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How do *C9ORF72* repeat expansions cause ALS and FTD: can we learn from other non-coding repeat expansion disorders?

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Abstract

Purpose of review—The aim of this review is to describe disease mechanisms by which chromosome 9 open reading frame 72 (*C9ORF72*) repeat expansions could lead to amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), and to discuss these diseases in relation to other non-coding repeat expansion disorders.

Recent findings—ALS and FTD are complex neurodegenerative disorders with a considerable clinical and pathological overlap, and this overlap is further substantiated by the recent discovery of *C9ORF72* repeat expansions. These repeat expansions are currently the most important genetic cause of familial ALS and FTD, accounting for approximately 34.2% and 25.9% of the cases. Clinical phenotypes associated with these repeat expansions are highly variable, and combinations with mutations in other ALS- and/or FTD-associated genes may contribute to this pleiotropy. It is challenging, however, to diagnose patients with *C9ORF72* expansions, not only because of large repeat sizes, but also due to somatic heterogeneity. Most other non-coding repeat expansion disorders share an RNA gain-of-function disease mechanism, a mechanism that could underlie the development of ALS and/or FTD as well.

Summary—The discovery of *C9ORF72* repeat expansions provides novel insights into the pathogenesis of ALS and FTD, and highlights the importance of non-coding repeat expansions and RNA toxicity in neurodegenerative diseases.

Keywords

Amyotrophic lateral sclerosis; frontotemporal dementia; non-coding repeat expansion disorders; *C9ORF72*; genetics

Introduction

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are fatal neurodegenerative disorders for which no effective treatments are available. ALS is the most frequent motor neuron disease, resulting in progressive weakness and death from respiratory failure, typically within three years of symptom onset [1*]. FTD is the second most common cause of early-onset dementia, and is characterized by behavior and personality changes and/or language dysfunction, due to degeneration of the frontal and temporal cortex [2–5]. Most FTD patients die 5–10 years after symptom onset.

Clinicopathological studies have long supported the concept that ALS and FTD may represent a disease continuum with a shared underlying pathogenesis [6*,7*,8]. They often

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co-occur in a family, and the prevalence of frontal lobe impairment in ALS populations may approach 50% [9–11]. Similarly, as many as half of FTD patients develop clinical symptoms of motor neuron dysfunction [12]. Especially important was the identification of the transactive response DNA-binding protein 43 (TDP-43) in 2006, as the major inclusion protein in the vast majority of ALS patients and in the most common pathological subtype of FTD, now referred to as frontotemporal lobar degeneration with TDP-43 pathology (FTLD-TDP) [13,14].

Both diseases are etiologically complex with genetic and presumably environmental factors contributing to its onset [15**,16]. A positive family history has been reported in ~10% of ALS patients, and up to 50% of FTD patients [2,17]. Nonetheless, no genes were identified that sufficiently explained the growing class of families in which affected members developed either ALS or FTD or both (ALS-FTD). Last year, however, two independent studies identified hexanucleotide repeat expansions in the chromosome 9 open reading frame 72 gene (*C9ORF72*) [18**,19**]. This important discovery raised new hope with clinicians and researchers for the development of treatments, and provided novel avenues for studying and understanding the disease pathogenesis of ALS and FTD.

In this review, we underscore the importance of the *C9ORF72* mutations in ALS and FTD, and review current hypotheses related to its disease mechanism(s) and associated phenotypes. We also discuss this novel mutation in relation to other repeat expansion disorders, especially myotonic dystrophy type 1 (DM1).

Identification of repeat expansions in *C9ORF72* and possible disease mechanism(s)

Previously, genetic studies convincingly linked ALS and FTD to a region on chromosome 9p21 [20–24,25*,26,27]. Although these studies were able to minimize the region, the genetic defect remained elusive until last year, when two independent groups described a GGGGCC hexanucleotide repeat in a non-coding region of *C9ORF72* [18**,19**]. One of these groups focused on family VSM-20, a large ALS-FTD family, and used primers flanking the repeat region to amplify the region and to determine the size of the repeat in affected and unaffected family members [18**]. Intriguingly, their results appeared to indicate that all affected individuals were homozygous for the repeat, while none of their affected children seemed to have inherited their alleles. Their findings underlined the need for an alternative methodology, since a pathogenic repeat expansion may not be amplified by a conventional PCR, and therefore, they developed a repeat-primed PCR assay, resulting in the identification of the pathogenic repeat expansion. The other group employed next-generation sequencing to study a Welsh ALS-FTD family [19**]. Their efforts revealed a drop off in the sequence coverage in the region of the repeat, emphasizing the polymorphic nature of this region, which eventually led to the detection of the pathogenic repeat expansion.

The GGGGCC hexanucleotide repeat is located between two five prime non-coding exons of *C9ORF72*, which encodes a completely uncharacterized protein with unknown function. Two different isoforms of the protein are predicted to be generated from a total of three or more different *C9ORF72* transcripts. Unexpectedly, several groups showed reduced levels of at least one *C9ORF72* transcript in expanded-repeat carriers, suggesting a possible loss-of-function disease mechanism [18**,19**,28*]. The accumulation of transcripts containing the GGGGCC repeat as nuclear RNA foci in the frontal cortex and spinal cord of *C9ORF72* mutation carriers, however, has also been demonstrated, favoring a toxic RNA gain-of-function disease mechanism in line with most other non-coding expansion disorders [18**].

Frequency of *C9ORF72* repeat expansions

Until the discovery of the pathogenic repeat expansion in *C9ORF72* only 20–30% of the familial ALS cases could be explained by mutations in the superoxide dismutase-1 gene (*SOD1*), and the genes encoding TDP-43 (*TARDBP*) and fused in sarcoma (*FUS*), while mutations in the microtubule-associated protein tau gene (*MAPT*), progranulin gene (*GRN*), and, less commonly, the valosin containing protein gene (*VCP*) and the charged multivesicular body protein 2b gene (*CHMP2B*) were responsible for 20–30% of the FTD cases [15**,29**,30*,31*]. Mutations in these genes were also present in 1–5% of the sporadic ALS and FTD cases.

To date, less than a year after the discovery, more than thirty articles have described frequencies of *C9ORF72* repeat expansions in ALS and FTD populations: from the United States of America to Europe, Australia and Asia [18**,19**,28*,29**,32**–41*,42**–46*,47**–49*,50**–52*,53**–56*,57**]. In Figure 1, we have provided a graphic representation of these frequencies. Even though heterogeneity between populations and study design hampers this comparison (Supplementary Table 1) our representation suggests that *C9ORF72* mutations account for 34.2% (standard error [SE] 4.5) of the familial ALS cases, 5.9% (SE 1.3) of the sporadic ALS cases, 25.9% (SE 5.9) of the familial FTD cases, 5.1% (SE 2.0) of the sporadic FTD cases, and 0.17% (SE 0.07) of the control subjects. Hence, these frequencies underline that *C9ORF72* repeat expansions are currently the major genetic cause of ALS and/or FTD worldwide.

Oligogenic etiology

Interestingly, more than twenty patients have been described that harbor mutations in *C9ORF72* in combination with mutations in other ALS- and/or FTD-associated genes [29**,33*,36*,42**,43*,51*,58*,59*]. In these patients, mutations were also detected in *SOD1*, *TARDBP*, *FUS*, angiogenin (*ANG*), optineurin (*OPTN*), ubiquilin-2 (*UBQLN2*), vesicle-associated membrane protein B (*VAPB*), D-amino-acid oxidase (*DAO*), peripherin (*PRPH*), *GRN* and presenilin-2 (*PSEN2*). It is important to note, however, that more than half of these additional mutations have also been reported in control subjects, and that their effects, as predicted by *in silico* programs, remain unclear (Table 1). Although it could therefore be argued that they merely represent benign polymorphisms, a recent ALS study has demonstrated that the frequency of multiple mutations is higher than expected on the basis of chance [29**]. This could indicate that these mutations act as disease modifiers, which could contribute to the pleiotropy that is encountered in patients with *C9ORF72* mutations.

Clinicopathological phenotypes associated with *C9ORF72* repeat expansions

Clinical data of patients with *C9ORF72* mutations demonstrates that approximately 55.8% (SE 2.4) is male; the mean age at onset is 56.1 years (SE 0.9), and the mean disease duration is 49.9 months (SE 4.9) (Table 2 and Supplementary Table 2) [18**,28*,36*–38*,42**,44*–47**,49*,50**–52*,53**–56*,57**,69*,70*]. Importantly, age at onset and disease duration are highly variable, even within a single family. Based on the current literature, the age at onset ranges from 27 to 83 years [46*,47**,56*], and the disease duration varies between 3 and 264 months [47**,52*,54*]. Nevertheless, there seems to be a tendency towards a younger age at onset and shorter disease duration in patients with *C9ORF72* repeat expansions, as compared to patients without them [28*,45*,46*,49*,51*,56*].

Approximately 29.3% (SE 3.3) of the patients with *C9ORF72* mutations displays symptoms of both ALS and FTD (Table 2). Of the patients with ALS, 44.2% (SE 5.0) presents with a

bulbar onset of symptoms, which is higher than the expected frequency of 19–30% [72]. In 81.0% (SE 5.0) of the FTD patients with *C9ORF72* mutations, the behavioral variant is detected, while the expected frequency is ~50% [31*].

Additional symptoms have also been described in patients with *C9ORF72* mutations, including signs of parkinsonism and psychotic phenomena [46*,50**,51*,54*,55*,69*, 73,74*–77*]. Furthermore, in patients with clinical diagnoses of Alzheimer's disease (AD), Parkinson disease (PD), corticobasal syndrome (CBS), and olivopontocerebellar degeneration (OPCD) *C9ORF72* mutations have been detected as well, but they appear to be rare, and may be due to clinical misdiagnoses (<3%) [75*,78*–80*,81]. All these findings highlight the substantial clinical heterogeneity that is detected in patients with *C9ORF72* mutations, both between and in families [82**].

Apart from clinical studies, neuropathological investigations have shown that repeat expansions in *C9ORF72* are characterized by TDP-43 pathology in various neuroanatomical regions, and ubiquitin-positive but TDP-43-negative neuronal cytoplasmic inclusions in the cerebellar granular layer, hippocampal pyramidal neurons and other neuroanatomical sites, which are unique to *C9ORF72* repeat expansions carriers [18**,28*,45*,50**,51*,52*,54*–57**,69*,70*,83*]. Several research groups are now focusing on identifying the nature of the ubiquitinated protein in these TDP-43 negative inclusions, as it may shed light on the disease mechanism(s) associated with *C9ORF72* expansions.

Critical issues associated with size and sequence composition of *C9ORF72* repeats

In the general population, the vast majority of the *C9ORF72* alleles contain two to thirty GGGGCC hexanuceotide repeats [18**,19**]. Affected individuals with *C9ORF72* mutations harbor one normal allele and one expanded allele with hundreds to thousands of these repeats [18**,19**]. Consequently, a cut-off of thirty repeats is commonly used to differentiate between pathogenic and non-pathogenic repeat sizes [19**]. It is important to realize, however, that repeat sizes of thirty or more are also present in approximately 0.17% of the control subjects (Supplementary Table 1) [18**,19**,28*,29**,33*,35*,38*,42**–44*,46*,47**,49*,51*,53**,54*,57**], and therefore, a cut-off of thirty repeats should be used with caution.

A second problem relates to the repeat-primed PCR method that is commonly used to screen ALS and FTD patients. Even though this method is fast and cost effective, it does not provide an accurate estimate of the number of repeats. Therefore, Southern blot analysis should be used to estimate the number of repeats in expanded repeat carriers, however, its application appears to be challenging, and currently only a handful of samples has been tested [18**,39*]. Moreover, neither repeat-primed PCR methods nor Southern blot analysis are able to reveal the actual DNA composition of the expanded repeat, and thus, new protocols and methods are needed to guarantee more reliable diagnostic testing.

Finally, somatic heterogeneity is likely to be common in repeat expansion carriers, resulting in varying repeat sizes in different tissues from a single patient. As a result, repeat sizes determined using DNA extracted from whole blood may not adequately reflect the *C9ORF72* repeat sizes in a patient's brain or spinal cord tissue, and may hamper genotype-phenotype correlations.

Can we learn from other repeat expansion disorders?

Pathogenic repeat expansions have already been identified in at least 24 other neurological disorders [84–86**] (Table 3). In coding regions, repeat expansions can cause long stretches of amino acids, for instance, of polyglutamine or polyalanine. These stretches can disrupt the normal function of encoded proteins and result in toxic aggregate formation [104,105**]. The underlying mechanism of non-coding repeat expansions disorders, on the other hand, most commonly involves an RNA gain-of-function, independently of the encoded proteins [106]. This mechanism has been thoroughly studied for DM1, which is caused by more than fifty CTG repeats in the three prime untranslated region of the dystrophia myotonica-protein kinase gene (*DMPK*) [107,108]. These CTG repeat expansions result in flawed RNA transcripts that prevent translation into proteins, and can cause nuclear retention in RNA foci [109,110]. It is thought that these RNA foci will alter the function of one or more RNA-binding proteins, such as muscleblind-like 1, resulting in downstream changes in gene expression and/or alternative splicing of a range of transcripts [111–113].

RNA foci have also been detected in a growing number of other non-coding repeat expansion disorders, including myotonic dystrophy type 2 (DM2), Fragile X-associated tremor ataxia syndrome (FXTAS), Huntington's disease-like 2 (HDL2), spinocerebellar ataxia type 36 (SCA36), spinocerebellar ataxia type 31 (SCA31), spinocerebellar ataxia type 8 (SCA8), spinocerebellar ataxia type 10 (SCA10) [85,86**,112,114–116], and now *C9ORF72*-associated ALS and FTD [18**,19**]. These findings support a common RNA gain-of-function mechanism in many non-coding repeat expansion disorders [104,105**, 117], and further support the importance of these foci in *C9ORF72*-positive patients [18**]. To better understand the role of RNA misprocessing in *C9ORF72* mutation carriers, total RNA sequencing studies in affected tissue should now be performed to identify the specific downstream targets affected in these patients.

Previous studies performed on DM1 can also help us learn about their diagnostic challenges and therapeutic strategies for *C9ORF72* repeat expansions. Patients with DM1 can harbor several thousands of repeats, and the number of repeats shows a high degree of instability [118]. This instability appears to predispose towards further expansion, and could be associated with the progressive nature of the disease [119]. It could also account for the differences in DM1 alleles that are detected both between and within tissues of the same patient [120–126]. Furthermore, it explains why children may inherit repeat lengths that are considerably longer than those of their parents [127], and why an earlier age at onset or increased severity are reported in successive generations (genetic anticipation) [108,121,128–133]. The degree of instability associated with *C9ORF72* repeat expansions still needs to be determined; however, some studies have suggested that families with *C9ORF72* mutations can display anticipation as well [18**,19**,28*,40*,43*,49*,50**,51*, 56*,69*,74*,77*].

For diagnostic purposes, DM1 flow charts have been developed. Firstly, a conventional PCR has to be performed to determine whether an individual has two alleles with a low number of repeats. If only one allele size is detected, then additional testing is necessary. Alleles up to ~100 CTG repeats can be identified with a repeat-primed PCR, both robustly and reliably [118,134,135**]. Because of extinction of the signal in higher size regions, however, no trustworthy information about the precise length of the expanded repeats can be obtained. Moreover, rare interruptions in the CTG repeats may result in a failure to detect expansions [135**]. Alleles containing 100 CTG repeats and over, can be assessed with Southern blot analysis. This method is the gold standard, but it is time-consuming and requires a large amount of genomic DNA [118,135**]. Southern blot analysis often results in a diffused

band, consistent with somatic heterogeneity and correlating with the age of the patient [136]. Therefore, diagnostic DM1 tests usually report a size range (5–35, 36–50, 50–150 and >150), instead of an exact repeat length, and are based on comparisons with molecular weight standards and/or characterized control samples [135**]. If more is known about the *C9ORF72* repeat sizes in control subjects and ALS and/or FTD patients, it would be possible to develop a similar flow chart and size range for diagnostic *C9ORF72* testing.

Lastly, promising therapeutic strategies are currently being developed for DM1, including antisense oligonucleotides that hybridize to cellular mRNAs, inhibit gene expression, and could target mRNA for degradation, which could halt progression or reverse damage induced by toxic RNA [137**]. It is the hope that a similar approach may one day be used in FTD and ALS patients with *C9ORF72* repeat expansions.

What is the role of other repeat expansions in FTD and ALS?

Previously, other repeat expansions have already been implicated in ALS and/or FTD, these include repeat expansions in the ataxin-2 gene (*ATXN2*) and in the non-imprinted Prader-Willi/Angelman syndrome region protein 1 gene (*NIPA1*) [138*–140*,141]. In addition, a rare intronic GGCCTG repeat expansion in the NOP56 ribonucleoprotein homolog gene (*NOP56*) has recently been identified in patients with spinocerebellar ataxia and motor neuron involvement [86**].

These expansions, however, may only represent the tip of the iceberg, and the actual contribution of repeat expansions to the etiology of ALS and/or FTD may be much higher. For example, the *C9ORF72* mutation is only one out of 109 repeats in the human genome with at least three GGGGCC repeat units, several of which are in non-coding regions, and expansions of either one of these repeats could be implicated in disease. Unfortunately, until novel methods are developed that are designed to systematically screen for repeat expansions on a genome-wide level, the actual contribution of repeat expansions to the pathogenesis of ALS and/or FTD remains undetermined.

Conclusions and future directions

Despite the excitement, it is important to acknowledge that it is still early days, and several key questions related to the *C9ORF72* mutation presently remain unanswered: Are the RNA foci observed in *C9ORF72* repeat carriers toxic and which downstream targets are affected? Which protein accumulates in the ubiquitinated TDP-43-negative inclusions and do they have a role in disease pathogenesis? What determines whether someone develops ALS, FTD or both? Which role does the repeat length play in determining disease onset and presentation, and is there a minimal number of repeats needed for pathogenicity?

In this review, we have provided an up-to-date overview of the current *C9ORF72* literature, and we have discussed this mutation in relation to other non-coding repeat expansion disorders, which emphasizes the crucial role of repeat expansions, and RNA toxicity, in a broad range of neurodegenerative disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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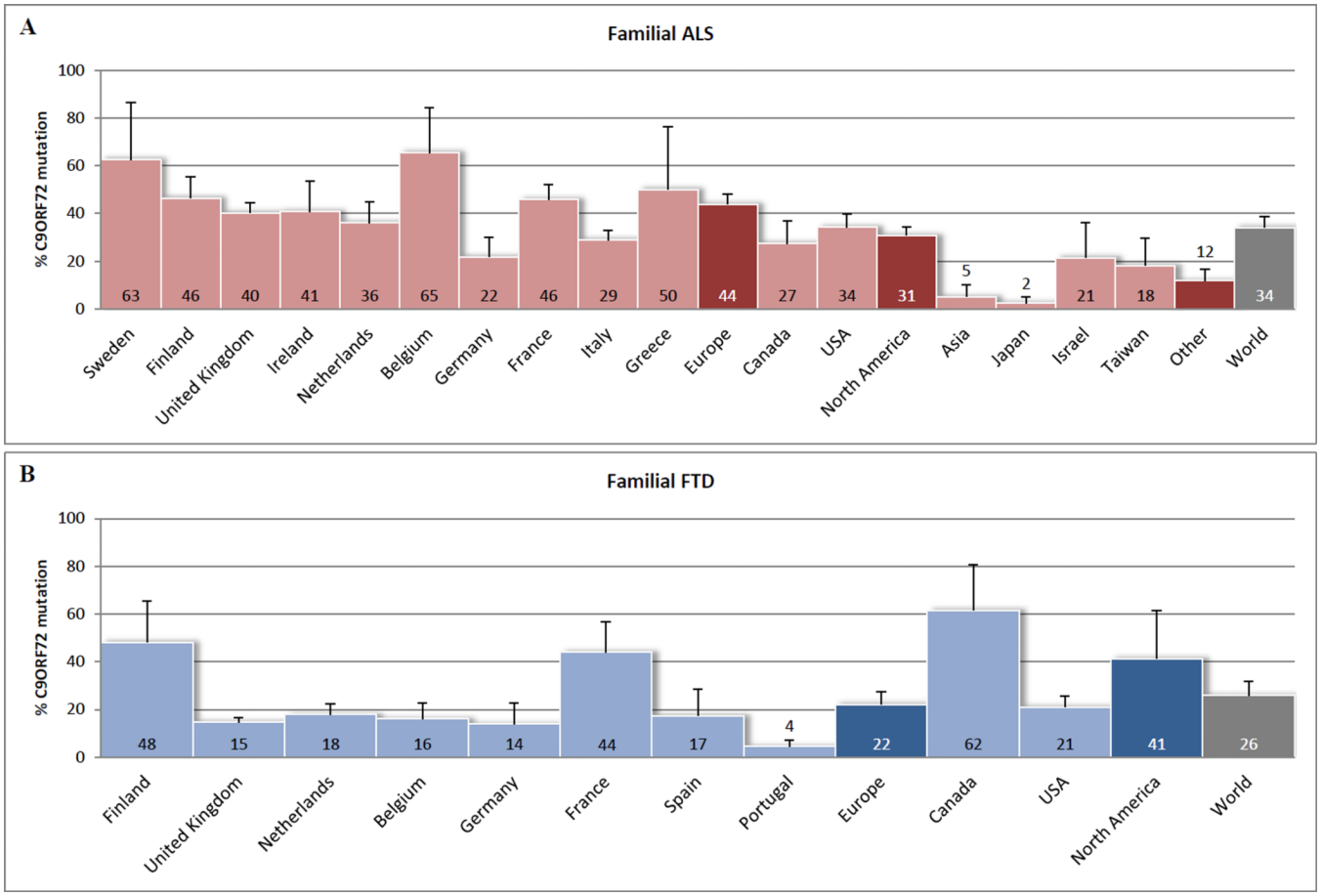
Key points

- *C9ORF72* repeat expansions are currently the major genetic cause of ALS and/or FTD worldwide, accounting for approximately 34.2% of the familial ALS cases, and 25.9% of the familial FTD cases.
- Mutations in other ALS- and/or FTD-associated genes may act as disease modifiers, which could contribute to the pleiotropy that is encountered in patients with *C9ORF72* mutations.
- Somatic heterogeneity is likely to be common in repeat expansion carriers, and as a result, repeat sizes determined using DNA extracted from whole blood may not adequately reflect the *C9ORF72* repeat sizes in a patient's brain or spinal cord tissue.
- *C9ORF72* expansions are probably pathogenic due to a toxic RNA gain-of-function mechanism in line with most other non-coding repeat expansion disorders.
- The actual contribution of repeat expansions to the etiology of ALS and/or FTD may be much higher; non-coding expansions that have been identified thus far may only represent the tip of the iceberg.



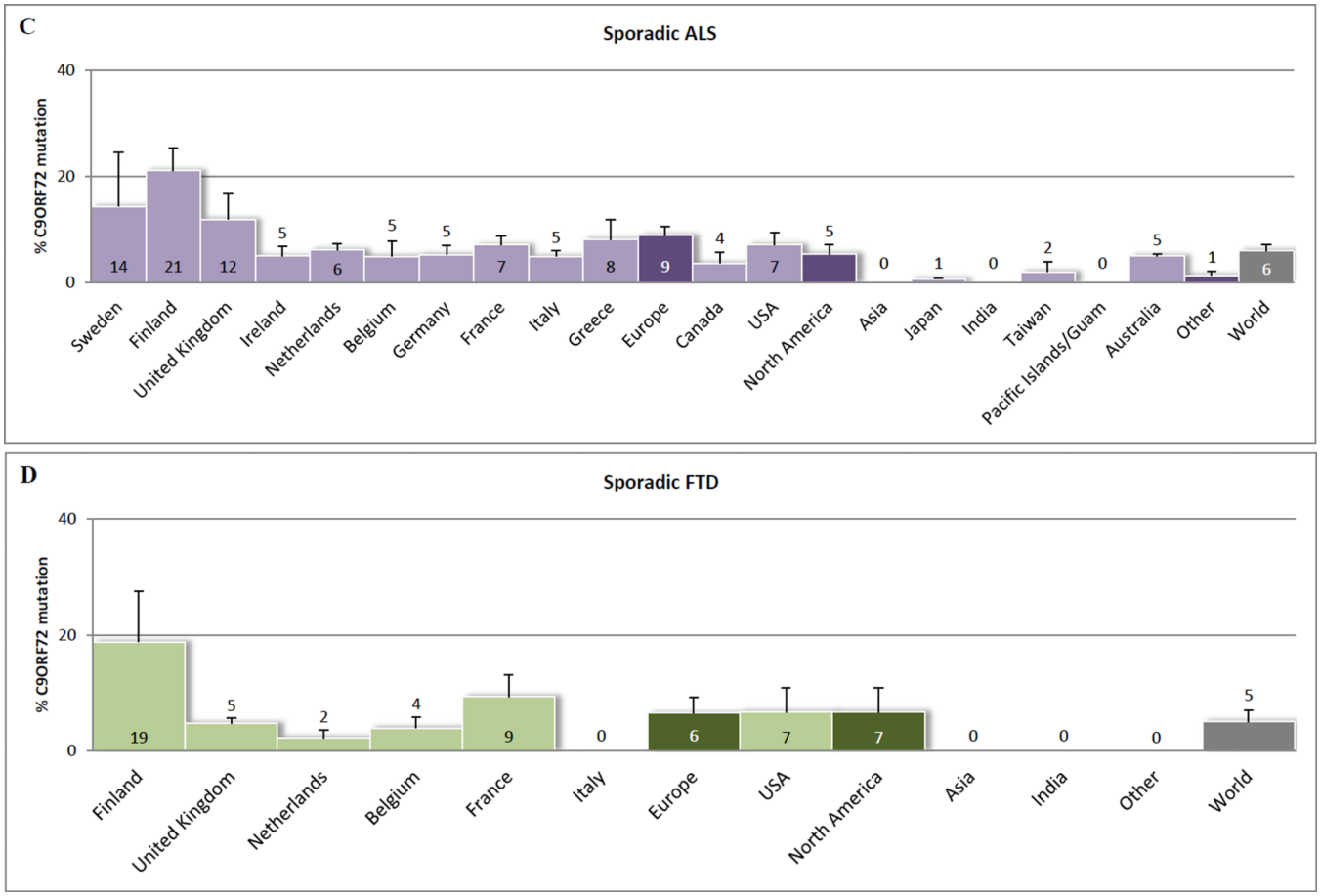


Figure 1.

C9ORF72 mutation frequencies worldwide. Percentages reported in (A) familial ALS patients, (B) familial FTD patients, (C) sporadic ALS patients, and (D) sporadic FTD patients. Cohorts of less than ten subjects were not included. Only reported subjects with more than 29 repeat expansions were considered to have a *C9ORF72* mutation. In general, error bars represent 95% confidence intervals, as calculated with the Wald method. If multiple studies were conducted for one country, then error bars represent standard errors. For these countries the average of all conducted studies was calculated, applying equal weight to all of them. The same method was used to calculate an average for all reported countries in Europe, countries in North America, other countries (Other), and an overall average (World). Graphpad Prism version 5.04 (http://www.graphpad.com) was used to perform these analyses. When mutation frequencies of other genes were reported, they were incorporated, to give a better impression of the actual mutation percentage in the general ALS/FTD population. If authors stated that the same (sub)group was used in multiple studies, then this (sub)group was only included in one of the studies. Majounie *et al.* [47**], Chio *et al.* [49*], and Renton *et al.* [19**], however, did include the same subset of 29 familial ALS samples without specification, and therefore, this relatively small amount of samples could not be excluded. More details about the studies included in our comparison can be found in Supplementary Table 1.

Table 1

Patients reported with *C9ORF72* repeat expansions in combination with mutations in other genes.

	Gene 1	Gene 2	Mutation 2	ALS	FTD	Gender	Age at Onset (y)	Site of Onset	Duration (m)	Reported in controls	Prediction PolyPhen-2	Prediction PMut	Additional information
Ratti 2012 [33*]	*C9ORF72*	*TARDBP*	p.A382T	FALS	N/A	M	47	Spinal	N/A	Yes	Benign	Neutral	[60]
	C9ORF72	*PRPH*	p.R133P	SALS	N/A	M	70	Spinal	24	No	Probably damaging	Pathological	[61*]
Lattante 2012 [36*]	*C9ORF72*	*ANG*	p.I46V	SALS	N/A	F	38	Spinal	> 38	Yes	Benign	Neutral	[36*]
Van Blitterswijk 2012 [29]**	*C9ORF72*	*TARDBP*	p.N352S	FALS	No	M	42	Cervical	> 91	No	Benign	Pathological	[29**][62*]
	C9ORF72	*TARDBP*	p.N352S	FALS	No	F	47	Cervical	> 15	No	Benign	Pathological	[29**][62*]
	C9ORF72	*SOD1*	p.D90A	FALS	No	F	51	Cervical	77	Yes	Benign	Pathological	[29**][62*]
	C9ORF72	*FUS*	p.Q210H	FALS	No	M	58	Lumbosacral	25	Yes	Probably damaging *	Neutral	[29**][62*]
Van Blitterswijk 2012b [58*]	*C9ORF72*	*VAPB*	p.V234I	FALS	No	F	65	Lumbosacral	34	No	Benign *	Neutral	[58*][62*]
Millecamps 2012 [42]**	*C9ORF72*	*ANG*	p.K41I (p.K17I)	FALS	No	F	47	Lumbosacral	27	Yes	Benign	Pathological	[63]
	C9ORF72	*DAO*	p.R38H	FALS	N/A	F	42	Lumbosacral	7	Yes	Benign	Pathological	[64]
	C9ORF72	*OPTN*	p.D128EfsX22	FALS	N/A	M	46	N/A	21	Yes	N/A	N/A	[65*]
	C9ORF72	*UBQLN2*	p.G502_I504del	FALS	No	F	52	Bulbar	27	Yes	N/A	N/A	[66*]
	C9ORF72	*SOD1*	p.D110Y	FALS	N/A	N/A	59	Cervical	42	No	Benign	Neutral	[63]
	C9ORF72	*FUS*	p.R521C	FALS	N/A	N/A	40	Bulbar	14	No	Benign	Pathological	[63]
Ferrari 2012 [43*]	*C9ORF72*	*GRN*	p.Y294C	No	FTD, familial	M	53	Behavioral-variant	> 96	No	Probably damaging	Pathological	[43*]
	C9ORF72	*PSEN2*	p.I146V	No	FTD, familial	M	59	Behavioral-variant	> 108	No	Benign	Neutral	[43*]
Cooper-Knock 2012 [51*]	*C9ORF72*	*TARDBP*	p.A321V	FALS	No	F	37	Cervical	58	No	Benign	Pathological	[67]
	C9ORF72	*FUS*	p.G174del	FALS	No	F	62	Bulbar	24	Yes	N/A	N/A	[68]
	C9ORF72	*OPTN*	p.E322K	FALS	No	F	50	Bulbar	29	Yes	N/A	N/A	[51*]
Chio 2012b [59*]	*C9ORF72*	*TARDBP*	p.A382T	FALS	FTD	M	43	Bulbar	34	Yes	Benign	Neutral	[59*]
	C9ORF72	*TARDBP*	p.A382T	FALS	No	M	35	Cervical	> 42	Yes	Benign	Neutral	[59*]
Total				**90% familial**	**21% FTD**	**47% M**	**50 y**	**25% bulbar**	**42 m**	**57% yes**			

Abbreviations: ALS = amyotrophic lateral sclerosis, FALS = familial ALS, SALS = sporadic ALS, FTD = frontotemporal dementia, N/A = not available, M = male, F = female, y = years, and m = months.

Predictions were performed on 07-18-2012 by PolyPhen-2 version 2.2 (http://genetics.bwh.harvard.edu/pph2/) and PMut (http://mmb2.pcb.ub.es:8080/PMut/).

*Multiple sequence alignments used by the previous version of PolyPhen-2 resulted in ‘unknown’ for p.Q210H in *FUS* and ‘possibly damaging’ for p.V234I in *VAPB*.

Table 2

Clinical characteristics of ALS and FTD patients with *C9ORF72* mutations.

Cohort	% Male (SE)	% Bulbar (SE)	Mean Age at Onset (SE)	Median Age at Onset (SE)	Mean Duration (SE)	Median Duration (SE)	% Dementia (SE)	% Behavioral (SE)	% FTD-MND (SE)
ALS[a]	55.31 (3.21)	34.58 (2.55)	56.28 (1.20)	56.50 (0.93)	35.43 (1.80)	31.40 (2.56)	26.95 (4.94)		
FTD[b]	56.91 (3.29)	85.08 (6.12)	55.75 (0.80)	54.10 (2.10)	75.33 (6.35)			80.95 (5.08)	30.57 (3.43)
Total	55.84 (2.37)	44.20 (4.99)	56.13 (0.88)	55.90 (0.88)	49.94 (4.87)				

Abbreviation: SE = standard error.

For ALS and FTD cohorts the average of studies reporting clinical characteristics was calculated, applying equal weight to all of them. Cohorts of less than five subjects were not included. Only subjects with more than 29 repeat expansions were considered to have a *C9ORF72* mutation.

For a more detailed overview we refer to Supplementary Table 2. Included cohorts are described in: [a][18**,28*,36*–38*,42**,44*–47**,49*,50**,51*,53**,56*,57**,69*,70*], and [b][18**,28*,47**, 50**,52*,54*,55*,57**,69*–71*].

Table 3

Neurological disorders caused by expanded repeats.

Disease	Repeat Unit	Repeat Locus	Repeat Location	Affected Gene	Disease Causing Repeat Length	Mechanisms of Pathogenesis
Myotonic Dystrophy type 1 (DM1)	CTG	19q13	3' UTR	*DMPK*	50–6500	Altered RNA function
Myotonic Dystrophy type 2 (DM2)	CCTG	3q21	Intron	*CNBP*	75–11,000	Altered RNA function
Spinocerebellar ataxia 1 (SCA1)	CAG	6p23	Coding	*ATXN1*	> 44	Polyglutamine gain-of-function
Spinocerebellar ataxia 2 (SCA2)	CAG	12q24	Coding	*ATXN2*	> 32	Polyglutamine gain-of-function
Spinocerebellar ataxia 3 (SCA3)	CAG	14q24-q32	Coding	*ATXN3*	> 52	Polyglutamine gain-of-function
Spinocerebellar ataxia 6 (SCA6)	CAG	19p13	Coding	*CACNA1A*	20–33	Polyglutamine gain-of-function
Spinocerebellar ataxia 7 (SCA7)	CAG	3q21	Coding	*ATXN7*	37–460	Polyglutamine gain-of-function
Spinocerebellar ataxia 8 (SCA8)	CTG/CAG	13q21	3' UTR	*ATXN8*	80–1300	Polyglutamine gain-of-function
Spinocerebellar ataxia 10 (SCA10)	ATTCT	22q13	Intron	*ATXN10*	800–4500	Altered RNA function
Spinocerebellar ataxia 12 (SCA12)	CAG	5q31-q33	5' UTR	*PPP2R2B*	55–78	Unknown
Spinocerebellar ataxia 17 (SCA17)	CAG	6q27	Coding	*TBP*	49–66	Polyglutamine gain-of-function
Spinocerebellar ataxia 31 (SCA 31)	TGGAA	16q21-q22	Intron	*TK2-BEAN*	2.5- to 3.8-kb	RNA gain-of-function
Spinocerebellar ataxia 36 (SCA 36)	GGCCTG	20p13	Intron	*NOP56*	1500–2500	RNA gain-of-function
Fragile X mental retardation 1 (FMR1)	CGG	Xq27	5' UTR	*FMR1*	> 200	Altered RNA function
Fragile X-associated tremor ataxia syndrome (FXTAS)	CGG	Xq27	5' UTR	*FMR1*	55–200	RNA gain-of-function
Fragile X mental retardation 2 (FMR2)	CCG	Xq28	5' UTR	*FMR2*	200–900	Loss of protein function
Huntington's disease (HD)	CAG	4p16	Coding	*HTT*	> 35	Polyglutamine gain-of-function
Huntington's disease-like 2 (HDL2)	CTG	16q24	3' UTR	*JPH3*	> 41	Altered RNA function
Friedreich's Ataxia (FRDA)	GAA	9q13	Intron	*FXN*	66–1700	Loss of protein function
Epilepsy progressive myoclonia (EPM1)	CCCCGCGCGCGCGCG	21q22	Promoter	*CSTB*	30–75	Loss of protein function
Oculopharyngeal muscular dystrophy (OPMD)	GCG	14q11	Coding	*PABPN1*	11–17	Polyalanine gain-of-function
Spinal and bulbar muscular atrophy (SBMA)	CAG	Xq12	Coding	*AR*	> 37	Polyglutamine gain-of-function
X-linked mental retardation	GCG	Xp21	Coding	*ARX*	17–23	Loss of protein function
Dentatorubral-pallidoluysian atrophy (DRPLA)	CAG	12p13	Coding	*ATN1*	48–93	Polyglutamine gain-of-function
ALS and/or FTD	GGGGCC	9p21	Intron	*C9ORF72*	Up to thousands	RNA gain-of-function?

For more information we refer to recent reviews [84,87–89,90*–92*,93–97], articles [85,86**,98–103], and GeneReviews (http://www.ncbi.nlm.nih.gov/sites/GeneTests/review).