



The C9orf72 GGGGCC Repeat Is Translated into Aggregating Dipeptide-Repeat Proteins in FTL/ALS

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of the *hgcAB* cluster in the genomes of several sequenced, but so far untested, microorganisms (table S4) leads us to hypothesize that these organisms are also capable of methylating mercury. The gene cluster appears to be quite sporadically distributed across two phyla of bacteria (Proteobacteria and Firmicutes) and one phylum of archaea (Euryarchaeota). Organisms possessing the two-gene cluster include 24 strains of Deltaproteobacteria, 16 Clostridia, 1 Negativicutes, and 11 Methanomicrobia. Interestingly, we also found these genes in a psychrophile (30), in a thermophile (31), and in a human commensal methanogen (32) (Fig. 3). The sparse phylogenetic gene distribution of the *hgcAB* system may be due to gene loss or lateral gene transfer (or both) across distant taxa and may be linked to environmental and community-structure factors. The sporadic distribution of these genes and the lack of an obvious selective advantage related to mercury toxicity (15) raise important questions regarding their physiological roles. Identification of these genes is a critical step linking specific microorganisms and environmental factors that influence microbial Hg methylation in aquatic ecosystems.

References and Notes

1. J. M. Wood, *Science* **183**, 1049 (1974).
2. H. Hintelmann, *Met. Ions Life Sci.* **7**, 365 (2010).
3. G. C. Compeau, R. Bartha, *Appl. Environ. Microbiol.* **50**, 498 (1985).
4. C. C. Gilmour, E. A. Henry, R. Mitchell, *Environ. Sci. Technol.* **26**, 2281 (1992).
5. E. J. Fleming, E. E. Mack, P. G. Green, D. C. Nelson, *Appl. Environ. Microbiol.* **72**, 457 (2006).
6. E. J. Kerin et al., *Appl. Environ. Microbiol.* **72**, 7919 (2006).
7. R. Q. Yu et al., *Environ. Sci. Technol.* **46**, 2684 (2012).
8. J. M. Wood, F. S. Kennedy, C. G. Rosen, *Nature* **220**, 173 (1968).
9. S. Hamelin, M. Amyot, T. Barkay, Y. P. Wang, D. Planas, *Environ. Sci. Technol.* **45**, 7693 (2011).
10. J. K. Schaefer et al., *Proc. Natl. Acad. Sci. U.S.A.* **108**, 8714 (2011).
11. S. C. Choi, T. Chase Jr., R. Bartha, *Appl. Environ. Microbiol.* **60**, 4072 (1994).
12. S. C. Choi, T. Chase Jr., R. Bartha, *Appl. Environ. Microbiol.* **60**, 1342 (1994).
13. E. B. Ekstrom, F. M. Morel, J. M. Benoit, *Appl. Environ. Microbiol.* **69**, 5414 (2003).
14. M. Ranchou-Peyruse et al., *Geomicrobiol. J.* **26**, 1 (2009).
15. C. C. Gilmour et al., *Appl. Environ. Microbiol.* **77**, 3938 (2011).
16. R. Q. Yu et al., *Environ. Sci. Technol.* **46**, 2684 (2012).
17. T. I. Doukov, T. M. Iverson, J. Seravalli, S. W. Ragsdale, C. L. Drinnan, *Science* **298**, 567 (2002).
18. Y. Kung et al., *Nature* **484**, 265 (2012).
19. S. W. Ragsdale, P. A. Lindahl, E. Münck, *J. Biol. Chem.* **262**, 14289 (1987).
20. T. Svetlichnaia, V. Svetlichnyi, O. Meyer, H. Dobbek, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 14331 (2006).
21. S. Goetzl, J. H. Jeoung, S. E. Hennig, H. Dobbek, *J. Mol. Biol.* **411**, 96 (2011).
22. S. D. Brown et al., *J. Bacteriol.* **193**, 2078 (2011).
23. M. Punta et al., *Nucleic Acids Res.* **40** (database issue), D290 (2012).
24. H. L. Drake, S. I. Hu, H. G. Wood, *J. Biol. Chem.* **256**, 11137 (1981).
25. T. Doukov, J. Seravalli, J. J. Stezowski, S. W. Ragsdale, *Structure* **8**, 817 (2000).
26. P. Cardiano, G. Falcone, C. Foti, S. Sammartano, *J. Chem. Eng. Data* **56**, 4741 (2011).

27. R. E. DeSimone et al., *Biochim. Biophys. Acta* **304**, 851 (1973).
28. H. A. O. Hill, J. M. Pratt, S. Ridsdale, F. R. Williams, *J. Chem. Soc. Chem. Commun.* **1970**, 341 (1970).
29. G. N. Schrauzer, J. W. Sibert, *J. Am. Chem. Soc.* **92**, 3509 (1970).
30. G. S. Zhang, N. Jiang, X. L. Liu, X. Z. Dong, *Appl. Environ. Microbiol.* **74**, 6114 (2008).
31. G. B. Slobodkina et al., *Int. J. Syst. Evol. Microbiol.* **62**, 2463 (2012).
32. B. Dridi, M. L. Fardeau, B. Ollivier, D. Raoult, M. Drancourt, *Int. J. Syst. Evol. Microbiol.* **62**, 1902 (2012).

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Supplementary Materials

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The *C9orf72* GGGGCC Repeat Is Translated into Aggregating Dipeptide-Repeat Proteins in FTL/ALS

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Expansion of a GGGGCC hexanucleotide repeat upstream of the *C9orf72* coding region is the most common cause of familial frontotemporal lobar degeneration and amyotrophic lateral sclerosis (FTLD/ALS), but the pathomechanisms involved are unknown. As in other FTLD/ALS variants, characteristic intracellular inclusions of misfolded proteins define *C9orf72* pathology, but the core proteins of the majority of inclusions are still unknown. Here, we found that most of these characteristic inclusions contain poly-(Gly-Ala) and, to a lesser extent, poly-(Gly-Pro) and poly-(Gly-Arg) dipeptide-repeat proteins presumably generated by non-ATG-initiated translation from the expanded GGGGCC repeat in three reading frames. These findings directly link the FTLD/ALS-associated genetic mutation to the predominant pathology in patients with *C9orf72* hexanucleotide expansion.

Frontotemporal lobar degeneration (FTLD) and amyotrophic lateral sclerosis (ALS) are the extreme ends of a spectrum of overlapping neurodegenerative disorders variably associated with dementia, personality changes, language abnormalities, and progressive muscle

weakness (1–3). The majority of patients show intracellular inclusions that are strongly positive for phosphorylated TDP-43 (classified as FTLD-TDP, FTLD/ALS-TDP, or ALS-TDP). Recently, expansion of a GGGGCC hexanucleotide repeat in the gene *C9orf72* has been identified as the

most common pathogenic mutation in families with autosomal dominant FTLD, FTLD/ALS, and ALS (4–6). The expansion is located upstream of the *C9orf72* coding region, either in the first intron or the promoter region, depending on the transcript isoform (fig. S1A). Although the extreme GC content precludes sequencing in patients, the number of GGGGCC repeat units is believed to be at least several hundred, compared with fewer than 25 in healthy controls (7).

Patients with a *C9orf72* repeat expansion mutation have clinical symptoms similar to other FTLD/ALS-TDP patients but show several unique

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pathological features (8–12). Aggregates of phosphorylated TDP-43 are accompanied by abundant dotlike and star-shaped phospho-TDP-43–negative neuronal cytoplasmic inclusions—in particular, in cerebellum, hippocampus, and frontotemporal neocortex—that can only be identified with antibodies for p62, ubiquitin, or the related ubiquilins (8–11). These phospho-TDP-43–negative aggregates are highly characteristic of diseased *C9orf72* mutation carriers and are absent in other variants of FTLN/ALS-TDP (9–11). The identity of the disease protein(s) in these inclusions and their relation to the *C9orf72* hexanucleotide repeat expansion have remained elusive. Proposed pathomechanisms include haploinsufficiency through impaired transcription or splicing of the mutant *C9orf72* allele and RNA toxicity through the sequestration of unidentified RNA-binding proteins (3–7).

We hypothesized that the intronic GGGGCC repeat might be aberrantly translated into dipeptide-repeat (DPR) proteins. Although quite rare, two mechanisms of non-ATG-initiated translation have been described: Initiation from alternative start codons with a good Kozak sequence is possible (13–15), and hairpin formation in the repeat region may trigger so-called repeat-associated non-ATG-initiated (RAN) translation, as described for CAG repeats in ataxin 8 (*ATXN8*) (16–18). *ATXN8* encodes a natural poly-Q stretch that can cause poly-Q inclusions upon repeat expansion in spinocerebellar ataxia type 8 (SCA8) patients. Strikingly, the expanded CAG repeat is translated in all three reading frames (poly-Q, poly-A, and poly-S) even after removal of the endogenous start codon.

Translation of the GGGGCC repeat in all reading frames would result in three DPR proteins: poly-(Gly-Ala), poly-(Gly-Pro), and poly-(Gly-Arg). poly-GA and poly-GP proteins are extremely hydrophobic and may form intracellular aggregates. We raised antibodies (anti-GA and anti-GP) against (GA)₁₅ and (GP)₁₅ peptides fused to maltose binding protein and tested a monoclonal antibody (anti-GR) that was originally raised against an EBNA2A epitope with a (GR)₆ repeat (19). All three affinity-purified DPR antibodies detected the respective repeat antigen by immunoblotting without cross-reaction with the other two DPR proteins (Fig. 1A).

To investigate whether such repeat proteins can be translated in the absence of a start codon, we cloned parts of the repeat region from *C9orf72* patients into a mammalian expression vector. For the longer constructs, we could only use restriction digest to estimate the repeat number ranging from ~28 to ~145 (Fig. 1B), because the extreme GC content precludes sequencing. The region upstream of the GGGGCC repeat lacks ATG start codons and contains four to five stop codons, depending on the reading frame. Upon transfection of these constructs into human embryonic kidney (HEK) 293 cells, anti-GA detected proteins of increasing size starting with a faint product from ~38 repeats, suggesting that the translation

mechanism becomes more efficient with increasing repeat length (Fig. 1B). We did not detect poly-GR products, and only the longest construct with ~145 repeats additionally expressed detectable amounts of poly-GP (Fig. 1B).

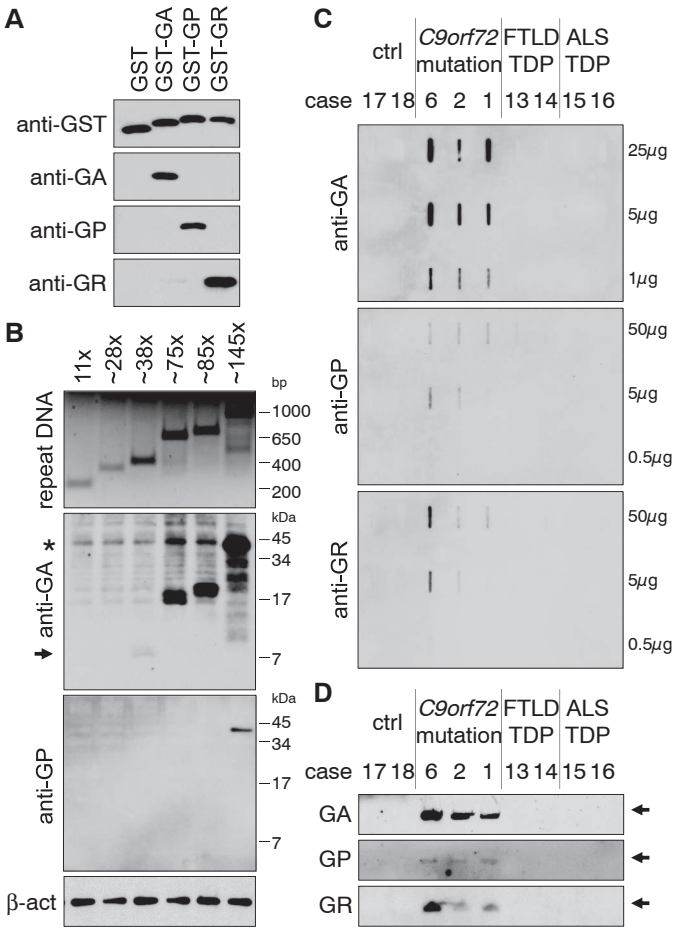
To analyze poly-GA aggregation, we performed filter trap assays (20) using 2% sodium dodecyl sulfate (SDS) extracts from human postmortem cerebellum of healthy controls and FTLN/ALS-TDP patients with and without pathological *C9orf72* hexanucleotide repeat expansion. We observed strong poly-GA signal only in FTLN/ALS patient with hexanucleotide repeat expansion (Fig. 1C), indicating that poly-GA forms SDS-insoluble aggregates in the cerebellum. We also detected insoluble poly-GP and poly-GR in *C9orf72* patients (Fig. 1C). The 2% SDS-insoluble material was partially solubilized upon boiling in 8% SDS and could be analyzed by immunoblotting. We detected high-molecular weight DPR aggregates in all three reading frames at the top of the gel specifically in patients with *C9orf72* mutation (Fig. 1D).

mRNA expression of the mutant *C9orf72* allele is reported to be repressed through impaired transcription or splicing (4, 6, 7). We also found

reduced *C9orf72* mRNA levels in patient cerebellum (fig. S1B). However, both sense and antisense transcripts containing intron 1 (where the GGGGCC repeat is located) were strongly increased in *C9orf72* patients (fig. S1C). This suggests a selective stabilization of repeat containing pre-mRNA (or the excised intron 1 alone) and raises the possibility that the antisense strand may be translated into poly-(Pro-Arg), poly-(Ala-Pro) and further poly-GP DPR proteins.

To determine the cellular distribution patterns of these DPR proteins in patients with pathological *C9orf72* repeat expansion, we focused on the cerebellum and hippocampus in the immunohistochemical analysis because these brain regions contain abundant inclusion pathology positive for p62 but negative for phospho-TDP-43 (9–11) (Fig. 2, A and B, and fig. S2A). In all patients with *C9orf72* mutation, poly-GA–specific antibodies detected dotlike neuronal cytoplasmic inclusions in the granular cell layer of the cerebellum (Fig. 2C and fig. S2B). Their shape and abundance were similar to the p62-positive/TDP-43-negative inclusions considered to be pathognomonic for *C9orf72* mutation patients.

Fig. 1. Extended GGGGCC repeats are translated into aggregating DPR proteins. (A) Validation of DPR-specific affinity-purified antibodies by immunoblotting with purified GST-fusion proteins containing (GA)₁₅, (GP)₁₅, or (GR)₁₅. (B) GGGGCC-repeat constructs with indicated repeat length lacking an upstream ATG were transfected into HEK293 cells. Restriction digest to estimate the repeat length of the transfected constructs (upper panel). Immunoblots show length-dependent expression of poly-GA and poly-GP proteins. Poly-GA products were detectable starting from ~38 repeats (arrow). Asterisk indicates nonspecific band. Poly-GR products were not detected (not shown). (C) Filter trap assay from patient cerebellum (table S1). Triton-X100 insoluble fractions were re-suspended in 2% SDS and filtered through cellulose acetate membranes, and retained proteins were detected with the indicated antibodies. (D) The SDS-insoluble fraction from (C) was boiled in 4x Laemmli buffer (containing 8% SDS) and analyzed by immunoblotting. Arrows mark the top of the gel. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.



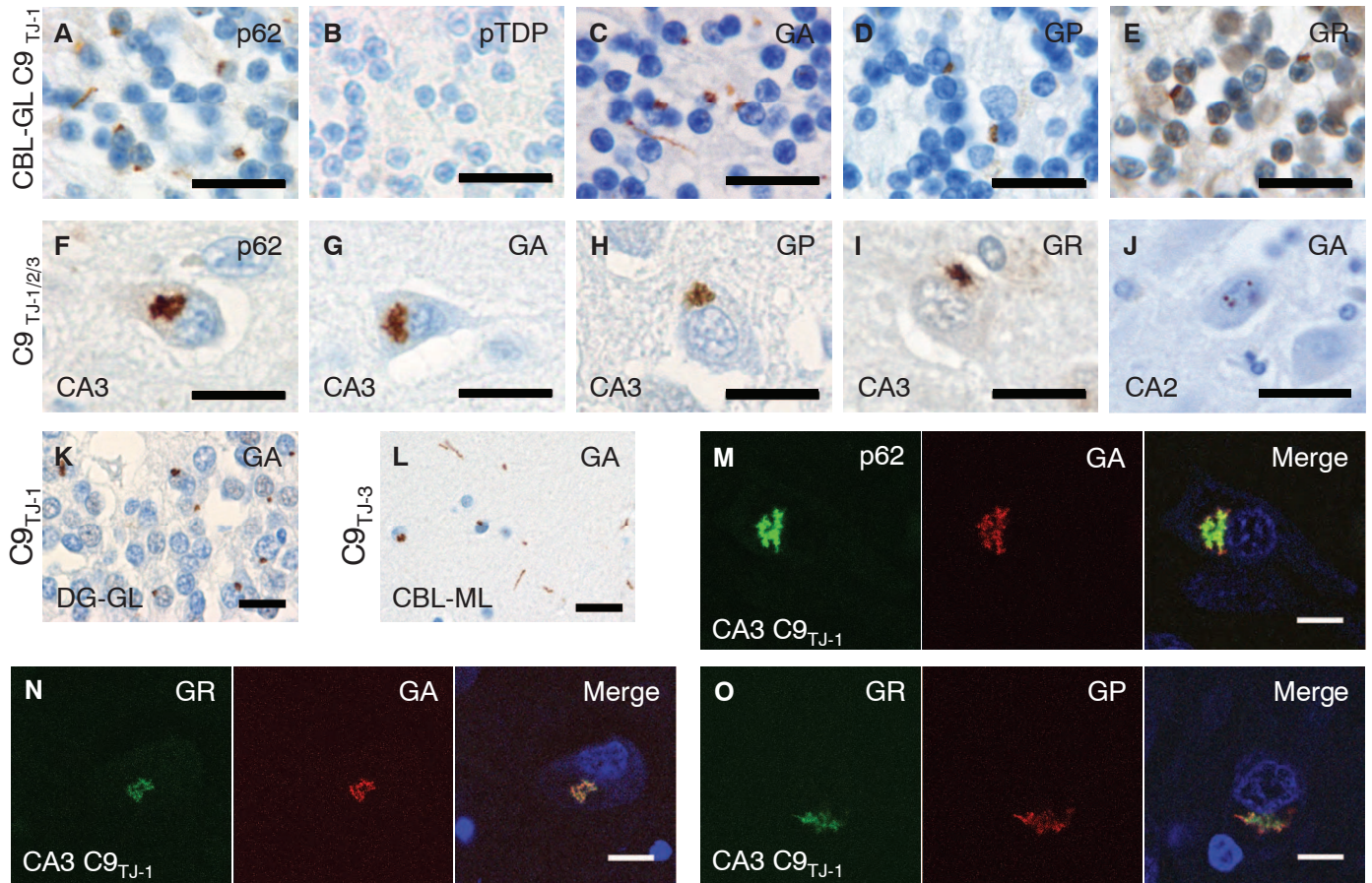
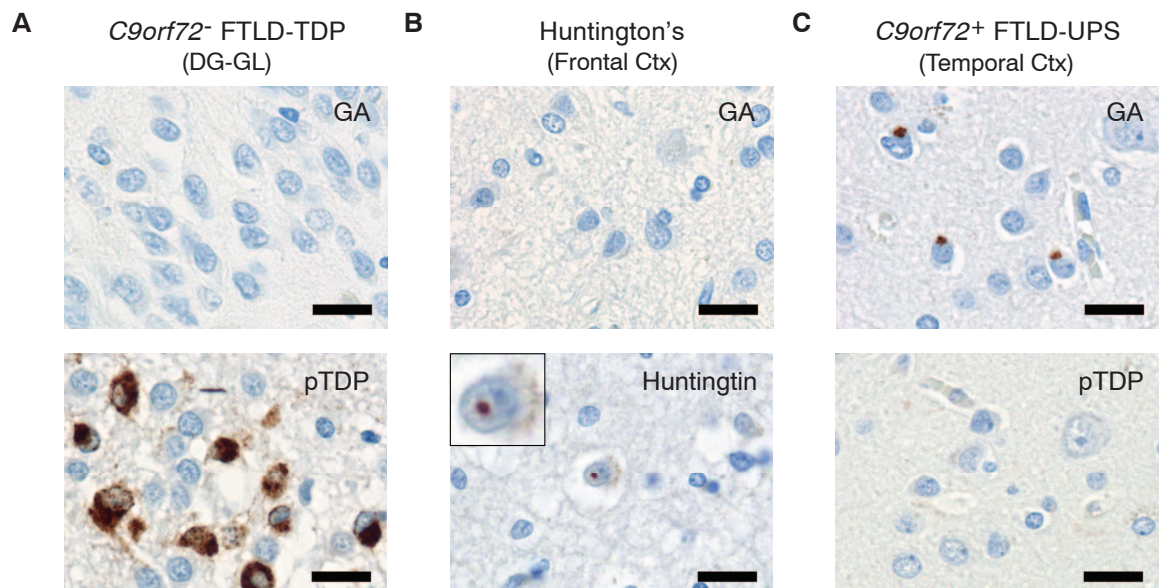


Fig. 2. DPR proteins form the characteristic TDP-43-negative inclusions in *C9orf72* patients. Immunohistochemistry with affinity-purified DPR-specific antibodies (GA, GP, and GR) reveals poly-GA, poly-GP, and poly-GR inclusions resembling the p62-positive aggregates in FTLD/ALS patients with *C9orf72* mutation (compare table S1). Dotlike and threadlike inclusions in cerebellar granular layer (CBL-GL) (A to E). Star-shaped cytoplasmic (F to I) and dot-like intranuclear (J) inclusions in hippocampal cornu ammonis regions 2 and 3 (CA2 and CA3). Inclusion of mixed morphology in dentate gyrus granular layer (DG-GL) and cerebellar molecular layer (CBL-ML) (K and

L). In patients and controls, poly-GR antibodies additionally showed faint nuclear and cytoplasmic staining. Scale bars, 20 μ m. Anti-GA and anti-GP specificity was confirmed by preincubation experiments with recombinant antigens (fig. S3, A to F). Validation of anti-GR was only possible by immunoblot experiments (Fig. 1A) because the poly-GR antigen itself bound to the tissue directly (fig. S3, G to K). (M to O) Double immunofluorescence reveals composition of DPR aggregates in *C9orf72* FTLD/ALS patient TJ-1. No colocalization of DPR proteins was observed with phospho-TDP-43 (fig. S4). Scale bars, 10 μ m.

Fig. 3. DPR pathology is specific to patients with *C9orf72* hexanucleotide repeat expansion. (A and B) Immunohistochemistry with poly-GA-specific antibodies (GA) detects no aggregates in an FTLD-TDP patient (TJ-13) without *C9orf72* repeat expansion and a case with Huntington's disease (TJ-11). Phospho-TDP-43 and Huntingtin inclusions are readily detectable. Granular layer of dentate gyrus (DG-GL) and frontal cortex, respectively. (C) Poly-GA-positive inclusions, but no phospho-TDP-43 inclusions, in temporal cortex of patient TJ-10 with *C9orf72* mutation diagnosed with FTLD-UPS (6). Scale bars, 20 μ m.



Furthermore, these types of inclusions were also detected by antibodies against poly-GP and poly-GR, however to a much lesser extent (Fig. 2, D and E, and fig. S2, C and D). In the hippocampus, most inclusions stained by the three DPR antibodies resembled the p62-positive star-shaped inclusions typical for *C9orf72* mutation patients (Fig. 2, F to I, and fig. S3). As reported for p62 stainings (9, 11), we also observed some DPR-positive neuronal intranuclear inclusions (Fig. 2J). Their relation to the RNA foci described previously remains to be determined (4). Similar DPR pathology was visible in other brain regions, including the granular layer of the dentate gyrus and the molecular layer of the cerebellum and neocortex (Fig. 2, K and L, and table S1).

We next analyzed whether the DPR-positive aggregates are identical to the p62-positive and phospho-TDP-43-negative aggregates. In hippocampal sections of a *C9orf72* mutation patient, poly-GA, poly-GP, and poly-GR colocalized with p62 in the characteristic starlike inclusions (Fig. 2, M to O, and fig. S4, D and E). However, there was no coaggregation of phospho-TDP-43 and DPR proteins (fig. S4, A to C). Occasionally, small spheric poly-GA aggregates were surrounded by aggregated phospho-TDP-43, forming a core inside phospho-TDP-43 inclusions (fig. S4F), which suggests that DPR aggregation may precede TDP-43 pathology. Quantitative analysis confirmed extensive colocalization of p62-positive inclusions with poly-GA aggregates and, to a lesser extent, with poly-GP and poly-GR (fig. S4G).

Consistent with the filter trap assay (Fig. 1C), such poly-GA aggregates were not detectable in FTLN-TDP patients without *C9orf72* mutation or with Huntington's disease, which features expanded poly-Q stretches (Fig. 3, A and B). In total, we identified poly-GA, poly-GP, and poly-GR aggregates in all 10 patients with a confirmed pathological *C9orf72* repeat expansion but not in 12 other cases with normal repeat length (table S1).

Some patients with *C9orf72* mutation show remarkably few phospho-TDP-43 inclusions throughout the brain. So far, only a single exceptional patient (TJ-10)—classified as FTLN-UPS (ubiquitin pro-

teasome system), with *C9orf72* mutation and prominent ubiquitin-pathology but without detectable TDP-43 pathology—has been reported (6, 7). We found abundant poly-GA and some poly-GP and poly-GR aggregates in the temporal cortex of this patient (Fig. 3C and table S1), suggesting that DPR proteins are crucial for FTLN pathogenesis in this case. Thus, we propose that poly-GA is the main aggregating species in FTLN-UPS patients with *C9orf72* repeat expansion.

Here, we have shown that non-ATG-initiated translation of the intronic GGGGCC-repeat expansion in FTLN/ALS patients leads to accumulation of insoluble DPR aggregates. In addition to DPR and TDP-43 pathology, the *C9orf72* expansion may lead to haploinsufficiency and trigger sequestration of GGGGCC-binding proteins. Such interacting proteins may even support nuclear export of the repeat RNA or its translation.

Ample evidence suggests a pathogenic role of DPR inclusions in FTLN patients with *C9orf72* hexanucleotide repeat expansion. First, DPR pathology is predominant in clinically relevant brain regions (hippocampus and frontotemporal neocortex) and may precede TDP-43 pathology. Second, *C9orf72* patients show cerebellar atrophy that does not occur in the other FTLN/ALS variants lacking cerebellar DPR inclusions (10, 12). Third, at least one *C9orf72* mutation carrier had abundant DPR pathology and behavioral-variant clinical FTLN but no detectable TDP-43 inclusion pathology. Finally, DPR pathology is a direct consequence of the pathological hexanucleotide repeat expansion, the most common genetic cause of FTLN/ALS. We therefore suggest the acronym FTLN/ALS-DPR to pathologically classify these patients in a revised nomenclature.

References and Notes

1. K. A. Josephs et al., *Acta Neuropathol.* **122**, 137 (2011).
2. I. R. Mackenzie, R. Rademakers, M. Neumann, *Lancet Neurol.* **9**, 995 (2010).
3. R. Rademakers, M. Neumann, I. R. Mackenzie, *Nat. Rev. Neurol.* **8**, 423 (2012).
4. M. DeJesus-Hernandez et al., *Neuron* **72**, 245 (2011).
5. A. E. Renton et al., *Neuron* **72**, 257 (2011).
6. I. Gijssels et al., *Lancet Neurol.* **11**, 54 (2012).
7. J. van der Zee et al., *Hum. Mutat.* **34**, 363 (2013).
8. A. L. Boxer et al., *J. Neurol. Neurosurg. Psychiatry* **82**, 196 (2011).

9. S. Al-Sarraj et al., *Acta Neuropathol.* **122**, 691 (2011).
10. C. J. Mahoney et al., *Brain* **135**, 736 (2012).
11. E. H. Bigio et al., *Neuropathology* (2012); 10.1111/j.1440-1789.2012.01332.x.
12. J. L. Whitwell et al., *Brain* **135**, 794 (2012).
13. I. P. Ivanov, A. E. Firth, A. M. Michel, J. F. Atkins, P. V. Baranov, *Nucleic Acids Res.* **39**, 4220 (2011).
14. D. S. Peabody, *J. Biol. Chem.* **264**, 5031 (1989).
15. C. Touriol et al., *Biol. Cell* **95**, 169 (2003).
16. T. Zu et al., *Proc. Natl. Acad. Sci. U.S.A.* **108**, 260 (2011).
17. C. E. Pearson, *PLoS Genet.* **7**, e1002018 (2011).
18. M. L. Moseley et al., *Nat. Genet.* **38**, 758 (2006).
19. E. Kremmer et al., *Virology* **208**, 336 (1995).
20. H. Li, T. Wyman, Z. X. Yu, S. H. Li, X. J. Li, *Hum. Mol. Genet.* **12**, 2021 (2003).

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