



Review article

The clinical and pathological phenotypes of frontotemporal dementia with C9ORF72 mutations



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ABSTRACT

An expanded hexanucleotide repeat in the chromosome 9 open reading frame 72 (C9ORF72), on chromosome 9p21, has recently been identified as a major cause of familial frontotemporal dementia (FTD). The neuropathology and clinical characteristics associated with C9ORF72 mutations are heterogeneous with the unknown pathomechanism. These cases were reported with a series of neuropathology, including TDP-43 pathology, ubiquilin (UBQLN) pathology, p62 pathology, microglial pathology, RNA-binding protein pathology and pathology associated with dipeptide-repeat (DPR) proteins. TDP-43 positive neuropathology was important in FTD patients with the mutations. Nevertheless, the majority of reports agree with a special pattern of neuropathology with p62 positive, TDP-43-negative inclusions being a consistent feature. Although subjects with the C9ORF72 mutations more frequently present with earlier onset age, earlier death, a shortened survival and a positive family history, most of the subjects present with typical clinical features of FTD. All these findings support that the C9ORF72 mutations become important newly recognized causes of FTD, providing a more detailed characterization of the associated clinical and pathological features. The following review summarizes the pathological development of FTD associated with C9ORF72, the clinical and pathological features of this cohort, some pathological mechanism hypotheses, and describes their phenotypic range and overlap with other neurodegenerative diseases.

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1. Introduction

Frontotemporal dementia (FTD) is the most common form of primary degenerative dementia after Alzheimer's disease that affects people in middle age, accounting for up to 20% of presenile dementia cases [1,2]. FTD is a term for a class of neurodegenerative disorders characterized by significant atrophy of the frontal and temporal lobes of the brain, which has been called frontotemporal lobar degeneration (FTLD). It presents with a spectrum of clinical manifestations: progressive changes in personality, behavior, insight, judgment, reasoning abilities, or language, with relative perseveration of episodic memory. There are several major clinical presentations of FTD, including behavioral variant frontotemporal dementia (bvFTD), progressive nonfluent aphasia, semantic dementia and corticobasal syndrome, as well as FTD with motor neuron disease (FTD-MND) [3,4]. BvFTD is the most frequent FTD phenotype, which is present with insidious changes in personality and interpersonal conduct [5]. Currently, FTD is pathologically classified based on three main proteins which aggregated in the central nervous systems, microtubule-associated protein tau, transactive response (TAR) DNA binding protein (TDP-43), and fused in sarcoma [6]. Thus, FTD can be pathologically divided into FTD-tau, FTD-TDP and the rarest type, FTD-FUS [7]. Besides, there are a small number of FTD patients with unknown inclusions which is referred to as FTD-ubiquitin proteasome system (FTD-UPS). The majority of FTD cases are sporadic and more likely caused by multiple factors including genetic and environmental factors. Thus, no genes are identified that sufficiently explained the growing class of families in which affected members developed FTD. A significant proportion of patients with FTD-TDP develop features of motor neuron dysfunctions, especially amyotrophic lateral sclerosis (ALS) [8–11]. On the other hand, frontal executive deficits and cognitive impairment are also reported in a number of ALS patients [12]. The overlap in TDP-43 pathology between ALS and FTD-TDP identified the linkage. FTD and ALS may form a disease spectrum, and the discovery of C9ORF72 mutations tied them together.

About 40% of the patients have at least one extra family member with dementia and in 13.4%, family history is consistent with an autosomal dominant inheritance [1,13–15]. The presence of familial aggregation suggested a genetic cause for FTD. To date, molecular genetic studies identified several genes associated with FTD, including microtubule-associated-protein-tau (MAPT) gene, the progranulin (GRN) gene, the gene fused in sarcoma (FUS), charged multivesicular body protein 2B (CHMP2B) gene, the 43-kDa transactive response (TAR)-DNA-binding protein (TARDBP) gene, valosin containing protein (VCP) gene and C9ORF72 gene [16–31] (Table 1). The majority of

families with autosomal dominant FTD have a mutation in one of the three most important genes for FTD including GRN, MAPT, and C9ORF72. A large hexanucleotide (GGGGCC) repeat expansion in the first intron of C9ORF72, a gene located on the short arm of chromosome 9, had recently been identified as the most common hereditary cause of chromosome 9p-linked amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) [8,9]. In the past two years, significant advances have been seen in the etiology, pathogenesis, pathology and clinical phenotype of FTD related with C9ORF72 gene. In this review, we summarize recent advances on the neuropathology and clinical phenotypes of FTD associated with C9ORF72 mutations.

2. Brief characterizations of C9ORF72 and underlying mechanisms

C9ORF72 is a gene of unknown function on chromosome 9p21 first detected in Finnish and North American familial FTD and ALS cohorts, and identified by two separate groups of researchers [8,9]. The C9ORF72 gene structure spans 27, 546, 543–27, 573, 864 base pair (bp) on chromosome 9p21 and encodes eleven exons (Fig. 1). In these two studies, the C9ORF72 mutation was particularly frequent in patients and families with FTD-ALS. Based on the analysis of this region, normal individuals usually carry up to 23 repeated GGGGCC repeats, whereas the repeat number in affected cases may be up to 1000 [8,9]. However, there is evidence to demonstrate that larger normal repeat numbers do not influence disease phenotype [32,33]. Nevertheless, there may be a threshold number of repeats exist before C9ORF72 mutations that can finally cause the associated diseases [32–34]. This intriguing discovery leads to further research on C9ORF72 mutations all over the world. Epidemiologic studies have identified C9ORF72 mutations in one-third of all FTD cases due to genetic mutations [35,36]. Although the C9ORF72 mutation seems to be one of the most frequent mutations associated with FTD, it is difficult to suspect the presence of this mutation partly because of the numerous sporadic cases. We reviewed several reports from different countries, and found the frequency was varied from different countries [8,9,35–42] (Figs. 2 and 3). Furthermore, recent reports also suggest that the frequency of C9ORF72 mutations is much higher than mutations in progranulin (GRN) and micro-tubule protein tau (MAPT) as a cause of autosomal dominant FTD at present [30,43].

The function of uncharacterized C9ORF72 protein in the nervous system is not fully understood yet. The C9ORF72 protein has two different isoforms, isoform a (481 amino acid protein) and isoform b (222 amino acid protein), which are predicted to be expressed from three different transcripts [8,9] (Fig. 1). Transcript variants 1 and 4 are predicted to encode for isoform a, whereas variant 2 is predicted to encode

Table 1
Genes associated with FTD.

Gene	Number of mutation types	Location	Year	First reported	Pathological inclusions	Frequency	Associated clinical phenotypes	Reference
MAPT	44	17q21.1	1998	[16,17]	Tau(+), Ubiquitin(+)	0–21%	bvFTD, ALS, PNFA, SD, Parkinsonism	[18–20]
CHMP2B	4	3p11.2	2002	[21]	Ubiquitin(+), TDP-43(–), NCI	Rare	ALS, FTD	[21]
VCP	17	9p13.3	2004	[22]	TDP-43(+), NCI, NII	<1	Paget disease, FTD, ALS	[22,31]
GRN	69	17q21.32	2006	[22,23]	TDP-43(+), Ubiquitin(+), NII and NCI	3–26	bvFTD, ALS, PNFA, SD, PLS, Parkinsonism,	[31]
TARDBP	NA	1p36.22	2009	[26]	TDP-43(+)	<1%	ALS, bvFTD, Parkinsonism	[22]
FUS	NA	16p11.2	2010	[27]	Ubiquitin(+), TDP-43(–), NCI, NII	<3%	FTD, ALS	[28,29]
C9ORF72	1	9p21.2	2011	[9,10]	TDP-43(+), UPS, RAN, P62(+), NCI, NII	14–48	bvFTD, PNFA, PSP, AD, ALS, Parkinsonism	[30]

MAPT, microtubule-associated-protein-tau gene; GRN, the progranulin gene; FUS, the gene fused in sarcoma; CHMP2B, charged multivesicular body protein 2B; VCP, valosin containing protein gene; TARDBP, TAR DNA-binding protein; UPS, ubiquitin-positive but TDP-43 negative; RAN, non-ATG translation immunoreactive inclusions; PSP, progressive supranuclear palsy; SD, semantic dementia; bvFTD, behavior variant frontotemporal dementia; ALS, amyotrophic lateral sclerosis; AD, Alzheimer's disease; NII, neuronal intranuclear inclusions; NCI, neuronal cytoplasmic inclusions; (+), positive; (–), negative; NA, not applicable or not analyzed.

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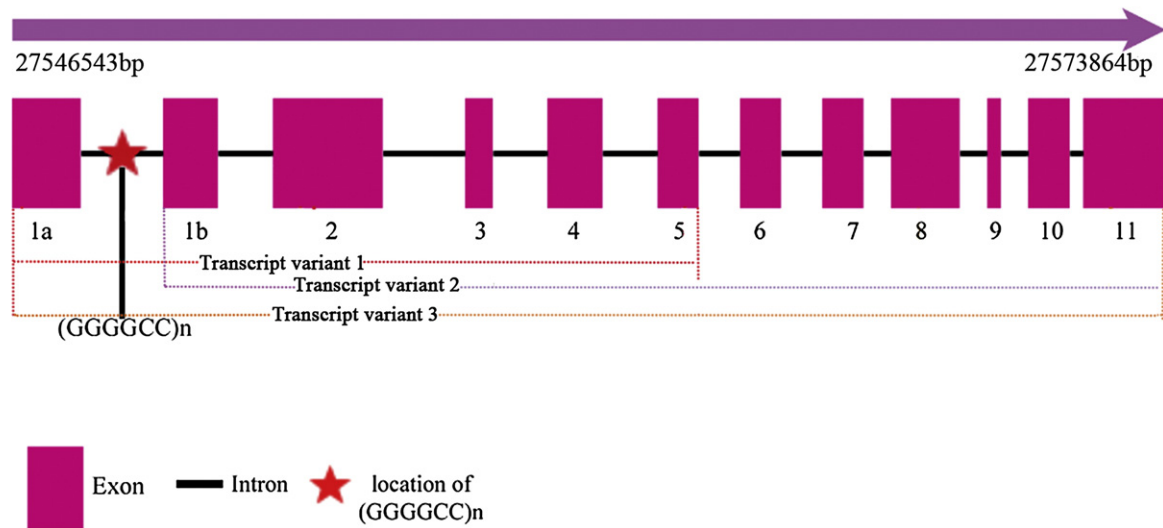


Fig. 1. Schematic representation of C9ORF72 gene and its major transcript variants. The C9ORF72 gene structure spans 27, 546, 543–27, 573, 864 base pair (bp) on chromosome 9p21 and encodes eleven exons (represented with magenta boxes). The position of the G_4C_2 repeat was represented as the red star. The letter “n” indicates G_4C_2 repeat length, with number of units ranging from 2 to 23 in healthy individuals and more than 30 are regarded as the expansion mutation. The three major transcript variants are numbered according to the NCBI RefSeq transcript collection (<http://www.ncbi.nih.gov/refseq/>). Coding regions of the three transcript variants: variant 1, exons between the red dotted line regions at the bottom; variant 2, exons between the purple dotted line regions; variant 3, exons between the orange dotted line regions. Transcript variants 1 and 4 are predicted to encode for a 481 amino acid long protein (isoform a), whereas variant 2 is predicted to encode a 222 amino acid short protein (isoform b).

isoform b (Fig. 1). C9ORF72 protein is largely a cytoplasmic protein in neurons. The unknown protein C9ORF72 was found to be predominantly localized within the nucleus in human control fibroblast cell lines. It is also indicated that the C9ORF72 RNA was detected across multiple CNS tissues obtained from neuropathologically normal individuals including spinal cord [9].

To date, nothing is known about the C9ORF72 protein's normal function or cellular distribution, but two possible disease mechanisms associated with the C9ORF72 mutation have thus far been proposed. One potential loss-of-function mechanism suggested that the repeat expansion is predicted to influence the normal expression of two alternative isoforms of the C9ORF72 protein [8]. Quantitative messenger RNA analysis has shown that the presence of the C9ORF72 mutation may result in loss of one alternatively spliced C9ORF72 transcript [8]. It is also demonstrated that the decreased transcript may influence the function of the unknown C9ORF72 protein [8,39]. Several groups have shown loss of the transcripts in C9ORF72 mutations carriers probably owing to the interference of the expanded mutations [8,9,39]. To address the loss-of-function hypothesis, a group developed a genetic model of C9ORF72 haploinsufficiency via knockdown of the zebrafish

orthologue of C9ORF72 (zC9ORF72) transcripts in the vertebrate model organism, zebrafish [44]. They found that the zC9ORF72 is selectively expressed in the developing nervous system at developmental stages; loss of the function of zC9ORF72 transcripts causes both behavioral and cellular deficits related to locomotion without major morphological abnormalities. All these results may suggest that loss of function of C9ORF72 mutation is sufficient to cause motor neuron defects in an in vivo setting. However, they also demonstrated another possible toxic RNA gain-of-function disease mechanism, which depends upon the accumulation of transcripts containing the GGGGCC repeat as nuclear RNA foci in the frontal cortex and spinal cord of C9ORF72 mutations carriers [8]. This accumulation mechanism of toxic RNA fragments composed of the repeated nucleotides has been shown in other non-coding expansion repeat disorders such as myotonic dystrophies, fragile-X associated tremor/ataxia syndrome, and several spinocerebellar ataxias [8]. Meanwhile, other groups also discovered the formation of nuclear RNA foci composed of the hexanucleotide repeat in brain tissue from the mutation carriers [45]. Although the pathogenicity of these RNA aggregates is still unclear, it is possible that they carry toxicity by sequestering essential cellular factors [46,47]. Alternatively, it has been

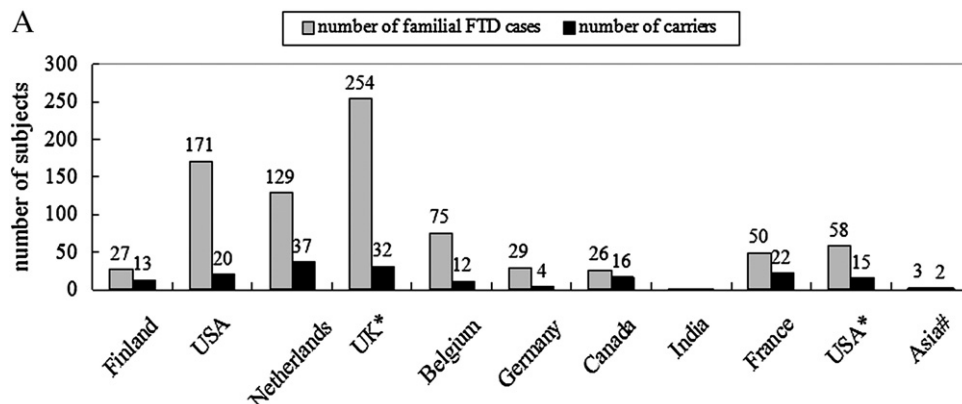


Fig. 2. Bar chart showing the frequency of the C9ORF72 repeat mutation in patients diagnosed with familial FTD varies across countries [8,9,35–42].

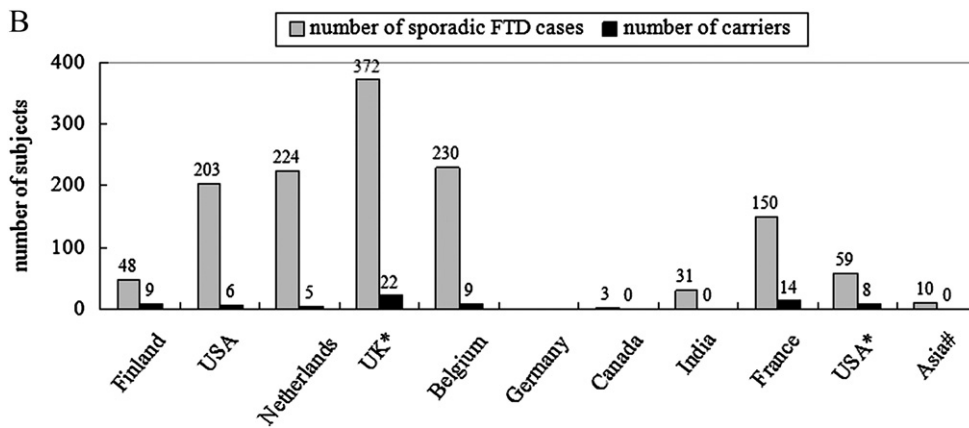


Fig. 3. Bar chart showing the frequency of the C9ORF72 repeat mutation in patients diagnosed with sporadic FTD varies across countries.

suggested that this accumulation of RNA foci within the cell nucleus may also lead to downstream messenger RNA splicing defects [48].

More quantitative approaches will be needed to definitively determine the localization of the different C9ORF72 isoforms in different tissues and at various stages of disease progression.

3. Neuropathology

The neuropathology associated with clinical FTD is heterogeneous. Furthermore, Murray and colleagues highlighted the pathological heterogeneity of patients with the C9ORF72 mutations [11]. Information about pathology in FTD with C9ORF72 mutations is limited. In many forms of neurodegenerative diseases, neuronal aggregations of dysfunctional proteins are regarded as an important neuropathological feature. It has also been identified that FTD is proteinopathy in which the toxic aggregation and deposition of characteristic proteins in specific brain areas are major etiologic and diagnostic hallmarks [49]; FTD is a key feature of both sporadic FTD-TDP and GRN-mutated FTD cases [50]. Staining using immunohistochemistry allows the pathology divided into specific proteinopathies based on the major constituent of the inclusions [37]. Immunohistochemistry analysis was always employed to detect the distributions and levels of UBQLN, TDP-43, and p62 in recent studies [11,37]. Neuronal cytoplasmic inclusions (NCIs) containing TDP-43 are regarded as a common pathological hallmark of FTD-TDP, and recognized as main components of the ubiquitin-positive inclusions of the FTD cases with GRN mutations [11,50]. Therefore, the relationship between TDP-43 inclusions and C9ORF72 mutations is unclear. Recently, the new discovery of p62 positive, TDP43 negative inclusions were mostly regarded as the consistent pathological feature in cases of the C9ORF72 mutations. We are not sure whether the neuropathological signature can distinguish FTD cases with and without the C9ORF72 expansion or not, but the neuropathological findings provide evidence that the ubiquilin (UBQLN) pathologies were sufficiently unique to allow correct prediction of cases that were later confirmed to have C9ORF72 expansion by genetic analysis. Notwithstanding the mechanism of FTD disease remains unknown, the neuropathological findings have significant implications for its pathomechanism.

3.1. TDP-43 positive pathology

TDP-43 has been identified as an important component of ubiquitinated inclusions in FTD patients [6]. TDP-43 can be classified into four types (type A, B, C, D) according to the new classification [51]. TDP-43 type A (Mackenzie type 1; Sampathu type 3) is characterized by cortical neuronal cytoplasmic inclusions and dystrophic neurites (DN) often with intranuclear inclusions and widespread involvement

of subcortical areas, while type B (Mackenzie type 3; Sampathu type 2) have many neuronal cytoplasmic, but few dystrophic neurites and more limited subcortical pathology. The TDP-43 type C (Mackenzie type 2; Sampathu type 1) is characterized by long thick dystrophic neurites and Pick-body-like NCI with minimal brainstem pathology, while type D (Mackenzie type 4; Sampathu type 4) is characterized by numerous short DN and frequent lentiform NII. In healthy individuals, both TDP-43 and FUS are primarily located in the nucleus of cells [52,53]. While in ALS, FTD-FUS and FTD-TDP cases, they are mislocalized and form neuronal and glial inclusions pathologically [54].

In a review of 16 unrelated families with FTD caused by the C9ORF72 mutations, this important pattern of pathology was reported [38]. All these affected members were found to have transactive response DNA binding protein with 43kD (TDP-43) pathology [38]. Although the degree of cerebral atrophy in these mutation carriers varied considerably, gross atrophy of the cerebral lobes was noted in 67% cases. The atrophy was described as symmetric and more frequently occurred in the frontal lobes. Loss of pigmentation of the substantia nigra was demonstrated in 57% affected cases. No-specific degenerative changes were present in affected regions of the cerebral cortex as well as striatum and substantia nigra. Besides, a majority of cases showed significant loss of pyramidal cell loss from the CA1 region of the hippocampus. Microscopic neuropathology also demonstrated the presence of TDP-43 immunoreactive cellular inclusions. In another report, FTD-TDP type B, characterized by compact neuronal cytoplasmic inclusions in all cortical layers with few dystrophic neurites, was identified in 62% of cases [51]. The remaining cases also showed a pattern consistent with FTD-TDP type A, characterized by FTD-TDP type B features combined with a superficial band of more compact small neuronal cytoplasmic inclusions and dystrophic neuritis, as well as rare lentiform neuronal intranuclear inclusions (NII).

Table 2

The TDP-43 positive findings of FTD cases associated with C9ORF72.

Reference	Associated clinical phenotype	TDP-43 Type A (n)	TDP-43 Type B (n)	TDP-43 Type C (n)	TDP-43 negative P62 positive
[11]	FTD-MND	1	6	1	0
[11]	FTD-TDP	6	0	1	0
[35]	FTD-TDP	4	2	0	6
[36]	FTD	1	3	0	4
[38]	FTD-TDP	8	13	0	18
[41]	FTD	0	10	0	NA
[42]	FTD	10	3	0	NA
[56]	Familial FTD	0	5	0	NA
[57]	FTD	0	11	0	NA
[58]	FTD-TDP	0	9	0	NA
[65]	FTD	0	2	0	0

FTD, frontotemporal dementia; TDP-43, TAR DNA-binding protein-43.

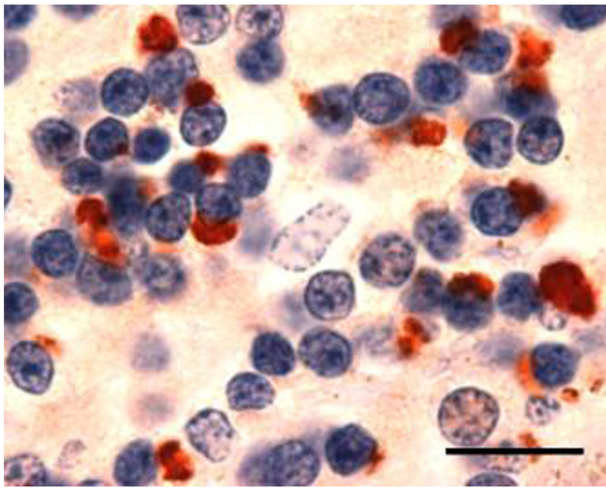


Fig. 4. Numerous neuronal cytoplasmic inclusions (ubiquitin positive, TDP-43 negative inclusions) and neurites in cerebellar granular layer in C9ORF72 mutations carriers (Scale bar: 12 μ m) [8].

This TDP-43 type B pathology has been found in other families with FTD \pm ALS with the pathogenic repeat expansion in C9ORF72 by other groups [8,9,36,41,42,55–57] (Table 2). In one report, the pathological examination of the 10 brains from patients carrying expanded repeats also revealed TDP-43 positive inclusions of variable type, size and morphology in all brains [41]. Postmortem examination usually shows the TDP-43-positive histopathological changes in a wide range of neuro-anatomical regions, including the extramotor cerebral cortex, hippocampus, basal ganglia, substantia nigra, and lower motor neurons of the brainstem and spinal cord. A research from Australia demonstrated that 41% of TDP-43-positive cases in the Australian FTD patient cohort harbored the repeat expansion [58]. Besides, these TDP-43-positive mutations carriers are detected with type B pathology in a large number, and besides, type A and type C in a small number. Dot-like TDP-43 positivity in the hippocampal CA2–4 region as well as TDP-43 positive fine dystrophic neurites (DNs) in the hippocampal CA1 region and subiculum in cases with the C9ORF72 mutations has been reported in several reports [11,59]. Irwin and colleagues examined the ubiquilin (UBQLN and TDP-43) neuropathology in a group of ALS and FTD patients [60]. They intriguingly found that greater neuronal loss in the mid-frontal cortex in FTD patients with the C9ORF72 mutations and mid-frontal cortex TDP-43 inclusion is related with the letter fluency performance occurred in the FTD patients. Neuronal cytoplasmic inclusions (NCIs) containing TDP-43 are regarded as the pathological hallmark of FTD-TDP [11], but the relationship between TDP-43 and C9ORF72 related FTD needs further research.

3.2. P62 and UBQLN pathology

In addition to TDP-43-positive neuronal and glial inclusions, ubiquitin and P62 immunohistochemistry demonstrated TDP-negative neuronal cytoplasmic inclusions, which can only be identified with antibodies for p62, ubiquitin or the related ubiquitins [61](Fig. 4). Moreover, mounting publications of additional families with C9ORF72 mutations have confirmed p62 positive, TDP-43 negative neuronal inclusions to be the consistent pathological feature in cases of the C9ORF72 mutations [8,10,11,35,36,38,41,42,48,59,61–67](Fig. 4; Table 3). These reported TDP-43 negative, p62-positive inclusions always presented as compact globular or star-shaped NCIs and spherical NIIs, and were abundant in the granular layer [68](Table 3). These special neuronal cytoplasmic inclusions and glial cytoplasmic inclusions were found in the frontotemporal neocortex, cerebellum, and hippocampus, which were relatively rare reported in cases without the C9ORF72 mutations [38,61,66]. Thus, these special phospho-TDP-43 negative inclusions are regarded as the highly special pathological characteristic of C9ORF72 mutation carriers [35,55,59,61]. The most consistent and specific of these p62 positive neuronal cytoplasmic inclusions (NCIs) were seen in the granular layer of the cerebellum, Purkinje cells and dentate fascia of the hippocampus [38,61]. P62 positive and TDP-43 negative NCIs were detected in the granular cell layer, molecular layer and Purkinje cells of the cerebellum and pyramidal cell layer of the hippocampus; p62 positive and TDP-43 negative spherical NIIs were found in the pyramidal cell layer of hippocampus and granular cell layer of the cerebellum [61]. They also demonstrated that these inclusions do not contain the C9ORF72 protein nor any other known FTD associated proteins such as fused in sarcoma (FUS). The immunohistological discovery of P62 positive, TDP-43 negative inclusions in a variety of neuro-anatomical regions may contribute to this specific feature of the C9ORF72 mutation. Besides, the presence of these special inclusions in the cerebellar granule cell layer may be of great use for distinguishing cases with the mutation. A group reported that these special p62 positive, TDP-43 negative inclusions were highly correlated with hippocampal atrophy [59]. The consistent presence of p62 positive pathology also implies the mismetabolism of some protein other than TDP-43 protein in cases with C9ORF72 mutations. In most cases, immunohistochemistry with antibodies against A β , tau proteins failed to demonstrate any additional pathology. But in a bvFTD patient with C9ORF72 and MAPT mutations, the dominant neuropathology was a tauopathy with Pick's disease-like features [63]. Furthermore, TDP-43 labeling in the cases was mainly confined to Pick bodies, but p62-positive, TDP-43-negative inclusions were present in the cerebellum and hippocampus [63]. Interestingly, the pathology in the patient's frontal and temporal lobes was predominantly 3 repeat tau (3-R tau) deposition which appears as numerous Pick bodies associated with MAPT mutations in the hippocampus and cerebral cortex. It is speculated that the presence of the C9ORF72 mutation might influence tau deposition in what was previously thought

Table 3
Summary of the p62 positive inclusions in various brain regions of FTD cases with C9ORF72 repeat expansion.

Reference	Frontotemporal neocortex	Cerebellum	Hippocampus	Purkinje cell
[10]	Many NCI, swollen DN, in pyramidal neurons	NCI; short DN, small round NII, in the granule cell layer	Small round NII in pyramidal neurons	(–)
[35]	(–)	NCI in the granule cells	NCI in the granule cells of dentate gyrus	(–)
[36]	NA	NCI in granule cells	(–)	NA
[38]	Small dot-like NCI, GCI, rare large swollen DN	Neuronal inclusions in the cortex	Focal cytoplasmic collection of granules	NA
[61]	Semi-lunar and dot-like NCIs positive for both TDP-43 and P62	NCIs, NII in the granular layer	Abundant globular and star-shaped NCIs in the pyramidal cell layer; occasional dystrophic neurites	(+)
[62]	Irregular and star-shaped NCIs in upper layers; abundant in the lower layers	Abundant NCIs in the granular cell layer; molecular layer	Abundant NCIs in the pyramidal cells; tiny NIIs in CA4 neurons	(+)
[63]	Pick body: HP-tau (+), 3-R tau (+), p62 (+)	No neuronal loss; numerous NCIs, neurites, occasional NIIs in granular cell layer	NCIs in the pyramidal cell layer, especially CA4	Occasional NCIs

HP-tau, hyperphosphorylated tau; 3-R tau, 3 repeat tau; NCI, neuronal cytoplasmic inclusion; NII, neuronal intranuclear inclusion; DN, dystrophic neurites; NA, not available; (–), no p62 positive inclusions; (+), positive results.

to be a “benign” variant in MAPT in addition to the aggregation of TDP-43 and other unidentified proteins decorated with ubiquitin and p62.

These inclusions were also immunoreactive for ubiquitin and select ubiquitin-binding proteins, most notably ubiquitin-2 [37,67]. It is also exemplified that ubiquilin (UBQLN) pathology, which was also found in ALS cases caused by mutations in the UBQLN2 gene, showed a highly distinct pattern in cases with the C9ORF72 mutations [37]. UBQLN pathology showed that UBQLN-positive cytoplasmic inclusions aggregated in the cerebellar granular layer. FTD-TDP cases with C9ORF72 expansions also showed dystrophic neurites and aggregate-like formations throughout the neocortex. The relationship between the p62 pathology and UBQLN pathology was discussed in this article. It was demonstrated that several inclusions exhibited colocalization of UBQLN and p62 pathology in both hippocampal aggregate-like formations as well as in cerebellar granular layer neuronal inclusions. However, UBQLN pathology was more extensive than p62 pathology. This finding suggests a pathophysiological link between C9ORF72 mutations and UBQLN pathology in FTD, which still needs to be confirmed in the future.

Thus, all these consistent findings provide further hypothesis that the mutation causes abnormal metabolism and one or more unidentified proteins other than TDP-43 were accumulating in the C9ORF72 mutations carriers.

3.3. Pathology related with repeat associated non-ATG translation

One possible pathogenic mechanism for C9ORF72 mutations is repeat associated non-ATG translation (RAN translation), an unconventional mode of translation that occurs in the absence of an initiating ATG codon [46]. RAN translation was first described by Ranum and colleagues, who reported that RAN translation across expanded CAG repeats occurs in all reading frames (CAG, AGC and GCA) to produce homopolymeric proteins, in patients with spinocerebellar ataxia type 8 and myotonic dystrophy type 1 [69]. RAN translation of (GGGGCC)_n transcripts in three alternate reading frames would produce three dipeptide-repeat (DPR) proteins: poly-(glycine–alanine), poly-(glycine–proline), and poly-(glycine–arginine) [70,71]. In a recent literature published in *Science*, researches also identified those three dipeptide-repeat (DPR) proteins aggregated in the intracellular inclusions of all ten patients with confirmed C9ORF72 expansion mutations [47]. They found that the C9ORF72 mRNA levels was reduced in patient cerebellum, while both sense and antisense transcripts containing the GGGGCC repeat were obviously increased in mutations carriers. It is therefore tempting to speculate that the antisense strand may be translated into poly-(Pro–Arg), poly-(Ala–Pro) and further poly-GP DPRs.

The insoluble anti-C9RANT-immunoreactive high molecular weight material was observed in samples from C9ORF72 mutations carriers, but showed negative result in other neurodegenerative diseases or in peripheral tissues of c9FTD/ALS [46]. Meanwhile, widespread neuronal cytoplasmic and intranuclear inclusions were revealed by the immunohistochemistry. These inclusions were morphologically similar to TDP-43-negative inclusions noted in the above and detected in most abundance in regions previously shown to be affected in c9FTD/ALS with p62 immunohistochemistry, including the neocortex, hippocampus, and cerebellum [46]. But C9RANT-immunoreactive inclusions were exclusively found in gray matter and within neurons. In the following researches, it was reported that dot-like neuronal cytoplasmic inclusions detected by poly-GA specific antibodies in the granular cell layer of the cerebellum were similar to the p62 positive/TDP-43 negative inclusions both in shape and abundance [47]. However, the following research demonstrated that there was no coaggregation of the two proteins. They found that occasionally small spheric poly-GA aggregates were surrounded by aggregated p63 positive, TDP-43 negative proteins forming a core inside phospho-TDP-43 inclusions. These findings highly suggested a possibility that DPR aggregation may precede TDP-43 pathology. This development of anti-C9RANT immunoassays in cerebrospinal fluid may help to be served as a potential marker of disease

activity or progression. Since the abnormal accumulation of insoluble proteins has been associated with neuronal dysfunction and degeneration in many neurodegenerative diseases, the identification of disease-specific C9RANT-positive pathology may provide a new insight on the pathological mechanism. This new discovery gives new insight into the pathology of FTD related with C9ORF72 mutations, and requires further confirmation in other racial and ethnic groups in numerous numbers.

3.4. Microglial pathology

Microglial pathology was assessed by IHC with 2 different antibodies (CD68, Iba1), myelin loss by Kluver–Barrera staining and myelin basic protein (MBP) IHC and axonal loss by neurofilament protein (TA51) IHC [70]. It was demonstrated that microglial pathology as depicted by CD68 and Iba1 was significantly more extensive in the CST of ALS cases with rapid progression of disease [70]. They reported that cases with C9ORF72 repeat expansion showed more extensive microglial pathology in the medulla and motor cortex. This new finding may give us new clue to find the pathological mechanism of FTD with C9ORF72 mutations.

3.5. RBM45 pathology

RNA-binding protein pathology represents one of the best characterized pathologic features of FTD patients with TDP-43 or FUS pathology. RNA-binding motif 45 (RBM45) is a 476 amino acid protein that exhibited structural similarities with TDP-43 and FUS. RBM45 contains three RNA recognition motifs and a C-terminal nuclear localization sequence. In one research, RBM45 pathology was observed in all researched FTD-TDP cases with the C9ORF72 mutations [71]. Collins and colleagues also detected that these RBM45 inclusions were in spinal cord motor neurons, glia and neurons of the dentate gyrus. The presence of RBM45 pathology in all FTD-TDP C9ORF72 mutations carriers may provide another pathway of the pathology. But the confirmed researches in lager cohort were needed to identify this relationship.

3.6. Other pathological clues

Apolipoprotein E (apoE), a lipid transport protein, is encoded by a gene with three alleles (ϵ 2, ϵ 3, and ϵ 4). As reported in the original reports, apoE4 isoform plays an important role in enhancing brain atrophy within vulnerable regions in behavioral variant FTD (bvFTD) [72]. Vessel and colleagues studied two siblings with C9ORF72-linked familial FTD-MND [73], and they predicted that apoE4 aggravates TDP-43 pathology by forming apoE-TDP-43 complexes which may disrupt normal nuclear localization and RNA processing functions of TDP-43 or impair cell survival. The interaction of apoE and TDP-43 may contribute to the survival length of neurodegenerative diseases, but needs more researches to identify.

In addition, patients with mutations in the progranulin (GRN) gene are typically associated with TDP-43 pathology [24]. As discussed above, cases with C9ORF72 mutations also showed TDP-43 pathology. There is evidence that plasma GRN levels reflect mutation factors and can be modulated by some additional factors [74]. All these findings encourage researchers to find the mechanism overlap between the two mutations [75]. It was reported that most GRN mutations cause protein haploinsufficiency, leading to a significant decrease in progranulin (GRN) levels that can be detected in GRN mutation carriers [76]. However, the analysis of plasma GRN levels in 65 FTD patients showed no differences in plasma GRN level between the C9ORF72 mutations carriers and noncarriers [75]. Plasma GRN levels are not associated with the hexanucleotide repeat expansion in C9ORF72 gene, and cannot be recognized as a participator in the pathology.

There are a number of antibodies against C9ORF72 protein, however, previous studies have failed to demonstrate any abnormal distribution or accumulation of this protein in mutations carriers [8,10,35–38,48]. Although C9ORF72 staining didn't reveal specific pathology, future

studies are needed to determine whether the level of expression or cellular distribution of C9ORF72 protein/RNA takes part in the pathology of cases with the C9ORF72 mutations. Besides, there were evident to note that no significant difference of tau, A β , or α -synuclein pathology was reported between FTD-TDP cases with and without C9ORF72 mutations [77].

All these findings above may direct to the correlation of C9ORF72 with the neurodegenerative diseases. Nevertheless, further detailed clinical pathological correlations of cellular and animal-model experiments are required to elucidate the complex pathology of diseases related with C9ORF72 mutations. Besides, further study with larger, prospective databases of C9ORF72 mutations related diseases and animal models would be beneficial to identify the pathology and disease mechanism.

4. Clinical phenotypes

To date, the spectrum of frontotemporal dementia with C9ORF72 mutations includes the behavioral variant (bvFTD), primary progressive aphasia (PPA; further sub-categorized as progressive non-fluent aphasia PNFA, semantic dementia SD), FTD-ALS, and movement disorders with extrapyramidal features such as Parkinsonism and corticobasal syndrome. The first reported linkage between 9p21-22 and FTD comes from a study carried out in families with FTD-MND patients in 2000 [78]. After more than a decade research, C9ORF72 is first identified in Finnish and North American familial FTD and ALS cohorts by two separate groups of researchers. Since its discovery in 2011, many groups from different countries have published descriptions of the demographic, clinical features of their cohorts with the C9ORF72 mutations. Regarding the large hexanucleotide (GGGGCC) repeat expansion in the first intron of C9ORF72 recently discovered, there are limited data about its frequency in FTD (Figs. 2 and 3). The frequency of C9ORF72 mutations in familial cases is higher than in sporadic cases. In a cross-sectional study, the pathological C9ORF72 repeat expansions were detected in 11.4% of the 1381 FTD patients of European origin, and 24.8% in familial patients [40]. Even in sporadic FTD, the frequency is reaching to 6.0% in the same report. It is significant to note that the C9ORF72 repeat expansion was most frequent in those with a diagnosis of FTD-ALS [11,58].

Previous reports find considerable heterogeneity in the clinical phenotype and demographic features of C9ORF72 mutations carriers as compared with the patients without the C9ORF72 mutations [38,41,65]. The clinical presentations of C9ORF72 mutations carriers are various, even in a family. Cases with the C9ORF72 mutations may present with FTD, ALS or other neurodegenerative diseases. However, there is considerable variation in age at onset and clinical presentation among FTD patients with C9ORF72 mutations. Although the age at onset varied from 27 to 83 years and the disease duration varied from 1 to 22 years, they more frequently present with earlier onset, earlier death, a shortened survival and a positive family history [10,38,39,42,79]. Moreover, further research demonstrated that more rapid rate of cognitive decline was occurred in FTD patients with the C9ORF72 mutations [60].

4.1. Behavioral variant frontotemporal dementia

BvFTD is the most frequent FTD phenotype, even in the C9ORF72 mutations carrier cohort. It is primarily characterized by behavioral changes and progressive deterioration of personality. Although there was substantial clinical heterogeneity among the FTD C9ORF72 mutations carriers, bvFTD is the most common presenting phenotype [35].

Mounting reports demonstrated that the bvFTD patients with the C9ORF72 mutations most frequently manifest early psychotic symptoms: delusions, hallucinations, paranoid ideation and disordered thinking [58,80]. Other cases also underline that the hexanucleotide repeat expansion in chromosome 9 could be associated with early onset psychiatric presentations [81–84]. Additional clinical features of C9ORF72 mutations

carriers included frequent memory deficit, hallucinations and delusions [55,85,86]. A group reported a case of a suicide attempt as the presenting symptom of early dementia was finally detected with the C9ORF72 expansion mutations [87]. Impaired differentiation of self from others (self-other differentiation), as a core cognitive operation, was reported in one bvFTD patient harboring the C9ORF72 mutations [88]. This psychopathological symptom category may be a marker for recognizing the mutation of C9ORF72. They also found that the behavioral characteristics of patients with C9ORF72 mutations are qualitatively different from those without.

4.2. Primary progressive aphasia

Primary progressive aphasia (PPA), which is characterized by early and progressive changes in language functions, represents the alternative presentation of FTD. No subject with a diagnosis of PPA was identified with the C9ORF72 mutations in several researches [42]. However, the mutations were detected in 6.8% (5/73) of PPA patients in a cohort of French population [30]. Meanwhile, five PPA patients were also detected with the C9ORF72 mutations in another report [60]. Primary progressive aphasia (PPA) phenotype has been reported in small number of patients with C9ORF72 mutations [35,36,38].

Progressive non-fluent aphasia (PNFA) subtype is a disorder predominantly of expressive language, in which severe problems in word retrieval occur in the context of preserved word comprehension. As is reported in a literature, 4.8% (3/62) of the PNFA patients and 18.2% (2/11) of the patients with semantic variant PPA were detected to carry the C9ORF72 mutations [30]. Semantic dementia (SD) subtype represents loss of knowledge about words and objects, anomia and single-word comprehension deficits. There were also reported C9ORF72 mutation carriers in patients diagnosed with semantic dementia [36,86].

4.3. FTD-ALS

Since the early 20th century, the comorbidity of amyotrophic lateral sclerosis (ALS) with behavior alterations, cognitive impairment or dementia has been reported [89–91]. Recently more significant clinical and neuropathological overlap gives more clues to show that ALS and FTD may represent a disease spectrum [92]; the hexanucleotide expansion in C9ORF72 tied them together [9–11,58,93,94]. The discovery of C9ORF72 made it the most frequent genetic cause of ALS and FTD (c9FTD/ALS). Just as demonstrated by DeJesus-Hernandez and colleagues, there are 26.9% FTD cases detected with concomitant ALS and more than 30% had relatives affected with ALS [8]. C9ORF72 was reported to be the most frequent mutation in the FTD-ALS patients at all ages when compared with patients with other genes [30]. Besides, they also illustrated that ALS is indicative of a C9ORF72 expansion. Nevertheless, not all cases of frontotemporal dementia with motor neuron disease (ALS) must be associated with C9ORF72 mutations. It is speculated that the C9ORF72 expansion, environmental factors or other gene's interaction plays a role in phenotypic expression in the cases with the C9ORF72 mutations [95]. It may help to explicate the phenomenon that some FTD cases with the C9ORF72 mutations have elements or the full clinical picture of ALS.

4.4. Phenotypic overlap with other neurological diseases

Since the discovery of C9ORF72 mutations, the overlap of FTD with motor neuron disease (FTD-MND or FTD-ALS) [36], Alzheimer's disease, as well as the Parkinsonism, progressive supranuclear palsy (PSP) and corticobasal syndrome (CBS) has been mentioned.

Although Alzheimer's disease and frontotemporal dementia are clinically distinguishable entities, there is a clinical and pathologic overlap between FTD and Alzheimer's disease. Because the patients who fulfill the current diagnostic criteria for AD might also meet the FTD criteria, the clinical overlap between AD and FTD is not surprising

[96]. In a recent research reported on the New England Journal of Medicine, the C9ORF72 repeat mutations were present in 3 of 342 families (<1%) which are apparently affected with Alzheimer's disease and also present in 6 of 771 subjects (<1%) who were probably Alzheimer's disease [97]. In a second series of 114 AD patients with early age at onset and cerebrospinal fluid (CSF) biomarker profile typical of AD, three patients were detected with the C9ORF72 mutations [98]. C9ORF72 mutation carriers might present as "mixed dementia" with features of both bvFTD and Alzheimer's disease [97,99]. A large cohort of 1184 AD patients was investigated by Kohli's group and resulted in the identification of 9 patients carrying the C9ORF72 expansion mutations [100]. Meanwhile, negative reports were also reported [79,101]. All these findings may confirm that C9ORF72 mutations are not a common cause of AD, but it could nevertheless underline a neurodegenerative process presenting with a clinical phenotype compatible with AD [97,102,103]. The most possible explanation is that these positive subjects had amnesic FTD that was misdiagnosed as probable Alzheimer's disease. But nine C9ORF72 mutation carriers who showed no signs of clinical or pathologic FTD were finally confirmed with definite AD diagnoses via brain autopsy [100]. The misdiagnosed explanation cannot fully illustrate the relationship between AD and C9ORF72 gene. Contrarily, another explanation is that many patients with clinical AD and C9 expansion do not have autopsy information, but they may present an atypical clinical phenotype associated with FTD-TDP/C9RAN pathology. The discovery of C9ORF72 mutations in AD patients may suggest, albeit rare, that the C9ORF72 mutations could act as a contributor to AD pathogenesis. In addition, mounting evidence indicated that the occurrence of C9ORF72 mutation may increase the incidence of Parkinson's disease in their relatives [38,66]. Many bvFTD or ALS cases were reported with some degree of Parkinsonism, which is typically of the akinetic-rigid type without tremor [42,84]. Visuospatial dysfunction is reported in several publications [35,36,38,42]. There is special phenotype of dementia with Lewy bodies reported in a few cases [11].

All these observations suggest that the FTD cases with C9ORF72 mutations can represent as a variety of phenotypes including bvFTD, PPA phenotype, and FTD-ALS. In addition to the FTD phenotype, emerging evidence has demonstrated that C9ORF72 mutations could be associated with a series of neurodegenerative diseases [104]. At the rapid rate new knowledge is currently learned in this field, the connection between C9ORF72, FTD and other neurodegenerative diseases will likely soon be identified.

4.5. Neuroimaging characteristics

It is known that neuroimaging plays a critical role in diagnosis of FTD. New neuroimaging investigations can help differentiate the C9ORF72 mutation carriers from the FTD cases. Assessment of patients with the C9ORF72 mutations by means of structural neuroimaging showed that the neuroimaging profile of the C9ORF72 expansion was significantly more symmetrically bilateral atrophy, primarily in frontotemporal regions [35,38,41,42,105]. In addition to the symmetric atrophy in dorsolateral, medial and orbitofrontal lobes, anterior temporal lobe, parietal lobes, occipital lobes and cerebellum were also reported to be associated with atrophy [105]. This specific pattern reported differed from that observed in MAPT and GRN mutations [105,106]. Furthermore, previous imaging studies have found that the C9ORF72 mutation carriers showed a regional profile of grey matter atrophy including thalamus bilaterally, left orbitofrontal cortex and bilateral posterior cerebellum, which is not typically involved in sporadic FTD cases [35]. It is speculated that involvement of thalamus and cerebellar connections could underpin the prominent neuropsychiatric features of these mutation carriers, as well as episodic memory deficits. It is noteworthy that the sites of p62 positive inclusions mentioned above, including hippocampal and cerebellum, are likely to be pathophysiologically relevant based on these neuroimaging findings in the same cohort [35].

However, this correlation between neuroimaging findings and pathological findings needs to be further identified in other cohorts.

The relationship between the C9ORF72 gene and the clinical FTD subtypes and proteinopathies was underlined in these above reports, but the strict relationship of proteinopathies and clinical phenotypes associated with C9ORF72 is lacking. Further research is required to improve our understanding of the correlation between neuropathology and clinical phenotypes of the FTD phenotypes related with C9ORF72 mutations.

5. Concluding remarks

Although the mutation was detected in September 2011, in this short space of time, further clinical and pathological details on neurodegenerative diseases related with C9ORF72 mutations were reported all over the world. The TDP-43 pathology is regarded as an important pathology in the initial researches. However, the relevance of TDP-43 accumulation in C9ORF72 mutations carriers has recently been challenged by the new identification of TDP-43 negative, ubiquitin-positive pathology which is more abundant than the TDP-43 pathology in distinct brain regions. Abundant reports suggest that in the C9ORF72 mutation carriers, other unidentified proteins other than neither TDP-43 proteins nor C9ORF72 proteins might exist to play an important role in the neuropathology. C9ORF72 staining didn't show significant differences between FTD cases with or without C9ORF72 mutations. The new discovery of dipeptide-repeat protein may help to explain the neuropathology associated with C9ORF72 mutations, and gives us new insight into it. When the disease mechanism is not well known or the role of C9ORF72 gene and mutations in the biology of disease is not established, the identification of the special proteins and their predicted function are useful indicators for the therapy of FTD. The discovery of C9ORF72 mutations improved our knowledge of the pathology and the mechanism of FTD. The identification of C9ORF72 in FTD also opened a new avenue of research on other neurodegenerative diseases. Despite the excitement, it is important to acknowledge that the knowledge of C9ORF72 mutations we mastered is only the iceberg, we need to explore it further. Identification of neuropathological biomarkers for clinical diagnosis warrants future studies. Meanwhile, the underlying C9ORF72-related mechanisms in FTD still need to be elucidated in further researches. More researches are required to improve our understanding of the clinical and pathological phenotypes associated with C9ORF72 mutations. Finally, we sincerely hope that patients with FTD will soon experience real benefits from this new discovery and future advances.

Conflicts of Interest

No conflicts of interest.

Acknowledgments

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