

Review Article

C9ORF72 hexanucleotide repeats in behavioral and motor neuron disease: clinical heterogeneity and pathological diversity

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Abstract: Hexanucleotide repeat expansion in *C9ORF72* is the most common genetic cause of frontotemporal dementia (FTD), a predominantly behavioral disease, and amyotrophic lateral sclerosis (ALS), a disease of motor neurons. The primary objectives of this review are to highlight the clinical heterogeneity associated with *C9ORF72* pathogenic expansion and identify potential molecular mechanisms underlying selective vulnerability of distinct neural populations. The proposed mechanisms by which *C9ORF72* expansion causes behavioral and motor neuron disease highlight the emerging role of impaired RNA and protein homeostasis in a spectrum of neurodegeneration and strengthen the biological connection between FTD and ALS.

Keywords: *C9ORF72*, frontotemporal dementia, amyotrophic lateral sclerosis, motor neuron disease, RNA, protein trafficking

Introduction

A GGGGCC hexanucleotide repeat expansion intronic to *chromosome 9 open reading frame 72 (C9ORF72)* was identified in 2011 [1, 2] as the most common genetic cause of amyotrophic lateral sclerosis (ALS, or Lou Gehrig's disease) and frontotemporal dementia (FTD) with or without concomitant motor neuron disease (MND). The literature on *C9ORF72* has expanded greatly in the ensuing two years, leading to characterization of the frequency of pathogenic expansion carriers (C9+) in diverse populations and to putative molecular mechanisms underlying the pathogenicity of such expansions.

In addition to two forms of TDP-43 pathology (harmonized [3] Type A and B), C9+ is also characterized by Ub+/p62+/TDP-43- inclusions, most notably in cerebellum, thalamus and hippocampus; the latter pathology is unique to C9+ and, in some cases, this may be the only form of pathology [4-8]. These pathological findings are broadly mirrored by neuroimaging findings describing diffuse cortical and subcor-

tical atrophy in all lobes of the brain, with cerebellar and thalamic atrophy emerging as distinguishing features of C9+ when compared to sporadic disease in FTD/FTD-MND (reviewed in [9]) and ALS [10, 11]. One notable observation has been the diversity of phenotype associated with C9+ patients [12], which will be highlighted below. The clinical heterogeneity associated with *C9ORF72* expansion is predicted to be a reflection of the well-established structural and pathological heterogeneity [13]. Identifying the molecular mechanisms responsible for the apparent morphological and pathological diversity will be critical for making predictions about clinical outcomes in carriers of this shared genetic risk factor.

Clinical features of *C9ORF72* expansion-mediated disease

Motor features

Pathologic expansion of *C9ORF72* is the most common genetic cause of ALS, estimated at around 34% of familial and 6% of sporadic ALS cases [13]. C9+ ALS patients may demonstrate

more bulbar onset of symptoms (reviewed in [13, 14]). MND occurs concomitantly in about 30% of C9+ FTD [13]. C9+ may be a rare cause of other motor neuron disorders as well. One study investigating a large Dutch cohort found N=4 individuals with progressive muscular atrophy and N=1 patient with primary lateral sclerosis with expanded repeats [15] highlighting the variability of upper and/or lower motor neuron involvement associated with C9ORF72 expansion.

Some C9+ patients also show Parkinsonism, with or without MND. Parkinsonism symptomatology usually appears after onset of FTD or ALS findings, and is likely explained by neurodegeneration of the substantia nigra in many C9+ cases [16]. Hexanucleotide expansion of C9ORF72 has also been associated with a handful of cases with clinical diagnoses of idiopathic Parkinson's disease (PD) [17-19]. Intermediate repeat length has also been suggested as a risk factor for sporadic PD in two studies surveying large numbers of patients: for >20-30+ repeats in N=889 Caucasian PD or essential tremor plus Parkinsonism patients, and for ≥ 7 repeats in N=911 Han Chinese PD patients [20, 21]. Further investigations in diverse populations are required to confirm these findings.

Isolated cases of progressive supranuclear palsy, corticobasal, and olivopontocerebellar degeneration syndromes have also been reported, further expanding the spectrum of phenotypes associated with C9+ [19, 22]. Whether these rare cases are associated with C9+ pathology specifically in the basal ganglia, brainstem, and cerebellum remain to be determined.

Regions expressing the C9ORF72 mouse ortholog (discussed in more detail in the next section) include the striatum (a component of the basal ganglia), brainstem, and cerebellum [23]. Neuroimaging and pathological studies show that the cerebellum, which plays a critical role in motor control, is particularly affected in C9+ disease. The thalamus—which appears uniquely involved in C9+ compared to sporadic disease—participates in both the direct and indirect pathways linking the striatum and motor cortex, resulting in motor stimulation and inhibition, respectively [24]. Thus, pathological changes in cerebellum and thalamus have the

potential to affect multiple aspects of motor control, which could lead to less common motor syndromes.

Behavioral features

Behaviorally, C9ORF72 expansion is most commonly associated with a clinical syndrome of behavioral variant (bv)FTD, characterized by deficits in social behavior and executive function. Less common diagnoses include primary progressive aphasia (PPA), predominant amnesic, and psychiatric clinical syndromes. Some individuals also show deficits in visuospatial function (reviewed in [25]). In addition, cognitive and behavioral impairments appear to be more common in ALS patients with C9+ versus sporadic ALS [11]. Some cases of bvFTD associated with C9+ have remarkably slow progression and little to no visible neuroanatomical involvement [16, 26-28]. In addition to lack of frank brain atrophy, self-awareness of disease remains relatively intact, and patients are sometimes able to make behavioral modifications to compensate for the deficits imparted by disease [26]. This insight is in contrast to the majority of bvFTD patients, where there is marked lack of awareness into social and emotional deficits [29].

In the context of these broader clinical syndromes, specific psychiatric symptoms may further differentiate C9+ patients from other patients with sporadic or genetic forms of FTD. In particular, psychotic features may be enriched in C9ORF72 expansion carriers, with delusions and hallucinations more common in C9+ versus matched sporadic cases [30, 31]. In one Swedish C9+ kindred, psychotic symptoms and somatic complaints were observed in the majority of affected individuals [32]. Anxiety and depressive symptoms [8] are also observed in C9+. These symptoms may relate to findings that C9ORF72 expansion is associated with unique pathology in critical regions of the limbic system such as the thalamus and hippocampus [4-8]. Similarly, in Alzheimer's disease (AD), degeneration of the hippocampus may allow 'release' of its regulation of the amygdala, resulting in higher levels of anxiety and emotional contagion [33]. In addition, degeneration of the cerebellum could result in 'disconnection' of the emotion-regulating portions of this brain region from the cortex [34].

Occasionally, C9+ patients present clinically with an AD-like dementia; in a recent screen of

FTD genes in early-onset AD patients, two individuals were found to harbor *C9ORF72* expansions [35]. In these cases, neuroimaging may be particularly informative if findings are atypical of AD but instead show frontotemporal involvement [36]. Amnesic presentation may be related to the hippocampal sclerosis and/or p62+ pathology observed in the hippocampus of many C9+ patients [4-8]. In addition, episodic memory deficits in C9+ correlate with atrophy in the frontal, temporal and parietal cortices, including the posterior cingulate cortex, and are distinct from the regions correlated with episodic memory in sporadic bvFTD (i.e., medial prefrontal, medial and lateral temporal cortices) [37]. Finally, visuospatial deficits are in line with observed parietal lobe involvement in C9+ (reviewed in [9]). This further highlights how anatomic heterogeneity in *C9ORF72* expansion-mediated disease may contribute to a diversity of clinical symptoms. The diversity of clinical behavioral syndromes associated with *C9ORF72* expansion strongly suggests the presence of pathology in distinct areas of the brain across individuals.

Molecular mechanisms of *C9ORF72* disease

If *C9ORF72* expansion is associated with altered structural organization of the brain that culminates in a wide-spectrum of clinical disease, what molecular mechanisms might explain these changes? The three primary models accounting for *C9ORF72* expansion-mediated toxicity [38] are: (1) loss of *C9ORF72* protein function [1, 2]; (2) accumulation of toxic RNA foci [39], which sequester RNA-binding proteins such as TDP-43, FUS, hnRNP A3 [40], and Pur α [41] and result in dysregulation of RNA splicing, trafficking and translation; (3) novel dipeptide aggregate formation resulting from non-ATG mediated (RAN) translation of the expanded GGGGCC hexanucleotide repeat [42, 43]. Additional mechanisms that could modify disease pathogenesis include differential expansion size of *C9ORF72* hexanucleotide repeats across different tissues and independent genetic modifiers that mediate any of the factors that lead to neuronal toxicity. Also of note, recent evidence suggests that C9+ toxicity may not necessarily occur cell-autonomously in neurons; any of the proposed mechanisms of toxicity may in fact occur first in astrocytes and subsequently spread to neurons [44]. Potentially, the large degree of clinical heterogeneity

observed within the C9+ patient population could be a result of distinct pathogenic mechanisms (or combinations thereof) occurring in different individuals.

C9ORF72 haploinsufficiency

Loss of *C9ORF72* protein function from reduced expression due to pathogenic expansion is one proposed mechanism of disease. The expanded copy of *C9ORF72* results in reduced gene expression due to histone trimethylation, as measured in blood [45, 46]. This gene is predominantly expressed in neural populations vulnerable in FTD and ALS. Specifically, the mouse ortholog of *C9ORF72* is expressed in the hippocampus, dentate gyrus, striatum, thalamus, brainstem nucleus, cerebellum, throughout the cortex, and in the spinal cord, as well as several peripheral tissues. In mouse, expression appears to be limited primarily to gray matter [23]. Recent studies in both *C. elegans* and zebrafish indicate that loss of *C9ORF72* function may be associated with motor neuron degeneration [47, 48].

The protein product of *C9ORF72* is predicted to be structurally similar to the Differentially Expressed in Normal and Neoplasia (DENN) family of guanine nucleotide exchange factors that activate Rab-GTPases (Rab-GEFs), which are important regulators of membrane traffic [49, 50]. The putative yeast ortholog of *C9ORF72*, Lst4p, prevents lysosomal delivery of cargo by redirecting endosome-localized proteins to cell surface [51]. If *C9ORF72* similarly serves to sort endosomal cargo to the plasma membrane in neurons, then mutations reducing its function would be predicted to augment lysosomal degradation of particular cargo proteins. Intriguingly, the membrane protein TMEM106B, which has recently been shown to be a genetic modifier of both progranulin- and C9-mediated FTD, appears to influence both lysosomal morphology and dendritic trafficking of lysosomes within neurons [52, 53]. In addition, homozygous loss-of-function mutations in progranulin result in neuronal ceroid lipofuscinosis, a lysosomal storage disorder [54]. Dysfunctional degradation within the endolysosomal pathway may thus represent a common molecular pathology associated with altered levels of *C9ORF72*, progranulin and TMEM106B. Consistent with this scenario, accumulation of ubiquitinated proteins down-

stream of impaired lysosomal degradation could explain the Ub+/p62+/TDP-43- pathology that discriminates C9+ from other forms of FTD and ALS.

van der Zee and colleagues found decreased expression of C9ORF72 with an increased number of repeats at intermediate repeat numbers [55]. rGGGGCC (but not rCCCCGG) repeats form stable, tract length- and RNA concentration-dependent unimolecular and multimolecular RNA G-quadruplexes [56, 57], which can affect promoter activity, genetic instability, RNA splicing, translation and mRNA localization within neurites. The dose-dependence of stability of these structures suggests a mechanism by which increased repeat length would be more toxic. These RNA structures are potentially amenable to intervention with small molecules that break up G-quadruplexes [58-60]. This repeat (and how it folds) may serve as a mechanism by which splice variation occurs (given redistribution of C9ORF72 splice variants with expansion); ASF/SF2 splicing factor can bind to this repeat [57].

One patient has been reported with a homozygous repeat expansion; this individual had an early onset of bvFTD but typical clinical and pathological presentation within the spectrum of C9+ heterozygous disease. The authors of this report suggest that this case provides evidence that haploinsufficiency is not the only mechanism of C9+ disease as one would expect a more severe or different clinical phenotype associated with homozygous loss of C9ORF72 expression compared to heterozygous loss [61]. Toxic gain of function would be in line with an earlier onset but phenotypically similar form of C9+ disease, though it is also possible that presence of genetic or environmental disease modifiers play a role in this individual.

Sequestration of RNA-binding proteins into RNA foci

Another potential mechanism of toxicity involves the GGGGCC expansion itself, whereby toxic RNA foci are formed that sequester RNA-binding proteins and splicing factors such as TDP-43 and FUS, the latter of which was identified in rGGGGCC binding screen [40]. Both sense and antisense RNA foci have been identified via *in situ* hybridization, where they are

most abundant in neurons of the frontal cortex, and to a lesser extent in astrocytes, microglia and oligodendrocytes [62]. Accumulation of expanded RNA into toxic foci is a disease mechanism implicated in other neurodegenerative expansion disorders such as several spinocerebellar ataxias and fragile-X associated with tremor/ataxia syndrome (FXTAS) [1]. Screening for point mutations in C9ORF72 via sequencing of 389 ALS samples did not render any pathogenic variants, further suggesting that C9ORF72 pathogenesis is caused by a toxic gain of function due to RNA foci resulting from the non-coding expansion [63]. These RNA foci have the potential to sequester other RNA-binding proteins, which could result in widespread effects on transcriptional regulation and protein expression.

One RNA-binding protein critically linked to C9+ disease is TDP-43. As one of the main protein aggregates found in C9+ FTD/ALS, TDP-43 is a DNA- and RNA-binding protein that cycles between the nucleus and cytosol (though it localizes primarily to the nucleus) and plays numerous roles in RNA metabolism, including transcription and regulation of splicing, transport and translation, miRNA processing, and stress granule formation (reviewed in [38]). Mutations in *TARDBP*, which encodes TDP-43, cause ALS (reviewed in [64]). TDP-43 binds and regulates hundreds of RNA targets, including an enrichment of genes involved in neuronal development and synaptic function [65, 66]. TDP-43 is critical for early embryonic development of the central nervous system [67, 68] and plays an important role in the association and size of stress granules, which form transiently in response to cellular stress (e.g., [69]; reviewed in [38, 70, 71]). This suggests a possible mechanism by which early sequestration of TDP-43 could cause alterations in multiple proteins involved in neuronal development and function that could ultimately result in altered structural and/or network architecture that is vulnerable to diffuse cortical and subcortical damage. This would then be exacerbated by alterations in the cellular stress response due to altered stress granule dynamics.

Identification of specific RNA-binding proteins that bind the C9ORF72 GGGGCC repeat expansion is currently underway. In a recent screen, Xu, *et al.* found that rGGGGCC binds the RNA-binding protein Pur α , and overexpression of

Pur α rescues rGGGGCC-mediated neurodegeneration in *Drosophila* [41]. Pur α is involved in modulation of gene transcription, translation, controls cell cycle and differentiation and is a component of RNA-transport granules [72, 73]. The putative disease mechanism would thus be a loss of function of Pur α due to binding to rGGGGCC. Of note, Pur α also binds the *FXTAS* GCC repeat [74]. This model of neurodegeneration in *Drosophila* would thus argue against a primary role for loss of C9ORF72 function in disease pathogenesis.

Another screen for rGGGGCC RNA-binding proteins identified hnRNP A3, which forms p62+/TDP-43- neuronal cytoplasmic and intranuclear inclusions in hippocampus, as well as cerebellum in a subset of C9+ [40]. hnRNP A3 cycles between the nucleus and cytoplasm and is involved in alternative pre-mRNA splicing, nuclear import and cytoplasmic trafficking of mRNA, as well as mRNA stability, turnover and translation [75]. Expressed primarily in the nucleus of neurons, it appears to be redistributed to cytosol in its pathological state, similarly to TDP-43 and FUS [76-79]. The hnRNP A3 finding was not replicated by Xu, *et al.* but this discrepancy could relate to differences in binding conditions and protein concentrations [41]. Also of note, a screen in *Drosophila* for *FXTAS*-repeat associated changes in miRNA expression identified miRNA-277; hnRNP A2/B1 can directly regulate miRNA-277, which modulates CGG repeat-mediated neurodegeneration in *FXTAS* [80]. In iPSCs derived from C9+ ALS patients, repeat-containing RNA foci colocalized with hnRNPA1 and Pur α [81].

The ability of RNA foci to sequester RNA-binding proteins and thus alter the processing and expression of hundreds of distinct genes in a stochastic nature [38, 39] could result in markedly diverse forms of disease across different individuals. With known genetic modifiers (*TMEM106B*, described in more detail below) and variability in the number of hexanucleotide repeats it is not surprising that C9ORF72 expansion results in a diverse set of anatomical, clinical and pathologic phenotypes. Utilizing large datasets to identify patterns of RNA expression change across multiple C9+ individuals with the same clinical syndrome may be useful for dissecting the spectrum of changes that are most likely to predict a particular set of symptomatology. Targeting the cause of the

expression changes—that is, reducing RNA foci formation—may prove beneficial for C9+ carriers with distinct clinical presentations. In support of this notion, antisense oligonucleotides (ASOs) targeting the C9ORF72 transcript suppressed RNA foci formation and reversed gene expression changes and aberrant cell excitability associated with the pathologic expansion [81, 82] suggesting a potential therapeutic intervention.

RAN-dependent translation of GGGGCC expansions

Repeat-associated non-ATG (RAN)-dependent translation of dipeptides from both sense and anti-sense strands of the expanded hexanucleotide repeat in C9ORF72 form insoluble aggregates [42, 43, 83]. RAN translation of the sense strand creates poly Gly-Arg (poly-GR), poly Gly-Pro (poly-GP), and poly Gly-Ala (poly-GA) dipeptides which are hydrophobic and aggregation-prone; anti-sense RAN translation results in Pro-Ala, Pro-Gly, and Pro-Arg dipeptides. Using an antibody binding the poly-GP dipeptides, Ash, *et al.* showed variability in pathological location [42]. Highest presence included hippocampal regions, motor cortex, temporal and frontal cortices, amygdala, anterior and lateral thalamus, and Purkinje cells of the cerebellum. RAN-translated dipeptides have been shown to colocalize with p62+ inclusions [42, 43] in granule cells of the cerebellum, cells in the dentate gyrus, and the CA4 of the hippocampus [84].

The presence of inclusion bodies of these dipeptides does not appear to correlate with clinical severity or neurodegeneration (whereas TDP-43 pathology does), and has been suggested by some to be a protective response to coping with large numbers of dipeptides rather than a driving force of neurodegenerative processes [85]. This evidence, however, does not preclude the possibility that soluble forms of the dipeptides, or variation in the distribution of the different types of dipeptides across brain tissue, could contribute to the clinical and/or pathological manifestations of C9+ disease.

Formation of RAN-translated dipeptides can also be partially ameliorated with ASOs in mouse models [86] and iPSC-differentiated neurons [82], however, ASO intervention in C9+ iPSCs appears to ameliorate gene expression

and cellular deficits despite continued presence of RAN translated dipeptides [82], further suggesting that RAN-translated products may be a secondary or downstream mechanism which has less influence on pathology. Additional testing of this type of intervention in the context of clinical disease may help to determine the role that RAN-translated dipeptides play in C9ORF72 expansion-mediated disease.

Notably, the amount of RAN translation that occurs could alter the availability of rGGGGCC repeats to sequester RNA-binding proteins, since RAN translation would be expected to reduce the binding of proteins such as TDP-43 and Pur α . Thus it is possible that the amount of RNA-binding protein sequestration versus RAN-mediated translation that occurs in each cell is variable, offering yet another source of disease heterogeneity. If indeed dipeptide aggregates are not toxic to the cell [82, 85], then it stands to reason that formation of dipeptides through RAN-mediated translation may be an adaptive mechanism by which the cell attempts to limit the formation of RNA foci and sequestration of RNA-binding proteins. In line with this theory, Gendron, *et al.* found that RAN-translated poly-GP peptides infrequently colocalized with RNA foci [83]. For neurons with long axons, such as motor neurons, alterations in RNA-binding proteins may be particularly problematic (e.g., myotonic dystrophy) [87]. Thus, the balance of RAN translation versus RNA foci formation in particular neuronal subtypes could potentially affect disease pathogenesis and thus clinical presentation.

Other variables that may play a role in C9+ disease

Expansion-size differences across tissue: The C9ORF72 hexanucleotide repeat expansion length is highly variable and likely unstable due to surrounding genomic architecture ([55]; reviewed in [14]). C9 expansion size varies across different brain regions [88-90] and between monozygotic twins [89], and larger expansions may contribute to more potent pathology in the affected network of neurons. Three studies have investigated this with varying results. One study of blood samples found that C9+ length did not correlate with diagnostic group when comparing FTD, ALS, and other neurodegenerative phenotypes, but longer

expansion correlated with older age of onset [90]. However, other studies showed that expansion length varies across tissues (e.g., blood versus brain [88, 89]) suggesting measures from periphery may not be representative of expansion size in the brain [13]. Another study found that C9ORF72 expansion length did not correlate with FTD, FTD-MND or MND diagnostic groups in frontal cortex, cerebellum or blood samples; they found that longer frontal cortex expansion length correlated with older age of onset in FTD only, and that longer cerebellar expansion length was associated with reduced survival [88]. A third study did not find correlations between C9+ length in cerebellum and age of onset or disease duration, but found that cerebellar expansion length was higher in ALS versus FTD [89]. Thus, it remains unclear what role expansion length in different brain regions plays in C9+ disease.

Finally, evidence suggests that intermediate repeat expansion lengths that fall under the “pathologic” cutoff of 30 repeats but are above what is considered normal (less than 20) may serve as a risk factor for sporadic FTD [91], ALS [92], and PD [20, 21]. This is in line with evidence suggesting that intermediate repeat lengths are associated with reduced C9ORF72 expression, if protein haploinsufficiency plays a role in C9+ pathogenesis. Further work will be required to characterize the role of expansion length in pathological and clinical heterogeneity.

Genetic modifiers of C9ORF72 expansion disease: It is likely that genetic variation plays a role in modifying the pathological and clinical manifestation of C9+ disease. Mutations in other ALS-associated genes have now been found in C9+ carriers suggesting a two-hit model of disease (e.g., [93-96]), in line with the oligogenic theory of ALS, which suggests that harboring multiple risk variants in different ALS-associated genes is sufficient to cause disease (reviewed in [97-99]). C9+ patients that also carried deleterious variation in other FTD genes (*GRN* or *MAPT*) demonstrated early disease onset, bvFTD clinical presentation, and no motor neuron involvement suggesting a parallel two-hit model for FTD [100].

Common variation in other neurodegenerative disease associated genes may also contribute to clinical heterogeneity in C9+ carriers. This

relationship has been observed in patients with *GRN* mutations, where carrying the AD risk allele *APOE-ε4* resulted in exacerbated disease progression, amnesic syndromes and accompanying amyloid pathology [101]. For example, pathologic *C9ORF72* expansion coupled with a high-risk genetic polymorphism in an ALS gene could represent a risk mechanism predisposing some C9+ individuals to MND, whereas individuals without these additional risk variants may have a predominantly behavioral form of disease. This remains an untested hypothesis worth exploring in the context of disease-modifying risk genes.

In addition to exacerbating clinical presentation, genetic variation also has the potential to reduce disease risk. A recent study by van Blitterswijk, *et al.* identified variation in *TMEM106B*, which was previously associated with protection from FTD with TDP-43 pathology (FTD-TDP) [102, 103] as protective in C9+ patients with FTD but not MND [104]. One study also found that variation in *TMEM106B* protected against cognitive change in ALS patients [105]. Taken together, these results suggest that *TMEM106B* may broadly modify behavioral/cognitive symptoms associated with TDP-43 pathology and may thus represent a robust therapeutic target [106].

C9ORF72 expansion as a disease of dysfunctional cellular trafficking

Recent studies in model organisms *C. elegans* and zebrafish provide compelling evidence that loss of *C9ORF72* function is pathogenic to motor neurons [47, 48] and leads to motor deficits. While it is currently unclear if loss of *C9ORF72* function contributes to disease in C9+ carriers, the observation that *C9ORF72* transcript levels are reduced in patients with FTD and FTD-MND suggests that loss of protein function should be seriously considered as a disease mechanism.

What cellular consequences might be expected due to loss of *C9ORF72* function? Sophisticated homology searches have revealed that *C9ORF72* is a full-length homolog of the DENN family of Rab-GEFs, as noted above [49, 50]. While nothing is known about the cell biological function of mammalian *C9ORF72*, its yeast ortholog has been implicated in the sorting of endosome-localized proteins to the cell sur-

face, such that they do not reach the lysosome. If this function is conserved in humans, reduced *C9ORF72* levels might be associated with defects in the endo-lysosomal pathway. In addition, *TMEM106B*, the genetic modifier of both progranulin- and C9 expansion-associated FTD, has recently been implicated in lysosomal trafficking in neurons [52, 53]. In particular, *TMEM106B* appears to negatively regulate retrograde transport of lysosomes within dendrites, with reductions in *TMEM106B* associated with movement of lysosomes toward the neuronal soma [52]. Since *TMEM106B* influences lysosome function and modulates progranulin levels [52, 107], it is tempting to speculate that its protective role in C9+ carriers might similarly involve the endo-lysosomal pathway, providing a common link to two genetic forms of FTD. Finally, the finding that some C9+ carriers harbor unique Ub+/p62+/TDP-43 pathology further implicates dysfunctional autophagy, as p62 is a ubiquitin-binding protein which accumulates when autophagy is impaired [108]. Since lysosomal degradation is the ultimate endpoint of autophagy, defects in lysosomal trafficking or degradation would be expected to produce the observed Ub+/p62+/TDP-43 pathology that is seen in C9+ carriers. A mutation in the multivesicular body protein *CHMP2B* leading to familial FTD in a Danish pedigree further implicates dysregulation of the endo-lysosomal system as a pathological mechanism leading to FTD [109, 110].

C9ORF72 pathogenesis spreads through neuroanatomical networks

The underlying pattern of neurodegeneration in C9+ may be the best starting point for understanding how one type of genetic variant can result in such heterogeneous clinical presentations. The pattern of diffuse gray and white matter involvement observed in C9+ FTD/FTD-MND (reviewed in [9]) and ALS [10, 11] patients stands in contrast to the idea of neurodegenerative processes spreading through specific, clearly defined functional brain networks [111, 112]. Two intriguing hypotheses suggest how these patterns may fit into the 'selective vulnerability' framework: 1) the epicenter of vulnerability in C9+ neurodegeneration is highly and diffusely interconnected to both cortical and subcortical regions of the brain; 2) functional brain networks in *C9ORF72* expansion carriers are less strongly defined (i.e., there is more

inter-network connectivity than intra-network connectivity).

The first theory proposes a 'central station' node that serves as a major hub for multiple different pathways throughout the brain such that degeneration of that network would result in a diffuse pattern of cortical atrophy and profound white matter integrity loss. One such centrally connected subcortical structure is the thalamus. Divided into numerous functionally distinct nuclei, the thalamus receives sensory and motor information from a variety of cortical, cerebellar, and brainstem efferent projections, and then relays it through afferent projections to the cortex for further processing and integration. Each nucleus has specific afferent and efferent projections associated with it, and the nuclei themselves are also highly connected (reviewed in [113]). One longitudinal study of C9+ patients found neuroimaging patterns consistent with spread through such a distributed subcortical network, with thalamic and cerebellar atrophy most prominent while cortical atrophy appeared diffuse and nonspecific [114].

Given the behavioral component of FTD, the dorsomedial nucleus is one tempting candidate given its interconnectivity with the prefrontal, cingulate, and association cortices, and its involvement could also contribute to memory deficits observed in a subset of C9+ carriers [115, 116]. Also, the pulvinar nucleus dominates the posterior portion of the thalamus and is highly interconnected with the occipital cortex, as well as adjacent areas of the parietal and temporal cortices. These two nuclei, along with the lateral posterior—which receives afferent projects from occipital cortex and projects to the parietal cortex—make up the 'associative' functional group of thalamic nuclei involved in high level cognition [34]. The ventral anterior and ventral lateral nuclei receive inputs from basal ganglia and cerebellum, and project to premotor and motor areas of the frontal cortex, respectively, and along with the ventral posterior nucleus compose the 'effector' group involved with movement and aspects of language [34]. Functionally and anatomically, these two groups of thalamic nuclei represent domains affected in the clinical syndromes associated with C9ORF72 expansion thus far: bvFTD, ALS/MND and PPA. The role of the thalamus in C9+ disease remains to be elucidated through careful pathological dissection and

characterization, and may benefit from studies of resting state connectivity seeded within specific thalamic nuclei and studies of thalamic microstructural connectivity [117, 118].

In contrast to the central node hypothesis, the second theory suggests that the diffuse pattern of neurodegeneration observed in C9+ patients may be a by-product of damage that is spreading throughout multiple functional networks rather than being isolated in a single, defined functional circuit, and implicates early systemic disorganization as the underlying cause of diffuse non-selective spread. Reduced network connectivity has been observed even prior to symptom onset in Huntington's disease (HD), another neurodegenerative disorder caused by DNA repeat expansion in the *HTT* gene. Pathogenic *HTT* expansion carriers show lower cortico-striatal functional connectivity as compared to controls, even prior to disease onset [119]. Early changes in brain organization have been suggested in a transgenic rat model of HD [120], with differential aging patterns observed in the brains of transgenic rats as compared to wildtype as early as the first year of life [121]. Microstructure alterations in brain regions relevant to HD were also seen in these transgenic rats during postnatal development [122], though further study is required to determine if similar changes occur in people.

Identifying early changes in brain structure and function in C9ORF72-expansion carriers may help to disentangle these two hypotheses, which are not necessarily mutually exclusive. For example, a highly connected node of C9+ neurodegeneration could be identified during prodromic stages of disease, with longitudinal follow-up demonstrating insidious spread across multiple, interconnected functional networks of the brain. On the other hand, early animal experiments established that retrograde degeneration of thalamic nuclei occurs when damage is inflicted upon the cortical area that specific nucleus projects to [123], suggesting a mechanism by which widespread cortical loss across multiple networks could result in thalamic neurodegeneration.

Contributions of C9ORF72 expansion to clinical heterogeneity

In addition to the phenotypic heterogeneity highlighted in preceding sections, C9+ disease is also associated with other aspects of pheno-

typic variability. As suggested by slowly progressive C9+ bvFTD cases, there is a large variation in the length of disease course; some groups have suggested that C9+ patients demonstrate longer disease courses than matched sporadic cases (e.g., [124]) whereas others have observed shorter durations of disease (reviewed in [13]). Age of onset is also highly variable, ranging from the 20's – 80's [13], with 50% penetrance by age 58 and nearly full penetrance by age 80 [125]. One report, however, described two C9+ carriers with no cognitive impairments as of ages 80 and 84, suggesting that C9ORF72 expansion has incomplete penetrance [126]. Whether slowly progressive forms of C9+ bvFTD and predominant psychiatric presentations are a result of reduced expansion size or other genetic or environmental modifiers remains to be established.

In sporadic neurodegenerative disease, there appears to be a sudden precipitous drop in cognitive function several years before a full clinical symptom manifests [127, 128]. However, in a genetically mediated adult-onset disease it is difficult to deny the neurodevelopmental aspect – how is the brain of a disease-causing gene carrier different from that of a non-carrier? Does the brain learn to 'adapt' to deficits, and only during the aging process—which weakens neural plasticity—does dysfunction become apparent? Is there slow, insidious accumulation of pathology in the neurons such that, only after 50+ years, it comes to the point where neurons are being killed? Or are there subtle signs that there is underlying dysfunction from the outset, but these go unrecognized until the symptoms become impossible to ignore?

Whether a prodrome of neurodegenerative disease exists remains unanswered; gene carriers may provide a unique opportunity to study disease in its earliest stages, prior to frank symptom onset. For example, early personality/behavioral changes have been described in some C9+ carriers [129]. In C9+ bvFTD patients, there is often emotional dysregulation reminiscent of cerebellar disconnection syndrome [8, 34]. If subtle alterations in emotional and/or physiological regulation reflect progressive neural dysfunction from a central node or due to systemic disorganization as proposed above, then measures of these features could provide a quantitative measure of these underlying pathological processes as they progress

into a full clinical syndrome. Studies of sporadic bvFTD suggest that patients often have psychiatric diagnoses years before referral to the neurology clinic [130]. Whether these are simply misdiagnoses of an underlying neurodegenerative process or are, in fact, early manifestations of FTD remain to be determined.

If C9+ pathogenesis begins in the thalamus, then the molecular mechanism of spread through the interconnected networks of the thalamic nuclei could involve physical spread of toxic TDP-43 pathology in a seeded fashion [131], or functional spread whereby changes in synaptic activity in the thalamus could result in downstream neuronal dysfunction. In mouse, C9ORF72 is robustly expressed in the thalamus [23], and unique Ub+/p62+/TDP-43-pathology is often found in the thalamus of C9+ carriers, supporting a mechanism whereby molecular changes resulting from C9ORF72 expansion could begin in this central subcortical region and then, over time, affect other regions of the brain through its interconnectedness with cerebellar and diffuse cortical structures.

Regardless of the mechanism, the fundamental leap to identifying effective biomarkers for making predictions of clinical prognosis and disease progression will require linking peripheral measures of disease with local pathological processes. This may include tracking changes in the expression of C9ORF72 transcripts or other genes dysregulated (directly or indirectly) by hexanucleotide-generated RNA foci and/or RAN-translated dipeptides. Multimodal neuroimaging may also serve as a sensitive measure of C9-specific changes in gray and white matter structures over time [114, 132], even in presymptomatic carriers.

Concluding remarks

In summary, C9ORF72-mediated disease is characterized by heterogeneous clinical presentations of motor and/or behavioral syndromes of ALS, bvFTD, or FTD-MND, as well as less common diagnoses of PPA, primary amnesic presentation and psychiatric disease such as depression or bipolar disorder. Parkinsonism is also a common symptom accompanying these clinical diagnoses. Three main molecular mechanisms of C9+ disease have emerged as potential contributors to this

observed clinical heterogeneity: haploinsufficiency resulting in a loss of C9ORF72 protein function, formation of RNA foci resulting in a toxic gain of function, and formation of dipeptide aggregates resulting from RAN-mediated sense and antisense translation of the hexanucleotide expansion. Variable expansion length across tissue types and brain regions as well as contributions of other genetic modifiers may provide additional sources of disease heterogeneity. In addition, C9+ diseases may be associated with alterations in cellular trafficking, particularly within the endo-lysosomal pathway. The mode of spread of one or more of these contributing pathological mechanisms could occur via a centrally located neural hub connecting multiple selectively vulnerable functional networks, or through multiple, interconnected networks converging on a common neuroanatomical region. The diversity of clinicopathology demonstrated by C9+ patients suggests a spectrum of disease manifestations that ultimately culminate in unique protein pathology (Ub+/p62+/TDP-43- in the cerebellum and hippocampus) and neuroanatomical damage (thalamic atrophy).

Elucidation of novel genetic and molecular modifiers of C9-mediated disease progression will provide the opportunity for development of therapeutic interventions. In addition, identification of biomarkers that predict future clinical syndrome will be critical for identification of candidates for clinical trials. Finally, gaining a better understanding of the preclinical manifestations of disease – whether they are behavioral, functional or physiological—will also provide deeper insight into the workings of the neuroanatomical system most vulnerable to C9ORF72 expansion disease.

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Disclosure of conflict of interest

None.

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References

- [1] DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H, Adamson J, Kouri N, Wojtas A, Sengdy P, Hsiung GYR, Karydas A, Seeley WW, Josephs KA, Coppola G, Geschwind DH, Wszolek ZK, Feldman H, Knopman DS, Petersen RC, Miller BL, Dickson DW, Boylan KB, Graff-Radford NR and Rademakers R. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 2011; 72: 245-256.
- [2] Renton AE, Majounie E, Waite A, Simón-Sánchez J, Rollinson S, Gibbs JR, Schymick JC, Laaksovirta H, van Swieten JC, Myllykangas L, Kalimo H, Paetau A, Abramzon Y, Remes AM, Kaganovich A, Scholz SW, Duckworth J, Ding J, Harmer DW, Hernandez DG, Johnson JO, Mok K, Ryten M, Trabzuni D, Guerreiro RJ, Orrell RW, Neal J, Murray A, Pearson J, Jansen IE, Sondervan D, Seelaar H, Blake D, Young K, Halliwell N, Callister JB, Toulson G, Richardson A, Gerhard A, Snowden J, Mann D, Neary D, Nalls MA, Peuralinna T, Jansson L, Isoviiita VM, Kaivorinne AL, Hölttä-Vuori M, Ikonen E, Sulikava R, Benatar M, Wu J, Chiò A, Restagno G, Borghero G, Sabatelli M, Consortium I, Heckerman D, Rogaeve E, Zinman L, Rothstein JD, Sendtner M, Drepper C, Eichler EE, Alkan C, Abdullaev Z, Pack SD, Dutra A, Pak E, Hardy J, Singleton A, Williams NM, Heutink P, Pickering-Brown S, Morris HR, Tienari PJ and Traynor BJ. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 2011; 72: 257-268.
- [3] Mackenzie IRA, Neumann M, Baborie A, Sampathu DM, Du Plessis D, Jaros E, Perry RH, Trojanowski JQ, Mann DMA and Lee VMY. A harmonized classification system for FTLD-TDP pathology. *Acta Neuropathologica* 2011; 122: 111-113.
- [4] Al-Sarraj S, King A, Troakes C, Smith B, Maekawa S, Bodi I, Rogelj B, Al-Chalabi A, Hortobágyi T and 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 Neuropathologica* 2011; 122: 691-702.
- [5] Bigio EH, Weintraub S, Rademakers R, Baker M, Ahmadian SS, Rademaker A, Weitner BB, Mao Q, Lee KH, Mishra M, Ganti RA and Mesulam MM. Frontotemporal lobar degeneration with TDP-43 proteinopathy and chromosome

C9ORF72 in behavioral and motor disease

- 9p repeat expansion in C9ORF72: clinicopathologic correlation. *Neuropathology* 2013; 33: 122-33.
- [6] Cooper-Knock J, Hewitt C, Highley JR, Brockington A, Milano A, Man S, Martindale J, Hartley J, Walsh T, Gelsthorpe C, Baxter L, Forster G, Fox M, Bury J, Mok K, McDermott CJ, Traynor BJ, Kirby J, Wharton SB, Ince PG, Hardy J and Shaw PJ. Clinico-pathological features in amyotrophic lateral sclerosis with expansions in C9ORF72. *Brain* 2012; 135: 751-764.
- [7] Hsiung GYR, DeJesus-Hernandez M, Feldman HH, Sengdy P, Bouchard-Kerr P, Dwosh E, Butler R, Leung B, Fok A, Rutherford NJ, Baker M, Rademakers R and Mackenzie IRA. Clinical and pathological features of familial frontotemporal dementia caused by C9ORF72 mutation on chromosome 9p. *Brain* 2012; 135: 709-722.
- [8] Mahoney CJ, Beck J, Rohrer JD, Lashley T, Mok K, Shakespeare T, Yeatman T, Warrington EK, Schott JM, Fox NC, Rossor MN, Hardy J, Collinge J, Revesz T, Mead S and Warren JD. Frontotemporal dementia with the C9ORF72 hexanucleotide repeat expansion: clinical, neuroanatomical and neuropathological features. *Brain* 2012; 135: 736-750.
- [9] Yokoyama JS and Rosen HJ. Neuroimaging features of C9ORF72 expansion. *Alzheimers Res Ther* 2012; 4: 45.
- [10] Bede P, Bokde AL, Byrne S, Elamin M, McLaughlin RL, Kenna K, Fagan AJ, Pender N, Bradley DG and Hardiman O. Multiparametric MRI study of ALS stratified for the C9orf72 genotype. *Neurology* 2013; 81: 361-369.
- [11] Byrne S, Elamin M, Bede P, Shatunov A, Walsh C, Corr B, Heverin M, Jordan N, Kenna K, Lynch C, McLaughlin RL, Iyer PM, O'Brien C, Phukan J, Wynne B, Bokde AL, Bradley DG, Pender N, Al-Chalabi A and Hardiman O. Cognitive and clinical characteristics of patients with amyotrophic lateral sclerosis carrying a C9orf72 repeat expansion: a population-based cohort study. *Lancet Neurol* 2012; 11: 232-240.
- [12] Irwin DJ, McMillan CT, Brettschneider J, Libon DJ, Powers J, Rascovsky K, Toledo JB, Boller A, Bekisz J, Chandrasekaran K, Wood EM, Shaw LM, Woo JH, Cook PA, Wolk DA, Arnold SE, Van Deerlin VM, McCluskey LF, Elman L, Lee VMY, Trojanowski JQ and Grossman M. Cognitive decline and reduced survival in C9orf72 expansion frontotemporal degeneration and amyotrophic lateral sclerosis. *J Neurol Neurosurg Psychiatr* 2013; 84: 163-169.
- [13] van Blitterswijk M, DeJesus-Hernandez M and Rademakers R. How do C9ORF72 repeat expansions cause amyotrophic lateral sclerosis and frontotemporal dementia: can we learn from other noncoding repeat expansion disorders? *Curr Opin Neurol* 2012; 25: 689-700.
- [14] Cooper-Knock J, Shaw PJ and Kirby J. The widening spectrum of C9ORF72-related disease; genotype/phenotype correlations and potential modifiers of clinical phenotype. *Acta Neuropathol* 2014; 127: 333-45.
- [15] van Rheenen W, van Blitterswijk M, Huisman MH, Vlam L, van Doormaal PT, Seelen M, Medic J, Dooijes D, de Visser M, van der Kooij AJ, Raaphorst J, Schelhaas HJ, van der Pol WL, Veldink JH and van den Berg LH. Hexanucleotide repeat expansions in C9ORF72 in the spectrum of motor neuron diseases. *Neurology* 2012; 79: 878-882.
- [16] Boeve BF, Boylan KB, Graff-Radford NR, DeJesus-Hernandez M, Knopman DS, Pedraza O, Vemuri P, Jones D, Lowe V, Murray ME, Dickson DW, Josephs KA, Rush BK, Machulda MM, Fields JA, Ferman TJ, Baker M, Rutherford NJ, Adamson J, Wszolek ZK, Adeli A, Savica R, Boot B, Kuntz KM, Gavrilova R, Reeves A, Whitwell J, Kantarci K, Jack CR, Parisi JE, Lucas JA, Petersen RC and Rademakers R. Characterization of frontotemporal dementia and/or amyotrophic lateral sclerosis associated with the GGGGCC repeat expansion in C9ORF72. *Brain* 2012; 135: 765-783.
- [17] Lesage S, Le Ber I, Condroyer C, Broussolle E, Gabelle A, Thobois S, Pasquier F, Mondon K, Dion PA, Rochefort D, Rouleau GA, Durr A and Brice A. C9orf72 repeat expansions are a rare genetic cause of parkinsonism. *Brain* 2013; 136: 385-391.
- [18] Cooper-Knock J, Frolov A, Highley JR, Charlesworth G, Kirby J, Milano A, Hartley J, Ince PG, McDermott CJ, Lashley T, Revesz T, Shaw PJ, Wood NW and Bandmann O. C9ORF72 expansions, parkinsonism, and Parkinson disease: a clinicopathologic study. *Neurology* 2013; 81: 808-811.
- [19] Lindquist SG, Duno M, Batbayli M, Puschmann A, Braendgaard H, Mardosiene S, Svenstrup K, Pinborg LH, Vestergaard K, Hjermand LE, Stokholm J, Andersen BB, Johannsen P and Nielsen JE. Corticobasal and ataxia syndromes widen the spectrum of C9ORF72 hexanucleotide expansion disease. *Clin Genet* 2013; 83: 279-283.
- [20] Jiao B, Guo JF, Wang YQ, Yan XX, Zhou L, Liu XY, Zhang FF, Zhou YF, Xia K, Tang BS and Shen L. C9orf72 mutation is rare in Alzheimer's disease, Parkinson's disease, and essential tremor in China. *Front Cell Neurosci* 2013; 7: 164.
- [21] Nuytemans K, Bademci G, Kohli MM, Beecham GW, Wang L, Young JL, Nahab F, Martin ER, Gilbert JR, Benatar M, Haines JL, Scott WK, Zuchner S, Pericak-Vance MA and Vance JM. C9ORF72 Intermediate Repeat Copies Are a

C9ORF72 in behavioral and motor disease

- Significant Risk Factor for Parkinson Disease. *Ann Hum Genet* 2013; 77: 351-363.
- [22] Le Ber I, Camuzat A, Guillot-Noel L, Hannequin D, Lacomblez L, Golfier V, Puel M, Martinaud O, Deramecourt V, Rivaud-Pechoux S, Millecamps S, Vercelletto M, Couratier P, Sellal F, Pasquier F, Salachas F, Thomas-Anterion C, Didic M, Pariente J, Seilhean D, Ruberg M, Wargon I, Blanc F, Camu W, Michel BF, Berger E, Sauvee M, Thauvin-Robinet C, Mondon K, Tournier-Lasserre E, Goizet C, Fleury M, Viennet G, Verpillat P, Meininger V, Duyckaerts C, Dubois B and Brice A. C9ORF72 repeat expansions in the frontotemporal dementias spectrum of diseases: a flow-chart for genetic testing. *J Alzheimers Dis* 2013; 34: 485-499.
- [23] Suzuki N, Maroof AM, Merkle FT, Koszka K, Intoh A, Armstrong I, Moccia R, Davis-Dusenbery BN and Eggan K. The mouse C9ORF72 ortholog is enriched in neurons known to degenerate in ALS and FTD. *Nat Neurosci* 2013; 16: 1725-1727.
- [24] Smith Y, Bevan MD, Shink E and Bolam JP. Microcircuitry of the direct and indirect pathways of the basal ganglia. *Neuroscience* 1998; 86: 353-387.
- [25] Boeve BF and Graff-Radford NR. Cognitive and behavioral features of c9FTD/ALS. *Alzheimers Res Ther* 2012; 4: 29.
- [26] Khan BK, Yokoyama JS, Takada LT, Sha SJ, Rutherford NJ, Fong JC, Karydas AM, Wu T, Kettelle RS, Baker MC, Hernandez MD, Coppola G, Geschwind DH, Rademakers R, Lee SE, Rosen HJ, Rabinovici GD, Seeley WW, Rankin KP, Boxer AL and Miller BL. Atypical, slowly progressive behavioural variant frontotemporal dementia associated with C9ORF72 hexanucleotide expansion. *J Neurol Neurosurg Psychiatr* 2012; 83: 358-364.
- [27] Simón-Sánchez J, Dopfer EGP, Cohn-Hokke PE, Hukema RK, Nicolaou N, Seelaar H, de Graaf JRA, de Koning I, van Schoor NM, Deeg DJH, Smits M, Raaphorst J, van den Berg LH, Schelhaas HJ, De Die-Smulders CEM, Majoor-Krakauer D, Rozemuller AJM, Willemsen R, Pijnenburg YAL, Heutink P and van Swieten JC. The clinical and pathological phenotype of C9ORF72 hexanucleotide repeat expansions. *Brain* 2012; 135: 723-735.
- [28] Suhonen NM, Kaivorinne AL, Moilanen V, Bode M, Takalo R, Hanninen T and Remes AM. Slowly progressive frontotemporal lobar degeneration caused by the C9ORF72 repeat expansion: a 20-year follow-up study. *Neurocase* 2014; [Epub ahead of print].
- [29] Williamson C, Alcantar O, Rothlind J, Cahn-Weiner D, Miller BL and Rosen HJ. Standardised measurement of self-awareness deficits in FTD and AD. *J Neurol Neurosurg Psychiatry* 2010; 81: 140-145.
- [30] Takada LT and Sha SJ. Neuropsychiatric features of C9orf72-associated behavioral variant frontotemporal dementia and frontotemporal dementia with motor neuron disease. *Alzheimers Res Ther* 2012; 4: 38.
- [31] Kertesz A, Ang LC, Jesso S, MacKinley J, Baker M, Brown P, Shoemsmith C, Rademakers R and Finger EC. Psychosis and hallucinations in frontotemporal dementia with the C9ORF72 mutation: a detailed clinical cohort. *Cogn Behav Neurol* 2013; 26: 146-154.
- [32] Landqvist Waldo M, Gustafson L, Nilsson K, Traynor BJ, Renton AE, Englund E and Passant U. Frontotemporal dementia with a C9ORF72 expansion in a Swedish family: clinical and neuropathological characteristics. *Am J Neurodegener Dis* 2013; 2: 276-286.
- [33] Sturm VE, Yokoyama JS, Seeley WW, Kramer JH, Miller BL and Rankin KP. Heightened emotional contagion in mild cognitive impairment and Alzheimer's disease is associated with temporal lobe degeneration. *Proc Natl Acad Sci U S A* 2013; 110: 9944-9949.
- [34] Schmahmann JD and Pandya DN. Disconnection syndromes of basal ganglia, thalamus, and cerebrocerebellar systems. *Cortex* 2008; 44: 1037-1066.
- [35] Wojtas A, Heggeli KA, Finch N, Baker M, DeJesus-Hernandez M, Younkin SG, Dickson DW, Graff-Radford NR and Rademakers R. C9ORF72 repeat expansions and other FTD gene mutations in a clinical AD patient series from Mayo Clinic. *Am J Neurodegener Dis* 2012; 1: 107-118.
- [36] Adeli A, Savica R, Lowe VJ, Vemuri P, Knopman DS, DeJesus-Hernandez M, Rademakers R, Fields JA, Crum BA, Jack CR, Petersen RC and Boeve BF. The GGGGCC repeat expansion in C9ORF72 in a case with discordant clinical and FDG-PET findings: PET trumps syndrome. *Neurocase* 2014; 20: 110-120.
- [37] Irish M, Devenney E, Wong S, Dobson-Stone C, Kwok JB, Piguet O, Hodges JR and Hornberger M. Neural substrates of episodic memory dysfunction in behavioural variant frontotemporal dementia with and without C9ORF72 expansions. *Neuroimage Clin* 2013; 2: 836-843.
- [38] Ling SC, Polymenidou M and Cleveland DW. Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. *Neuron* 2013; 79: 416-438.
- [39] Polymenidou M, Lagier-Tourenne C, Hutt KR, Bennett CF, Cleveland DW and Yeo GW. Misregulated RNA processing in amyotrophic lateral sclerosis. *Brain Res* 2012; 1462: 3-15.
- [40] Mori K, Lammich S, Mackenzie IRA, Forné I, Zilow S, Kretzschmar H, Edbauer D, Janssens J, Kleinberger G, Cruts M, Herms J, Neumann M, Van Broeckhoven C, Arzberger T and Haass

- C. 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.
- [41] Xu Z, Poidevin M, Li X, Li Y, Shu L, Nelson DL, Li H, Hales CM, Gearing M, Wingo TS and Jin P. Expanded GGGGCC repeat RNA associated with amyotrophic lateral sclerosis and frontotemporal dementia causes neurodegeneration. *Proc Natl Acad Sci U S A* 2013; 110: 7778-83.
- [42] Ash PEA, Bieniek KF, Gendron TF, Caulfield T, Lin WL, DeJesus-Hernandez M, van Blitterswijk MM, Jansen-West K, Paul JW, Rademakers R, Boylan KB, Dickson DW and Petrucelli L. Unconventional translation of C9ORF72 GGGGCC expansion generates insoluble polypeptides specific to c9FTD/ALS. *Neuron* 2013; 77: 639-646.
- [43] Mori K, Weng SM, Arzberger T, May S, Rentzsch K, Kremmer E, Schmid B, Kretzschmar HA, Cruts M, Van Broeckhoven C, Haass C and Edbauer D. The C9orf72 GGGGCC Repeat Is Translated into Aggregating Dipeptide-Repeat Proteins in FTL/ALS. *Science* 2013; 339: 1335-8.
- [44] Meyer K, Ferraiuolo L, Miranda CJ, Likhite S, McElroy S, Rensch S, Ditsworth D, Lagier-Tourenne C, Smith RA, Ravits J, Burghes AH, Shaw PJ, Cleveland DW, Kolb SJ and Kaspar BK. Direct conversion of patient fibroblasts demonstrates non-cell autonomous toxicity of astrocytes to motor neurons in familial and sporadic ALS. *Proc Natl Acad Sci U S A* 2014; 111: 829-832.
- [45] Belzil VV, Bauer PO, Prudencio M, Gendron TF, Stetler CT, Yan IK, Pregent L, Daugherty L, Baker MC, Rademakers R, Boylan K, Patel TC, Dickson DW and Petrucelli L. Reduced C9orf72 gene expression in c9FTD/ALS is caused by histone trimethylation, an epigenetic event detectable in blood. *Acta Neuropathol* 2013; 126: 895-905.
- [46] Xi Z, Zinman L, Moreno D, Schymick J, Liang Y, Sato C, Zheng Y, Ghani M, Dib S, Keith J, Robertson J and Rogaeva E. Hypermethylation of the CpG island near the G4C2 repeat in ALS with a C9orf72 expansion. *Am J Hum Genet* 2013; 92: 981-989.
- [47] Ciura S, Lattante S, Le Ber I, Latouche M, Tosvint H, Brice A and Kabashi E. Loss of function of C9orf72 causes motor deficits in a zebrafish model of Amyotrophic Lateral Sclerosis. *Ann Neurol* 2013; [Epub ahead of print].
- [48] Therrien M, Rouleau GA, Dion PA and Parker JA. Deletion of C9ORF72 Results in Motor Neuron Degeneration and Stress Sensitivity in *C. elegans*. *PLoS One* 2013; 8: e83450.
- [49] Zhang D, Iyer LM, He F and Aravind L. Discovery of Novel DENN Proteins: Implications for the Evolution of Eukaryotic Intracellular Membrane Structures and Human Disease. *Front Genet* 2012; 3: 283.
- [50] Levine TP, Daniels RD, Gatta AT, Wong LH and Hayes MJ. The product of C9orf72, a gene strongly implicated in neurodegeneration, is structurally related to DENN Rab-GEFs. *Bioinformatics* 2013; 29: 499-503.
- [51] Rubio-Teixeira M and Kaiser CA. Amino acids regulate retrieval of the yeast general amino acid permease from the vacuolar targeting pathway. *Mol Biol Cell* 2006; 17: 3031-3050.
- [52] Schwenk BM, Lang CM, Hogl S, Tahirovic S, Orozco D, Rentzsch K, Lichtenthaler SF, Hoogenraad CC, Capell A, Haass C and Edbauer D. The FTL/ALS risk factor TMEM106B and MAP6 control dendritic trafficking of lysosomes. *EMBO J* 2014; 33: 450-67.
- [53] Brady OA, Zheng Y, Murphy K, Huang M and Hu F. The frontotemporal lobar degeneration risk factor, TMEM106B, regulates lysosomal morphology and function. *Hum Mol Genet* 2013; 22: 685-695.
- [54] Smith KR, Damiano J, Franceschetti S, Carpenter S, Canafoglia L, Morbin M, Rossi G, Pareyson D, Mole SE, Staropoli JF, Sims KB, Lewis J, Lin WL, Dickson DW, Dahl HH, Bahlo M and Berkovic SF. Strikingly different clinicopathological phenotypes determined by progranulin-mutation dosage. *Am J Hum Genet* 2012; 90: 1102-1107.
- [55] Van Der Zee J, Gijssels I, Dillen L, Van Langenhove T, Theuns J, Engelborghs S, Philtjens S, Vandenbulcke M, Sleegers K, Sieben A, Bäumer V, Maes G, Corsmit E, Borroni B, Padovani A, Archetti S, Pernecky R, Diehl-Schmid J, De Mendonça A, Miltenberger-Miltenyi G, Pereira S, Pimentel J, Nacmias B, Bagnoli S, Sorbi S, Graff C, Chiang HH, Westerlund M, Sanchez-Valle R, Llado A, Gelpi E, Santana I, Almeida MR, Santiago B, Frisoni G, Zanetti O, Bonvicini C, Synofzik M, Maetzler W, Vom Hagen JM, Schöls L, Heneka MT, Jessen F, Matej R, Parobkova E, Kovacs GG, Ströbel T, Sarafov S, Tournev I, Jordanova A, Danek A, Arzberger T, Fabrizi GM, Testi S, Salmon E, Santens P, Martin JJ, Cras P, Vandenberghe R, De Deyn PP, Cruts M, Van Broeckhoven C, Van Der Zee J, Gijssels I, Dillen L, Van Langenhove T, Theuns J, Philtjens S, Sleegers K, Bäumer V, Maes G, Corsmit E, Cruts M, Van Broeckhoven C, Van Der Zee J, Gijssels I, Dillen L, Van Langenhove T, Philtjens S, Theuns J, Sleegers K, Bäumer V, Maes G, Cruts M, Van Broeckhoven C, Engelborghs S, De Deyn PP, Cras P, Engelborghs S, De Deyn PP, Vandenbulcke M, Vandenbulcke M, Borroni B, Padovani A, Archetti S,

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- Perneczky R, Diehl-Schmid J, Synofzik M, Maetzler W, Müller Vom Hagen J, Schöls L, Synofzik M, Maetzler W, Müller Vom Hagen J, Schöls L, Heneka MT, Jessen F, Ramirez A, Kurzwelly D, Sachtleben C, Mairer W, De Mendonça A, Miltenberger-Miltenyi G, Pereira S, Fermo C, Pimentel J, Sanchez-Valle R, Llado A, Antonell A, Molinuevo J, Gelpi E, Graff C, Chiang HH, Westerlund M, Graff C, Kinhult Ståhlbom A, Thonberg H, Nennesmo I, Börjesson-Hanson A, Nacmias B, Bagnoli S, Sorbi S, Bessi V, Piaceri I, Santana I, Santiago B, Santana I, Helena Ribeiro M, Rosário Almeida M, Oliveira C, Massano J, Garret C, Pires P, Frisoni G, Zanetti O, Bonvicini C, Sarafov S, Tournev I, Jordanova A, Tournev I, Kovacs GG, Ströbel T, Heneka MT, Jessen F, Ramirez A, Kurzwelly D, Sachtleben C, Mairer W, Jessen F, Matej R, Parobkova E, Danel A, Arzberger T, Maria Fabrizio G, Testi S, Ferrari S, Cavallaro T, Salmon E, Santens P, Cras P; European Early-Onset Dementia Consortium. A pan-European study of the C9orf72 repeat associated with FTLD: geographic prevalence, genomic instability, and intermediate repeats. *Hum Mutat* 2013; 34: 363-373.
- [56] Fratta P, Mizielinska S, Nicoll AJ, Zloh M, Fisher EMC, Parkinson G and Isaacs AM. C9orf72 hexanucleotide repeat associated with amyotrophic lateral sclerosis and frontotemporal dementia forms RNA G-quadruplexes. *Sci Rep* 2012; 2: 1016.
- [57] Reddy K, Zamiri B, Stanley SYR, Macgregor RB Jr and Pearson CE. The disease-associated r(GGGGCC)_n repeat from the C9ORF72 gene forms tract length-dependent uni- and multi-molecular RNA G-quadruplex structures. *J Biol Chem* 2013; 288: 9860-6.
- [58] Siddiqui-Jain A, Grand CL, Bearss DJ and Hurley LH. Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. *Proc Natl Acad Sci U S A* 2002; 99: 11593-11598.
- [59] Neidle S. Human telomeric G-quadruplex: the current status of telomeric G-quadruplexes as therapeutic targets in human cancer. *FEBS J* 2010; 277: 1118-1125.
- [60] Faudale M, Cogoi S and Xodo LE. Photoactivated cationic alkyl-substituted porphyrin binding to g4-RNA in the 5'-UTR of KRAS oncogene represses translation. *Chem Commun (Camb)* 2012; 48: 874-876.
- [61] Fratta P, Poulter M, Lashley T, Rohrer JD, Polke JM, Beck J, Ryan N, Hensman D, Mizielinska S, Waite AJ, Lai MC, Gendron TF, Petrucelli L, Fisher EM, Revesz T, Warren JD, Collinge J, Isaacs AM and Mead S. Homozygosity for the C9orf72 GGGGCC repeat expansion in frontotemporal dementia. *Acta Neuropathol* 2013; 126: 401-409.
- [62] Mizielinska S, Lashley T, Norona FE, Clayton EL, Ridler CE, Fratta P and Isaacs AM. C9orf72 frontotemporal lobar degeneration is characterised by frequent neuronal sense and antisense RNA foci. *Acta Neuropathol* 2013; 126: 845-857.
- [63] Harms MB, Cady J, Zaidman C, Cooper P, Bali T, Allred P, Cruchaga C, Baughn M, Libby RT, Pestronk A, Goate A, Ravits J and Baloh RH. Lack of C9ORF72 coding mutations supports a gain of function for repeat expansions in amyotrophic lateral sclerosis. *Neurobiol Aging* 2013; 34: 2234, e2213-2239.
- [64] Lattante S, Conte A, Zollino M, Luigetti M, Del Grande A, Marangi G, Romano A, Marcaccio A, Meleo E, Bisogni G, Rossini PM and Sabatelli M. Contribution of major amyotrophic lateral sclerosis genes to the etiology of sporadic disease. *Neurology* 2012; 79: 66-72.
- [65] Polymenidou M, Lagier-Tourenne C, Hutt KR, Huelga SC, Moran J, Liang TY, Ling SC, Sun E, Wancewicz E, Mazur C, Kordasiewicz H, Sedghat Y, Donohue JP, Shiue L, Bennett CF, Yeo GW and Cleveland DW. Long pre-mRNA depletion and RNA missplicing contribute to neuronal vulnerability from loss of TDP-43. *Nat Neurosci* 2011; 14: 459-68.
- [66] Sephton CF, Cenik C, Kucukural A, Dammer EB, Cenik B, Han Y, Dewey CM, Roth FP, Herz J, Peng J, Moore MJ and Yu G. Identification of neuronal RNA targets of TDP-43-containing ribonucleoprotein complexes. *J Biol Chem* 2011; 286: 1204-1215.
- [67] Sephton CF, Cenik B, Cenik BK, Herz J and Yu G. TDP-43 in central nervous system development and function: clues to TDP-43-associated neurodegeneration. *Biol Chem* 2012; 393: 589-594.
- [68] Sephton CF, Good SK, Atkin S, Dewey CM, Mayer P 3rd, Herz J and Yu G. TDP-43 is a developmentally regulated protein essential for early embryonic development. *J Biol Chem* 2010; 285: 6826-6834.
- [69] Dewey CM, Cenik B, Sephton CF, Dries DR, Mayer P 3rd, Good SK, Johnson BA, Herz J and Yu G. TDP-43 is directed to stress granules by sorbitol, a novel physiological osmotic and oxidative stressor. *Mol Cell Biol* 2011; 31: 1098-1108.
- [70] Dewey CM, Cenik B, Sephton CF, Johnson BA, Herz J and Yu G. TDP-43 aggregation in neurodegeneration: are stress granules the key? *Brain Res* 2012; 1462: 16-25.
- [71] Bentmann E, Haass C and Dormann D. Stress granules in neurodegeneration—lessons learnt from TAR DNA binding protein of 43 kDa and fused in sarcoma. *FEBS J* 2013; 280: 4348-4370.

C9ORF72 in behavioral and motor disease

- [72] White MK, Johnson EM and Khalili K. Multiple roles for Puralpha in cellular and viral regulation. *Cell Cycle* 2009; 8: 1-7.
- [73] Kanai Y, Dohmae N and Hirokawa N. Kinesin transports RNA: isolation and characterization of an RNA-transporting granule. *Neuron* 2004; 43: 513-525.
- [74] Aumiller V, Graebisch A, Kremmer E, Niessing D and Förstemann K. Drosophila Pur- α binds to trinucleotide-repeat containing cellular RNAs and translocates to the early oocyte. *RNA Biol* 2012; 9: 633-643.
- [75] He Y and Smith R. Nuclear functions of heterogeneous nuclear ribonucleoproteins A/B. *Cell Mol Life Sci* 2009; 66: 1239-1256.
- [76] Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, Mann D, Tsuchiya K, Yoshida M, Hashizume Y and Oda T. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* 2006; 351: 602-611.
- [77] Kwiatkowski TJ Jr, Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, Russ C, Davis A, Gilchrist J, Kasarskis EJ, Munsat T, Valdmanis P, Rouleau GA, Hosler BA, Cortelli P, de Jong PJ, Yoshinaga Y, Haines JL, Pericak-Vance MA, Yan J, Ticozzi N, Siddique T, McKenna-Yasek D, Sapp PC, Horvitz HR, Landers JE and Brown RH Jr. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* 2009; 323: 1205-1208.
- [78] Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, McCluskey LF, Miller BL, Masliah E, Mackenzie IR, Feldman H, Feiden W, Kretzschmar HA, Trojanowski JQ and Lee VM. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 2006; 314: 130-133.
- [79] Vance C, Rogelj B, Hortobagyi T, De Vos KJ, Nishimura AL, Sreedharan J, Hu X, Smith B, Ruddy D, Wright P, Ganesalingam J, Williams KL, Tripathi V, Al-Saraj S, Al-Chalabi A, Leigh PN, Blair IP, Nicholson G, de Bellerocche J, Gallo JM, Miller CC and Shaw CE. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* 2009; 323: 1208-1211.
- [80] Tan H, Poidevin M, Li H, Chen D and Jin P. MicroRNA-277 modulates the neurodegeneration caused by Fragile X premutation rCGG repeats. *PLoS Genet* 2012; 8: e1002681.
- [81] Sareen D, O'Rourke JG, Meera P, Muhammad AK, Grant S, Simpkinson M, Bell S, Carmona S, Ornelas L, Sahabian A, Gendron T, Petrucelli L, Baughn M, Ravits J, Harms MB, Rigo F, Bennett CF, Otis TS, Svendsen CN and Baloh RH. Targeting RNA foci in iPSC-derived motor neurons from ALS patients with a C9ORF72 repeat expansion. *Sci Transl Med* 2013; 5: 208ra149.
- [82] Donnelly Christopher J, Zhang PW, Pham Jacqueline T, Haeusler Aaron R, Mistry Nipun A, Vidensky S, Daley Elizabeth L, Poth Erin M, Hoover B, Fines Daniel M, Maragakis N, Tienari Pentti J, Petrucelli L, Traynor Bryan J, Wang J, Rigo F, Bennett CF, Blackshaw S, Sattler R and Rothstein Jeffrey D. RNA Toxicity from the ALS/FTD C9ORF72 Expansion Is Mitigated by Antisense Intervention. *Neuron* 2013; 80: 415-428.
- [83] Gendron TF, Bieniek KF, Zhang YJ, Jansen-West K, Ash PE, Caulfield T, Daugherty L, Dunmore JH, Castanedes-Casey M, Chew J, Cosio DM, van Blitterswijk M, Lee WC, Rademakers R, Boylan KB, Dickson DW and Petrucelli L. 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.
- [84] Mann DM, Rollinson S, Robinson A, Bennion Callister J, Thompson JC, Snowden JS, Gendron T, Petrucelli L, Masuda-Suzukake M, Hasegawa M, Davidson Y and Pickering-Brown S. Dipeptide repeat proteins are present in the p62 positive inclusions in patients with frontotemporal lobar degeneration and motor neuron disease associated with expansions in C9ORF72. *Acta Neuropathol Commun* 2013; 1: 68.
- [85] Mackenzie IR, Arzberger T, Kremmer E, Troost D, Lorenzl S, Mori K, Weng SM, Haass C, Kretzschmar HA, Edbauer D and Neumann M. Dipeptide repeat protein pathology in C9ORF72 mutation cases: clinico-pathological correlations. *Acta Neuropathol* 2013; 126: 859-879.
- [86] Lagier-Tourenne C, Baughn M, Rigo F, Sun S, Liu P, Li HR, Jiang J, Watt AT, Chun S, Katz M, Qiu J, Sun Y, Ling SC, Zhu Q, Polymenidou M, Drenner K, Artates JW, McAlonis-Downes M, Markmiller S, Hutt KR, Pizzo DP, Cady J, Harms MB, Baloh RH, Vandenberg SR, Yeo GW, Fu XD, Bennett CF, Cleveland DW and Ravits J. 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: E4530-4539.
- [87] Roy S, Zhang B, Lee VM and Trojanowski JQ. Axonal transport defects: a common theme in neurodegenerative diseases. *Acta Neuropathol* 2005; 109: 5-13.
- [88] van Blitterswijk M, DeJesus-Hernandez M, Niemannsverdriet E, Murray ME, Heckman MG, Diehl NN, Brown PH, Baker MC, Finch NA, Bauer PO, Serrano G, Beach TG, Josephs KA, Knopman DS, Petersen RC, Boeve BF, Graff-

C9ORF72 in behavioral and motor disease

- Radford NR, Boylan KB, Petrucelli L, Dickson DW and Rademakers R. Association between repeat sizes and clinical and pathological characteristics in carriers of C9ORF72 repeat expansions (Xpansize-72): a cross-sectional cohort study. *Lancet Neurol* 2013; 12: 978-988.
- [89] Dols-Icardo O, Garcia-Redondo A, Rojas-Garcia R, Sanchez-Valle R, Noguera A, Gomez-Tortosa E, Pastor P, Hernandez I, Esteban-Perez J, Suarez-Calvet M, Anton-Aguirre S, Amer G, Ortega-Cubero S, Blesa R, Fortea J, Alcolea D, Capdevila A, Antonell A, Llado A, Munoz-Blanco JL, Mora JS, Galan-Davila L, Rodriguez De Rivera FJ, Lleo A and Clarimon J. Characterization of the repeat expansion size in C9orf72 in amyotrophic lateral sclerosis and frontotemporal dementia. *Hum Mol Genet* 2014; 23: 749-754.
- [90] Beck J, Poulter M, Hensman D, Rohrer JD, Mahoney CJ, Adamson G, Campbell T, Uphill J, Borg A, Fratta P, Orrell RW, Malaspina A, Rowe J, Brown J, Hodges J, Sidle K, Polke JM, Houlden H, Schott JM, Fox NC, Rossor MN, Tabrizi SJ, Isaacs AM, Hardy J, Warren JD, Collinge J and Mead S. Large C9orf72 Hexanucleotide Repeat Expansions Are Seen in Multiple Neurodegenerative Syndromes and Are More Frequent Than Expected in the UK Population. *Am J Hum Genet* 2013; 92: 345-53.
- [91] Benussi L, Rossi G, Glionna M, Tonoli E, Piccoli E, Fostinelli S, Paterlini A, Flocco R, Albani D, Pantieri R, Cereda C, Forloni G, Tagliavini F, Binetti G and Ghidoni R. C9ORF72 hexanucleotide repeat number in frontotemporal lobar degeneration: a genotype-phenotype correlation study. *J Alzheimers Dis* 2014; 38: 799-808.
- [92] Byrne S, Heverin M, Elamin M, Walsh C and Hardiman O. Intermediate repeat expansion length in C9orf72 may be pathological in amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Frontotemporal Degener* 2014; 15: 148-50.
- [93] Kaivorinne AL, Moilanen V, Kervinen M, Renton AE, Traynor BJ, Majamaa K and Remes AM. Novel TARDBP Sequence Variant and C9ORF72 Repeat Expansion in a Family With Frontotemporal Dementia. *Alzheimer Dis Assoc Disord* 2012; [Epub ahead of print].
- [94] Chio A, Restagno G, Brunetti M, Ossola I, Calvo A, Canosa A, Moglia C, Floris G, Tacconi P, Marrosu F, Marrosu MG, Murru MR, Majounie E, Renton AE, Abramzon Y, Pugliatti M, Sotgiu MA, Traynor BJ and Borghero G. ALS/FTD phenotype in two Sardinian families carrying both C9ORF72 and TARDBP mutations. *J Neurol Neurosurg Psychiatry* 2012; 83: 730-733.
- [95] Millecamps S, Boillee S, Le Ber I, Seilhean D, Teyssou E, Giraudeau M, Moigneu C, Vandenberghe N, Danel-Brunaud V, Corcia P, Pradat PF, Le Forestier N, Lacomblez L, Bruneteau G, Camu W, Brice A, Cazeneuve C, Leguern E, Meininger V and Salachas F. Phenotype difference between ALS patients with expanded repeats in C9ORF72 and patients with mutations in other ALS-related genes. *J Med Genet* 2012; 49: 258-263.
- [96] Williams KL, Fifita JA, Vucic S, Durnall JC, Kieran MC, Blair IP and Nicholson GA. Pathophysiological insights into ALS with C9ORF72 expansions. *J Neurol Neurosurg Psychiatr* 2013; 84: 931-5.
- [97] van Blitterswijk M, van Es MA, Hennekam EA, Dooijes D, van Rheenen W, Medic J, Bourque PR, Schelhaas HJ, van der Kooij AJ, de Visser M, de Bakker PI, Veldink JH and van den Berg LH. Evidence for an oligogenic basis of amyotrophic lateral sclerosis. *Hum Mol Genet* 2012; 21: 3776-3784.
- [98] Turner MR, Hardiman O, Benatar M, Brooks BR, Chio A, de Carvalho M, Ince PG, Lin C, Miller RG, Mitsumoto H, Nicholson G, Ravits J, Shaw PJ, Swash M, Talbot K, Traynor BJ, Van den Berg LH, Veldink JH, Vucic S and Kiernan MC. Controversies and priorities in amyotrophic lateral sclerosis. *Lancet Neurol* 2013; 12: 310-322.
- [99] Renton AE, Chio A and Traynor BJ. State of play in amyotrophic lateral sclerosis genetics. *Nat Neurosci* 2014; 17: 17-23.
- [100] van Blitterswijk M, Baker MC, DeJesus-Hernandez M, Ghidoni R, Benussi L, Finger E, Hsiung GY, Kelley BJ, Murray ME, Rutherford NJ, Brown PE, Ravenscroft T, Mullen B, Ash PE, Bieniek KF, Hatanpaa KJ, Karydas A, Wood EM, Coppola G, Bigio EH, Lippa C, Strong MJ, Beach TG, Knopman DS, Huey ED, Mesulam M, Bird T, White CL 3rd, Kertesz A, Geschwind DH, Van Deerlin VM, Petersen RC, Binetti G, Miller BL, Petrucelli L, Wszolek ZK, Boylan KB, Graff-Radford NR, Mackenzie IR, Boeve BF, Dickson DW and Rademakers R. C9ORF72 repeat expansions in cases with previously identified pathogenic mutations. *Neurology* 2013; 81: 1332-1341.
- [101] Perry DC, Lehmann M, Yokoyama JS, Karydas A, Lee JJ, Coppola G, Grinberg LT, Geschwind D, Seeley WW, Miller BL, Rosen H and Rabinovici G. Progranulin mutations as risk factors for Alzheimer disease. *JAMA Neurol* 2013; 70: 774-778.
- [102] Finch N, Carrasquillo MM, Baker M, Rutherford NJ, Coppola G, DeJesus-Hernandez M, Crook R, Hunter T, Ghidoni R, Benussi L, Crook J, Finger E, Hatanpaa KJ, Karydas AM, Sengdy P, Gonzalez J, Seeley WW, Johnson N, Beach TG, Mesulam M, Forloni G, Kertesz A, Knopman DS, Uitti R, White CL, Caselli R, Lippa C, Bigio EH, Wszolek ZK, Binetti G, Mackenzie IR, Miller BL,

- Boeve BF, Younkin SG, Dickson DW, Petersen RC, Graff-Radford NR, Geschwind DH and Rademakers R. TMEM106B regulates progranulin levels and the penetrance of FTL in GRN mutation carriers. *Neurology* 2011; 76: 467-74.
- [103] Van Deerlin VM, Sleiman PMA, Martinez-Lage M, Chen-Plotkin A, Wang LS, Graff-Radford NR, Dickson DW, Rademakers R, Boeve BF, Grossman M, Arnold SE, Mann DMA, Pickering-Brown SM, Seelaar H, Heutink P, van Swieten JC, Murrell JR, Ghetti B, Spina S, Grafman J, Hodges J, Spillantini MG, Gilman S, Lieberman AP, Kaye JA, Woltjer RL, Bigio EH, Mesulam M, Al-Sarraj S, Troakes C, Rosenberg RN, White CL, Ferrer I, Lladó A, Neumann M, Kretzschmar HA, Hulette CM, Welsh-Bohmer KA, Miller BL, Alzualde A, Lopez de Munain A, McKee AC, Gearing M, Levey AI, Lah JJ, Hardy J, Rohrer JD, Lashley T, Mackenzie IRA, Feldman HH, Hamilton RL, Dekosky ST, Van Der Zee J, Kumar-Singh S, Van Broeckhoven C, Mayeux R, Vonsattel JPG, Troncoso JC, Kril JJ, Kwok JBJ, Halliday GM, Bird TD, Ince PG, Shaw PJ, Cairns NJ, Morris JC, McLean CA, DeCarli C, Ellis WG, Freeman SH, Frosch MP, Growdon JH, Perl DP, Sano M, Bennett DA, Schneider JA, Beach TG, Reiman EM, Woodruff BK, Cummings J, Vinters HV, Miller CA, Chui HC, Alafuzoff I, Hartikainen P, Seilhean D, Galasko D, Masliah E, Cotman CW, Tuñón MT, Martínez MCC, Munoz DG, Carroll SL, Marson D, Riederer PF, Bogdanovic N, Schellenberg GD, Hakonarson H, Trojanowski JQ and Lee VMY. Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. *Nat Genet* 2010; 42: 234-239.
- [104] van Blitterswijk M, Mullen B, Nicholson AM, Bieniek KF, Heckman MG, Baker MC, DeJesus-Hernandez M, Finch NA, Brown PH, Murray ME, Hsiung GY, Stewart H, Karydas AM, Finger E, Kertesz A, Bigio EH, Weintraub S, Mesulam M, Hatanpaa KJ, White Iii CL, Strong MJ, Beach TG, Wszolek ZK, Lippa C, Caselli R, Petrucelli L, Josephs KA, Parisi JE, Knopman DS, Petersen RC, Mackenzie IR, Seeley WW, Grinberg LT, Miller BL, Boylan KB, Graff-Radford NR, Boeve BF, Dickson DW and Rademakers R. TMEM106B protects C9ORF72 expansion carriers against frontotemporal dementia. *Acta Neuropathol* 2014; 127: 397-406.
- [105] Vass R, Ashbridge E, Geser F, Hu WT, Grossman M, Clay-Falcone D, Elman L, McCluskey L, Lee VMY, Van Deerlin VM, Trojanowski JQ and Chen-Plotkin AS. Risk genotypes at TMEM106B are associated with cognitive impairment in amyotrophic lateral sclerosis. *Acta Neuropathol* 2011; 121: 373-80.
- [106] Van Der Zee J and Van Broeckhoven C. TMEM106B a novel risk factor for frontotemporal lobar degeneration. *J Mol Neurosci* 2011; 45: 516-521.
- [107] Chen-Plotkin AS, Unger TL, Gallagher MD, Bill E, Kwong LK, Volpicelli-Daley L, Busch JI, Akle S, Grossman M, Van Deerlin V, Trojanowski JQ and Lee VMY. TMEM106B, the Risk Gene for Frontotemporal Dementia, Is Regulated by the microRNA-132/212 Cluster and Affects Progranulin Pathways. *J Neurosci* 2012; 32: 11213-11227.
- [108] Moscat J and Diaz-Meco MT. p62 at the crossroads of autophagy, apoptosis, and cancer. *Cell* 2009; 137: 1001-1004.
- [109] Skibinski G, Parkinson NJ, Brown JM, Chakrabarti L, Lloyd SL, Hummerich H, Nielsen JE, Hodges JR, Spillantini MG, Thusgaard T, Brandner S, Brun A, Rossor MN, Gade A, Johannsen P, Sorensen SA, Gydesen S, Fisher EM and Collinge J. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. *Nat Genet* 2005; 37: 806-808.
- [110] Urwin H, Authier A, Nielsen JE, Metcalf D, Powell C, Froud K, Malcolm DS, Holm I, Johannsen P, Brown J, Fisher EMC, van der Zee J, Bruylant M, Consortium F, Van Broeckhoven C, Collinge J, Brandner S, Futter C and Isaacs AM. Disruption of endocytic trafficking in frontotemporal dementia with CHMP2B mutations. *Hum Mol Genet* 2010; 19: 2228-2238.
- [111] Seeley WW, Crawford RK, Zhou J, Miller BL and Greicius MD. Neurodegenerative diseases target large-scale human brain networks. *Neuron* 2009; 62: 42-52.
- [112] Zhou J, Gennatas Efstathios D, Kramer Joel H, Miller Bruce L and Seeley William W. Predicting Regional Neurodegeneration from the Healthy Brain Functional Connectome. *Neuron* 2012; 73: 1216-1227.
- [113] Schmahmann JD. Vascular syndromes of the thalamus. *Stroke* 2003; 34: 2264-2278.
- [114] Mahoney CJ, Downey LE, Ridgway GR, Beck J, Clegg S, Blair M, Finnegan S, Leung KK, Yeatman T, Golden H, Mead S, Rohrer JD, Fox NC and Warren JD. Longitudinal neuroimaging and neuropsychological profiles of frontotemporal dementia with C9ORF72 expansions. *Alzheimers Res Ther* 2012; 4: 41.
- [115] Klein JC, Rushworth MF, Behrens TE, Mackay CE, de Crespigny AJ, D'Arceuil H and Johansen-Berg H. Topography of connections between human prefrontal cortex and mediodorsal thalamus studied with diffusion tractography. *Neuroimage* 2010; 51: 555-564.
- [116] Mitchell AS and Chakraborty S. What does the mediodorsal thalamus do? *Front Syst Neurosci* 2013; 7: 37.

C9ORF72 in behavioral and motor disease

- [117] Johansen-Berg H, Behrens TEJ, Sillery E, Ciccarelli O, Thompson AJ, Smith SM and Matthews PM. Functional-Anatomical Validation and Individual Variation of Diffusion Tractography-based Segmentation of the Human Thalamus. *Cerebral Cortex* 2005; 15: 31-39.
- [118] Hughes EJ, Bond J, Svrckova P, Makropoulos A, Ball G, Sharp DJ, Edwards AD, Hajnal JV and Counsell SJ. Regional changes in thalamic shape and volume with increasing age. *Neuroimage* 2012; 63: 1134-42.
- [119] Unschuld PG, Joel SE, Liu X, Shanahan M, Margolis RL, Biglan KM, Bassett SS, Schretlen DJ, Redgrave GW, van Zijl PC, Pekar JJ and Ross CA. Impaired cortico-striatal functional connectivity in prodromal Huntington's Disease. *Neurosci Lett* 2012; 514: 204-209.
- [120] von Horsten S, Schmitt I, Nguyen HP, Holzmann C, Schmidt T, Walther T, Bader M, Pabst R, Kobbe P, Krotova J, Stiller D, Kask A, Vaarmann A, Rathke-Hartlieb S, Schulz JB, Grasshoff U, Bauer I, Vieira-Saecker AM, Paul M, Jones L, Lindenberg KS, Landwehrmeyer B, Bauer A, Li XJ and Riess O. Transgenic rat model of Huntington's disease. *Hum Mol Genet* 2003; 12: 617-624.
- [121] Blockx I, Van Camp N, Verhoye M, Boisgard R, Dubois A, Jegu B, Jonckers E, Raber K, Siquier K, Kuhnast B, Dolle F, Nguyen HP, Von Horsten S, Tavitian B and Van der Linden A. Genotype specific age related changes in a transgenic rat model of Huntington's disease. *Neuroimage* 2011; 58: 1006-1016.
- [122] Blockx I, De Groof G, Verhoye M, Van Audekerke J, Raber K, Poot D, Sijbers J, Osmand AP, Von Horsten S and Van der Linden A. Microstructural changes observed with DKI in a transgenic Huntington rat model: evidence for abnormal neurodevelopment. *Neuroimage* 2012; 59: 957-967.
- [123] Rose JE. Some Modern Aspects of the Functional Anatomy of the Thalamus. *Mschr Psychiatr Neurol* 1950; 120: 378-387.
- [124] Sha SJ, Takada LT, Rankin KP, Yokoyama JS, Rutherford NJ, Fong JC, Khan B, Karydas A, Baker MC, DeJesus-Hernandez M, Pribadi M, Coppola G, Geschwind DH, Rademakers R, Lee SE, Seeley W, Miller BL, Boxer AL. Frontotemporal dementia due to C9ORF72 mutations: Clinical and imaging features. *Neurology* 2012; 79: 1002-11.
- [125] Majounie E, Renton AE, Mok K, Dopper EG, Waite A, Rollinson S, Chio A, Restagno G, Nicolaou N, Simon-Sanchez J, van Swieten JC, Abramzon Y, Johnson JO, Sendtner M, Pampillet R, Orrell RW, Mead S, Sidle KC, Houlden H, Rohrer JD, Morrison KE, Pall H, Talbot K, An-sorge O, Hernandez DG, Arepalli S, Sabatelli M, Mora G, Corbo M, Giannini F, Calvo A, Englund E, Borghero G, Floris GL, Remes AM, Laakso-virta H, McCluskey L, Trojanowski JQ, Van Deerlin VM, Schellenberg GD, Nalls MA, Drory VE, Lu CS, Yeh TH, Ishiura H, Takahashi Y, Tsuji S, Le Ber I, Brice A, Drepper C, Williams N, Kirby J, Shaw P, Hardy J, Tienari PJ, Heutink P, Morris HR, Pickering-Brown S and Traynor BJ. 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.
- [126] Galimberti D, Arosio B, Fenoglio C, Serpente M, Cioffi SM, Bonsi R, Rossi P, Abbate C, Mari D and Scarpini E. Incomplete Penetrance of the C9ORF72 Hexanucleotide Repeat Expansions: Frequency in a Cohort of Geriatric Non-Demented Subjects. *J Alzheimers Dis* 2014; 39: 19-22.
- [127] Small BJ and Bäckman L. Longitudinal Trajectories of Cognitive Change in Preclinical Alzheimer's Disease: A Growth Mixture Modeling Analysis. *Cortex* 2007; 43: 826-834.
- [128] Amieva H, Jacqmin-Gadda H, Orgogozo JM, Le Carret N, Helmer C, Letenneur L, Barberger-Gateau P, Fabrigoule C and Dartigues JF. The 9 year cognitive decline before dementia of the Alzheimer type: a prospective population-based study. *Brain* 2005; 128: 1093-1101.
- [129] Savica R, Adeli A, Vemuri P, Knopman DS, DeJesus-Hernandez M, Rademakers R, Fields JA, Whitwell J, Jack CR, Lowe V, Petersen RC and Boeve BF. Characterization of a family with c9FTD/ALS associated with the GGGGCC repeat expansion in C9ORF72. *Arch Neurol* 2012; 69: 1164-1169.
- [130] Woolley JD, Khan BK, Murthy NK, Miller BL and Rankin KP. The diagnostic challenge of psychiatric symptoms in neurodegenerative disease: rates of and risk factors for prior psychiatric diagnosis in patients with early neurodegenerative disease. *J Clin Psychiatry* 2011; 72: 126-133.
- [131] Guo JL and Lee VM. Cell-to-cell transmission of pathogenic proteins in neurodegenerative diseases. *Nat Med* 2014; 20: 130-138.
- [132] Whitwell JL, Weigand SD, Boeve BF, Senjem ML, Gunter JL, DeJesus-Hernandez M, Rutherford NJ, Baker M, Knopman DS, Wszolek ZK, Parisi JE, Dickson DW, Petersen RC, Rademakers R, Jack CR and Josephs KA. Neuroimaging signatures of frontotemporal dementia genetics: C9ORF72, tau, progranulin and sporadics. *Brain* 2012; 135: 794-806.