Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Neurobiology

An amyloid-like cascade hypothesis for *C9orf72* ALS/FTD

Dieter Edbauer[1,2,3] and Christian Haass[1,2,4]

Expansion of a *GGGGCC* repeat in *C9orf72* causes amyotrophic lateral sclerosis, frontotemporal dementia, or a combination of both. Bidirectional repeat transcripts sequester RNA-binding proteins into nuclear RNA foci. The repeat is translated into dipeptide repeat (DPR) proteins that are crucial for repeat-induced toxicity. DPRs inhibit the proteasome and sequester other proteins. These changes are accompanied by widespread brain atrophy and subclinical cognitive impairment before disease onset. Both repeat RNA and DPRs impair nucleocytoplasmic transport and promote TDP-43 mislocalization and aggregation. Thus, repeat RNA and DPRs may gradually trigger TDP-43 pathology and subsequent region-specific neurodegeneration in a cascade similar to amyloid-β peptide in Alzheimer's disease. The key components of the *C9orf72* cascade are promising therapeutic targets in different disease stages.

Addresses
[1] German Center for Neurodegenerative Diseases (DZNE), Munich, Feodor-Lynen-Str. 17, 81377 Munich, Germany
[2] Munich Cluster of Systems Neurology (SyNergy), Feodor-Lynen-Str. 17, 81377 Munich, Germany
[3] Ludwig-Maximilians University Munich, 81377 Munich, Germany
[4] Biomedical Center (BMC), Ludwig-Maximilians University Munich, Feodor-Lynen-Str. 17, 81377 Munich, Germany

Corresponding author: Edbauer, Dieter (dieter.edbauer@dzne.de)

Current Opinion in Neurobiology 2016, **36**:99–106

This review comes from a themed issue on **Neurobiology of disease**

Edited by **Dennis J Selkoe** and **Daniel R Weinberger**

For a complete overview see the Issue and the Editorial

Available online 8th November 2015

http://dx.doi.org/10.1016/j.conb.2015.10.009

Introduction

Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are two fatal neurodegenerative diseases that overlap clinically, genetically and pathologically [1,2]. Degeneration of the frontotemporal lobes results in behavioral disinhibition, dementia and speech and language impairments, depending on which regions are most affected. Loss of the upper and lower motoneurons in the motor cortex and the spinal cord causes progressive paralysis and ultimately respiratory failure. Genetic and neuropathological findings suggest that both diseases are opposite ends of a disease spectrum and many patients suffer from combined symptoms. Neuropathologically, ALS and FTD are classified depending on the key aggregating proteins. TDP-43 inclusions are found in 60% of FTD and about 90% of ALS patients, specifically in neurons and glia in the areas with the most severe neurodegeneration [3]. Rare TDP-43 mutations have been found to cause predominantly ALS [4], but most inherited forms of ALS or FTD with TDP-43 pathology are due to mutations in several other genes, most commonly in GRN (in FTD) and *C9orf72* (in ALS and FTD) [5,6]. A massive expansion of a *GGGGCC* repeat (with up to several thousand repeats compared to fewer than 25 in controls) upstream of the coding region of *C9orf72* (Figure 1a) is the most common ALS/FTD causing mutation among Caucasians [7–9]. Repeat expansions cause equally likely ALS, FTD or a mixture of both and are found in 5–40% of familial ALS and FTD cases and in ~5% seemingly sporadic patients [10,11].

Pathomechanisms

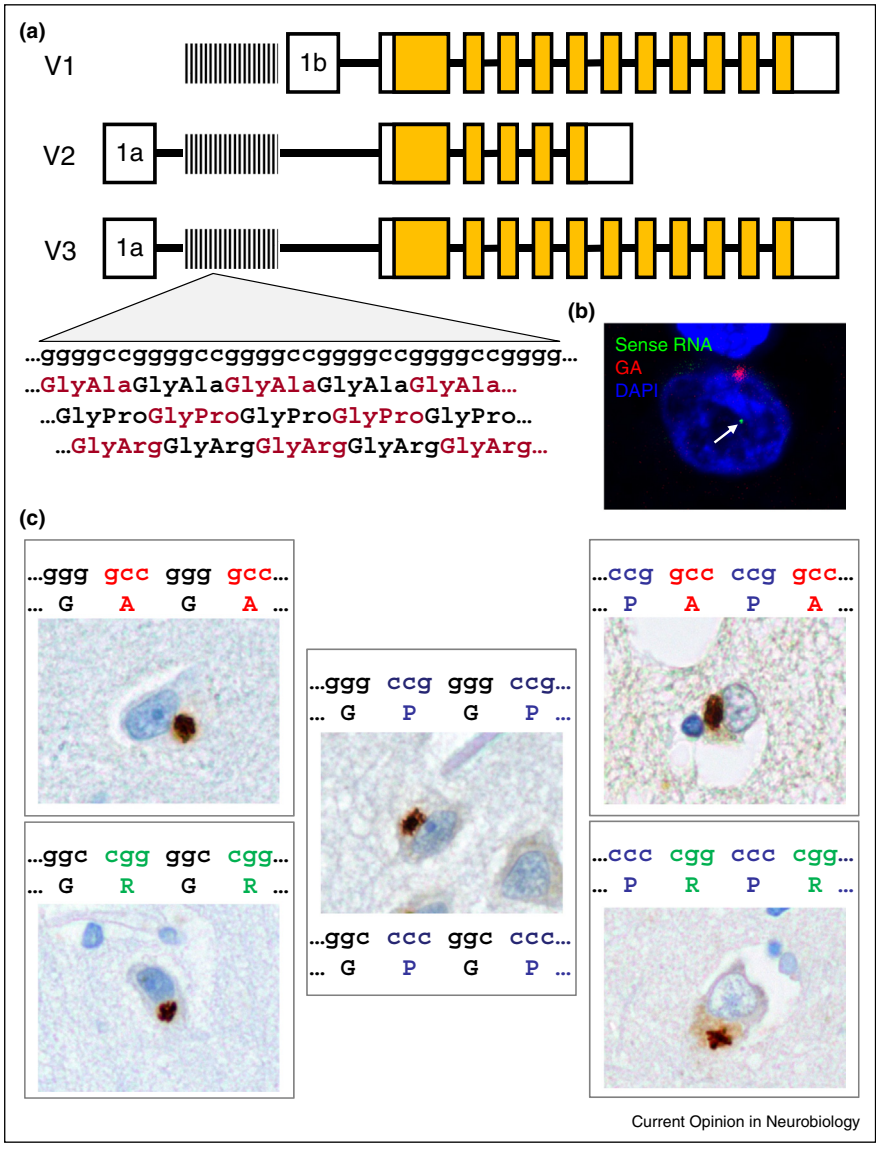
Several pathomechanisms have been proposed for *C9orf72* ALS and FTD that may act in concert. These include: Firstly, haploinsufficiency; secondly, RNA toxicity, and finally, toxicity of highly unusual protein deposits.

Many studies show reduced mRNA expression in *C9orf72* ALS/FTD cases [7–9] that was recently confirmed on the C9orf72 protein level [12], suggesting haploinsufficiency as a cause of ALS/FTD. However, the physiological function of the C9orf72 protein is poorly understood and neuron specific knockout mice develop no disease like pathology [13] making it unlikely that haploinsufficiency significantly contributes to the disease mechanism.

The presence of nuclear foci of the repeat RNA (Figure 1b) suggests that sense and antisense transcripts of the repeat sequester specific RNA-binding proteins, which may profoundly alter RNA-metabolism. Several $(ggggcc)_n$ and $(ccccgg)_n$ interacting proteins (lower case denotes RNA) have been identified *in vitro*, including hnRNPA3 [14], SRSF2, hnRNP H1/F, ALYREF [15], ADARB2 [16•] and Nucleolin [17].

Pathologically, *C9orf72* mutation cases show specific star-shaped or dot-like TDP-43 negative, p62-positive cytoplasmic inclusions, which are not found in other ALS/FTD cases [18]. The core components of these

Figure 1



Genomic structure of *C9orf72* and key pathological findings. **(a)** Depiction of the three main transcript of *C9orf72*. The *GGGGCC*-repeat expansion (about 100–5000 repeats compared to <30 repeat in controls) is either located upstream of the coding region (orange), in the promoter region (isoform V1) or in the first intron (isoforms V2 and V3). V1 and V3 encode the same ~54 kDa isoform, while V2 encodes a C-terminally truncated ~25 kDa isoform. The sense transcript (ggggcc RNA repeat in lower case) is translated in all three reading frames into abundant DPR proteins. **(b)** RNA foci formed by the sense strand (arrow) are detectable by *in situ* hybridization in the nucleus. **(c)** Specific antibodies detect DPR inclusions derived from non-ATG translation of sense and antisense repeat transcripts in all reading frames.

inclusions are derived from unconventional translation of the intronic repeat RNA in all reading frames into five aggregating dipeptide repeat (DPR) proteins (Figure 1a,c) [19••,20••,21–23]. Virtually all TDP-43 negative inclusions contain poly-GA. The other DPR species coaggregate to a variable extent in these inclusions. Poly-GP and poly-GR are found in about half of the aggregates, while the antisense transcript-derived poly-PA and poly-PR are much rarer.

Repeat-associated non-ATG (RAN) translation was first discovered for $(cag)_n$ expansions and is thought to involve hairpin formation of the repeat mRNA [24]. Recently, RAN translation of an expanded $(cgg)_n$ repeat was also observed in fragile X tremor ataxia syndrome [25]. It is still unclear how repetitive RNA hairpins can overcome the tight control mechanisms during translation initiation, but interfering with the secondary structure has been shown to reduce translation of the *C9orf72* repeat [26•].

In this review we try to reconcile mechanistic insights from cellular and animal models and findings from neuropathological and clinical studies. Furthermore, we unify these findings into a pathological cascade with surprising similarities to the amyloid cascade in Alzheimer's disease (AD).

A major role of C9orf72 loss of function in ALS/FTD is unlikely

Although reduction of *C9orf72* transcripts has been described already in the initial reports of the *C9orf72* repeat expansion (and widely replicated afterwards), it remains unclear whether haploinsufficiency contributes to *C9orf72* pathogenesis. Data on promoter hypermethylation even suggest that reduced C9orf72 expression is neuroprotective, because methylation correlates with prolonged survival [27].

Neuron specific conditional knockout of *C9orf72* in mice leads to an unexplained weight loss, but does not cause motor symptoms, TDP-43 pathology, neurodegeneration, neuroinflammation or premature death [13], although this does not exclude a non-neuronal component of haploinsufficiency. Moreover, sustained reduction of *C9orf72* expression by antisense oligonucleotides (ASO) in mouse brain did not result in ALS/FTD like symptoms [28•]. Thus, C9orf72 loss of function does not seem to cause TDP-43 associated neurodegeneration and is unlikely to be the main cause of ALS/FTD.

To fully address a possible loss of function component, a better understanding of the C9orf72 function is crucial. Unfortunately, antibodies detecting endogenous C9orf72 are notoriously difficult to generate, which may be due to the low endogenous expressions levels. C9orf72 protein levels are about 200-fold lower than the levels of TDP-43 and FUS in motor neurons and various cell lines [29]. Homology searches identified a putative DENN (Differentially Expressed in Normal and Neoplasia) domain in C9orf72 suggesting it acts as a guanine nucleotide exchange factor (GEF) for small GTPases, particularly within the Rab protein family [30]. Indeed, partial colocalization of C9orf72 with Rab1/5/7/11 endo-lysosomes was reported, which suggests that C9orf72 might regulate protein degradation and autophagy [31]. In contrast, antibodies to the short isoform have been reported to label the nuclear envelop in healthy controls and the plasma membrane in ALS cases with or without *C9orf72* mutation indicating a general defect in nuclear trafficking in ALS [32] which may be interesting in regard to the nuclear transport deficiencies recently reported as a pathological consequence of *C9orf72* repeat expansions (see below).

Gain of function models resemble patient pathology best

Many groups have reported toxic gain of function of the *C9orf72* repeat expansion in cellular and animal models that to various degrees invoke either DPR, RNA toxicity, or both. iPSC-derived neurons from *C9orf72* patients consistently show RNA foci, but lack abundant compact DPR inclusions, while DPR protein expression is often detectable at low level [16•,33–35]. Most likely inclusion formation is a slow process due to inefficient non-ATG translation of the DPR proteins. Several groups reported subtle changes in gene expression in patient derived fibroblasts and neurons, but the different studies show little overlap [16•,28•,35]. Neurons carrying the repeat expansion are initially hyperexcitable [16•] and become hypoexcitable later in development [34,35] which may lead to the functional inactivation of motoneurons before cell death. In patient derived neurons, ASOs targeting the repeat or the *C9orf72* gene reduce RNA foci surprisingly without affecting poly-GP expression and restored normal gene expression arguing for predominant RNA toxicity [16•], although this may also be explained by a long half-life of poly-GP. Similarly, ASOs have been shown to inhibit RNA foci formation in patient fibroblasts, but did not correct altered gene expression in this case [28•].

To dissect the role of RNA and protein toxicity, several groups expressed endogenous ggggcc-repeat sequence or used ATG-initiated synthetic genes where the ggggcc-repeat was replaced by alternative codons for expression of individual DPR proteins in cellular models, suggesting poly-GA is the most aggregation prone DPR protein within cells [21,36–40]. Nearly all reports observed p62-positive cytoplasmic inclusions like those found in *C9orf72* patients only upon poly-GA expression [21,36–39]. In many reports this was associated with toxicity. Poly-GA neurotoxicity has been attributed to impaired function of the ubiquitin proteasome system and sequestration of Unc119, a protein regulating the trafficking of myristoylated cargo proteins [36–38]. Two inhibitors of ER stress, salubrinal and TUDCA, were able to rescue poly-GA toxicity [37]. In contrast, poly-GR and poly-PR expression typically resulted in nucleolar inclusions and also induced toxicity [38,39,41]. While poly-GR and poly-PR toxicity was stronger than poly-GA toxicity in many systems, we could not detect such nucleolar poly-GR/PR inclusions in patient tissue using monoclonal antibodies [42]. Poly-GR/PR may interfere with RNA metabolism and protein synthesis by directly binding RNA, particularly in the nucleolus through repetitive arginine residues [39,41]. Importantly, co-expression of poly-GA and poly-GR recruits poly-GR into cytoplasmic inclusions and partially rescues its toxicity in a model with predominantly diffuse cytoplasmic GR_{80} expression [43] indicating that the toxicity of poly-GR depends is on its localization. Sense strand repeat expression without start codon had little effect in most cellular models [36,39], while antisense repeat expression has been linked to toxicity [21].

In Drosophila, however, expression of ggggcc-repeats of increasing length causes degeneration in the eye that is

largely caused by DPR protein expression, because it is completely prevented by stop codons interspaced in the repeat [44••]. While expression of poly-GR and poly-PR from synthetic genes throughout the nervous system caused early lethality, poly-GA expression still significantly shortened the life span of transgenic flies. In another report, diffuse cytoplasmic GR_{80} expression specifically in the fly wing causes a classic notching phenotype that can be rescued by expressing the membrane-tethered transcription factor Notch [43]. At least in flies, cytoplasmic ggggcc-repeat RNA derived from poly-A tailed expression is far more toxic than nuclear repeat RNA derived from intronic expression, which has been attributed to enhanced DPR expression of the RNA exported from the nucleus [45].

Three recent reports found that modulation of nuclear transport in flies and yeast suppresses toxicity of repeat RNA and/or poly-GR/PR [46••,47••,48••]. Expression of the sense repeat in the fly eye induced toxicity with partial mislocalization of the fly TDP-43 homology (TBPH) protein, although poly-GR and poly-GP expression was not detected in this model [46••]. The sense repeat RNA binds to and inhibits RanGAP and thus disturbs the nucleocytoplasmic Ran gradient critical for the localization of many other proteins and RNAs, because only RanGDP but not RanGTP is imported into the nucleus. Enhancing nuclear import using overexpression of RanGAP or Importin-α suppresses neurodegeneration. Interestingly, RanGAP normally shows smooth perinuclear staining whereas RanGAP aggregates are found along the nuclear envelope in iPSC derived neurons and tissue from *C9orf72* mutation cases [46••]. Moreover, in an unbiased screen in ggggcc-expressing flies Freibaum and colleagues identified 18 enhancers and suppressors that encode components of the nuclear pore complex (NPC) [47••]. This group observed poly-GP and poly-GR, but no poly-GA expression. Boosting nuclear import by expression of karyopherins also rescues poly-PR toxicity in yeast suggesting that the ggggcc-repeat RNA and poly-GR/PR cause toxicity by disrupting nucleocytoplasmic transport [48••]. Interestingly, nucleocytoplasmic transport seems to be affected in ALS patients without *C9orf72* mutation and may therefore be a common feature of ALS [32,49]. In line with that, ALS causing mutations affect the PY-NLS of FUS and lead to its accumulation within the cytosol and its subsequent aggregation [50].

The best mouse model so far is based on AAV-mediated $(ggggcc)_{66}$ expression [51••]. Six months after neonatal intracerebroventricular injection, nuclear RNA foci and intranuclear and cytoplasmic poly-GA/GP/GR inclusions were detected together with diffuse nuclear poly-GP and diffuse cytoplasmic poly-GR. These mice show mild neuronal loss and deficits in the Rota-Rod assay, suggesting impaired motor coordination or balance. Importantly, this is the first *C9orf72* model displaying prominent TDP-43 aggregates and thus most closely resembles *C9orf72* ALS/FTD patients. Cells with TDP-43 aggregates always had visible RNA foci, while poly-GA inclusions were present in 75% of these cells. Similarly, we recently generated a cell culture model by high-level expression of a $(ggggcc)_{80}$ repeat without start codons that co-expresses all DPRs and showed cytoplasmic redistribution of TDP-43 (Mori *et al.*, unpublished).

The first cellular and animal models clearly highlight the importance of toxic gain of function as the origin of TDP-43 aggregation and neurodegeneration. Particularly, poly-GR/PR and to a lesser extent poly-GA are neurotoxic *in vitro* and *in vivo*, but only aggregates in the poly-GA models resemble the inclusions found in patients. It will be critical to determine, which of the mechanisms of RNA and DPR toxicity seen in model systems contribute to pathogenesis in patients.

Lack of spatial correlation of DPR deposits with TDP-43 pathology and neurodegeneration

The distribution of sense and antisense RNA foci and interacting proteins has not been rigorously studied in large cohorts of ALS and FTD cases. One possible reason might be that these tiny structures are only visible in large magnifications and would require great effort for quantitative analysis of several brain regions in many patients. A recent study found that only antisense, but not sense RNA foci correlated with pathological nuclear clearance of TDP-43 in motoneurons [52].

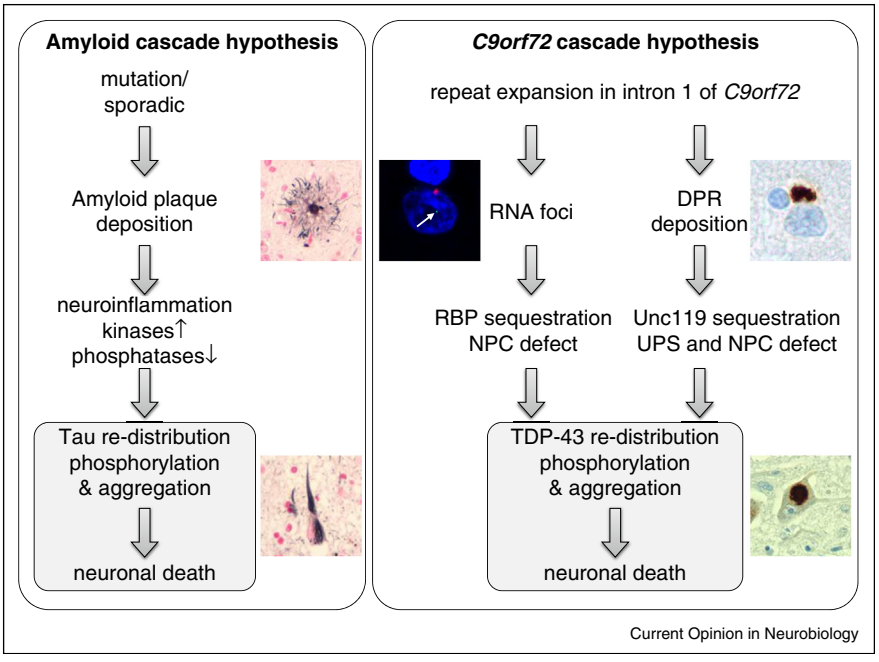
Regardless of clinical or neuropathological diagnosis, poly-GA/GP/GR inclusions are widespread throughout the neocortex, hippocampus, cerebellum and thalamus and rare in the spinal cord including motor neurons [23,42,53•,54,55]. Thus, DPR distribution is not spatially correlated with the areas of most prominent neurodegeneration in ALS and FTD cases. Interestingly, the distribution of amyloid plaques also poorly correlates with the neurodegeneration and clinical progression in AD cases [56]. However, recently some clinical correlates of DPR deposits have been found in *C9orf72* mutant cohorts. Higher poly-GA load is associated with earlier age of onset [54]. Cases with FTD with or without additional signs of motoneuron disease show more cerebellar poly-GA inclusions than cases with pure motoneuron disease [42]. Poly-PR has been reported to preferentially aggregate in motor neurons [52], but this has not been confirmed by us and others [42,55]. Several FTD cases with abundant DPR but without TDP-43 pathology have been identified, strongly arguing that DPR pathology contributes at least to the early symptoms of *C9orf72* patients [19••,57,58].

In contrast to DPR pathology, the distribution of TDP-43 pathology is highly correlated with areas of neurodegeneration and clinical symptoms [3,53•]. Thus, TDP-43 mislocalization and/or aggregation are the most likely effectors of *C9orf72* repeat associated toxicity, although a causal relation is not formally proven and the mechanistic link is not fully resolved. Interestingly, pathogenic TDP-43 mutations cause predominantly regional TDP-43 pathology similar to sporadic ALS/FTD cases indicating that motoneurons and the frontotemporal cortex are especially susceptible to cytoplasmic TDP-43 aggregation [59]. Therefore, Dormann and Haass formulated a multiple hit hypothesis where additional stressors must induce the irreversible deposition of TDP-43 following initial cytoplasmic mislocalization [1]. We speculate that DPR proteins and repeat RNA are such stressors that promote TDP-43 pathology in *C9orf72* patients.

Cross-sectional analysis of *C9orf72* patients reveals widespread brain atrophy and early cognitive deficits

Recent clinical data may help to reconcile the conflicting findings on DPR toxicity in model systems and neuropathological analysis. In Alzheimer's disease, cross-sectional analysis of individuals carrying pathogenic mutations revealed changes in biomarkers, imaging parameters and cognitive tests many years before clinical dementia and established the concept of prodromal disease stages [60]. Similarly, the GENFI consortium analyzed presymptomatic changes in individuals carrying FTD-causing mutations in *C9orf72*, *GRN* or *MAPT* and normalized the data to the average age of onset within each family [61•]. While all carriers showed impairment in neuropsychological tests several years before developing clinical FTD/ALS, *C9orf72* carriers showed detectable impairment 5–15 years earlier than the *GRN* or *MAPT* patients. Moreover, while volumetric imaging data support discrete atrophy in the insula and temporal cortex 5–10 years before the expected age of onset independent of the mutation, *C9orf72* patients showed widespread atrophy in the neocortex and thalamus already 20–25 years before the expected disease onset. Since TDP-43 pathology is largely limited to the frontotemporal cortex and the motor cortex, the clinical findings argue for an early role of the widely expressed DPR inclusions and/or RNA

Figure 2



Cascade model for *C9orf72* ALS/FTD compared to the amyloid cascade hypothesis in Alzheimer's disease. Left panel: In Alzheimer's disease Aβ plaques accumulate years or even decades before clinical onset of dementia. Aβ triggers Tau aggregation through altering its phosphorylation, neuroinflammation and several other mechanisms. Tau-dependent neurodegeneration in advanced AD patients may be independent of Aβ. Insets show Gallyas silver staining of neuritic Aβ plaques and Tau tangles in an Alzheimer cases. Right panel: Sense and antisense transcripts of the *C9orf72* repeat are found in nuclear foci containing sequestered RNA-binding proteins (RBP). The repeat RNA is translated by an unconventional mechanism into five DPR species that interfere with the function of Unc119 and the ubiquitin proteasome system (UPS). Both repeat RNA and DPR proteins impair function of the nuclear pore complex (NPC). All these pathways may already impair neuronal function and induce widespread presymptomatic brain atrophy. In combination these pathways trigger TDP-43 mislocalization and aggregation, which ultimately leads to rapid neurodegeneration. Insets show RNA foci (arrow, like in Figure 1b) and immunostainings of poly-GA aggregates and phospho-TDP-43 aggregates. Reduced C9orf72 protein expression may further contribute to pathogenesis by unidentified mechanisms.

foci in the presymptomatic brain atrophy in *C9orf72* cases.

An amyloid-like cascade model of *C9orf72* pathogenesis

Together these findings led us to postulate a cascade hypothesis for *C9orf72* associated ALS/FTD in approximate analogy to the amyloid cascade hypothesis for Alzheimer's disease [56,62,63]. Increased production, aggregation or failure of clearance of the amyloid β-peptide (Aβ) is the most likely initial trigger of the disease (Figure 2a). Accumulation of soluble Aβ species, subtle synaptic dysfunction and insoluble Aβ plaques induce an inflammatory response, which may result in microglial dysfunction and an early cognitive dysfunction in neuropsychological tests [60]. Then, a misbalance of kinases and phosphates boosts abnormal phosphorylation of tau and its subsequent aggregation and deposition in tangles. Tau aggregates or oligomers further impair synaptic function and cause neuronal loss by incompletely defined mechanisms. Importantly, Aβ pathology arises decades before the onset of dementia in the neocortex and then spreads centripetally towards the brain stem. By the time of diagnosis Aβ pathology has reached a plateau and poorly correlates with disease progression similar to DPR pathology in ALS/FTD. In contrast, the progression of Tau pathology from the (trans)entorhinal cortex to the neocortex correlates well with the clinical progression of dementia.

In line with Aβ plaques, we postulate that DPR proteins and toxic RNA may trigger a deadly cascade decades before clinical symptoms occur. Most likely, several cell autonomous and non-cell autonomous mechanisms act in concert. Sequestration of RNA-binding proteins, impairment of nuclear import and transcriptional alterations may lead to TDP-43 hyperphosphorylation, mislocalization and reduced excitability of neurons. DPR proteins may promote TDP-43 aggregation by inhibiting the proteasome and further altering RNA metabolism and nuclear transport. This is in line with the multiple hit model for FUS and TDP-43 aggregation in ALS/FTD [1]. We speculate that DPR aggregates could induce neuroinflammation which may further enhance TDP-43 aggregation. Neuronal cell death could then be induced by cytosolic accumulation of abnormally phosphorylated TDP-43 in analogy to abnormally phosphorylated tau. Thus, the lack of correlation of Aβ plaques and DPR inclusion density with neurodegeneration may be explained by triggering a slow cascade before clinical disease onset that can later sustain itself independent of its trigger as it has been postulated for AD [56].

According to our cascade model, three strategic points appear most suited for therapeutic intervention. First, preventing initiation of the disease cascade right at the top, for example, by enhancing repeat RNA degradation or preventing its translation using ASOs [16•,28•]. Second, preventing the conversions from presymptomatic to symptomatic stage by targeting DPR or RNA toxicity to block initiation of TDP-43 mislocalization, for example, by boosting DPR degradation or by modulating the structure of the repeat RNA-repeat or by restoring nuclear transport [26•,46••,47••,48••]. Third, targeting common downstream mechanisms not limited to ALS and FTD cases with *C9orf72* mutation, for example, by inhibiting TDP-43 aggregation and toxicity or modulating inflammation. This concept highlights the need for combination of different drugs in the prodromal and later stages of *C9orf72* ALS/FTD.

Conflict of interest statement

Nothing declared.

Acknowledgements

We thank Thomas Arzberger and Martin Schludi for the pathology images and Carina Lehmer, Stephanie May, Kohji Mori, Martin Schludi and Qihui Zhou for critical comments. DE and CH are supported by the European Research Council under the European Union's Seventh Framework Program (FP7/2007–2013) under grant agreement no. 617198 [DPR-MODELS] and no. 321366 [Amyloid], respectively. Open access publication was funded by the Munich cluster of Systems Neurology (SyNergy).

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Dormann D, Haass C: **TDP-43 FUS: a nuclear affair**. *Trends Neurosci* 2011, **34**:339-348.

2. Rademakers R, Neumann M, Mackenzie IR: **Advances in understanding the molecular basis of frontotemporal dementia**. *Nat Rev Neurol* 2012, **8**:423-434.

3. Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM *et al.*: **Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis**. *Science* 2006, **314**:130-133.

4. Kabashi E, Valdmanis PN, Dion P, Spiegelman D, McConkey BJ, Vande Velde C, Bouchard JP, Lacomblez L, Pochigaeva K, Salachas F *et al.*: **TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis**. *Nat Genet* 2008, **40**:572-574.

5. Scotter EL, Chen HJ, Shaw CE: **TDP-43 proteinopathy and ALS: insights into disease mechanisms and therapeutic targets**. *Neurotherapeutics* 2015, **12**:352-363.

6. Irwin DJ, Cairns NJ, Grossman M, McMillan CT, Lee EB, Van Deerlin VM, Lee VM, Trojanowski JQ: **Frontotemporal lobar degeneration: defining phenotypic diversity through personalized medicine**. *Acta Neuropathol* 2015, **129**:469-491.

7. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H, Adamson J *et al.*: **Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS**. *Neuron* 2011, **72**:245-256.

8. Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, Schymick JC, Laaksovirta H, van Swieten JC, Myllykangas L *et al.*: **A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD**. *Neuron* 2011, **72**:257-268.

9. Gijselinck I, Van Langenhove T, van der Zee J, Sleegers K, Philtjens S, Kleinberger G, Janssens J, Bettens K, Van Cauwenberghe C, Pereson S *et al.*: **A C9orf72 promoter repeat expansion in a Flanders–Belgian cohort with disorders of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum: a gene identification study**. *Lancet Neurol* 2012, **11**:54-65.

10. van der Zee J, Gijselinck I, Dillen L, Van Langenhove T, Theuns J, Engelborghs S, Philtjens S, Vandenbulcke M, Sleegers K, Sieben A *et al.*: **A Pan-European study of the C9orf72 repeat associated with FTLD: geographic prevalence, genomic instability and intermediate repeats**. *Hum Mutat* 2013, **34**:363-373.

11. Majounie E, Renton AE, Mok K, Dopper EG, Waite A, Rollinson S, Chio A, Restagno G, Nicolaou N, Simon-Sanchez J *et al.*: **Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study**. *Lancet Neurol* 2012, **11**:323-330.

12. Waite AJ, Baumer D, East S, Neal J, Morris HR, Ansorge O, Blake DJ: **Reduced C9orf72 protein levels in frontal cortex of amyotrophic lateral sclerosis and frontotemporal degeneration brain with the C9ORF72 hexanucleotide repeat expansion**. *Neurobiol Aging* 2014, **35** 1779 e1775–1779 e1713.

13. Koppers M, Blokhuis AM, Westeneng HJ, Terpstra ML, Zundel CA, Vieira de Sa R, Schellevis RD, Waite AJ, Blake DJ, Veldink JH *et al.*: **C9orf72 ablation in mice does not cause motor neuron degeneration or motor deficits**. *Ann Neurol* 2015, **78**:426-438.

14. Mori K, Lammich S, Mackenzie IR, Forne I, Zilow S, Kretzschmar H, Edbauer D, Janssens J, Kleinberger G, Cruts M *et al.*: **hnRNP A3 binds to GGGGCC repeats and is a constituent of p62-positive/TDP43-negative inclusions in the hippocampus of patients with C9orf72 mutations**. *Acta Neuropathol* 2013, **125**:413-423.

15. Cooper-Knock J, Walsh MJ, Higginbottom A, Robin Highley J, Dickman MJ, Edbauer D, Ince PG, Wharton SB, Wilson SA, Kirby J *et al.*: **Sequestration of multiple RNA recognition motif-containing proteins by C9orf72 repeat expansions**. *Brain* 2014, **137**:2040-2051.

16. • Donnelly CJ, Zhang PW, Pham JT, Haeusler AR, Mistry NA, Vidensky S, Daley EL, Poth EM, Hoover B, Fines DM *et al.*: **RNA toxicity from the ALS/FTD C9ORF72 expansion is mitigated by antisense intervention**. *Neuron* 2013, **80**:415-428.
First demonstration that antisense treatment may have therapeutic relevance in this disease.

17. Haeusler AR, Donnelly CJ, Periz G, Simko EA, Shaw PG, Kim MS, Maragakis NJ, Troncoso JC, Pandey A, Sattler R *et al.*: **C9orf72 nucleotide repeat structures initiate molecular cascades of disease**. *Nature* 2014, **507**:195-200.

18. Al-Sarraj S, King A, Troakes C, Smith B, Maekawa S, Bodi I, Rogelj B, Al-Chalabi A, Hortobagyi T: **Shaw CE: p62 positive, TDP-43 negative, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of C9orf72-linked FTLD and MND/ALS**. *Acta Neuropathol* 2011, **122**:691-702.

19. •• Mori K, Weng SM, Arzberger T, May S, Rentzsch K, Kremmer E, Schmid B, Kretzschmar HA, Cruts M, Van Broeckhoven C *et al.*: **The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTLD/ALS**. *Science* 2013, **15**: 1335-1338.
First description of DPR generation in *C9orf72* patients and cellular models.

20. •• Ash PE, Bieniek KF, Gendron TF, Caulfield T, Lin WL, Dejesus-Hernandez M, van Blitterswijk MM, Jansen-West K, Paul JW 3rd, Rademakers R *et al.*: **Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS**. *Neuron* 2013, **77**:639-646.
First description of DPR generation in *C9orf72* patients.

21. Zu T, Liu Y, Banez-Coronel M, Reid T, Pletnikova O, Lewis J, Miller TM, Harms MB, Falchook AE, Subramony SH *et al.*: **RAN proteins and RNA foci from antisense transcripts in C9ORF72 ALS and frontotemporal dementia**. *Proc Natl Acad Sci U S A* 2013, **110**:E4968-E4977.

22. Gendron TF, Bieniek KF, Zhang YJ, Jansen-West K, Ash PE, Caulfield T, Daughrity L, Dunmore JH, Castanedes-Casey M, Chew J *et al.*: **Antisense transcripts of the expanded C9ORF72 hexanucleotide repeat form nuclear RNA foci and undergo repeat-associated non-ATG translation in c9FTD/ALS**. *Acta Neuropathol* 2013, **126**:829-844.

23. Mori K, Arzberger T, Grasser FA, Gijselinck I, May S, Rentzsch K, Weng SM, Schludi MH, van der Zee J, Cruts M *et al.*: **Bidirectional transcripts of the expanded C9orf72 hexanucleotide repeat are translated into aggregating dipeptide repeat proteins**. *Acta Neuropathol* 2013, **126**:881-893.

24. Zu T, Gibbens B, Doty NS, Gomes-Pereira M, Huguet A, Stone MD, Margolis J, Peterson M, Markowski TW, Ingram MA *et al.*: **Non-ATG-initiated translation directed by microsatellite expansions**. *Proc Natl Acad Sci U S A* 2011, **108**:260-265.

25. Todd PK, Oh SY, Krans A, He F, Sellier C, Frazer M, Renoux AJ, Chen KC, Scaglione KM, Basrur V *et al.*: **CGG repeat-associated translation mediates neurodegeneration in fragile X tremor ataxia syndrome**. *Neuron* 2013, **78**:440-455.

26. • Su Z, Zhang Y, Gendron TF, Bauer PO, Chew J, Yang WY, Fostvedt E, Jansen-West K, Belzil VV, Desaro P *et al.*: **Discovery of a biomarker and lead small molecules to target r(GGGGCC)-associated defects in c9FTD/ALS**. *Neuron* 2014, **83**:1043-1050.
Discovery of DPRs in patient CSF as potential biomarker. First compounds modifying DPR expression.

27. Russ J, Liu EY, Wu K, Neal D, Suh E, Irwin DJ, McMillan CT, Harms MB, Cairns NJ, Wood EM *et al.*: **Hypermethylation of repeat expanded C9orf72 is a clinical and molecular disease modifier**. *Acta Neuropathol* 2015, **129**:39-52.

28. • Lagier-Tourenne C, Baughn M, Rigo F, Sun S, Liu P, Li HR, Jiang J, Watt AT, Chun S, Katz M *et al.*: **Targeted degradation of sense and antisense C9orf72 RNA foci as therapy for ALS and frontotemporal degeneration**. *Proc Natl Acad Sci U S A* 2013, **110**:E4530-E4539.
See annotation to Ref. [16•].

29. Hornburg D, Drepper C, Butter F, Meissner F, Sendtner M, Mann M: **Deep proteomic evaluation of primary and cell line motoneuron disease models delineates major differences in neuronal characteristics**. *Mol Cell Proteomics* 2014, **13**:3410-3420.

30. Levine TP, Daniels RD, Gatta AT, Wong LH, Hayes MJ: **The product of C9orf72, a gene strongly implicated in neurodegeneration, is structurally related to DENN Rab-GEFs**. *Bioinformatics* 2013, **29**:499-503.

31. Farg MA, Sundaramoorthy V, Sultana JM, Yang S, Atkinson RA, Levina V, Halloran MA, Gleeson PA, Blair IP, Soo KY *et al.*: **C9ORF72, implicated in amytrophic lateral sclerosis and frontotemporal dementia, regulates endosomal trafficking**. *Hum Mol Genet* 2014, **23**:3579-3595.

32. Xiao S, MacNair L, McGoldrick P, McKeever PM, McLean JR, Zhang M, Keith J, Zinman L, Rogaeva E, Robertson J: **Isoform specific antibodies reveal distinct subcellular localizations of C9orf72 in amyotrophic lateral sclerosis**. *Ann Neurol* 2015, **78**:568-583.

33. Almeida S, Gascon E, Tran H, Chou HJ, Gendron TF, Degroot S, Tapper AR, Sellier C, Charlet-Berguerand N, Karydas A *et al.*: **Modeling key pathological features of frontotemporal dementia with C9ORF72 repeat expansion in iPSC-derived human neurons**. *Acta Neuropathol* 2013, **126**:385-399.

34. Devlin AC, Burr K, Borooah S, Foster JD, Cleary EM, Geti I, Vallier L, Shaw CE, Chandran S, Miles GB: **Human iPSC-derived motoneurons harbouring TARDBP or C9ORF72 ALS mutations are dysfunctional despite maintaining viability**. *Nat Commun* 2015, **6**:5999.

35. Sareen D, O'Rourke JG, Meera P, Muhammad AK, Grant S, Simpkinson M, Bell S, Carmona S, Ornelas L, Sahabian A *et al.*: **Targeting RNA foci in iPSC-derived motor neurons from ALS patients with a C9ORF72 repeat expansion**. *Sci Transl Med* 2013, **5**:208ra149.

36. May S, Hornburg D, Schludi MH, Arzberger T, Rentzsch K, Schwenk BM, Grasser FA, Mori K, Kremmer E, Banzhaf-Strathmann J *et al.*: **C9orf72 FTLD/ALS-associated Gly-Ala**

dipeptide repeat proteins cause neuronal toxicity and Unc119 sequestration. *Acta Neuropathol* 2014, **128**:485-503.

37. Zhang YJ, Jansen-West K, Xu YF, Gendron TF, Bieniek KF, Lin WL, Sasaguri H, Caulfield T, Hubbard J, Daughrity L *et al.*: **Aggregation-prone c9FTD/ALS poly(GA) RAN-translated proteins cause neurotoxicity by inducing ER stress**. *Acta Neuropathol* 2014, **128**:505-524.

38. Yamakawa M, Ito D, Honda T, Kubo KI, Noda M, Nakajima K, Suzuki N: **Characterization of the dipeptide repeat protein in the molecular pathogenesis of c9FTD/ALS**. *Hum Mol Genet* 2014, **24**:1630-1645.

39. Wen X, Tan W, Westergard T, Krishnamurthy K, Markandaiah SS, Shi Y, Lin S, Shneider NA, Monaghan J, Pandey UB *et al.*: **Antisense proline-arginine RAN dipeptides linked to C9ORF72-ALS/FTD form toxic nuclear aggregates that initiate in vitro and in vivo neuronal death**. *Neuron* 2014, **84**:1213-1225.

40. Tao Z, Wang H, Xia Q, Li K, Jiang X, Xu G, Wang G, Ying Z: **Nucleolar stress and impaired stress granule formation contribute to C9orf72 RAN translation-induced cytotoxicity**. *Hum Mol Genet* 2015, **24**:2426-2441.

41. Kwon I, Xiang S, Kato M, Wu L, Theodoropoulos P, Wang T, Kim J, Yun J, Xie Y, McKnight SL: **Poly-dipeptides encoded by the C9orf72 repeats bind nucleoli, impede RNA biogenesis, and kill cells**. *Science* 2014, **345**:1139-1145.

42. Schludi MH, May S, Grasser FA, Rentzsch K, Kremmer E, Küpper C, Klopstock T, German Consortium for Frontotemporal Lobar Degeneration, Bavarian Brain Banking Alliance, Arzberger T *et al.*: **Distribution of dipeptide repeat proteins in cellular models and C9orf72 mutation cases suggests link to transcriptional silencing**. *Acta Neuropathol* 2015, **130**:537-555.

43. Yang D, Abdallah A, Li Z, Lu Y, Almeida S, Gao FB: **FTD/ALS-associated poly(GR) protein impairs the Notch pathway and is recruited by poly(GA) into cytoplasmic inclusions**. *Acta Neuropathol* 2015, **130**:525-535.

44. •• Mizielinska S, Gronke S, Niccoli T, Ridler CE, Clayton EL, Devoy A, Moens T, Norona FE, Woollacott IO, Pietrzyk J *et al.*: **C9orf72 repeat expansions cause neurodegeneration in Drosophila through arginine-rich proteins**. *Science* 2014, **345**:1192-1194.
First *C9orf72* Drosophila model. Repeat toxicity is mediated predominantly by DPR expression.

45. Tran H, Almeida S, Moore J, Gendron TF, Chalasani U, Lu Y, Du X, Nickerson JA, Petrucelli L, Weng Z *et al.*: **Differential toxicity of nuclear RNA foci versus dipeptide repeat proteins in a drosophila model of C9ORF72 FTD/ALS**. *Neuron* 2015, **87**:1207-1214.

46. •• Zhang K, Donnelly CJ, Haeusler AR, Grima JC, Machamer JB, Steinwald P, Daley EL, Miller SJ, Cunningham KM, Vidensky S *et al.*: **The C9orf72 repeat expansion disrupts nucleocytoplasmic transport**. *Nature* 2015, **525**:56-61.
Repeat RNA and DPRs disrupt nucleocytoplasmic transport by an overlaping mechanism.

47. •• Freibaum BD, Lu Y, Lopez-Gonzalez R, Kim NC, Almeida S, Lee KH, Badders N, Valentine M, Miller BL, Wong PC *et al.*: **GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport**. *Nature* 2015, **525**:129-133.
See annotation to Ref. [46••].

48. •• Jovicic A, Mertens J, Boeynaems S, Bogaert E, Chai N, Yamada SB, Paul JW 3rd, Sun S, Herdy JR, Bieri G *et al.*: **Modifiers of C9orf72 dipeptide repeat toxicity connect nucleocytoplasmic transport defects to FTD/ALS**. *Nat Neurosci* 2015, **18**:1226-1229.
See annotation to Ref. [46••].

49. Zhang J, Ito H, Wate R, Ohnishi S, Nakano S, Kusaka H: **Altered distributions of nucleocytoplasmic transport-related proteins in the spinal cord of a mouse model of amyotrophic lateral sclerosis**. *Acta Neuropathol* 2006, **112**:673-680.

50. Dormann D, Rodde R, Edbauer D, Bentmann E, Fischer I, Hruscha A, Than ME, Mackenzie IR, Capell A, Schmid B *et al.*: **ALS-associated fused in sarcoma (FUS) mutations disrupt Transportin-mediated nuclear import**. *EMBO J* 2010, **29**:2841-2857.

51. •• Chew J, Gendron TF, Prudencio M, Sasaguri H, Zhang YJ, Castanedes-Casey M, Lee CW, Jansen-West K, Kurti A, Murray ME *et al.*: **C9ORF72 repeat expansions in mice cause TDP-43 pathology, neuronal loss, and behavioral deficits**. *Science* 2015, **348**:1151-1154.
Best current animal model for *C9orf72* pathology related phenotypes.

52. Cooper-Knock J, Higginbottom A, Stopford MJ, Highley JR, Ince PG, Wharton SB, Pickering-Brown S, Kirby J, Hautbergue GM, Shaw PJ: **Antisense RNA foci in the motor neurons of C9ORF72-ALS patients are associated with TDP-43 proteinopathy**. *Acta Neuropathol* 2015, **130**:63-75.

53. • Mackenzie IR, Arzberger T, Kremmer E, Troost D, Lorenzl S, Mori K, Weng SM, Haass C, Kretzschmar HA, Edbauer D *et al.*: **Dipeptide repeat protein pathology in C9ORF72 mutation cases: clinico-pathological correlations**. *Acta Neuropathol* 2013, **126**:859-879.
Lack of correlation of DPR distribution with clinical phenotypes and neurodegeneration.

54. Davidson YS, Barker H, Robinson AC, Thompson JC, Harris J, Troakes C, Smith B, Al-Saraj S, Shaw C, Rollinson S *et al.*: **Brain distribution of dipeptide repeat proteins in frontotemporal lobar degeneration and motor neurone disease associated with expansions in C9ORF72**. *Acta Neuropathol Commun* 2014, **2**:70.

55. Gomez-Deza J, Lee YB, Troakes C, Nolan M, Al-Sarraj S, Gallo JM, Shaw CE: **Dipeptide repeat protein inclusions are rare in the spinal cord and almost absent from motor neurons in C9ORF72 mutant amyotrophic lateral sclerosis and are unlikely to cause their degeneration**. *Acta Neuropathol Commun* 2015, **3**:38.

56. Musiek ES, Holtzman DM: **Three dimensions of the amyloid hypothesis: time, space and 'wingmen'**. *Nat Neurosci* 2015, **18**:800-806.

57. Proudfoot M, Gutowski NJ, Edbauer D, Hilton DA, Stephens M, Rankin J, Mackenzie IR: **Early dipeptide repeat pathology in a frontotemporal dementia kindred with C9ORF72 mutation and intellectual disability**. *Acta Neuropathol* 2014, **127**:451-458.

58. Mann DM: **Dipeptide repeat protein toxicity in frontotemporal lobar degeneration and in motor neurone disease associated with expansions in C9ORF72 — a cautionary note**. *Neurobiol Aging* 2015, **36**:1224-1226.

59. Van Deerlin VM, Leverenz JB, Bekris LM, Bird TD, Yuan W, Elman LB, Clay D, Wood EM, Chen-Plotkin AS, Martinez-Lage M *et al.*: **TARDBP mutations in amyotrophic lateral sclerosis with TDP-43 neuropathology: a genetic and histopathological analysis**. *Lancet Neurol* 2008, **7**:409-416.

60. Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, Marcus DS, Cairns NJ, Xie X, Blazey TM *et al.*: **Clinical and biomarker changes in dominantly inherited Alzheimer's disease**. *N Engl J Med* 2012, **367**:795-804.

61. • Rohrer JD, Nicholas JM, Cash DM, van Swieten J, Dopper E, Jiskoot L, van Minkelen R, Rombouts SA, Cardoso MJ, Clegg S *et al.*: **Presymptomatic cognitive and neuroanatomical changes in genetic frontotemporal dementia in the Genetic Frontotemporal dementia Initiative (GENFI) study: a cross-sectional analysis**. *Lancet Neurol* 2015, **14**:253-262.
First demonstration of decade-long prodromal phase in *C9orf72* patients.

62. Hardy JA, Higgins GA: **Alzheimer's disease: the amyloid cascade hypothesis**. *Science* 1992, **256**:184-185.

63. Hardy J, Selkoe DJ: **The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics**. *Science* 2002, **297**:353-356.