How can we define the presymptomatic C9orf72 disease in 2022? An overview on the current definitions of preclinical and prodromal phases

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An overview on the current definitions of preclinical and prodromal phases

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Abstract

Repeat expansions in *C9orf72* gene are the main genetic cause of frontotemporal dementia, amyotrophic lateral sclerosis and related phenotypes. With the advent of disease-modifying treatments, the presymptomatic disease phase is getting increasing interest as an ideal time window in which innovant therapeutic approaches could be administered. Recommendations issued from international study groups distinguish between a preclinical disease stage, during which lesions accumulate in absence of any symptoms or signs, and a prodromal stage, marked by the appearance the first subtle cognitive, behavioral, psychiatric and motor signs, before the full-blown disease. This paper summarizes the current definitions and criteria for these stages, in particular focusing on how fluid-based, neuroimaging and cognitive biomarkers can be useful to monitor disease trajectory across the presymptomatic phase, as well as to detect the earliest signs of clinical conversion. Continuous advances in the knowledge of *C9orf72* pathophysiology, and the integration of biomarkers in the clinical evaluation of mutation carriers will allow a better diagnostic definition of *C9orf72* disease spectrum from the earliest stages, with relevant impact on the possibility of disease prevention.

Keywords

Frontotemporal dementia; frontotemporal lobar degeneration; amyotrophic lateral sclerosis; *C9orf72*; biomarker; presymptomatic disease.

1. Introduction

Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are neurodegenerative diseases lying along a clinical continuum and sharing common pathophysiological and genetic mechanisms. One of the most relevant advances in the understanding of how these two diseases are related has been the identification, in 2011, of a hexanucleotide repeat expansion in *C9orf72* in families concerned by FTD, ALS, or the combination of the two [1,2].

C9orf72 expansions turned out to be the most frequent cause of genetic FTD and ALS in most countries, explaining up to 25% of familial FTD cases and around 40% of familial ALS cases [3–5]. When both disorders coexist, *C9orf72* expansions can be found in up to 80% of cases [3,4]. Besides, they can occur also in FTD or ALS patients without overt family history of neurodegenerative diseases, at a frequency estimated between 6% and 20% [5,6] thus underscoring the importance of genetic testing even in apparently sporadic cases. Notably, no such overlap exists with other relatively frequent genes after *C9orf72*, such as progranulin gene (*GRN*) and microtubule associated protein tau gene (*MAPT*), identified in FTD phenotypes, or superoxide dysmutase 1 (*SOD1*), responsible of pure ALS. Less common disease-causing genes can be involved in both cognitive, motor, or complex phenotypes [7].

The age at onset in *C9orf72* disease is extremely variable, ranging between the 2nd and the 9th decade, with a peak at 58 years [8]. There is increasing evidence about the heterogeneity of clinical phenotypes, encompassing cognitive, behavioral and motor syndromes. In addition to the behavioral variant of FTD (bvFTD), ALS and the association FTD/ALS, *C9orf72* patients may occasionally present with psychiatric phenotypes, mainly qualifying as atypical, late-onset psychoses [9–11]. Less common presentations, identifiable in less than 5% of carriers, include primary progressive aphasia (PPA) variants [8,12], and parkinsonian syndromes (corticobasal syndrome, progressive supranuclear palsy, and, rarely, typical parkinsonism) [13–15].

Knowledge about the implications of *C9orf72* repeat expansion in disease pathophysiology has been continuously increasing since the discovery of the gene. The first intron of *C9orf72* contains a G_4C_2 sequence which in healthy individuals mostly ranges between 2 and 8 repeats, and in any case below 30, which has been conventionally fixed as a pathogenic threshold [2,16]. The majority of affected individuals carry a pathologic expansion in the range of hundreds or thousands of repeats [9]; interestingly, the repeat length does not significantly affect the disease phenotype or the age at onset [17]. In addition, expansions of intermediate length (between 20 and 30 repeats), have been suggested to increase the risk of developing parkinsonian syndromes or ALS [18,19]. The biological functions of the C9ORF72 protein are not completely understood, but it has been determined to act as a GTPase activating protein (GAP), in partnership with two other subunits [20].

Three main pathogenic mechanisms have been hypothesized in *C9orf72*-associated disease, including loss of physiological role of C9ORF72 protein, accumulation of RNA foci in the nuclei and toxicity from dipeptide repeat proteins (DPR) generated from repeat-associated non-ATG (RAN) translation [21]. Hence, RNA foci and cytoplasmic DPRs accumulations coupled with p62-positive inclusions represent pathological hallmarks specific to *C9orf72* disease, in addition to diffuse neuronal and oligodendroglial TDP-43 positive inclusions [22,23].

Insights in the disease mechanisms have paved the way to the development of disease-modifying treatments, mainly acting to contrast the deleterious effect of the *C9orf72* expansion. Among them, one of the most developed so far consists of antisense oligonucleotides (ASOs), small DNA or RNA molecules binding to a complementary RNA sequence and leading to its degradation, thus modulating gene expression [24]. Phase 1 and 2 trials targeting *C9orf72*-associated FTD and ALS have started in recent years (www.clinicaltrials.gov NCT03626012, NCT04288856, NCT04931862, NCT04993755). These interventions should be eventually developed to block the pathological cascade and, ideally, prevent or significantly delay the

occurrence of clinical symptoms. Therefore, the presymptomatic disease phase is getting more and more interest as an ideal time window in which innovant therapeutic – or rather preventive – approaches should be tested. At present, the definitions and the time references of the presymptomatic phase, as well as the tools of choice for the longitudinal monitoring of the disease trajectory are a matter of intensive research. This review provides an overview on the current knowledge about the presymptomatic phase of the *C9orf72* disease, particularly focusing on: i) the proposed classification of presymptomatic stages, ii) the contribution of biomarkers to trace preclinical disease trajectory, and iii) the earliest clinically relevant changes associated with disease onset, and their interest in ongoing and future clinical trials tackling *C9orf72*-associated pathology.

2. Current framework for defining the presymptomatic disease stages

At present there is limited information about the sequential ordering of events occurring in FTD and ALS before clinical onset. In the field of neurodegenerative diseases, studies focusing on Alzheimer's disease (AD), particularly in its monogenic forms, have substantially contributed to define the concept of a presymptomatic disease stage, characterized by progressive lesion accumulation [25–27], up to a prodromal, oligosymptomatic stage defined as mild cognitive impairment (MCI) [28]. In Huntington's disease (HD), longitudinal studies on presymptomatic carriers allowed to depict the progressive changes occurring during the pre-manifest phase, with the useful contribution of repeat length and other genetic modifiers in the prediction of the age at onset [29–31].

In the case of FTD and ALS, a privileged point of view to study the presymptomatic phase is offered by carriers of disease-causative mutations, identified among first-degrees relatives of genetic patients. Over the recent years, international consortia have been built to assemble and study large cohorts of presymptomatic carriers in a standardized manner, to increase our

knowledge on presymptomatic stage. The Genetic Frontotemporal Initiative (GENFI), has been developed in Europe and Canada (www.genfi.org), and the ARTFL (Advancing Research and Treatment for Frontotemporal Lobar degeneration) and LEFFTDS (Longitudinal Evaluation of Familial FrontoTemporal Dementia Subjects) (www.allftd.org), as well as the presymptomatic familial ALS (Pre-fALS) study [32], have been initiated in US. Besides, multiple national initiatives have assembled country-based cohorts, such as the Australian DINAD (Dominantly Inherited Non-Alzheimer Dementias, www.ecdc.org.au/genetic-ftd-trials); the New Zealand Genetic FTD (FTDGeNZ) [33], the French PREV-DEMALS and Predict-PGRN [34,35] cohorts, the Belgian presymptomatic *C9orf72* cohort [36], and the Multi-partner Consortium to expand Dementia Research in Latin America (ReDLat) [37]. All these initiatives highlight the important challenge and critical need to better characterize this stage of the disease.

Overall, research results coming from the abovementioned initiatives contributed to generate a conceptual framework useful to define and further classify the presymptomatic phases of FTD and ALS, which could be thus employed in the context of *C9orf72* expansions, responsible of both these diseases. Key recommendations have been recently defined in the works by Benussi *et al.* (2021) [38] and Benatar *et al.* (2021) [39], and are summarized below (Figure 1).

The **preclinical disease stage** defines the period between the start of the neurodegenerative process and the appearance of the first signs and symptoms of disease. Theoretically, with *C9orf72* expansions this phase should correspond to progressive accumulation of DPR proteins, RNA foci and, sequentially, TDP-43 pathology [40,41]. However, the limited information obtainable from pathological biomarkers *in vivo* does not allow to determine how early these degenerative changes occur. Therefore, it is currently unclear whether a "**no disease**" stage, characterized by the absence of any pathological lesions exists, and the boundary between this latter and the subsequent preclinical phase is particularly hard to identify [38]. During the preclinical stage, clinical symptoms are completely absent, and no ongoing denervation changes

should be found on EMG [39]. Different biomarkers can variably contribute to inform on preclinical disease trajectory, as it will be discussed further [36,42].

The **prodromal disease stage** is defined by the appearance the first subtle cognitive, behavioral and motor signs, and lasts up to the onset of full-blown disease. *Prodromal FTD* is characterized by gradual changes affecting social cognition, executive functions or language, as well as recent behavioral modifications including reduced initiative, diminished empathy, change in dietary habits, repetitive or ritualized actions or behaviors [38]. These changes from the individual's baseline status should be of such intensity as to preserve independence in daily living, albeit a mild impact on close relationships or highly demanding professional tasks cannot be excluded [38]. From a quantitative approach, the Clinical Dementia Rating (CDR) plus National Alzheimer's Disease Coordinating Center (NACC) Frontotemporal Lobar Degeneration (FTLD) rating scale (CDR+NACC FTLD) is one of the tools of choice to score the severity of symptoms in FTD patients [43,44]. Preclinical, asymptomatic stage is defined by a CDR+NACC FTLD global score equal to 0, whereas prodromal subjects should present a score of 0.5. *Prodromal ALS* is characterized by mild motor complaints (cramps, early fatigue), subtle signs at neurological examination (fasciculations, changes in reflexes) or isolated EMG signs of ongoing denervation, without overt muscular weakness. "Phenotransition" is the term proposed by the ALS community to indicate the passage from the preclinical to the prodromal stage [39].

Overall, to account for the heterogeneity of the cognitive/clinical manifestations of the prodromal stage, especially with the occurrence of *C9orf72* expansions, the unifying concept of **mild cognitive/behavioral/motor impairment (MCBMI)** has been proposed [38]. However, several confounding factors should be ruled out before affirming that a MCBMI is due to underlying FTD or ALS. Cognitive impairment, especially affecting domains which are atypical for FTD, could result from degenerative processes unrelated to mutational status, as

well as from non-degenerative conditions (cerebrovascular lesions or sleep disturbances among many others). Subtle changes in behavior or personality are not specific for FTD, and could derive from unrelated psychiatric conditions or substance abuse. When evaluating motor signs and symptoms, common confounding conditions such as radiculopathies should be carefully looked for. Moreover, *C9orf72* disease offers another source of uncertainty, due to the existence of long-standing psychiatric phenotypes, hardly distinguishable from primary psychiatric disorders, which could precede the onset of FTD or ALS by years or decades [45,46].

The term "**phenoconversion"** indicates the transition from the prodromal to the full-blown clinical stage. Clear boundaries between the two stages are barely definable, therefore some operational criteria for phenoconversion have been proposed, including: 1) fulfillment of diagnostic criteria for bvFTD, PPA, ALS or other associated syndromes; 2) CDR+NACC FTLD score equal or greater than 1; 3) loss of independence in daily living; 4) significant impact on social/professional activities despite preserved autonomy (e.g., because of language deficits or inappropriate social behavior) [38].

It should be kept in mind, however, that some inconsistencies may emerge when applying this general framework to individual disease carriers, because of the differences in the temporal course across distinct genotypes [8,47], the interindividual variability in presymptomatic trajectories and the role of disease modifiers whose role is only partially understood [48,49].

3. The role of biomarkers across the presymptomatic phases

3.1. Definition and types of biomarkers

The term "biomarker" indicates an observable and measurable feature whose levels serve as objective indicators of different physiological or pathological states, or are associated to the response to therapeutic interventions [50]. In the context of presymptomatic *C9orf72* carriers, biomarkers are intended to monitor disease evolution form the presymptomatic to the full-

blown stages, predict the proximity to phenoconversion and, eventually, serve as outcome measures in therapeutic trials [51,52]. It has to kept in mind that the analytical variability of a biomarker should be appropriate for its context of use. For instance, during the preclinical phase an optimal disease-tracking biomarker should be sensitive enough to capture the evolution of the underlying pathophysiological cascade, which could last several years or decades. On the other hand, a valuable biomarker to predict clinical onset should stay as stable as possible in non-progressing carriers, and display clear and sustained changes close to phenoconversion. Different approaches can be used to provide biomarkers, and those which contribute the most to monitor presymptomatic *C9orf72* disease are summarized in Table 1.

3.2. Biomarkers to define the preclinical disease trajectory

As already stated, it is hard to define the beginning of lesional accumulation in most neurodegenerative diseases. However, *C9orf72* pathophysiology offers a privileged point of view, as DPR proteins, and in particular poly(GP) proteins (originated from both sense and antisense expanded transcripts) can be detectable in the CSF of asymptomatic carriers [53,54], without being associated with biomarkers of ongoing degeneration. There is a modest but significant increase of poly(GP) over time, and overall their levels are higher in patients than in presymptomatic carriers [54,55]. Autoptic studies have shown that accumulation of RNA foci and DPR-positive inclusions precede TDP-43 nuclear delocalisation and cytoplasmic deposition, underscoring that these changes are more closely related to the expansion itself, than to downstream degenerative events [56].

The model of the preclinical cascade in AD suggests that brain metabolic changes may antedate structural modifications [26,57]. A limited number of studies have explored the role of fluorodeoxyglucose (FDG) PET in the preclinical phase of genetic FTD; for what concerns *C9orf72* carriers, clusters of significant hypometabolism have been found in frontotemporal

cortices, basal ganglia and thalami, at a time in which volume loss is already detectable [36]. However, information about temporal dynamics of these alterations is currently lacking. Similarly, few studies have investigated the potential of fMRI to detect functionally compensated network dysregulations in asymptomatic carriers [58,59]. Salience network and thalamic-seeded network alterations are identifiable early, and are somewhat reminiscent of what observed in *C9orf72*-associated bvFTD [58,60]. The profile of connectivity changes extends over time, spreading towards the areas mostly affected in the symptomatic phase, which could herald impending neurodegeneration [59,61].

A number of structural MRI studies elegantly depicted the profile of brain changes identifiable at different points of the preclinical phase and, more recently, investigated their rate of change over time [35,58,62–66]. Overall, *C9orf72* carriers display significant and widespread cortical volume loss compared to non-carriers, including frontal areas, temporo-insular cortices, associative parieto-temporal regions, and hippocampi approximately 20 to 25 years before their estimated disease onset [35,58,62,64,66]. At the subcortical level, there is a diffuse and massive volume loss in the thalamus, with prominent involvement of the pulvinar subnucleus [65], a profile which is coherent with what observed in the clinical phase [67]. Early cerebellar involvement has been also evidenced, in particular in lobules VIIa-Crus II and VIIb, connected to the dorsolateral prefrontal cortex *via* the thalamus [65]. Notably, the precocity of grey matter alterations and the degree of subcortical involvement are more important in *C9orf72* carriers compared to *MAPT* or *GRN* carriers [62,65]. Individual baseline atrophy at the asymptomatic phase (CDR+NACC FTLD = 0) may be implemented in predictive models of progression towards the prodromal and clinical disease stages [68].

Gray matter changes are associated with, and often preceded by, white matter tracts degeneration. In *C9orf72* carriers, diffusion tensor imaging (DTI) analyses identified reduced fractional anisotropy (FA), a marker of microstructural integrity, in thalamic radiation, corpus

callosum, frontotemporal and corticospinal tracts (CST) [35,58,63,66,69], which are critically involved in the development of FTD and ALS.

Structural changes predictably appear more pronounced and diffuse among the older carriers, namely over 40 years of age [35,66]. However, a greater progression compared to noncarriers is barely detectable [63], and only one longitudinal study found a trend towards accelerated cortical thinning in presymptomatic carriers, tough not reaching statistical significance [64]. This is in line with the modest rates of atrophy observed during the clinical phase of *C9orf72* disease [70].

Spinal cord imaging features have been proposed as biomarkers for the development of ALS treatments [71], with measurable progression throughout the disease course [72]. Preysmptomatic *C9orf72* carriers display white matter atrophy at the cervical level, with progressive reduction of FA in CST occurring in individuals over 40 years old [73]. This is particularly relevant taking into account that no neurophysiological measures proved usefulness to detect motor neuron degeneration in asymptomatic *C9orf72* carriers [74].

In summary, the preclinical phase of *C9orf72* disease is marked by diffuse changes, mostly identifiable by means of neuroimaging approaches, which appear early and progress smoothly over the years. However, there is no sufficient evidence to ascertain if and how these changes could predict the subsequent clinical phenotype an individual carrier will manifest.

3.3. Biomarkers to support proximity to clinical onset

In neurodegenerative disorders, one of the most relevant contribution of biomarkers is the aid to identify those individuals who are going to develop the first symptoms and signs of the disease, hence predicting phenoconversion [26,42,75]. This information could enhance the stratification of carriers for clinical trials, and possibly provide outcome measures for treatment response [76].

Among the fluid-based biomarkers investigated for this purpose, neurofilaments turned out to be particularly useful [77–80]. Neurofilaments are structural proteins highly expressed in axons, composed of three main subunits, heavy (NfH), medium and light chain (NfL). Both NfL and NfH are released in extracellular fluids in proportion to neuronal loss in several neurological disorders, including neurodegenerative conditions [81–83]. With the development of the highly sensitive Simoa technique, neurofilament dosage can be easily performed in plasma or serum, whose levels are extremely correlated to CSF ones, thus allowing less invasive, repeatable dosages [84,85]. NfL levels in preclinical *C9orf72* carriers are comparable to controls at a group level [77,84], with steady, low-amplitude increases over the years [79,80]. NfL levels and their annualized rates of change increase during or just before the prodromal phase [47,77,79], thus allowing to identify those who are at risk of short-term progression to clinical disease, in the subsequent 2 to 5 years [78]. NfH are particularly stable during the presymptomatic phase, while mostly increasing at the moment of the phenoconversion and during the symptomatic stage [80]. Their changes occur earlier and are more pronounced in individuals displaying a phenotype of ALS [47,75].

Other proteomic biomarkers have been investigated in the presymptomatic phase of genetic FTD, including those linked to synaptic function, astrogliosis, inflammation, and complement activation [42,52,86]. Overall, levels of neuronal pentraxin 2 (NPTX2) decrease, while glial fibrillary acidic protein (GFAP) and complement proteins C3b and C1q sequentially increase, along with NfL and NfH, at the transition between presymptomatic and symptomatic FTD associated with *GRN* mutations, whereas their trajectory is much less clear for *C9orf72* expansions [42]. Preliminary evidence on novel putative biomarkers identified in the CSF of *C9orf72* patients such as chitotriosidase (CHIT1) has not been validated yet in the presymptomatic phase [87].

The expression profile of circulating miRNAs has been found to be altered in several neurodegenerative conditions [88,89]. In particular, a signature of four miRNA is dynamically altered throughout the presymptomatic and clinical phase of *C9orf72* disease, yielding an added value in the prediction of phenoconversion [90].

The transition towards the prodromal and clinical disease stage is marked by progressive cognitive and behavioral modifications, and the identification of the most appropriate neuropsychological/behavioral measures is the subject of active research. Longitudinal assessments of cognitive functions in presymptomatic cohorts of FTD mutation carriers provided information about the cognitive trajectories occurring in the main genetic groups [62]. One study showed that *C9orf72* carriers display worse scores in verbal fluencies since young age, without relevant changes over time, thus pointing towards a general neurodevelopmental disorder [91]. Episodic memory deficits have been also identified in the presymptomatic phase of *C9orf72* disease, with features more closely reflecting a profile of executive impairment rather than a true amnestic syndrome of hippocampal type [66,92].

Among the cognitive functions most closely related to FTD spectrum, social cognition and cognitive inhibition deficits mostly occur in the late presymptomatic phase and are potential predictive biomarkers of phenoconversion [93–95]. This underscores the usefulness of the Social Cognition and Emotional Assessment, shortened version, (mini-SEA) [96] and the Hayling test [97] to capture relevant, prodromal cognitive changes in *C9orf72* carriers. Additional changes observed in presymptomatic carriers include deficits in the praxis scores [35], and impairment in semantic knowledge occurring more closely to onset [98], in line with a profile of semantic dysfunction observed in some *C9orf72* patients [9,12].

As for cognitive changes, the earliest, subtle behavioral alterations, far from fulfilling bvFTD criteria, are remarkably difficult to identify. A commonly encountered difficulty is to discriminate between true new-onset behavioral changes and personality traits or attitudes

which are typical of the individual and are present since long time. When feasible, repeated assessments with appropriate and sensitive tools, such as the Cambridge Behavioral Inventory (CBI-R) [99], could aid to provide quantitative assessments of this often elusive constellation of symptoms [62]. Among the behavioral dimensions investigated in presymptomatic *C9orf72* carriers, apathy scores are higher than controls and increase over time, predicting subsequent cognitive impairment [100].

Overall, core criteria to define the threshold of cognitive and/or behavioral impairments to comply with a diagnosis of MCBMI due to FTD have not been defined yet, apart from a global score of CDR+NACC FTLD equal to 0.5 [38]. This useful tool presents some limitations, however, as it may not reliably identify the core symptoms attributable to prodromal FTD, and discrete cognitive and behavioral impairments (such as those found in a depressed individual showing attentional deficits) are a possible source of bias.

For carriers developing an ALS phenotype, neurophysiological biomarkers support the identification of early signs of motor neuron dysfunction. Isolated denervation signs at EMG, such as positive sharp waves in a single limb muscle or in paraspinal muscles, can indicate early lower motor neuron degeneration, in absence of confounding factors [39]. On the other hand, increased cortical excitability at TMS points towards upper motor neuron involvement [74]. Quantitative measures of motor unit loss, such as Motor Unit Number Index (MUNIX) at EMG, only investigated at the clinical stage so far [101], could represent a promising approach to better define prodromal ALS.

Finally, other biomarker modalities have contributed to illustrate different pathophysiological changes occurring in the presymptomatic phase of *C9orf72* disease, and it is possible that their use will be extended to investigational protocols in the upcoming years. These include a reduction of synaptic density in thalamic and frontotemporal regions observed with UCB-J PET [102], executive oculomotor abnormalities at video-oculographic examination [103], and

progressive changes in cerebral blood flow measured by arterial spin labeling (ASL) MRI sequences [104].

4. Long-standing psychopathological and personality features in *C9orf72* carriers blur the definition of prodromal stage

One of the difficulties in defining the prodromal stage in *C9orf72* carriers is due to the frequent presence of a prominent psychopathological, rather than cognitive, symptomatology at disease onset. Patients with *C9orf72*-associated bvFTD often show a constellation of psychiatric symptoms and syndromes [4,105–107], which can be inaugural of the cognitive disorder. Even more noteworthy, a subset of *C9orf72* carriers may present with isolated psychiatric phenotypes preceding dementia onset by several years or decades [46]. These are usually young adult individuals displaying psychotic syndromes, starting usually at a later age compared to primary psychiatric disorders [10,45,46]. The usually long disease history, in the absence of patent biomarkers of neuronal loss [45,79], favors the hypothesis of a dysfunctional brain disorder, eventually resulting in a degenerative syndrome. Other psychiatric disturbances display increased frequency among the presymptomatic *C9orf72* carriers, including mania [45], depression, substance abuse/dependence, and post-traumatic stress disorder [108]. Collectively, these findings point to life-long psychiatric vulnerability in the presence of the *C9orf72* expansion.

This susceptibility could also translate into several atypical behavioral and personality features present since early life, including fixed behavioral patterns, reduced empathy, tendency to hoarding and excessive sporting [107]. The importance of recognizing these traits in presymptomatic carriers is two-fold: first, it provides a baseline behavioral assessment to be accounted for when evaluating the occurrence of relevant behavioral changes in the prodromal or manifest disease stage; second, it draws attention on the impact *C9orf72* expansion may have

on brain development and maturation. This is of fundamental importance to correctly define the age of disease onset in patients.

The presence of long-standing structural signatures in the brains of *C9orf72* carriers, changing little over the years [58,91,109,110], provides additional evidence in favor of a neurodevelopmental hypothesis. The identification of low gyrification, index of immature cortical development, in the regions which are commonly atrophied during the disease also suggests that these abnormalities might confer vulnerability to future degeneration [109]. This is in line with the proposed role for C9ORF72 protein in nervous system maturation and synaptic modelling [111,112].

Overall, several lines of evidence support an influence of *C9orf72* repeat expansion on the brain which extends beyond the promotion of neurodegeneration, and the boundaries between developmental and degenerative manifestations appear to be quite blurred. The existence of international initiatives such as the Neuropsychiatric International Consortium on FTD (NIC-FTD) will hopefully expand the knowledge on these dimensions of the disease and raise clinician's awareness to atypical presentations occurring with *C9orf72*.

5. Conclusion

Research on genetic FTD and ALS is progressing at an impressive pace. In particular, major advances have been made in the understanding of the pathophysiology of *C9orf72* disease since the discovery of the gene, and the events occurring in the presymptomatic phase have been partially elucidated. A shared conceptual framework, and the major acquisitions on the use of biomarkers, are undoubtably helpful for clinicians and researchers to stratify presymptomatic carriers according to their presumed proximity to disease onset. Some unresolved issues still remain, notably for what concerns the translation of evidence collected from large cohort studies to the individual level for clinical purposes. The implementation of those concepts in

current practice could hopefully contribute to overcome these obstacles. Additionally, a revision of the currently adopted diagnostic criteria shall be considered, aiming to capture also earlier and milder forms, with obvious impact on disease prevention.

Table legend

Table 1. Main biomarkers studied to monitor *C9orf72* disease. ASL: arterial spin labeling; CHIT1: chitotriosidase; FA: fractional anisotropy; FDG: fluorodeoxyglucose; miRNA: micro-RNA; NfH: neurofilament heavy chain; NfL: neurofilament light chain; UCB-J: synaptic vesicle glycoprotein 2A PET tracer; WM: white matter.

Type of biomarker	Measure	Relevance	References
Fluid-based	Poly(GP) proteins (CSF)	High levels since the earliest stages	[53,54]
	NfL and NfH (CSF and plasma)	Increase in prodromal phase, ≤5 years before phenoconversion	[47,77–80]
	MiRNA	Altered profile, mostly in prodromal carriers	[90]
	CHIT1 (CSF)	Increase in patients	[87]
Neuroimaging	Cortical thickness, brain volumes, WM microstructure	Altered from 20-25 years before onset, slowly progressive	[35,58,62–66]
	Functional connectivity	Early salience and thalamic-seeded network dysregulation	[58,59]
	Cerebral blood flow (ASL)	Decreased from 12.5 years before onset	[104]
	Gyrification index	Congenitally reduced in C9orf72 carriers	[109]
	Cervical spinal cord WM volume and FA	Altered metrics mostly in carriers >40 years	[73]
	FDG PET uptake	Hypometabolism consistent with structural changes	[36]
	UCB-J PET uptake	Reduced synaptic density in preclinical phase	[102]
Neuropsychological	Verbal fluencies	Reduced scores since young age	[91]
	Episodic memory	Preclinical changes reflecting executive dysfunction	[92]
	Social cognition	Deficits in late presymptomatic phase	[93]
	Cognitive inhibition	Deficits mostly in >40 years	[94]
	Semantic knowledge	Deficits in late presymptomatic phase	[98]
	Gestural praxis	Impaired before 40 years	[35]
Neurophysiological	Cortical excitability	Increase in ALS patients	[74]

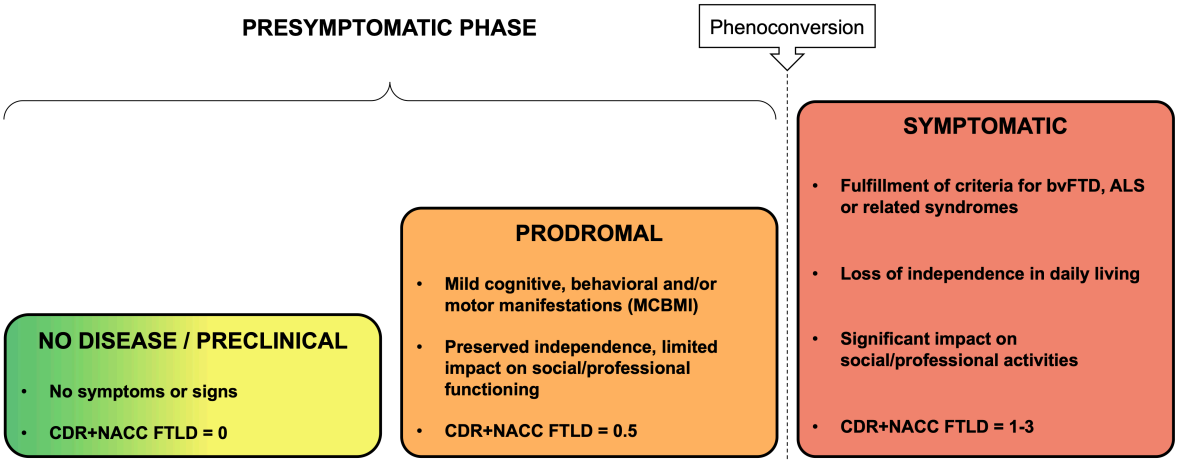

Figure 1. Overview on the presymptomatic and symptomatic stages in *C9orf72* disease. ALS: amyotrophic lateral sclerosis; bvFTD: behavioral variant of frontotemporal dementia; CDR+NACC FTLD: Clinical Dementia Rating plus National Alzheimer's Disease Coordinating Center Frontotemporal Lobar Degeneration (FTLD) rating scale, global score; MCBMI: mild cognitive/behavioral/motor impairment.

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