Genetically engineered mouse models of neurodegenerative diseases

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Recent research has significantly advanced our understanding of the molecular mechanisms of neurodegenerative diseases, including Alzheimer's disease (AD) and motor neuron disease. Here we emphasize the use of genetically engineered mouse models that are instrumental for understanding why AD is a neuronal disease, and for validating attractive therapeutic targets. In motor neuron diseases, Cu/Zn superoxide dismutase and survival motor neuron mouse models are useful in testing disease mechanisms and therapeutic strategies for amyotrophic lateral sclerosis (ALS) and spinal motor atrophy, respectively, but the mechanisms that account for selective motor neuron loss remain uncertain. We anticipate that, in the future, therapies based on understanding disease mechanisms will be identified and tested in mouse model systems.

The neurodegenerative diseases represent a challenge for science and medicine because of their prevalence, cost, lack of mechanism-based treatments, and impact on individuals and caregivers[1–6]. Genetic risk factors influence these age-associated, chronic illnesses, such as Alzheimer's disease, motor neuron diseases, Parkinson's disease (PD), trinucleotide repeat diseases and prion disorders. They are characterized by dysfunction and death of specific populations of neurons and by the presence, in many instances, of intracellular or extracellular protein aggregates. Although symptomatic treatments are available, there are no mechanism-based treatments. Recent research, particularly in animal models, has begun to provide new insights into the mechanisms of these disorders and has identified new targets for therapy.

The identification of mutations in specific genes causing each of these neurodegenerative diseases has provided new opportunities to investigate the molecular participants in disease processes and to explore pathogenic mechanisms using transgenic approaches. In autosomal dominant genetic disorders, the mutant proteins often do not exhibit reductions in their normal functions, but instead acquire toxic properties that directly or indirectly affect the functions and viability of neurons. Introducing mutant genes into mice can reproduce some features of these diseases[1,6–11]. Autosomal recessive diseases, which usually lack the functional protein encoded by the mutant gene, can often be modeled by gene knockout strategies. In both groups of disorders, gene knockout or overexpression of the genes that influence pathogenic pathways have provided insight into disease mechanisms and potential therapeutic targets. These model systems can also be used to evaluate novel treatments and expedite the path to clinical trials.

Here we focus on the familial forms of AD (FAD) and two forms of motor neuron disease, familial amyotrophic lateral sclerosis (FALS) and spinal muscular atrophy (SMA). In the case of FAD and FALS, we anticipate that understanding the inherited illnesses will shed light on the more common sporadic forms of AD and ALS. In the case of SMA, all cases are attributable to the same genetic mechanism.

Alzheimer's disease

Problems with memory and cognition appear during the seventh decade in most individuals with AD, but may appear earlier, particularly in familial cases[12]. Mental functions and activities of daily living become progressively impaired. The clinical signs of AD result from selective degeneration of neurons in brain regions critical for memory, cognitive performance and personality[13,14]. Dysfunction and death of these neurons lead to reduced numbers of synaptic markers in their target fields[14,15]; the disruption of synaptic communication is manifested by mental impairments and, finally, severe dementia[14].

Two types of intracellular and extracellular protein aggregates found in the brain are a pathological hallmark of AD (**Fig. 1**). Neurofibrillary tangles are inclusions located within cell bodies and proximal dendrites, and within filamentous swellings in distal axons and synaptic terminals. Hyperphosphorylated isoforms of the microtubule-associated protein tau, which assemble into poorly soluble paired helical filaments, are central feature of these neurofibrillary tangles[10]. The extracellular aggregates in the brain of individuals with AD result from elevated levels of Aβ, a 4 kD amyloid peptide derived by cleavage of the amyloid precursor protein (APP). Aβ monomers form oligomers and multimers, which assemble into protofilaments and then fibrils[7,14,16,17]. Eventually, Aβ fibrils are deposited as the amyloid cores of neuritic or senile plaques (amyloidosis), which are complex structures also containing dystrophic neurites, astrocytes and microglia. Both neurofibrillary lesions and plaques are preferentially localized to the cortex, hippocampus and amygdala.

In some individuals with early-onset AD, the illness may be inherited as an autosomal dominant (that is, only a single copy of

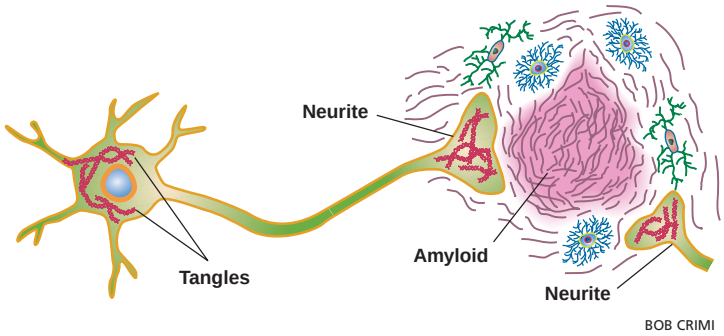


Fig. 1. Pathological features of Alzheimer's disease. Schematic diagram of a neuron showing an extracellular amyloid plaque in the target field with a central core of Aβ fibrils surrounded by dystrophic neurites, astrocytes and microglia. Within the cell body, proximal dendrites and distal axons are intracellular protein aggregates, called neurofibrillary tangles, which are paired helical filaments assembled from hyperphosphorylated tau protein.

the mutant gene is necessary to cause the disease). Such mutations are identified in at least three different genes: *APP*, *PS1* and *PS2* (refs. 1,6,18,19). APP is a type I transmembrane protein expressed in many different cell types, but particularly abundant in neurons (**Fig. 2a**). Pathogenic Aβ peptides are generated via cleavage of APP by BACE1 (β-site APP cleaving enzyme 1)[20–24] and γ-secretase[1,7] (**Fig. 2b**). The levels and distributions of APP and these pro-amyloidogenic cleavage enzymes in neurons, and in particular BACE1, are hypothesized to be the principal determinants of high levels of Aβ in the brain[25]. Formation of Aβ is precluded by cleavage of APP within the Aβ domain by α-secretase or BACE2 (ref. 26; **Fig. 2c**).

A variety of APP mutations reported in cases of FAD are near cleavage sites involved in formation of Aβ (**Fig. 2d**). The APP 717 mutation is located near the C-terminus of Aβ and facilitates γ-secretase activity, leading to increased secretion of the longer and more toxic Aβ peptide, Aβ42 (ref. 7; **Fig. 2b**). This longer Aβ42 peptide is thought to promote the formation of Aβ aggregates and amyloid plaques. The APPswe mutation, a double mutation at the N-terminus of Aβ, enhances BACE1 cleavage and is associated with elevated levels of Aβ peptides, including Aβ42. In contrast, APP mutations within the Aβ peptide domain (for example, APP-E693Q, A692G or E693G) do not elevate the level of Aβ but may cause amyloidosis by increasing Aβ oligomer or protofibril formation[27]. Thus, a common feature of all FAD-linked *APP* mutations is the enhancement or facilitation of Aβ protofibril formation.

Another family of genes identified in FAD is the *presenilins*[28–30]. *PS1* and *PS2* encode highly homologous 43- to 50-kD multipass transmembrane proteins that are processed to stable N-terminal and C-terminal fragments[31], and are widely expressed at low abundance in the central nervous system. PS1 influences APP processing, but it is not clear whether PS1 itself acts as the protease (γ-secretase), functions as a cofactor critical for the activity of γ-secretase, or exerts its influence via trafficking of APP to the proper compartment for γ -secretase cleavage[32–36]. Whatever the case, PS1 is now recognized as one of the critical element of the γ-secretase complex. Nicastrain, a type I transmembrane glycoprotein, is another important component of this complex[37]. The *PS1* gene has been reported to harbor more than 80 different FAD mutations (AD mutation database, http://molgen-www.uia.ac.be), whereas only a small number of mutations have been found in *PS2*-linked families. The vast majority of abnormalities in *PS* genes are missense mutations that result in single amino acid substitutions, which in general seem to influence γ-secretase activity and increase the generation of the Aβ42 peptide.

Mouse models relevant to AD

To generate animal models of Aβ amyloidosis, many groups have produced transgenic mice that express wild-type APP, APP fragments, Aβ and FAD-linked mutant APP and PS1. Some of the mutant APP mice, although they do not reproduce the full phenotype of AD, represent excellent models of Aβ amyloidosis.

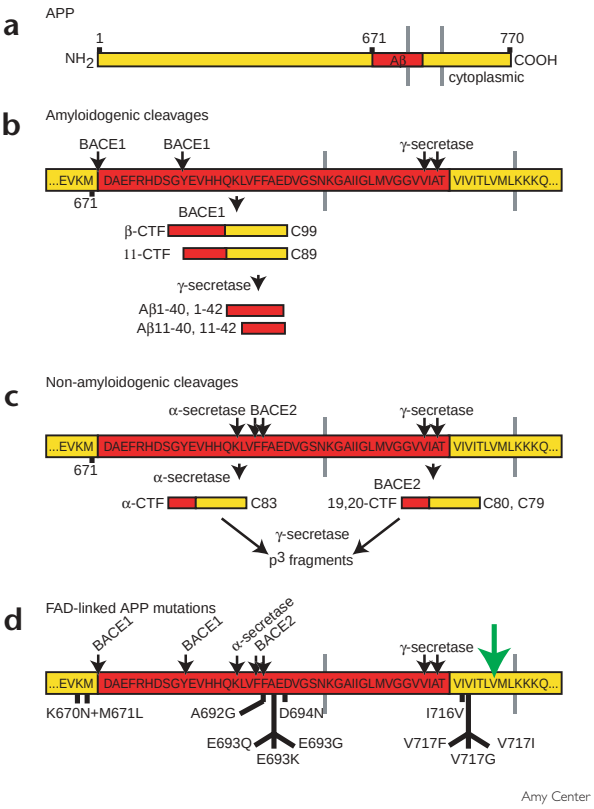
Expression of APP minigenes that encode FAD-linked APP mutants (swe and 717), using several different promoters, leads to elevated levels of Aβ, diffuse Aβ deposits and plaques in the hippocampus and cortex of these mice[38]. The severity is influenced by the level of transgene expression and the specific mutation. In addition to Aβ deposits, these plaques contain neurites (some showing hyperphosphorylated tau immunoreactivity), astrocytes and microglia; however, neurofibrillary tangles are not present. A variety of defects are found in different mutant lines, including mild loss of neurons, learning deficits, problems in object recognition memory, and problems with alternation-spatial reference and working memory[39]. Interestingly, synaptic abnormalities in hippocampal circuits seem to precede the deposition of Aβ into plaques[40,41].

Appropriate mouse models that display both amyloid plaques and neurofibrillary tangles have not been entirely successful. In an attempt to obtain mice with both plaques and tangles, mutant APP transgenic mice were mated to mice expressing the P301L tau mutant[42], a mutation linked to familial frontotemporal dementia with parkinsonism (FTDP)[2]. Although these lines do have more tangles, mice bearing both mutant tau and APP are problematic as a faithful model of FAD because the FTDP mutation alone is associated with increased tangles. Similarly, tau pathology can be induced by introducing Aβ fibril into P301L tau mutant mice[43]. More appropriate models of AD might require co-expression of mutant APP and all six isoforms of wild type human tau.

Mice expressing both mutant PS1 and mutant APP develop accelerated Aβ amyloidosis in the central nervous system. Coexpression of the human A246E mutant PS1 and APPswe elevates levels of Aβ in brain, and these mice develop numerous amyloid deposits, dystrophic neuritis, and glial responses in the hippocampus and cortex[44]. Mutations known to be more malignant in the human disorder also produce accelerated Aβ deposition in mouse models. These mice also demonstrate that the key participants in Aβ amyloidosis (APP, PS1 and BACE1) are colocalized in neurites immediately proximal to sites of Aβ formation in brain, supporting the concept of a neuronal origin for Aβ.

In an effort to understand the functions of the Alzheimer's related genes, researchers have ablated most of them. This approach is somewhat problematic with regard to *APP* because of the homologous amyloid precursor-like proteins, APLP1 and APLP2. Homozygous $APP^{-/-}$ mice are viable and fertile, but seem to have subtle decreases in locomotor activity and forelimb grip strength[45]. The absence of substantial phenotypes in $APP^{-/-}$ mice may be related to functional redundancy of APLP1 and APLP2. Consistent with this idea, $APLP2^{-/-}$ mice appear normal, but mice with either both *APP* and *APLP2* targeted alleles or both *APLP1* and *APLP2* null alleles show significant postnatal lethality[46].

Similar approaches have focused on the proteins implicated in β- and γ-secretase activities. Gene targeting of the presenilins



Amy Center

Fig. 2. Amyloid precursor protein (APP) and secretase cleavages. (**a**) Schematic of APP, a type I transmembrane protein. The Aβ region is indicated in red. Transmembrane segment is demarcated by the gray lines. (**b**) Amyloidogenic cleavages. Aβ region indicated in (**a**) is expanded to show the amino acid sequences of Aβ and flanking regions. The sequential actions of β-site APP-cleaving enzyme (BACE1) and γ-secretase generate Aβ1-40, 1-42 and Aβ 11-40, 11-42. Arrows indicate cleavage sites for BACE1 and γ-secretase. (**c**) Non-amyloidogenic cleavages. The sequential actions of α-secretase or BACE2 and γ-secretase generate p3 fragments. Arrows indicate α-secretase, BACE2, and γ-secretase cleavage sites. Note that α-secretase or BACE2 cleaves APP within the Aβ domain, precluding the formation of amyloidogenic Aβ peptides. In non-neuronal cells, APP is primarily processed by α-secretase or BACE2. (**d**) FAD-linked mutations in APP. The Swedish mutation (K670N+M671L) increased the cleavage efficiency of BACE1, whereas mutations within the transmembrane domain promoted the cleavage by γ-secretase to increase Aβ42. Mutations within the Aβ domain seemed to enhance Aβ oligomer or protofibril formation. Large arrow indicates cleavage by γ-secretase–like protease to generate APP intracellular domain.

is complicated because PS1 interacts functionally with Notch, a receptor protein involved in critical cell-fate decisions during development[47]. *PS1*$^{-/-}$ mice do not survive beyond the early postnatal period and show severe perturbations in the axial skeleton, ribs and spinal ganglia—all defects in somitogenesis that resemble a partial Notch1 null phenotype[48]. However, it is clear that PS1 is involved in γ-secretase activity; in cell culture, deletion of PS1, or substitution of particular aspartate residues, leads to reduced levels of γ-secretase cleavage products and levels of Aβ[36]. PS2 null mice are viable and fertile, though they develop age-associated mild pulmonary fibrosis and hemorrhage. Mice heterozygous for PS1 and lacking PS2 survive in relatively good health, but mice lacking PS1 and heterozygous for PS2 die midway through gestation with full Notch1 null-like phenotype[49].

To study the role of PS1 *in vivo* in adult mice, two groups generated conditional PS1-targeted mice lacking PS1 expression in the forebrain after embryonic development[50,51]. As expected, the absence of PS1 resulted in decreased Aβ generation, further establishing that PS1 is critical for γ-secretase activity in the brain. The finding that these forebrain-specific PS1 knockout mice do not have significant morphological, physiological or overt behavioral abnormalities suggest that γ-secretase inhibitors may be useful as therapeutic agents for Aβ amyloidosis.

BACE1 null mice are viable and healthy, have no obvious phenotype or pathology, and can mate successfully[52–54]. Importantly, in cortical neurons from *BACE1* null embryos, there is no cleavage at the +1 and + 11 sites of Aβ[52], and the secretion of Aβ peptides is abolished even in the presence of elevated level of exogenous APP. Moreover, Aβ peptides are not produced in brains of *BACE1* null mice. These results establish that BACE1 is the β-secretase required to cleave APP to generate the N-termini of Aβ. Although behavioral studies on *BACE1* null mice are necessary to determine the effect of the absence of BACE1, results thus far indicate that BACE1 is an excellent therapeutic target for drug development for AD.

These mouse models have revealed that neurons are the major source for Aβ production in the brain. Because BACE1 is the principal β-secretase in neurons, and BACE2 may serve to limit the secretion of Aβ peptides (**Fig. 2**), we hypothesized that the relative levels of BACE1 and BACE2 activities are major determinants of Aβ amyloidosis[52]. In this model, the secretion of Aβ peptides would be expected to be the highest in neurons and brain as compared to other cell types or tissues because neurons express high levels of BACE1 coupled with low expression of BACE2. Seemingly inconsistent with this hypothesis is a study showing high levels of BACE1 mRNA expression in the pancreas[24]. Given that APP is expressed in pancreas, why do AD and diabetes mellitus not occur together? It now appears that some of the pancreatic BACE1 mRNAs are alternatively spliced to generate a BACE1 isoform that is incapable of cleaving APP[55]. Taken together with the observations that pancreas possesses low levels of BACE1 protein and activity[20], these results are consistent with the view that a high ratio of BACE1 to BACE2 activity leads to selective vulnerability of neurons to Aβ amyloidosis, whereas pancreatic cells are spared.

Potential therapeutics for AD

Model systems have great value for evaluating experimental treatments. Although they do not model the full phenotype of AD, these mutant transgenic mice represent excellent models of Aβ amyloidois and are highly suitable for identification of therapeutic targets. Although both β-and γ-secretase activities represent therapeutic targets for the development of novel protease inhibitors for AD, the demonstration that BACE1 is the principal β-secretase in cultured neurons[52] and in brain[53] provides an excellent rationale for focusing on the design of novel therapeutics to inhibit BACE1 activity in brain. Importantly, in contrast to *PS1*$^{-/-}$ mice, the *BACE1*$^{-/-}$ mice seem to be normal[52–54]. Furthermore, BACE1-deficient neurons fail to secrete Aβ even when co-expressing the APPswe and mutant PS1 genes, and these mice do not exhibit Aβ plaques in the brain (H.C., D.L.P. and P.C.W., unpublished data).

Given the role of PS1 in γ-secretase activity[56], development of PS1 inhibitors is an important avenue of investigation for potential therapeutics. However, because of presenilins' role in Notch pro-

cessing[57], it may be valuable to try to design therapeutics that inhibit the γ-secretase activity of PS1 selectively, without affecting the activity involved in Notch1 processing[58]. This is important because several cell populations, hematopoetic stem cells in particular, use Notch signaling for cell-fate decisions even in the adult[59].

Both Aβ immunization (with Freund's adjuvant) and passive transfer of Aβ antibodies reduce levels of Aβ and plaque burden in mutant APP transgenic mice in both prevention and treatment trials[60,61]. Efficacy seems to be related to antibody titer. The mechanisms of enhanced clearance are not certain, but one possibility is that a small amount of Aβ antibody reaches the brain, binds to Aβ peptides, promotes the disassembly of fibrils, and via the Fc antibody domain, attracts activated microglia to remove Aβ[60]. Another possibility, not mutually exclusive, is that serum antibodies serve as a sink to draw the amyloid peptide from the brain into the circulation, thus changing the equilibrium of Aβ in different compartments and promoting removal from the brain[62]. Immunization seems to attenuate learning and behavioral deficits in at least two cohorts of mutant APP mice[63,64]. It is clear from the studies in mice that achieving adequate levels of antibody titer is critical because these levels predict amyloid clearance in mutant transgenic mice. Unfortunately, recently initiated Phase 2 trials have been suspended because of severe "inflammation" in the CNS of a subset of patients ($n > 15$). This is particularly disappointing, given that Phase 1 trials with Aβ and adjuvant were not associated with any adverse events, and considering the success of immunotherapy in transgenic mice.

Amyotrophic lateral sclerosis

ALS manifests as muscle weakness and atrophy, along with spastic paralysis, which result from selective degeneration of spinal and corticospinal motor neurons, respectively. The disease affects the size, appearance and metabolism of these cells and evolves in stages. First, neurofilamentous swellings occur in proximal axonal segments accompanied by ubiquitin-positive aggregates[65]. Next, motor axons retract and become disconnected from the denervated muscles. At this stage, trophic support is compromised, and cell bodies shrink and dendrites are attenuated. Neurons die in the final stages and exhibit several characteristics of apoptosis[66]. Ultimately, the numbers of motor neurons in brainstem nuclei and spinal cord are reduced, and large pyramidal neurons in motor cortex are lost. The clinical signs are most closely linked to the disconnection of the synaptic terminals of these neuronal populations from their targets.

Approximately 10% of cases of ALS are familial, and, in most of these cases, the disease is inherited in an autosomal dominant pattern[67]. Approximately 15–20% of patients with autosomal dominant FALS (~2% of all ALS cases) have mutations in the gene that encodes cytosolic Cu/Zn superoxide dismutase (SOD1), an antioxidant enzyme that catalyzes the conversion of $\cdot O^-_2$ to O_2 and H_2O_2. To date, ~90 different missense mutations have been identified in the SOD1 gene[67]. These mutations are scattered throughout the protein and are not preferentially localized near the active site or the dimer interface. Although some FALS SOD1 mutants show reduced enzymatic activities, many retain full activity. The mutant enzyme causes selective neuronal degeneration through a gain of toxic property, consistent with autosomal dominant inheritance (reviewed in ref. 68). The presence of ubiquitin aggregates containing mutant SOD1 protein in affected neurons raises the possibility that the defect is due to essential molecules being sequestered away by the aggregates. Alternatively, the misfolded mutant SOD1 could catalyze aberrant reactions.

Other chromosomal loci linked to FALS show autosomal dominant, autosomal recessive or X-linked inheritance patterns[67]. A gene termed *ALS2*, on chromosome 2, is linked to juvenile ALS in several families. *ALS2* encodes a protein termed alsin, which shares homology to GTPase regulatory proteins (guanine-nucleotide exchange factors) that participate in critical cellular functions including signal transduction, regulation of the cytoskeleton and intracellular trafficking[69,70]. Mutations in *ALS2* are inherited as autosomal recessive and lead to a premature truncation, suggesting that the disease is associated with a loss of ALS2 function. Thus, genetic approaches to ablate the gene encoding ALS2 may provide useful mouse models of this rare form of ALS.

SOD1 mutant mice

Mice expressing a variety of mutant SOD1s (found in FALS) develop progressive weakness and muscle atrophy, and show the prototypical cellular stages of ALS[71–73]. The G37R SOD1 transgenic mice provide an excellent illustration of these models. G37R SOD1, which retains full SOD activity, accumulates to 3–12 times the endogenous levels in the spinal cord, and the levels of the mutant protein influence the age of onset. Toxic SOD1 is transported anterograde in axons, and early on, it accumulates in axons, where it is associated with structural pathology. Approximately 2–3 months before the appearance of clinical signs, SOD1 accumulates in irregular, swollen, intraparenchymal portions of motor axons, and the axonal cytoskeleton and axonal transport are abnormal. Vacuoles, thought to represent degenerating mitochondria, are present in enlarged axons and in dendritic swellings. (The latter is reminiscent of changes seen in excitotoxicity, which is suggested to be involved in ALS[74,75].) The cell bodies of some neurons show SOD1, ubiquitin and phosphorylated NF-H immunoreactive inclusions[76]. Once Wallerian degeneration (characterized by degenerating myelin) of large axons is obvious, the mice are usually weak. Eventually, the number of motor neurons is reduced. These mouse models recapitulate the major clinical and pathological hallmarks of ALS.

Potential therapies for ALS

Mutant SOD1 mice have been used to test pharmacological and gene-based therapies[68,77–80]. Several potential therapeutics have been tested with unencouraging results, including vitamin E and selenium, riluzole and gabapentin, and the copper chelator d-penicillamine. At present, treatment with creatine seems to have the most robust pharmacological influence on the disease[80]; oral administration of creatine to G93A SOD1 mice resulted in a dose-dependent improvement in motor tasks and extended survival.

The molecular mechanisms whereby mutant SOD1 causes selective motor neuron death have not yet been defined. One proposal is that the toxic property of mutant SOD1 involves mutation-induced conformational changes in SOD1 that result in aberrant oxidative activities[81]. In this scenario, cell dysfunction and death could be initiated by aberrant oxidative chemistries catalyzed by the copper atom bound in the active site of mutant SOD1 (ref. 81). To test this hypothesis, multiple lines of mutant SOD1 mice were crossed with mice lacking the specific copper chaperone protein (CCS) required for Cu loading of SOD1. Inactivation of the CCS gene in mice demonstrates that CCS is required for efficient copper incorporation into SOD1 in mammals, and the phenotypes of the CCS null mice resemble those of the SOD1 null mice[82]. Metabolic [^{64}]Cu labeling studies in mutant SOD1 mice lacking CCS show that copper incorporation into wild-type and mutant SOD1 is significantly diminished without the CCS[83]. Motor neurons in mice lacking the CCS have an

increased rate of death after facial nerve axotomy, a response previously shown for mice deficient in SOD1. Thus, CCS is necessary for efficient copper incorporation into SOD1 in motor neurons. However, although the absence of the CCS results in a significant reduction in the level of copper-loaded mutant SOD1, it has no effect on the onset, progression or pathology of motor neuron disease in mutant SOD1 mice[83]. These results demonstrate that aberrant, Cu-dependent activity of mutant SOD1 is unlikely to be involved in the pathogenesis of FALS.

Although the molecular mechanisms underlying mutant SOD1-linked familial ALS remain unclear, several pathogenic mechanisms other than the copper hypothesis have been proposed. Mutant SOD1-containing aggregates participate in the pathogenesis of SOD1-linked FALS[84–86]. The identification of mutant SOD1-containing aggregates early in the pathogenesis of mutant SOD1 mice supports their importance in disease progression.

A pathological hallmark of ALS is the accumulation of neurofilaments in proximal axons and cell bodies of motor neurons. To test the role of neurofilaments in mutant SOD1–induced motor neuron disease, SOD1 mutant mice were crossbred to several lines of mice with altered distributions of neurofilaments. The progeny of SOD1 mutant mice crossed to mice expressing an NF-H-β-galactosidase fusion protein (NF-H-lacZ), which crosslinks neurofilaments and prevents their export to axons, has no effect on disease progression[87]. In contrast, the lifespan of SOD1 mutant mice was moderately increased in the absence of neurofilaments when *NF-L* (neurofilament light chain) was ablated[88]. However, crosses with mice over-expressing wild-type NF-L or NF-H (neurofilament heavy chain) resulted in sparing of motor neurons, attenuation of disease progression and increased life span[79,89]. Given the conflicting results, it remains unclear how the distribution of neurofilaments influences motor neuron disease induced by mutant SOD1 (ref. 68).

Spinal muscular atrophy

SMA is an autosomal recessive disease characterized by muscle weakness and atrophy in infants and children[90]. SMA is classified as Type I, II or III based on the of age of onset and the degree of functional disability. Infants with Type I SMA become weak before six months of age and die before two years. The incidence of Type I SMA (also known as Werdnig–Hoffmann disease) is estimated at ~1:10,000 live births, with a carrier rate frequency between 1:50 and 1:80. At the cellular level, motor neurons show chromatolysis and accumulations of phosphorylated neurofilaments in cell bodies, and motor roots degenerate, leading to denervation of skeletal muscle. The final result is apoptosis and loss of large motor neurons[90].

SMA Type I, II and III are linked to a single highly complex genetic locus on chromosome 5. Investigators initially identified two SMA candidate genes: survival motor neuron (*SMN*)[91] and neuronal apoptosis inhibitory protein (*NAIP*)[92]. The region of chromosome 5 containing these two genes is duplicated. The copy of the *SMN* gene closer to the telomere is termed *SMN1*, and the homologous copy closer to the centromere is termed *SMN2*. SMN is expressed from both *SMN* genes, however, and whereas *SMN1* produces full-length transcripts, *SMN2* transcripts are alternatively spliced, resulting in mainly truncated transcripts lacking exon 7. *SMN1* is now recognized as the SMA-determining gene, with *NAIP* possibly involved in modifying the severity of the disease. Mutations in *SMN1* are present in nearly 100% of affected individuals[90], and partial or complete deletions are detected in over 95% of cases. Homozygous deletion of *SMN1* has not been found in unaffected individuals, and *SMN2* has not been shown to be deleted in any affected individual. The level of full-length SMN protein in spinal motor neurons governs whether the individual has SMA Type I, II or III (Type I having the least).

SMN is expressed in large motor neurons, but also in many other cells, with the highest levels seen in the hippocampus and cerebellum. SMN is part of a multiprotein complex involved in biogenesis of small nuclear ribonucleoprotein (snRNP), and is thought to be involved in the processing of small nuclear RNAs. In the cytoplasm, the SMN complex is associated with snRNP Sm core proteins that are involved in the assembly of spliceosomal snRNP complexes[93]. The complex represents a functional unit of the splicesomal machinery.

SMA-linked SMN mutants are defective in binding to Sm proteins because mutant SMN cannot form the large oligomers that are essential for high-affinity binding. Although abnormalities of spliceosomal snRNP biogenesis and metabolism are thought to be involved in the pathogenesis of SMA, it is not clear how SMN deletions cause the abnormalities in motor neurons associated with SMA.

SMN knockout mice

Given the causative role of SMN loss in over 90% of SMA cases, deletion of SMN is an appropriate approach toward the development of a mouse model for the disease. *SMN* is highly conserved between mice (single copy) and humans (two copies, *SMN1* and *SMN2*), with 82% identity[94]. However, *SMN* null embryos do not survive past the peri-implantation stage, corresponding to the initiation of embryonic RNA transcription. These results are consistent with the view that SMN function is essential, most likely due to its role in the biogenesis of spliceosomal snRNPs and pre-mRNA splicing. $Smn^{+/-}$ heterozygous mice[95] showed approximately 50% reduction of SMN protein in the spinal cord, which resulted in a progressive loss of motor neurons between birth and 6 months of age. The phenotype of these mice resembles SMA type III.

To test whether human *SMN2* can complement the embryonic lethality of $Smn^{-/-}$ embryos, and to generate a mouse model for SMA, several groups generated transgenic mice expressing human *SMN2* and crossbred them to $Smn^{+/-}$ mice to produce *SMN2* transgenic mice lacking the endogenous mouse *SMN*[96,97]. These $Smn^{-/-}$; *SMN2* mice have abnormalities in the spinal cord and skeletal muscles similar to those seen in cases of SMA. Thus, *SMN2* can partially compensate for the endogenous mouse SMN, and the variable phenotypes observed in $Smn^{-/-}$; *SMN2* mice recapitulate those seen in SMA Type I, II or III. The level of full-length SMN protein in these $Smn^{-/-}$; *SMN2* mice correlates with the severity of the disease. $Smn^{-/-}$; *SMN2* mice exhibit several clinical characteristics: Type I mice do not develop fur and die by postnatal day 10; Type II mice are inactive and die between 2 to 4 weeks of age; and Type III mice survive and breed normally but have defects in tail size. These studies strongly support the idea that the level of intact SMN protein determines the severity of phenotypes in SMA.

These mouse models of SMA are useful in understanding disease mechanisms and for testing therapeutic strategies. It is clear from the human and mouse pathology that the level of full-length SMN protein correlates with the severity of the disease. Sodium butyrate is effective in elevating the level of SMN protein (from the *SMN2* gene) in lymphoid cell lines derived from SMA patients[98]. This compound was thus used to test for therapeutic effects in mouse models of SMA[98]. Treatment of SMA-like mice with sodium butyrate led to an increase in the level of SMN pro-

tein in spinal cord and a concomitant amelioration of disease phenotype[98]. These studies indicate that sodium butyrate may be an effective therapeutic for SMA.

Conclusions

The identification of genes mutated or deleted in the inherited forms of neurodegenerative diseases has allowed investigators to create *in-vivo* and *in-vitro* model systems relevant to a wide variety of human neurological disorders. Genetically engineered mice that recapitulate some of the features of human diseases can provide important new information about the neurobiology of these diseases. These new models allow investigators to examine the molecular mechanisms by which mutant proteins cause selective dysfunction and death of neurons. Moreover, they can be used to test pathogenic pathways by crossing these mice with either mutated or deleted alleles of other molecular players in the pathogenic process. The results of these approaches provide us with a better understanding of the pathogenic mechanisms of the diseases, and should lead to the design of new therapeutic strategies.

In this review, we emphasized the value of transgenic and gene targeted models and the lessons they provided for understanding these and other neurodegenerative diseases. Specifically, BACE1$^{-/-}$ mice and APP/PS1 transgenic models have provided extraordinary new insights into the mechanisms of amyloidogenesis and the reasons why AD is a brain amyloidosis. For ALS, SOD1 transgenic models lacking CCS demonstrate that aberrant activities dependent on copper-loaded SOD1 are unlikely to be the pathogenic mechanism.

The mouse models have not only provided substantial progress in understanding the molecular mechanisms of neurodegeneration, they are also instrumental in identifying targets for mechanism-based therapeutics, such as BACE1. These genetically engineered models are valuable for testing a variety of therapeutic approaches, including Aβ immunotherapy in AD, creatine in ALS, and sodium butyrate in SMA. In summary, investigation of the pathogeneses of neurodegenerative diseases using transgenic models and other approaches has made spectacular progress over the past few years, and we anticipate that more promising therapies based on our present understanding of the disease mechanisms will continue to be identified. Transgenic models should provide a highly useful tool for quickly assessing which therapies should be pursued.

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1. Price, D. L., Tanzi, R. E., Borchelt, D. R. & Sisodia, S. S. Alzheimer's disease: genetic studies and transgenic models. *Annu. Rev. Genet.* **32**, 461–493 (1998).
2. Lee, V. M. Y. & Trojanowski, J. Q. Neurodegenerative taupathies: human disease and transgenic mouse models. *Neuron* **24**, 507–510 (1999).
3. Lin, X., Cummings, C. J. & Zoghbi, H. Y. Expanding our understanding of polyglutamine diseases through mouse models. *Neuron* **24**, 499–502 (1999).
4. Dunnett, S. B. & Bjorklund, A. Prospects for new restorative and neuroprotective treatments in Parkinson's Disease. *Nature* **399**, A32–A39 (1999).
5. Yamamoto, A., Lucas, J. J. & Hen, R. Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. *Cell* **101**, 57–66 (2000).
6. Hardy, J. & Gwinn-Hardy, K. Genetic classification of primary neurodegenerative disease. *Science* **282**, 1075–1079 (1998).
7. Selkoe, D. J. Alzheimer's disease: genes, proteins and therapy. *Physiol. Rev.* **81**, 741–766 (2001).
8. Price, D. L., Sisodia, S. S. & Borchelt, D. R. Genetic neurodegenerative diseases: the human illness and transgenic models. *Science* **282**, 1079–1083 (1998).
9. Schilling, G. *et al.* Intranuclear inclusions and neuritic pathology in transgenic mice expressing a mutant N-terminal fragment of huntingtin. *Hum. Mol. Genet.* **8**, 397–407 (1999).
10. Goedert, M., Spillantini, M. G. & Davies, S. W. Filamentous nerve cell inclusions in neurodegenerative diseases. *Curr. Opin. Neurobiol.* **8**, 619–632 (1998).
11. Zoghbi, H. Y. & Orr, H. T. Polyglutamine diseases: protein cleavage and aggregation. *Curr. Opin. Neurobiol.* **9**, 566–570 (1999).
12. Albert, M. S. & Drachman, D. A. Alzheimer's disease. What is it, how many people have it, and why do we need to know? *Neurology* **55**, 166–168 (2000).
13. Mesulam, M. M. Neuroplasticity failure in Alzheimer's disease: bridging the gape between plaques and tangles. *Neuron* **24**, 521–529 (1999).
14. Price, D. L. & Sisodia, S. S. Mutant genes in familial Alzheimer's disease and transgenic models. *Annu. Rev. Neurosci.* **21**, 479–505 (1998).
15. Beach, T. G. *et al.* The cholinergic deficit coincides with Aβ deposition at the earliest histopathologic stages of Alzheimer disease. *J. Neuropathol. Exp. Neurol.* **59**, 308–313 (2000).
16. Serpell, L. C., Blake, C. C. F. & Fraser, P. E. Molecular structure of a fibrillar Alzheimer's Aβ fragment. *Biochemistry* **39**, 13269–13275 (2000).
17. Lansbury, P. T. Jr. Evolution of amyloid: what normal protein folding may tell us about fibrillogenesis and disease. *Proc. Natl. Acad. Sci. USA* **96**, 3342–3344 (1999).
18. Tanzi, R. E. New frontiers in Alzheimer's disease genetics. *Neuron* **32**, 181–184 (2001).
19. Selkoe, D. J. Clearing the brain's amyloid cobwebs. *Neuron* **32**, 177–180 (2001).
20. Sinha, S. *et al.* Purification and cloning of amyloid precursor protein β-secretase from human brain. *Nature* **402**, 537–540 (1999).
21. Hussain, I. *et al.* Identification of a novel aspartic protease (Asp 2) as β-secretase. *Mol. Cell. Neurosci.* **14**, 419–427 (1999).
22. Lin, X. *et al.* Human aspartic protease memapsin 2 cleaves the β-secretase site of β-amyloid precursor protein. *Proc. Natl. Acad. Sci. USA* **97**, 1456–1460 (2000).
23. Yan, R. *et al.* Membrane-anchored aspartyl protease with Alzheimer's disease β-secretase activity. *Nature* **402**, 533–537 (1999).
24. Vassar, R. *et al.* β-secretase cleavage of Alzheimer's amyloid precusor protein by the transmembrane aspartic protease BACE. *Science* **286**, 735–741 (1999).
25. Wong, P. C., Price, D. L. & Cai, H. The brain's susceptibility to amyloid plaques. *Science* **293**, 1434–1435 (2001).
26. Farzan, M., Schnitzler, C. E., Vasilieva, N., Leung, D. & Choe, H. BACE2, a β-secretase homolog, cleaves at the β site and within the amyloid-β region of the amyloid-β precursor protein. *Proc. Natl. Acad. Sci. USA* **97**, 9712–9717 (2000).
27. Nilsberth, C. *et al.* The 'Arctic' APP mutation (E693G) causes Alzheimer's disease by enhanced Aβ protofibril formation. *Nat. Neurosci.* **4**, 887–893 (2001).
28. Sherrington, R. *et al.* Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* **375**, 754–760 (1995).
29. Levy-Lahad, E. *et al.* Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* **269**, 973–977 (1995).
30. Rogaev, E. I. *et al.* Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* **376**, 775–778 (1995).
31. Thinakaran, G. *et al.* Endoproteolysis of presenilin 1 and accumulation of processed derivatives *in vivo*. *Neuron* **17**, 181–190 (1996).
32. Sisodia, S. S. & George-Hyslop, P. H. gamma-secretase, Notch, Aβ and Alzheimer's disease: where do the presenilins fit in? *Nat. Rev. Neurosci.* **3**, 281–290 (2002).
33. Esler, W. P. & Wolfe, M. S. A portrait of Alzheimer secretases—new features and familiar faces. *Science* **293**, 1449–1454 (2001).
34. Vassar, R. & Citron, M. Aβ-generating enzymes: recent advances in β- and γ-secretase research. *Neuron* **27**, 419–422 (2000).
35. De Strooper, B. *et al.* Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature* **391**, 387–390 (1998).
36. Wolfe, M. S. *et al.* Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and secretase activity. *Nature* **398**, 513–517 (1999).
37. Kopan, R. & Goate, A. Aph-2/Nicastrin: an essential component of gamma-secretase and regulator of notch signaling and presenilin localization. *Neuron* **33**, 321–324 (2002).
38. Calhoun, M. E. *et al.* Neuronal overexpression of mutant amyloid precursor

protein results in prominent deposition of cerebrovascular amyloid. *Proc. Natl. Acad. Sci. USA* **96**, 14088–14093 (1999).
39. Chen, G. *et al.* A learning deficit related to age and β-amyloid plaques in a mouse model of Alzheimer's disease. *Nature* **408**, 975–979 (2000).
40. Mucke, L. *et al.* High-level neuronal expression of $A\beta_{1-42}$ in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. *J. Neurosci.* **20**, 4050–4058 (2000).
41. Hsia, A. Y. *et al.* Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proc. Natl. Acad. Sci. USA* **96**, 3228–3233 (1999).
42. Lewis, J. *et al.* Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science* **293**, 1487–1491 (2001).
43. Götz, J., Chen, F., Barmettler, R. & Nitsch, R. M. Tau filament formation in transgenic mice expressing P301L tau. *J. Biol. Chem.* **276**, 529–534 (2001).
44. Borchelt, D. R. *et al.* Accelerated amyloid deposition in the brains of transgenic mice co-expressing mutant presenilin 1 and amyloid precursor proteins. *Neuron* **19**, 939–945 (1997).
45. Zheng, H. *et al.* β-amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. *Cell* **81**, 525–531 (1995).
46. Heber, S. *et al.* Mice with combined gene knock-outs reveal essential and partially redundant functions of amyloid precursor protein family members. *J. Neurosci.* **20**, 7951–7963 (2000).
47. Struhl, G. & Greenwald, I. Presenilin is required for activity and nuclear access of notch in *drosophila*. *Nature* **398**, 522–525 (1999).
48. Wong, P. C. *et al.* Presenilin 1 is required for *Notch1* and *Dll1* expression in the paraxial mesoderm. *Nature* **387**, 288–292 (1997).
49. Donoviel, D. *et al.* Mice lacking both presenilin genes exhibit early embryonic patterning defects. *Genes Dev.* **13**, 2801–2810 (1999).
50. Yu, H. *et al.* APP processing and synaptic plasticity in *presenilin-1* conditional knockout mice. *Neuron* **31**, 713–726 (2001).
51. Feng, R. *et al.* Deficient neurogenesis in forebrain-specific *presenilin-1* knockout mice is associated with reduced clearance of hippocampal memory traces. *Neuron* **32**, 911–926 (2001).
52. Cai, H. *et al.* BACE1 is the major β-secretase for generation of Aβ peptides by neurons. *Nat. Neurosci.* **4**, 233–234 (2001).
53. Luo, Y. *et al.* Mice deficient in BACE1, the Alzheimer's β-secretase, have normal phenotype and abolished β-amyloid generation. *Nature* **4**, 231–232 (2001).
54. Roberds, S. L. *et al.* BACE knockout mice are healthy despite lacking the primary β-secretase activity in brain: implication for Alzheimer's disease therapeutics. *Hum. Mol. Genet.* **10**, 1317–1324 (2001).
55. Bodendorf, U., Fischer, F., Bodian, D., Multhaup, G. & Paganetti, P. A splice variant of β-secretase deficient in the amyloidogenic processing of the amyloid precursor protein. *J. Biol. Chem.* **276**, 12019–12023 (2001).
56. Li, Y. M. *et al.* Photoactivated γ-secretase inhibitors directed to the active site convalently label presenilin 1. *Nature* **405**, 689–693 (2000).
57. Huppert, S. S. *et al.* Embryonic lethality in mice homozygous for a processing-deficient allele of Notch 1. *Nature* **405**, 966–970 (2000).
58. Petit, A. *et al.* New protease inhibitors prevent γ-secretase-mediated production of Aβ40/42 without affecting Notch clevage. *Nat. Cell Biol.* **3**, 507–511 (2001).
59. Hadland, B. K. *et al.* γ -secretase inhibitors repress thymocyte development. *Proc. Natl. Acad. Sci. USA* **98**, 7487–7491 (2001).
60. Bard, F. *et al.* Peripherally administered antibodies against amyloid β-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer's disease. *Nat. Med.* **6**, 916–919 (2000).
61. Schenk, D. *et al.* Immunization with amyloid attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* **400**, 173–177 (1999).
62. DeMattos, R. B. *et al.* Peripheral anti-Aβ antibody alters CNS and plasma Aβ clearance and decreases brain Aβ burden in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* **98**, 8850–8855 (2001).
63. Janus, C. *et al.* Aβ peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* **408**, 979–982 (2000).
64. Morgan, D. *et al.* A β peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* **408**, 982–985 (2000).
65. Ince, P. G. in *Amyotrophic Lateral Sclerosis* (eds. Brown, R. H. Jr., Meininger, V. & Swash, M.) 83–112 (Martin Dunitz, London, 2000).
66. Martin, L. J. Neuronal death in amyotrophic lateral sclerosis is apoptosis: possible contribution of a programmed cell death mechanism. *J. Neuropathol. Exp. Neurol.* **58**, 459–471 (1999).
67. Andersen, P. M., Morita, M. & Brown, R. H. Jr. in *Amyotrophic Lateral Sclerosis* (eds. Brown, R. H. Jr., Meininger, V. & Swash, M.) 223–250 (Martin Dunitz, London, 2000).
68. Cleveland, D. W. & Rothstein, J. D. From Charcot to Lou Gehrig: deciphering selective motor neurons death in ALS. *Nature* **2**, 806–819 (2001).
69. Hadano, S. *et al.* A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. *Nat. Genet.* **29**, 166–173 (2001).
70. Yang, Y. *et al.* The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nat. Genet.* **29**, 160–165 (2001).
71. Gurney, M. E. *et al.* Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. *Science* **264**, 1772–1775 (1994).
72. Wong, P. C. *et al.* An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. *Neuron* **14**, 1105–1116 (1995).
73. Bruijn, L. I. *et al.* ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. *Neuron* **18**, 327–338 (1997).
74. Jackson, M. & Rothstein, J. D. in *Amyotrophic Lateral Sclerosis* (eds. Brown, R. H. Jr., Meininger, V. & Swash, M.) 263–278 (Martin Dunitz, London, 2000).
75. Shaw, P. J. in *Amyotrophic Lateral Sclerosis* (eds. Brown, R. H. Jr., Meininger, V. & Swash, M.) 113–144 (Martin Dunitz, London, 2000).
76. Williamson, T. L. & Cleveland, D. W. Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. *Nat. Neurosci.* **2**, 50–56 (1999).
77. Julien, J.-P. Amyotrophic lateral sclerosis: unfolding the toxicity of the misfolded. *Cell* **104**, 581–591 (2001).
78. Pasinelli, P., Borchelt, D. R., Houseweart, M. K., Cleveland, D. W. & Brown, R. H. Caspase-1 is activated in neural cells and tissue with amyotrophic lateral sclerosis-associated mutations in copper-zinc superoxide dismutase. *Neurobiology* **95**, 15763–15768 (1998).
79. Couillard-Després, S. *et al.* Protective effect of neurofilament NF-H overexpression in motor neuron disease induced by mutant superoxide dismutase. *Proc. Natl. Acad. Sci. USA* **95**, 9626–9630 (1998).
80. Klivenyi, P. *et al.* Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. *Nat. Med.* **5**, 347–350 (1999).
81. Estévez, A. G. *et al.* Induction of nitric oxide-dependent apoptosis in motor neurons by zinc-deficient superoxide dismutase. *Science* **286**, 2498–2500 (1999).
82. Wong, P. C. *et al.* Copper chaperone for superoxide dismutase is essential to activate mammalian Cu/Zn superoxide dismutase. *Proc. Natl. Acad. Sci. USA* **97**, 2886–2891 (2000).
83. Subramaniam, J. R. *et al.* Mutant SOD1 causes motor neuron disease independent of copper chaperone-mediated copper loading. *Nat. Neurosci.* **5**, 301–307 (2002).
84. Johnston, J. A., Dalton, M. J., Gurney, M. E. & Kopito, R. R. Formation of high molecular weight complexes of mutant Cu, Zn-superoxide dismutase in a mouse model for familial amyotrophic lateral sclerosis. *Proc. Natl. Acad. Sci. USA* **97**, 12571–12576 (2000).
85. Bruijn, L. I. *et al.* Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science* **281**, 1851–1854 (1998).
86. Beaulieu, J. M., Jacomy, H. & Julien, J. P. Formation of intermediate filament protein aggregates with disparate effects in two transgenic mouse models lacking the neurofilament light subunit. *J. Neurosci.* **20**, 5321–5328 (2000).
87. Eyer, J., Cleveland, D. W., Wong, P.C. & Peterson, A. C. Pathogenesis of two axonopathies does not require axonal neurofilaments. *Nature* **391**, 584–587 (1998).
88. Williamson, T. L. *et al.* Absence of neurofilaments reduces the selective vulnerability of motor neurons and slows disease caused by a familial amyotrophic lateral sclerosis-linked superoxide dismutase 1 mutant. *Proc. Natl. Acad. Sci. USA* **95**, 9631–9636 (1998).
89. Kong, J. & Xu, Z. Overexpression of neurofilament subunit NF-L and NF-H extends survival of a mouse model for amyotrophic lateral sclerosis. *Neurosci. Lett.* **281**, 72–74 (2000).
90. Crawford, T. & Pardo, C. A. The neurobiology of childhood spinal muscular atrophy. *Neurobiol. Dis.* **3**, 97–110 (1996).
91. Lefebvre, S. *et al.* Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* **80**, 155–165 (1995).
92. Roy, N. *et al.* The gene for neuronal apoptosis inhibitory protein is partially deleted in individuals with spinal muscular atrophy. *Cell* **80**, 167–178 (1995).
93. Fischer, U., Liu, Q. & Dreyfuss, G. The SMN-SIP1 complex has an essential role in spliceosomal snRNP biogenesis. *Cell* **90**, 1023–1029 (1997).
94. Pagliardini, S. *et al.* Subcellular localization and axonal transport of the survival motor neuron (SMN) protein in the developing rat spinal cord. *Hum. Mol. Genet.* **9**, 47–56 (2000).
95. Jablonka, S., Schrank, B., Kralewski, M., Rossoll, W. & Sendtner, M. Reduced survival motor neuron (Smn) gene dose in mice leads to motor neuron degeneration: an animal model for spinal muscular atrophy type III. *Hum. Mol. Genet.* **9**, 341–346 (2000).
96. Hsieh-Li, H. M. *et al.* A mouse model for spinal muscular atrophy. *Nat. Genet.* **24**, 66–70 (2000).
97. Monani, U. R. *et al.* The human centromeric survival motor neuron gene (SMN2) rescues embryonic lethality in Smn–/– mice and results in a mouse with spinal muscular atrophy. *Hum. Mol. Genet.* **9**, 333–339 (2000).
98. Chang, J.-G. *et al.* Treatment of spinal muscular atrphy by sodium butyrate. *Proc. Natl. Acad. Sci. USA* **98**, 9808–9813 (2001).