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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) [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 β-amyloid protein (Aβ) 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 M_r 43 kD (TDP-43). [5] 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*. [6]

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 [7]. 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 [8]. 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).[3] 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. [9] Mutations in the microtubule associated protein tau *(MAPT* [10] and progranulin [*GRN])* [11], both on chromosome 17, and the *C9ORF72* gene on chromosome 9, [6] 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

family. [12] Mutations in the FTLD pathogenic proteinsTDP-43 *(TARDBP)* and *FUS* have mostly been associated with ALS. [13] 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*). [14] 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 [6] 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). [10] 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. [11] 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. [11] 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. [15] TAR-DNA Binding Protein 43 (TDP-43) colocalizes with ubiquitinated protein deposits in the brain of FTD and ALS patients. [5] *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 vesicules (ILVs) for lysosomal degradation and binding the Vps4 protein responsible for the dissociation of ESCRT components. [16] *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. [6]

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. [9] 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. [17] 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. [18] This technology relies on the expression of four genes, Oct3/4, SOX2, NANOG, and c-Myc with a retroviral system [19]. Other reprogramming methods without retroviral integration have also been developed, including through the expression of the miR-302/367 cluster [20]. 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 autosmal 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 [26] and the *wingless* pathway [27]. 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. [28]

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 [29]). 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 [29]. 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 [30] or in stable transgenic lines [31,32] 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 [30,32]. 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 [31,32], 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 [32], 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 [33], 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 [34]. Studies in mice further have shown that neurodegeneration and cognitive deficits can be dissociated from neurofibrillary pathology [35]. These findings point to the importance of identifying the species of tau responsible for synapto- and neuro-toxicity in mice and determining whether these tau species also occur in human neurodegenerative diseases and call into question whether therapies aimed at reducing neurofibrillary 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 [33]), although the number of studies is small compared to the number of pre-clinical studies in APP transgenic models relevant to AD. [36] 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 [36]. 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 levels of TDP-43, suggesting that some form of autoregulation of TDP-43 expression occurs [37]. 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 [38]. 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 [39]. 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 [40]. 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 [41]. 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 [42] 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 [43]. With advanced age *GRN* KO mice develop neuropathology characterized by accumulation of ubiquitinated proteins, lipofuscinosis, microgliosis, and astrocytosis. [44].

Analysis of synaptic transmission in these GrnKO mice identified disrupted synaptic connectivity and impaired synaptic plasticity (long-term potentiation in the hippocampus). [45] 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 [11,17]. PGRN regulates microglial function and *GRN* knockout mice demonstrate increased microglial proliferation and other abnormalities. [46] 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 [34]. 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 [47]; and 3) lack of CX3CR1 in mice worsened neurodegenerative phenotypes in mouse models of both Parkinson's disease and amyotrophic lateral sclerosis [48].

To examine the role of CX3CR1-CX3CL1 signaling in FTLDs, *Cx3cr1* knockout mice were crossed with hTau mice [49]. Notably, hTau;*Cx3cr1*$^{-/-}$ mice exhibited increased MAPT

phosphorylation when compared to age-matched hTau;*Cx3cr1*$^{+/+}$ mice. Furthermore, biochemical analysis revealed elevated levels of aggregated MAPT in hTau;*Cx3cr1*$^{-/-}$ mice that was confirmed by Gallyas silver staining of the brain sections. In addition, hTau;*Cx3cr1*$^{-/-}$ mice exhibited deficits in working memory when compared to age-matched hTau;*Cx3cr1*$^{+/+}$ controls. Finally, CX3CR1 deficiency was associated with enhanced microglial activation in hTau;*Cx3cr1*$^{-/-}$ mice when compared to hTau;*Cx3cr1*$^{+/+}$ 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 [50], 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. [51] Some FTD models have already been found to exhibit abnormalities on these tests. [43] Of particular importance to modeling FTD is the observation that social dysfunction in mice can arise as a result of abnormalities in frontal cortex. [52] 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. [53] 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. [54]

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 trial 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. [55,56] 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 [57]. 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 β-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 β-amyloid fail to produce tau deposits and are not associated with evidence of neuronal loss that is seen in human AD. [56] 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. [58] 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. [59]

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. [59] 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. [58] 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. [60] 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

1. Gorno-Tempini ML, Hillis AE, Weintraub S, et al. Classification of primary progressive aphasia and its variants. Neurology. Mar 15; 2011 76(11):1006–1014. [PubMed: 21325651]
2. Rascovsky K, Hodges JR, Knopman D, et al. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. Brain: a journal of neurology. Aug 2.2011
3. Mackenzie IR, Neumann M, Bigio EH, et al. Nomenclature and nosology for neuropathologic subtypes of frontotemporal lobar degeneration: an update. Acta Neuropathol. Jan; 2010 119(1):1–4. [PubMed: 19924424]
4. Cairns NJ, Lee VM, Trojanowski JQ. The cytoskeleton in neurodegenerative diseases. J Pathol. Nov; 2004 204(4):438–449. [PubMed: 15495240]
5. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science. Oct 6; 2006 314(5796):130–133. [PubMed: 17023659]
6. Dejesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC Hexanucleotide Repeat in Noncoding Region of C9ORF72 Causes Chromosome 9p-Linked FTD and ALS. Neuron. Sep 21.2011
7. Arai T, Mackenzie IR, Hasegawa M, et al. Phosphorylated TDP-43 in Alzheimer's disease and dementia with Lewy bodies. Acta Neuropathol. Feb; 2009 117(2):125–136. [PubMed: 19139911]
8. Mackenzie IR, Munoz DG, Kusaka H, et al. Distinct pathological subtypes of FTLD-FUS. Acta Neuropathol. Feb; 2011 121(2):207–218. [PubMed: 21052700]
9. Goldman JS, Rademakers R, Huey ED, et al. An algorithm for genetic testing of frontotemporal lobar degeneration. Neurology. Feb 1; 2011 76(5):475–483. [PubMed: 21282594]
10. van Swieten J, Spillantini MG. Hereditary frontotemporal dementia caused by Tau gene mutations. Brain Pathol. Jan; 2007 17(1):63–73. [PubMed: 17493040]
11. van Swieten JC, Heutink P. Mutations in progranulin (GRN) within the spectrum of clinical and pathological phenotypes of frontotemporal dementia. Lancet Neurol. Oct; 2008 7(10):965–974. [PubMed: 18771956]
12. Skibinski G, Parkinson NJ, Brown JM, et al. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. Nat Genet. Aug; 2005 37(8):806–808. [PubMed: 16041373]
13. Kwiatkowski TJ Jr, Bosco DA, Leclerc AL, et al. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science. Feb 27; 2009 323(5918):1205–1208. [PubMed: 19251627]
14. Watts GD, Wymer J, Kovach MJ, et al. Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. Nat Genet. Apr; 2004 36(4):377–381. [PubMed: 15034582]
15. Vance C, Rogelj B, Hortobagyi T, et al. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. Science. Feb 27; 2009 323(5918):1208–1211. [PubMed: 19251628]
16. Williams RL, Urbe S. The emerging shape of the ESCRT machinery. Nat Rev Mol Cell Biol. May; 2007 8(5):355–368. [PubMed: 17450176]
17. Tang W, Lu Y, Tian QY, et al. The growth factor progranulin binds to TNF receptors and is therapeutic against inflammatory arthritis in mice. Science. Apr 22; 2011 332(6028):478–484. [PubMed: 21393509]
18. Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. Science. Dec 21; 2007 318(5858):1917–1920. [PubMed: 18029452]

19. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. Nov 30; 2007 131(5):861–872. [PubMed: 18035408]
20. Anokye-Danso F, Trivedi CM, Juhr D, et al. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell. Apr 8; 2011 8(4):376–388. [PubMed: 21474102]
21. Ash PE, Zhang YJ, Roberts CM, et al. Neurotoxic effects of TDP-43 overexpression in C. elegans. Hum Mol Genet. Aug 15; 2010 19(16):3206–3218. [PubMed: 20530643]
22. Liachko NF, Guthrie CR, Kraemer BC. Phosphorylation promotes neurotoxicity in a Caenorhabditis elegans model of TDP-43 proteinopathy. J Neurosci. Dec 1; 2010 30(48):16208–16219. [PubMed: 21123567]
23. Brandt R, Gergou A, Wacker I, Fath T, Hutter H. A Caenorhabditis elegans model of tau hyperphosphorylation: induction of developmental defects by transgenic overexpression of Alzheimer's disease-like modified tau. Neurobiol Aging. Jan; 2009 30(1):22–33. [PubMed: 17590239]
24. Guthrie CR, Schellenberg GD, Kraemer BC. SUT-2 potentiates tau-induced neurotoxicity in Caenorhabditis elegans. Hum Mol Genet. May 15; 2009 18(10):1825–1838. [PubMed: 19273536]
25. Kao AW, Eisenhut RJ, Martens LH, et al. A neurodegenerative disease mutation that accelerates the clearance of apoptotic cells. Proc Natl Acad Sci U S A. Mar 15; 2011 108(11):4441–4446. [PubMed: 21368173]
26. Loewen CA, Feany MB. The unfolded protein response protects from tau neurotoxicity in vivo. PLoS ONE. 2010; 5(9)
27. Jackson GR, Wiedau-Pazos M, Sang TK, et al. Human wild-type tau interacts with wingless pathway components and produces neurofibrillary pathology in Drosophila. Neuron. May 16; 2002 34(4):509–519. [PubMed: 12062036]
28. Kosmidis S, Grammenoudi S, Papanikolopoulou K, Skoulakis EM. Differential effects of Tau on the integrity and function of neurons essential for learning in Drosophila. J Neurosci. Jan 13; 2010 30(2):464–477. [PubMed: 20071510]
29. Sager JJ, Bai Q, Burton EA. Transgenic zebrafish models of neurodegenerative diseases. Brain Struct Funct. Mar; 2010 214(2–3):285–302. [PubMed: 20162303]
30. Tomasiewicz HG, Flaherty DB, Soria JP, Wood JG. Transgenic zebrafish model of neurodegeneration. J Neurosci Res. Dec 15; 2002 70(6):734–745. [PubMed: 12444595]
31. Bai Q, Garver JA, Hukriede NA, Burton EA. Generation of a transgenic zebrafish model of Tauopathy using a novel promoter element derived from the zebrafish eno2 gene. Nucleic Acids Res. 2007; 35(19):6501–6516. [PubMed: 17897967]
32. Paquet D, Bhat R, Sydow A, et al. A zebrafish model of tauopathy allows in vivo imaging of neuronal cell death and drug evaluation. J Clin Invest. May; 2009 119(5):1382–1395. [PubMed: 19363289]
33. Noble W, Hanger DP, Gallo JM. Transgenic mouse models of tauopathy in drug discovery. CNS Neurol Disord Drug Targets. Aug; 2010 9(4):403–428. [PubMed: 20522014]
34. Yoshiyama Y, Higuchi M, Zhang B, et al. Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. Neuron. Feb 1; 2007 53(3):337–351. [PubMed: 17270732]
35. Santacruz K, Lewis J, Spires T, et al. Tau suppression in a neurodegenerative mouse model improves memory function. Science. Jul 15; 2005 309(5733):476–481. [PubMed: 16020737]
36. Zahs KR, Ashe KH. 'Too much good news' - are Alzheimer mouse models trying to tell us how to prevent, not cure, Alzheimer's disease? Trends Neurosci. Aug; 2010 33(8):381–389. [PubMed: 20542579]
37. Kraemer BC, Schuck T, Wheeler JM, et al. Loss of murine TDP-43 disrupts motor function and plays an essential role in embryogenesis. Acta Neuropathol. Apr; 2010 119(4):409–419. [PubMed: 20198480]
38. Xu YF, Gendron TF, Zhang YJ, et al. Wild-type human TDP-43 expression causes TDP-43 phosphorylation, mitochondrial aggregation, motor deficits, and early mortality in transgenic mice. J Neurosci. Aug 11; 2010 30(32):10851–10859. [PubMed: 20702714]

39. Tsai KJ, Yang CH, Fang YH, et al. Elevated expression of TDP-43 in the forebrain of mice is sufficient to cause neurological and pathological phenotypes mimicking FTLD-U. J Exp Med. Aug 2; 2010 207(8):1661–1673. [PubMed: 20660618]
40. Igaz LM, Kwong LK, Lee EB, et al. Dysregulation of the ALS-associated gene TDP-43 leads to neuronal death and degeneration in mice. J Clin Invest. Feb 1; 2011 121(2):726–738. [PubMed: 21206091]
41. Petkau TL, Neal SJ, Orban PC, et al. Progranulin expression in the developing and adult murine brain. J Comp Neurol. Oct 1; 2010 518(19):3931–3947. [PubMed: 20737593]
42. Kayasuga Y, Chiba S, Suzuki M, et al. Alteration of behavioural phenotype in mice by targeted disruption of the progranulin gene. Behav Brain Res. Dec 28; 2007 185(2):110–118. [PubMed: 17764761]
43. Yin F, Dumont M, Banerjee R, et al. Behavioral deficits and progressive neuropathology in progranulin-deficient mice: a mouse model of frontotemporal dementia. FASEB J. Jul 28.2010
44. Ahmed Z, Sheng H, Xu YF, et al. Accelerated lipofuscinosis and ubiquitination in granulin knockout mice suggest a role for progranulin in successful aging. Am J Pathol. Jul; 2010 177(1): 311–324. [PubMed: 20522652]
45. Tapia L, Milnerwood A, Guo A, et al. Progranulin deficiency decreases gross neural connectivity but enhances transmission at individual synapses. J Neurosci. Aug 3; 2011 31(31):11126–11132. [PubMed: 21813674]
46. Yin F, Banerjee R, Thomas B, et al. Exaggerated inflammation, impaired host defense, and neuropathology in progranulin-deficient mice. J Exp Med. Jan 18; 2010 207(1):117–128. [PubMed: 20026663]
47. Mizuno T, Kawanokuchi J, Numata K, Suzumura A. Production and neuroprotective functions of fractalkine in the central nervous system. Brain Res. Jul 25; 2003 979(1–2):65–70. [PubMed: 12850572]
48. Cardona AE, Pioro EP, Sasse ME, et al. Control of microglial neurotoxicity by the fractalkine receptor. Nat Neurosci. Jul; 2006 9(7):917–924. [PubMed: 16732273]
49. Bhaskar K, Konerth M, Kokiko-Cochran ON, Cardona A, Ransohoff RM, Lamb BT. Regulation of tau pathology by the microglial fractalkine receptor. Neuron. Oct 6; 2010 68(1):19–31. [PubMed: 20920788]
50. Cagnin A, Rossor M, Sampson EL, Mackinnon T, Banati RB. In vivo detection of microglial activation in frontotemporal dementia. Annals of Neurology. Dec; 2004 56(6):894–897. [PubMed: 15562429]
51. Crawley JN. Mouse behavioral assays relevant to the symptoms of autism. Brain Pathol. Oct; 2007 17(4):448–459. [PubMed: 17919130]
52. Scearce-Levie K, Roberson ED, Gerstein H, et al. Abnormal social behaviors in mice lacking Fgf17. Genes Brain Behav. 2008; 7(3):344–354. [PubMed: 17908176]
53. Peça J, Feliciano C, Ting JT, et al. Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. Nature. Apr 28; 2011 472(7344):437–442. [PubMed: 21423165]
54. Alavijeh MS, Chishty M, Qaiser MZ, Palmer AM. Drug metabolism and pharmacokinetics, the blood-brain barrier, and central nervous system drug discovery. Neuro Rx. Oct; 2005 2(4):554–571. [PubMed: 16489365]
55. van der Worp HB, Howells DW, Sena ES, et al. Can animal models of disease reliably inform human studies? PLoS Med. Mar.2010 7(3):e1000245. [PubMed: 20361020]
56. Jucker M. The benefits and limitations of animal models for translational research in neurodegenerative diseases. Nat Med. Nov; 2010 16(11):1210–1214. [PubMed: 21052075]
57. Hackam DG, Redelmeier DA. Translation of research evidence from animals to humans. Jama. Oct 11; 2006 296(14):1731–1732. [PubMed: 17032985]
58. Scott S, Kranz JE, Cole J, et al. Design, power, and interpretation of studies in the standard murine model of ALS. Amyotrophic lateral sclerosis: official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases. 2008; 9(1):4–15. [PubMed: 18273714]
59. Fillit, H.; Shineman, D.; Morse, I., et al. Accelerating Drug Discovery for Alzheimer's Disease: Best Practices for Preclinical Animal Studies. New York: 2010.

60. Sena ES, van der Worp HB, Bath PM, Howells DW, Macleod MR. Publication bias in reports of animal stroke studies leads to major overstatement of efficacy. PLoS biology. Mar.2010 8(3):e1000344. [PubMed: 20361022]

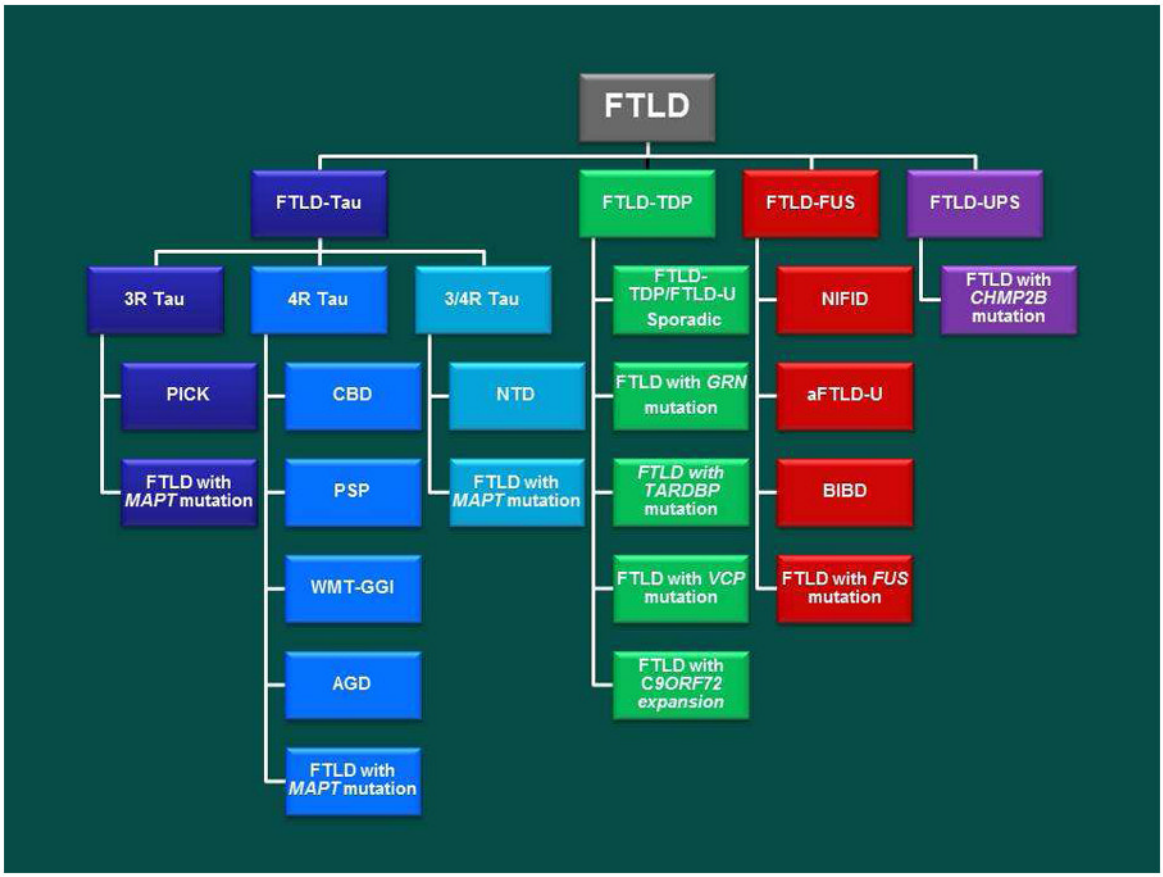

Figure 1. Neuropathological classification of FTD subtypes

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.

Table 1

Targets and investigational therapies in FTD and related disorders

Target	Agent	Mechanism(s)	Status	Limitations	Reference/NCT*
Tau	Lithium	GSK inhibitor	Phase 2 CBD/PSP completed	Toxicity	NCT00703677
	NP12 (tideglusib)	GSK inhibitor	Phase 2 AD, PSP	Toxicity	NCT01350362, NCT01049399
	Riluzole	Na Channel blocker	Phase 2 PSP completed	Not efficacious	1
	Co-Q10	Improve mitochondrial function	Phase 2 in PSP completed, Phase 3 underway	Mechanism?	2,3 NCT00382824
	rasagaline	MAO inhibitor	Phase 3 underway	Mechanism?	NCT01187888
	davunetide	microtubule stabilizer	Phase 2/3 underway in PSP	Specificity	NCT01110720, NCT01056965
	methylene blue	Inhibits aggregation	Completed phase 2 in AD	Mechanism?	NCT00515333
	epothilones	Microtubule stabilizer	Pre-clinical	Toxicity	4
	Anti-tau mAb or vaccines	Block transmission, increase clearance	Pre-clinical	Safety?	5–7
	Hsp90 inhibitors	Increased clearance	Pre-clinical	N/A	8
	Chloroquine	Enhance autophagy	Pre-clinical	N/A	8
	RNA binding drugs, antisense oligonucleotides	Alter tau exon 10 splicing to decrease 4R, decrease tau mRNA	Pre-clinical	BBB permeability, feasibility?	9
PGRN	Chloroquine	Increase secretion/vacuolar alkalinization	Pre-clinical, clinical trial planned	Toxicity, BBB penetration	10
	Amiodarone	Increase secretion/vacuolar alkalinization	Pre-Clinical	Toxicity, mechanism	10
	SAHA	Increase PGRN expression (HDAC inhibitor)	Pre-clinical	Toxicity, BBB penetration	11
	Resveratrol	Increase PGRN expression	Pre-clinical	N/A	11

Table 2

Preclinical models for FTD Drug Development—potential uses and limitations

Organism	Genes	Potential uses	Strengths	Limitations	Refs.
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 & supressors • 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

Organism	Genes	Potential uses	Strengths	Limitations	Refs.
		• Preclinical testing of compounds	• Can test disease-related behaviors	• Pharmacokinetic differences with humans	

References for Tables 1 and 2

1 Bensimon G, Ludolph A, Agid Y, Vidailhet M, Payan C, Leigh PN. Riluzole treatment, survival and diagnostic criteria in Parkinson plus disorders: the NNIPPS study. *Brain.* Jan 2009;132(Pt 1):156–171.

2 Stamelou M, Reuss A, Pilatus U, et al. Short-term effects of coenzyme Q10 in progressive supranuclear palsy: a randomized, placebo-controlled trial. *Mov Disord.* May 15 2008;23(7):942–949.

3 Elipenahli C, Stack C, Jainuddin S, et al. Behavioral Improvement after Chronic Administration of Coenzyme Q10 in P301S Transgenic Mice. *Journal of Alzheimer's disease: JAD.* Oct 4 2011.

4 Brunden KR, Zhang B, Carroll J, et al. Epothilone D improves microtubule density, axonal integrity, and cognition in a transgenic mouse model of tauopathy. *J Neurosci.* Oct 13 2010;30(41):13861–13866.

5 Asuni AA, Boutajangout A, Quartermain D, Sigurdsson EM. Immunotherapy targeting pathological tau conformers in a tangle mouse model reduces brain pathology with associated functional improvements. *J Neurosci.* Aug 22 2007;27(34):9115–9129.

6 Boutajangout A, Ingadottir J, Davies P, Sigurdsson EM. Passive immunization targeting pathological phospho-tau protein in a mouse model reduces functional decline and clears tau aggregates from the brain. *Journal of neurochemistry.* Aug 2011;118(4):658–667.

7 Chai X, Wu S, Murray TK, et al. Passive Immunization with Anti-Tau Antibodies in Two Transgenic Models: REDUCTION OF TAU PATHOLOGY AND DELAY OF DISEASE PROGRESSION. *The Journal of biological chemistry.* Sep 30 2011;286(39):34457–34467.

8 Brunden KR, Trojanowski JQ, Lee VM. Advances in tau-focused drug discovery for Alzheimer's disease and related tauopathies. *Nat Rev Drug Discov.* Oct 2009;8(10):783–793.

9 Zhou J, Yu Q, Zou T. Alternative splicing of exon 10 in the tau gene as a target for treatment of tauopathies. *BMC neuroscience.* 2008;9 Suppl 2:S10.

10 Capell A, Liebscher S, Fellerer K, et al. Rescue of progranulin deficiency associated with frontotemporal lobar degeneration by alkalizing reagents and inhibition of vacuolar ATPase. *J Neurosci.* Feb 2 2011;31(5):1885–1894.

11 Cenik B, Sephton CF, Dewey CM, et al. Suberoylanilide hydroxamic acid (vorinostat) up-regulates progranulin transcription: rational therapeutic approach to frontotemporal dementia. *J Biol Chem.* May 6 2011;286(18):16101–16108.

12 Yu J, Vodyanik MA, Smuga-Otto K, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science.* Dec 21 2007;318(5858):1917–1920.

13 Loewen CA, Feany MB. The unfolded protein response protects from tau neurotoxicity in vivo. *PLoS ONE.* 2010;5(9).

14 Jackson GR, Wiedau-Pazos M, Sang TK, et al. Human wild-type tau interacts with wingless pathway components and produces neurofibrillary pathology in Drosophila. *Neuron.* May 16 2002;34(4):509–519.

15 Kosmidis S, Grammenoudi S, Papanikolopoulou K, Skoulakis EM. Differential effects of Tau on the integrity and function of neurons essential for learning in Drosophila. *J Neurosci.* Jan 13 2010;30(2):464–477.

16 Ash PE, Zhang YJ, Roberts CM, et al. Neurotoxic effects of TDP-43 overexpression in C. elegans. *Hum Mol Genet.* Aug 15 2010;19(16):3206–3218.

17 Liachko NF, Guthrie CR, Kraemer BC. Phosphorylation promotes neurotoxicity in a Caenorhabditis elegans model of TDP-43 proteinopathy. *J Neurosci.* Dec 1 2010;30(48):16208–16219.

[18] Chitramuthu BP, Baranowski DC, Kay DG, Bateman A, Bennett HP. Progranulin modulates zebrafish motoneuron development in vivo and rescues truncation defects associated with knockdown of Survival motor neuron 1. *Mol Neurodegener.* 2010;5:41.

[19] Brandt R, Gergou A, Wacker I, Fath T, Hutter H. A Caenorhabditis elegans model of tau hyperphosphorylation: induction of developmental defects by transgenic overexpression of Alzheimer's disease-like modified tau. *Neurobiol Aging.* Jan 2009;30(1):22–33.

[20] Guthrie CR, Schellenberg GD, Kraemer BC. SUT-2 potentiates tau-induced neurotoxicity in Caenorhabditis elegans. *Hum Mol Genet.* May 15 2009;18(10):1825–1838.

[21] Kao AW, Eisenhut RJ, Martens LH, et al. A neurodegenerative disease mutation that accelerates the clearance of apoptotic cells. *Proc Natl Acad Sci U S A.* Mar 15 2011;108(11):4441–4446.

[22] Bai Q, Garver JA, Hukriede NA, Burton EA. Generation of a transgenic zebrafish model of Tauopathy using a novel promoter element derived from the zebrafish eno2 gene. *Nucleic Acids Res.* 2007;35(19):6501–6516.

[23] Paquet D, Bhat R, Sydow A, et al. A zebrafish model of tauopathy allows in vivo imaging of neuronal cell death and drug evaluation. *J Clin Invest.* May 2009;119(5):1382–1395.

[24] Noble W, Hanger DP, Gallo JM. Transgenic mouse models of tauopathy in drug discovery. *CNS Neurol Disord Drug Targets.* Aug 2010;9(4):403–428.

[25] Ramsden M, Kotilinek L, Forster C, et al. Age-dependent neurofibrillary tangle formation, neuron loss, and memory impairment in a mouse model of human tauopathy (P301L). *J Neurosci.* Nov 16 2005;25(46):10637–10647.

[26] Yoshiyama Y, Higuchi M, Zhang B, et al. Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron.* Feb 1 2007;53(3):337–351.

[27] Clavaguera F, Bolmont T, Crowther RA, et al. Transmission and spreading of tauopathy in transgenic mouse brain. *Nat Cell Biol.* Jul 2009;11(7):909–913.

[28] Santacruz K, Lewis J, Spires T, et al. Tau suppression in a neurodegenerative mouse model improves memory function. *Science.* Jul 15 2005;309(5733):476–481.

[29] Zahs KR, Ashe KH. 'Too much good news' - are Alzheimer mouse models trying to tell us how to prevent, not cure, Alzheimer's disease? *Trends Neurosci.* Aug 2010;33(8):381–389.

[30] Xu YF, Gendron TF, Zhang YJ, et al. Wild-type human TDP-43 expression causes TDP-43 phosphorylation, mitochondrial aggregation, motor deficits, and early mortality in transgenic mice. *J Neurosci.* Aug 11 2010;30(32):10851–10859.

[31] Tsai KJ, Yang CH, Fang YH, et al. Elevated expression of TDP-43 in the forebrain of mice is sufficient to cause neurological and pathological phenotypes mimicking FTLD-U. *J Exp Med.* Aug 2 2010;207(8):1661–1673.

[32] Petkau TL, Neal SJ, Milnerwood A, et al. Synaptic dysfunction in progranulin-deficient mice. *Neurobiology of disease.* Oct 25 2011.

[33] Tapia L, Milnerwood A, Guo A, et al. Progranulin deficiency decreases gross neural connectivity but enhances transmission at individual synapses. *J Neurosci.* Aug 3 2011;31(31):11126–11132.