* can be used to characterize novel functions of disease-related proteins. Kao et al. \(^{25}\) took advantage of the completely mapped lineage of all 959 somatic cells in the *C. elegans* hermaphrodite and the transparent cuticle of the animal to conduct real-time observations of the 131 cell death events that normally occur during development. They showed that absence of progranulin causes apoptotic cells to be engulfed and cleared about twice as quickly as in wild type animals \(^{25}\). They then showed that mouse macrophages lacking endogenous progranulin also engulfed apoptotic cells more
quickly. These findings suggest that PGRN normally functions to slow the rate of dying cell clearance.

5.3. Drosophila melanogaster

*Drosophila* is a powerful model system for dissecting neurodegenerative disease and identifying potential genetic modifiers of disease-associated mutations. Homologues of several genes causing human autosomal FTD syndromes including valosin-containing protein (VCP), TDP-43 and tau have been identified and modified in *Drosophila*. *Drosophila* models are particularly useful for dissecting the pathogenic mechanisms associated with particular mutations and identifying possible therapeutic targets because they are relatively inexpensive to produce and have rapid life cycles. Transgenic *Drosophila* carrying human disease-associated tau mutations have been used to identify mechanisms that suppress tau toxicity such as the unfolded protein response and the *wingless* pathway. The ability to conduct assays of learning and memory in *Drosophila* along with analyses of known anatomical substrates of memory formation such as mushroom bodies, has revealed important aspects of dynamic regulation of tau protein phosphorylation in development and memory formation.

5.4. Zebrafish

The zebrafish (*Danio rerio*) has been extensively used as a model for studying vertebrate development; embryos develop externally and are transparent, allowing direct observation of embryogenesis under the microscope and visualization of labeled cells using fluorescent reporter proteins. Zebrafish are prolific breeders and large numbers can be housed practically, facilitating large-scale genetic and chemical modifier screens. The zebrafish brain shares its basic organization with other vertebrates including mammals and contains neurochemical systems and specialized neuronal and glial cell populations of relevance to human neurodegenerative disorders (reviewed in ). Many of the genes implicated in human neurological disorders have highly conserved orthologues in zebrafish, suggesting that molecular mechanisms involved in neurodegeneration may be recapitulated in zebrafish models. This may allow use of models for the identification of drug targets and evaluation of therapeutic compounds. Since zebrafish larvae can be readily exposed to chemicals in multiwell plate formats, these models may provide an effective means for screening drugs for FTD in their early stages of development - from screens for novel chemical modifiers of disease phenotypes to rapid evaluation of panels of structural analogues for investigation of activity and toxicity in vivo.

Zebrafish expressing human tau either transiently or in stable transgenic lines have been reported, and provide evidence that zebrafish models can replicate biochemical, histological and neurobehavioral aspects of FTD. Human tau is a substrate for zebrafish kinases, resulting in its phosphorylation in vivo. This was abrogated by inhibitors of human GSK3β, suggesting sufficient phylogenetic conservation that compounds optimized for activity in a mammalian system were effective in the zebrafish model, and supporting the idea that zebrafish models could be predictive of efficacy in other systems. Human Tau accumulated in the somato-dendritic compartment of zebrafish neurons, reflecting a characteristic abnormality seen in FTD. In one model, using a conditionally-expressing system to achieve high expression levels of the P301L FTLD mutant, motor abnormalities and enhanced cell death in the CNS were observed and tau accumulations became argyrophilic, resembling NFTs. The detailed characterization of other tau transgenic lines and zebrafish progranulin mutants are ongoing.
5.5. Transgenic mice

5.5.1. Tau transgenic models—More than 25 lines of transgenic mice have been created that express human tau with mutations linked to FTD. Alternative splicing of the MAPT gene encoding tau gives rise to six isoforms in the adult human central nervous system. With the exception of a few lines that express tau mini-genes, most tau transgenic mice express cDNAs encoding a single splice variant, 4R tau with or without N-terminal inserts. In a recent comprehensive review, Noble et al. described the phenotypes of the several tau transgenic mice that have been created, and a frequently updated list of transgenic lines can be found in the Alzforum compendium of research models (http://www.alzforum.org/res/com/tra/). Not surprisingly, the spatio-temporal pattern and level of expression of transgenic protein, and hence the neuropathological and behavioral phenotypes, vary with the promoter used to drive the transgene and among specific lines.

Tau transgenic mice have provided important insights and raised new questions about mechanisms of tau-mediated neurotoxicity. Not all lines display the pronounced neurodegeneration seen in the human disease, but among those that do, deficits in synaptic plasticity and cognitive dysfunction precede neurodegeneration. Studies in mice further have shown that neurodegeneration and cognitive deficits can be dissociated from neurofibrillary pathology. These findings point to the importance of identifying the species of tau responsible for synapto- and neurotoxicity in mice and determining whether these tau species also occur in human neurodegenerative diseases and call into question whether therapies aimed at reducing neurofibrillar tangles will have any clinical benefit.

Tau transgenic mice have been used for pre-clinical testing of potential therapies, including kinase inhibitors, tau-related immunotherapy, and anti-inflammatory drugs (reviewed by), although the number of studies is small compared to the number of pre-clinical studies in APP transgenic models relevant to AD. A challenge in translating results from APP mice to humans is that APP mice show little neurodegeneration and mimic the asymptomatic phase of the disease, while the majority of clinical trials have been conducted in people with clinically diagnosed disease and accompanying neurodegeneration. A parallel situation is likely to exist for FTD, and therapies intended as treatments for people with symptomatic disease should be tested in tau transgenic mice that exhibit neurodegeneration.

5.5.2. Progranulin and TDP-43 transgenic mice—in an attempt to understand TDP-43 function researchers have generated mouse models. Models with targeted deletion of TDP-43 are embryonic lethal early in gestation (E7.5 or earlier), whereas hemizygous null TDP-43 animals show normal levels of TDP-43, suggesting that some form of autoregulation of TDP-43 expression occurs. Transgenic mice that overexpress wild-type human TDP-43 develop dose-dependent down regulation of endogenous mouse TDP-43 and the highest expressing lines develop motor dysfunction and die by 2 months of age. A transgenic mouse line expressing CAMKII-driven full-length mouse TDP have learning and memory impairment at 2 months, with motor deficits and mild impairment in LTP by 6 months of age. Transgenic mice with inducible CAMKII-driven human wild-type TDP-43 or nuclear localization signal mutant (NLSmutant) TDP-43 over-expression have been reported recently. Following induction of TDP-43, progressive cell loss in the dentate gyrus and cortex occurs with more acute neuronal loss and massive gliosis seen in the NLS mutant mouse. Rare TDP-43 protein aggregates were found in neurons, and were correlated with level of over-expression, but did not appear to be required for cell loss.

Progranulin (PGRN) haploinsufficiency has recently been identified as a cause of familial frontotemporal dementia (FTD), but the normal function of PGRN in the brain is currently not well understood. Recent work using mouse models has defined the expression of progranulin in the brain. PGRN is expressed late in neurodevelopment, co-localizing with
markers of mature neurons. PGRN is expressed in neurons in most brain regions, with high expression in the thalamus, hippocampus, and cortex. Microglia also express progranulin, and the level of expression is up-regulated by microglial activation. To functionally examine the role of progranulin in the CNS, several groups have generated knockout mice targeting the progranulin locus (GrnKO mice). GrnKO mice show sex-specific alterations in behavior and increased anxiety suggesting that progranulin is involved in sexual development in the brain. Other subtle behavioral abnormalities have also been reported including depression- and/or disinhibition-like behavior, deficits in social recognition, and impaired spatial learning. With advanced age GRN KO mice develop neuropathology characterized by accumulation of ubiquitinated proteins, lipofuscinosis, microgliosis, and astrogliosis.

Analysis of synaptic transmission in these GrnKO mice identified disrupted synaptic connectivity and impaired synaptic plasticity (long-term potentiation in the hippocampus). Pyramidal cells in the CA1 region of the hippocampus have an altered dendritic morphology and decreased spine density compared to wild-type mice. The observed changes in behavior, synaptic transmission, and neuronal morphology in GrnKO mice occur prior to gross neuropathology that is not apparent until 18 months of age. These studies suggest that progranulin deficiency leads to reduced synaptic connectivity and impaired plasticity that precedes overt neuropathological changes or cell loss. Synaptic dysfunction may be one of the earliest deficits caused by a lack of progranulin, and may contribute to FTD pathology in human patients. Strategies aimed at increasing or maintaining synaptic transmission may prove useful in the treatment of FTD.

5.5.3. Transgenic models to study the role of neuroinflammation in FTD—There is increasing evidence favoring a significant neuroinflammatory component in FTD. First, a large number of inflammatory cells (microglia and astrocytes) and molecules (cytokines, chemokines, complement components, etc.) are present at elevated levels in the brains of individuals with FTDs. Second, haploinsufficiency for GRN, a gene involved in immune regulation in the periphery, is a major cause of tau-negative FTD. PGRN regulates microglial function and GRN knockout mice demonstrate increased microglial proliferation and other abnormalities. Third, there is recent evidence that there are alterations in inflammatory cells/molecules prior to the aggregation of the microtubule-associated protein tau in several different mouse models of FTD. While these largely correlative studies suggest a link between FTDs and neuroinflammation, the exact contribution of inflammatory cells and molecules to the pathogenesis of FTDs and the therapeutic potential of targeting neuroinflammatory pathways for FTD remains to be established.

Microglia, the resident inflammatory cells of the brain, monitor the brain for pathological alterations and become activated in most neurodegenerative diseases, including FTDs. Microglial activation can be beneficial or detrimental, contingent on context, and involves morphological alterations, proliferation, phagocytosis, migration, enhanced expression of cell surface receptors and production of cytokines. One significant way that neuroinflammation is regulated is through neuronal-microglial signaling through the chemokine fractalkine (CX3CL1), and its receptor, CX3CR1. Several lines of evidence suggest a specific role for this chemokine in neuroinflammation: 1) CX3CL1 is highly expressed by neurons and CX3CR1 is exclusively expressed by microglia; 2) CX3CL1 is neuroprotective in several different models of neuroinflammation; and 3) lack of CX3CR1 in mice worsened neurodegenerative phenotypes in mouse models of both Parkinson’s disease and amyotrophic lateral sclerosis.

To examine the role of CX3CR1-CX3CL1 signaling in FTLDs, Cx3cr1 knockout mice were crossed with hTau mice. Notably, hTau;Cx3cr1−/− mice exhibited increased MAPT
phosphorylation when compared to age-matched hTau;Cx3cr1<sup>+/−</sup> mice. Furthermore, biochemical analysis revealed elevated levels of aggregated MAPT in hTau;Cx3cr1<sup>−/−</sup> mice that was confirmed by Gallyas silver staining of the brain sections. In addition, hTau;Cx3cr1<sup>−/−</sup> mice exhibited deficits in working memory when compared to age-matched hTau;Cx3cr1<sup>+/+</sup> controls. Finally, CX3CR1 deficiency was associated with enhanced microglial activation in hTau;Cx3cr1<sup>−/−</sup> mice when compared to hTau;Cx3cr1<sup>+/+</sup> or non-transgenic controls. Taken together, these results demonstrate that the absence of CX3CR1 results in enhanced tau phosphorylation, aggregation, microglial activation and working memory deficits in the hTau mice. Additional experiments utilizing cultured neurons and microglia demonstrated that CX3CR1 deficiency acts via microglial activation to accelerate tau phosphorylation and aggregation in hTau mice potentially via an interleukin 1 (IL-1)-dependent pathway. These studies suggest neuroinflammatory pathways directly contribute to the pathogenesis of FTDs and that CX3CL1-CX3CR1 signaling and/or IL-1 are potentially intriguing therapeutic targets for FTD. Since microglial-mediated neuroinflammation is measurable in living humans with FTD, using the PET ligand [11C](R)-PK11195<sup>49</sup>, which can also be used in transgenic mice, rapid translation of microglial drugs from mouse to humans may be possible.

5.5.4. Assessment of FTD-like behaviors in transgenic mice—When using animal models to study a disease, the choice of outcome measure is as important as the model itself. For mouse models, the most common outcome measures are behavioral or pathological. Behavioral outcome measures provide a significant advantage because they reflect function, and thus obviate difficulties in interpreting whether a given pathological change is “good” or “bad”. Behavioral measures are widely used for AD mouse models, including the Morris water maze which tests hippocampus-dependent memory. Given the differences between the two diseases, measures ideal for AD may not be the best choices for FTD.

Although it might seem challenging to find behavioral assays for FTD mouse models given the complex nature of the disease, several features of FTD are in fact amendable to behavioral analysis in mice. One aspect of FTD that can be examined in mice is social dysfunction. Mouse models of autism, another disorder with prominent social dysfunction, have demonstrated the usefulness of several social tests. Some FTD models have already been found to exhibit abnormalities on these tests. Of particular importance to modeling FTD is the observation that social dysfunction in mice can arise as a result of abnormalities in frontal cortex. Repetitive behavior is another symptom of FTD that may also serve as a useful outcome measure in mouse models. Repetitive behavior is common and disabling in FTD, and includes complex compulsive behaviors, motor and vocal stereotypies, and self-injurious pathological grooming. Repetitive grooming in FTD seems to relate to striatal dysfunction, and interestingly, mice lacking certain striatal genes exhibit repetitive grooming. Amygdala dysfunction, which is associated with impaired fear conditioning in FTD, should also be amenable to study in mouse models. In summary, behavioral assays that reflect dysfunction of the networks involved in FTD are available and may be useful as outcome measures for mouse models of the disease.

6. Improving predictive value of FTD animal models for human therapies: limitations of transgenic animals

Drug development for the treatment of neurological or psychiatric disorders is particularly challenging and is known to have a low success rate compared to other therapeutic areas. There have been significant challenges in CNS drug development, especially for the treatment of neurodegenerative diseases. Compared to other therapeutic areas, CNS drugs
have the lowest success rate in all phases of drug development. Only 1–2% of phase I, 2–3% of phase II, and 15% of phase III CNS drugs ever reach market.\(^5^4\)

It is likely that a considerable proportion of CNS drug development failures for neurological disorders relate to problems with the predictive values of the pre-clinical models that are used to justify human clinical trials. Because FTD is a relatively uncommon disease, the ability to conduct multiple, large, concurrent human clinical trials will be limited. Recent clinical failures in other neurodegenerative indications such as AD and ALS that were not adequately predicted by pre-clinical animal studies suggest that three of the most important limitations of preclinical animal models relate to their: 1) relevance to the human disease state, 2) pharmacological applicability, and 3) how well they were initially validated.\(^5^5,5^6\) FTD drug development will need to transfer more risk of a drug’s ultimate failure from the clinical development stage back to the pre-clinical stage. By taking into account these limitations and designing more rigorous models and study procedures, it is hoped that FTD drug development efforts will be more successful in translating potential drugs into successful human treatments.

### 6.1. Relevance to the human disease state

One of the central questions facing CNS drug development is whether the pathology or pathophysiology seen in animal models is a fair representation of the human condition that is being studied.\(^5^7\) Many transgenic models at best recapitulate rare genetic causes of more common human sporadic disease, and at worst may produce novel mechanisms of disease in animal models that are not relevant to the human disease. An excellent example of this issue is the reliance on transgenic models for the development of amyloid-related treatments for AD. All these models depend on some form of genetic mutation or combinations of mutations leading to the over-production of \(\beta\)-amyloid. These models are representative of the familial forms of AD, not necessarily of the late-onset sporadic form of AD. Models relying on over-expression of normal \(\beta\)-amyloid fail to produce tau deposits and are not associated with evidence of neuronal loss that is seen in human AD.\(^5^6\) Similarly, most ALS drug development studies have relied on a transgenic mouse model that carries 23 copies of the human SOD1G93A mutation, whereas only a single copy of this mutation is found in the approximately 3% of human patients who have SOD1-related ALS.\(^5^8\) Proposals to improve the predictive power of such imperfect animal models to select a lead candidate to move into clinical trials include using at least two different transgenic models as well as non-transgenic animal models to independently confirm pharmacological activity at doses predicted to be effective in humans.\(^5^9\)

### 6.2. Pharmacological applicability

Even if the pathophysiology in the animal model is relevant and generalizable to the human condition, one of the greatest values of an animal model is the validation of pharmacological effect at the intended target. Early human clinical trials help to define right dose range and dosing paradigm to move into later-stage, pivotal trials. Therefore, pre-clinical models need to be able to provide informative data on a wide range of doses for both pharmacokinetic and pharmacodynamics outcome measures. Models or species that fail to yield these types of data (i.e., those that require intrathecal administration, or display intractable kinetics) require drug developers to rely on simplifying assumptions in clinical trials and consequently transfer a substantial portion of risk into human clinical trials which is undesirable.

Since some models may have more use in target validation than in verification of pre-clinical therapeutic efficacy, it has been suggested that two types of animal model studies be conducted: exploratory studies, focused on the mechanism and target engagement, and
therapeutic studies focused on a lead compound. Therapeutic preclinical studies should incorporate rigorous study designs similar to human clinical trials such as randomization, placebo control and multiple drug doses, with pre-stated endpoints and power calculations. For maximal value, such studies should also incorporate pharmacokinetic and pharmacodynamic as well as absorption, distribution, metabolism and excretion (ADME) assays whenever possible. Finally, the use of biochemical, imaging, physiological and behavioral tests as potential biomarkers should be considered in pre-clinical animal trials with the aim of identifying a set of pharmacodynamics markers that are translatable into man. Using a consistent set of biomarkers throughout the pre-clinical to clinical transition would provide a more efficient determination of potential efficacy in man.

6.3. Technical validation

While the variety of potential animal models based on a given model of disease is rapidly expanding due to advances in the methods of transgenic model construction (e.g., knock-ins, knock-outs, conditional mutants and multiple concurrent gene defects) as well as pharmacological manipulations (selective toxins, target manipulation and others), many of these models have not undergone appropriate technical validation to determine the stability of the model from animal to animal, and generation to generation. For example it has been suggested that some previous positive outcomes in the SOD1 ALS mouse might be attributable to phenotypic variability within the mice that were studied due to either environmental (e.g., diet or health status) or biological factors (age, sex, genetic drift or background strain) that were not taken into account. A corollary of these concerns is that models should have good evidence that there is a predictable relationship between phenotype and disease pathophysiology. The use of standard, positive control compounds or other manipulations that have known pharmacologic effects within the disease-relevant pathway can be applied in multiple model systems and are ideal tools that may accelerate technical validation.

An example of additional forms of technical validation that animal models should undergo is represented by the work undertaken by the ALS-Therapeutic Development Institute (TDI) in the SOD-1 model (http://www.als.net) in which rigorous, large scale animal clinical trials are carried out. Admittedly, very few researchers will have the resources to validate their models to the same extent as the ALS-TDI, so there is a clear need for a transparent effort to identify the best models and come up with a funding model that allows the validation of the most promising models in a sustainable fashion. Negative drug efficacy results in animal models are often not published leading to a bias towards flawed, positive studies and wasted resources as multiple laboratories repeat experiments with ineffective compounds. Replication of potentially beneficial drug effects in a given pre-clinical model should be independently confirmed before such results are used to make development decisions. A database of well-conducted animal studies (with adequate positive controls) of potential therapeutic molecules of interest, including those that fail to demonstrate benefit in a given model, would likely accelerate the development of the most promising compounds to prioritize for human clinical trials.

7. Conclusion

The molecular underpinnings of FTD are becoming increasingly clear. Although much work still needs to be done to relate certain clinical phenotypes to pathogenic molecules, the understanding of two molecular forms of FTD, FTD associated with tau pathology and FTD associated with PGRN haploinsufficiency, has reached sufficient maturity to begin to consider clinical trials of tau- and PGRN-related therapeutics. Development of such therapeutics will be greatly facilitated by the existence of FTD-specific animal models derived from expression of human FTD causing genes in flies, worms, fish and mice as well.
as human neurons with the same mutations derived using iPS technology. FTD drug development efforts will also benefit from experience in AD and ALS, in which problems with the translation from transgenic mouse models to human clinical trials have been revealed. Such difficulties have led to new standards for animal experiments that should improve the translational process for FTD.

References


Figure 1. Neuropathological classification of FTD subtypes

Frontotemporal lobar degeneration (FTLD) encompasses three distinct neuropathologic categories which are identified by the molecular pathology of the misfolded protein within the inclusion: FTLD-Tau, FTLD-TDP, and FTLD-FUS; the molecular pathology of a fourth category, FTLD with epitopes of the ubiquitin-proteasome system (FTLD-UPS), remains indeterminate. 3R, 4R, 3R/4R the predominant tau isoform within the inclusion; PICK, Pick disease; FTLD with microtubule-associated protein tau (MAPT) mutation with inclusions of 3R, 4R, or 3R and 4R tau protein; CBD, corticobasal degeneration; PSP, progressive supranuclear palsy; WMT-GGI, white matter tauopathy with globular glial inclusions; AGD, argyrophilic grain disease; NFT Dementia, neurofibrillary tangle-predominant dementia; FTLD-U, FTLD with ubiquitin-immunoreactive inclusions, now called FTLD-TDP; FTLD with progranulin (GRN) mutation; FTLD with TAR DNA-binding protein of 43 kDa (TARDBP) mutation; FTLD with valosin-containing protein (VCP) mutation; FTLD with C9ORF72 expansion; NIFID, neuronal intermediate filament inclusion disease; aFTLD-U, atypical FTLD with ubiquitin inclusions; BIBD, basophilic inclusion body disease; FTLD with fused in sarcoma (FUS) mutation; FTLD with charged multivesicular body protein 2B (CHMP2B) mutation. Within each molecular pathology there may be unclassified entities.
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<tr>
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| Induced pluripotent stem (iPS) cells; (Humans) | Tau PGRN Chr9p | • Investigating pathogenesis of specific proteins  
• High throughput drug screens  
• Optimization of lead drug candidates | • Human cells and neurons  
• Pathogenic mutations in situ  
• Same genetic background as patients | • in vitro cellular model  
• High cost  
• Some differences from hESCs | 12     |
| Drosophila melanogaster                 | Tau PGRN VCP  
CHMPB2       | • Investigating pathogenesis of specific proteins  
• Drug target identification | • Rapid life cycle  
• Identification of genetic enhancers & suppressors  
• Survival and behavioral effects can be tested | • Limited usefulness in drug screening or testing of lead compounds | 13–15  |
| C. elegans                              | Tau PGRN TDP-43 VCP | • Genetic pathway discovery  
• Investigating pathogenesis of specific proteins  
• Drug target identification | • Rapid interrogation of genetic/molecular interactions  
• Unbiased screening for genetic enhancers and suppressors  
• In vivo fluorescence and Nomarski microscopy  
• Well-described nervous system with techniques to study behavior, learning, memory and forgetting | • Phylogenetic distance from humans  
• Lack of acquired immunity (only innate immunity) | 16–21  |
| Zebrafish                               | Tau PGRN TDP   | • Investigating pathogenesis of specific proteins  
• Drug target identification  
• High throughput drug screens | • In vivo vertebrate model  
• Discovery of genetic and chemical modifiers  
• Ease of testing drugs in meaningful sample sizes  
• Survival and behavioral, morphological and biochemical end points can be measured | • models still under development  
• Requires aquatics facility and expertise  
• Phylogenetic distance from human currently unclear | 22,23  |
| Mice                                    | Tau PGRN TDP-43 | • Investigating pathogenesis of specific genes/proteins  
• Target validation | • Neuroanatomical conservation with human brain  
• Genetic homology with humans | • Relatively high cost  
• Need to wait months–years for mice to age | 24–31 3233 |
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References for Tables 1 and 2


