

**Quantitative analysis and clinico-pathological correlations of different dipeptide repeat protein pathologies in *C9ORF72* mutation carriers**

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**Supplementary online material:**

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**Supplementary Table 1: Demographic and clinical data of cases used in this study**

| Case | FH  | Age at onset (years) | disease duration (years) | Sex | Dementia   | MND                    | other clinical features                                          |
|------|-----|----------------------|--------------------------|-----|------------|------------------------|------------------------------------------------------------------|
| 1    | yes | 74                   | 2                        | F   | -          | ALS                    |                                                                  |
| 2    | no  | 57                   | 2                        | F   | -          | ALS                    |                                                                  |
| 3    | yes | 68                   | 2                        | F   | -          | ALS                    |                                                                  |
| 4    | yes | 60                   | 1                        | M   | -          | PLS                    | gaze palsy, unsteady gait, poor coordination, parkinsonism (PSP) |
| 5    | no  | 59                   | 1                        | F   | -          | ALS                    | developmental delay, psychosis                                   |
| 6    | yes | 50                   | 1                        | F   | -          | ALS                    | psychosis                                                        |
| 7    | yes | 59                   | 3                        | M   | -          | ALS                    |                                                                  |
| 8    | yes | 65                   | 3                        | M   | -          | ALS                    |                                                                  |
| 9    | yes | 58                   | 4                        | M   | bvFTD      | ALS                    | tremor, unsteady gait                                            |
| 10   | yes | 66                   | 5                        | M   | bvFTD      | dysarthria, dysphagia  |                                                                  |
| 11   | yes | 67                   | 9                        | M   | PNFA       | fasciculations         | incontinence                                                     |
| 12   | yes | 52                   | 3                        | M   | bvFTD/PNFA | ALS                    |                                                                  |
| 13   | yes | 54                   | 2                        | M   | PNFA       | ALS                    |                                                                  |
| 14   | yes | 39                   | 2                        | F   | PNFA       | ALS                    | parkinsonism (CBS), gaze palsy                                   |
| 15   | yes | 46                   | 1                        | M   | (bvFTD)    | ALS                    |                                                                  |
| 16   | yes | 56                   | 3                        | F   | bvFTD/PNFA | ALS                    |                                                                  |
| 17   | yes | 50                   | 6                        | M   | bvFTD      | ALS                    |                                                                  |
| 18   | yes | 52                   | 10                       | M   | PNFA       | hyperreflexia, choking | parkinsonism, poor balance                                       |
| 19   | yes | 35                   | 8                        | F   | bvFTD      | dysphagia, spasticity  | parkinsonism, ataxia, dystonia, incontinence                     |
| 20   | yes | 61                   | 8                        | F   | bvFTD      | ALS                    | psychosis                                                        |
| 21   | yes | 72                   | 3                        | M   | bvFTD      | ALS                    |                                                                  |
| 22   | yes | 39                   | 3                        | F   | bvFTD      | ALS                    |                                                                  |
| 23   | yes | 58                   | 3                        | F   | bvFTD      | muscle weakness        | depression, hallucinations, fluctuations,                        |
| 24   | na  | 65                   | 1                        | M   | bvFTD      | ALS                    |                                                                  |
| 25   | yes | 56                   | 12                       | M   | bvFTD      | -                      |                                                                  |
| 26   | yes | 56                   | 16                       | M   | bvFTD      | -                      |                                                                  |
| 27   | yes | 58                   | 8                        | M   | bvFTD      | -                      |                                                                  |
| 28   | na  | 74                   | 10                       | M   | bvFTD      | -                      | parkinsonism, unsteady gait                                      |
| 29   | yes | 53                   | 3                        | M   | bvFTD      | -                      |                                                                  |
| 30   | yes | 49                   | 2                        | F   | bvFTD      | -                      | gaze palsy, psychosis                                            |
| 31   | no  | 56                   | 8                        | M   | dementia   | -                      | parkinsonism                                                     |
| 32   | yes | 58                   | 6                        | M   | dementia   | -                      |                                                                  |
| 33   | na  | 47                   | 11                       | M   | bvFTD      | -                      | mild rigor, hallucinations, Schizophrenia                        |
| 34   | yes | 35                   | 6                        | M   | bvFTD      | -                      |                                                                  |
| 35   | yes | 59                   | 7                        | F   | bvFTD      | -                      |                                                                  |

ALS amyotrophic lateral sclerosis, *bvFTD* behavioral variant of frontotemporal dementia, (*bvFTD*) mild features of bvFTD, CBS corticobasal syndrome, *F* female, *FH* positive family history, *M* male, *MND* motor neuron disease, *na* not available, *PLS* primary lateral sclerosis, *PNFA* progressive non-fluent aphasia, *PSP* progressive supranuclear palsy

**Supplementary table 2: Semiquantitative assessment of degeneration and TDP-43 pathology**

|         | Case | FTLD-TDP subtype | Degeneration <sup>a</sup> |        |    | TDP-43 <sup>b</sup> |     |    |        |     |    |    |
|---------|------|------------------|---------------------------|--------|----|---------------------|-----|----|--------|-----|----|----|
|         |      |                  | FC                        | SC/XII | CE | FC                  |     |    | SC/XII |     |    | CE |
|         |      |                  |                           |        |    | NCI                 | NII | DN | NCI    | NII | DN |    |
| MND     | 1    | B                | 0                         | 3      | 1  | 2                   | 0   | 2  | 4      | 0   | 2  | 0  |
|         | 2    | na               | 0                         | 3      | 0  | 0                   | 0   | 1  | 4      | 0   | 2  | 0  |
|         | 3    | B                | 1                         | 2      | 0  | 3                   | 0   | 2  | 4      | 0   | 2  | 0  |
|         | 4    | B                | 1                         | 0      | 0  | 2                   | 0   | 3  | 1      | 0   | 1  | 0  |
|         | 5    | B                | 0                         | 3      | 1  | 1                   | 0   | 1  | 4      | 0   | 3  | 0  |
|         | 6    | B                | 1                         | 2      | 1  | 4                   | 0   | 4  | 4      | 0   | 1  | 0  |
|         | 7    | B                | 0                         | 3      | na | 0                   | 0   | 0  | 3      | 0   | 1  | na |
|         | 8    | na               | 0                         | 3      | 1  | 1                   | 0   | 1  | 4      | 0   | 2  | 0  |
| FTD/MND | 9    | A&B              | 1                         | 3      | 0  | 2                   | 0   | 3  | 3      | 0   | 2  | 0  |
|         | 10   | B&C              | 1                         | 1      | 0  | 3                   | 0   | 4  | 2      | 0   | 2  | 0  |
|         | 11   | na               | 1                         | 0      | 0  | 1                   | 0   | 1  | 0      | 0   | 0  | 0  |
|         | 12   | B                | 1                         | 3      | 1  | 3                   | 0   | 2  | 4      | 0   | 1  | 0  |
|         | 13   | B                | 1                         | 3      | na | 2                   | 0   | 1  | 3      | 0   | 1  | na |
|         | 14   | B                | 3                         | 3      | 0  | 3                   | 0   | 2  | 3      | 0   | 2  | 0  |
|         | 15   | B                | 1                         | 3      | 0  | 2                   | 1   | 1  | 4      | 0   | 2  | 0  |
|         | 16   | B                | 3                         | 3      | 0  | 3                   | 0   | 2  | 3      | 0   | 1  | 0  |
|         | 17   | B                | 2                         | 2      | 0  | 3                   | 1   | 2  | 2      | 0   | 0  | 0  |
|         | 18   | A&B              | 2                         | 1      | 0  | 3                   | 1   | 4  | 1      | 0   | 1  | 0  |
|         | 19   | A&B              | 3                         | 2      | 2  | 4                   | 1   | 4  | 0      | 0   | 1  | 0  |
|         | 20   | B                | 2                         | 3      | 0  | 3                   | 0   | 4  | 3      | 0   | 1  | 0  |
|         | 21   | B                | 1                         | 1      | 1  | 1                   | 0   | 0  | 3      | 0   | 1  | 0  |
|         | 22   | B                | 2                         | 2      | 0  | 3                   | 0   | 3  | 3      | 0   | 1  | 0  |
|         | 23   | B                | 1                         | 1      | 1  | 2                   | 0   | 1  | 3      | 0   | 0  | 0  |
|         | 24   | B                | 2                         | 2      | 0  | 3                   | 1   | 3  | 3      | 0   | 1  | 0  |
| FTD     | 25   | A&B              | 3                         | 1      | 0  | 3                   | 1   | 4  | 0      | 0   | 1  | 0  |
|         | 26   | A&B              | 2                         | 1      | 1  | 2                   | 1   | 2  | 0      | 0   | 1  | 0  |
|         | 27   | B                | 1                         | 1      | 1  | 3                   | 0   | 2  | 2      | 0   | 1  | 0  |
|         | 28   | A&B              | 1                         | 0      | na | 2                   | 1   | 3  | 1      | 0   | 1  | na |
|         | 29   | B                | 2                         | 1      | 1  | 3                   | 0   | 1  | 3      | 0   | 1  | 0  |
|         | 30   | B                | 3                         | 1      | 1  | 3                   | 0   | 2  | 3      | 0   | 1  | 0  |
|         | 31   | B                | 2                         | 1      | 1  | 2                   | 1   | 3  | 0      | 0   | 1  | 0  |
|         | 32   | A&B              | 2                         | 1      | 0  | 3                   | 1   | 4  | 0      | 0   | 1  | 0  |
|         | 33   | A&B              | 3                         | 0      | 1  | 3                   | 2   | 2  | 0      | 0   | 0  | 0  |
|         | 34   | B                | 3                         | 1      | 1  | 4                   | 0   | 3  | 2      | 0   | 0  | 0  |
|         | 35   | B                | 3                         | 0      | 0  | 4                   | 0   | 3  | 0      | 0   | 0  | 0  |

<sup>a</sup> 4-tier grading system for degeneration: 0=absent, 1=mild, 2=moderate, 3=severe

<sup>b</sup> 5-tier grading system for TDP-43 pathology: 0=absent, 1=rare, 2=occasional, 3=moderate, 4=abundant

CE cerebellum, DN dystrophic neurites, FC frontal cortex, FTD frontotemporal dementia, MND motor neuron disease, na not available, NCI neuronal cytoplasmic inclusions, NII neuronal intranuclear inclusions, SC/XII spinal cord or hypoglossal nucleus.

**Supplementary table 3: Assessment of poly-GA pathology**

| Case    |    | Quantitative (inclusions per 0.95mm <sup>2</sup> ) |     |    |                          |     |    |        |     |    | Semiquantitative <sup>a</sup> |
|---------|----|----------------------------------------------------|-----|----|--------------------------|-----|----|--------|-----|----|-------------------------------|
|         |    | FC upper cortical layers                           |     |    | FC lower cortical layers |     |    | SC/XII |     |    | Cerebellum                    |
|         |    | NCI                                                | NII | DN | NCI                      | NII | DN | NCI    | NII | DN | Total                         |
| MND     | 1  | 68                                                 | 12  | 2  | 85                       | 8   | 4  | 0      | 0   | 0  | 4                             |
|         | 2  | 69                                                 | 5   | 1  | 81                       | 8   | 0  | 1      | 0   | 0  | 4                             |
|         | 3  | 76                                                 | 4   | 1  | 58                       | 1   | 1  | 1      | 0   | 0  | 4                             |
|         | 4  | 36                                                 | 6   | 0  | 86                       | 2   | 1  | 0      | 0   | 0  | 4                             |
|         | 5  | 97                                                 | 6   | 0  | 61                       | 3   | 1  | 1      | 0   | 0  | 4                             |
|         | 6  | 91                                                 | 4   | 0  | 102                      | 11  | 7  | 3      | 0   | 0  | 4                             |
|         | 7  | 87                                                 | 6   | 2  | 97                       | 2   | 2  | 1      | 0   | 0  | na                            |
|         | 8  | 32                                                 | 2   | 0  | 52                       | 5   | 0  | 0      | 0   | 0  | 3                             |
| FTD/MND | 9  | 75                                                 | 2   | 0  | 45                       | 1   | 2  | 0      | 0   | 0  | 4                             |
|         | 10 | 62                                                 | 8   | 4  | 66                       | 1   | 0  | 0      | 0   | 0  | 4                             |
|         | 11 | 71                                                 | 7   | 1  | 51                       | 6   | 0  | 0      | 0   | 0  | 4                             |
|         | 12 | 72                                                 | 8   | 8  | 110                      | 4   | 6  | 1      | 0   | 0  | 4                             |
|         | 13 | 22                                                 | 0   | 0  | 23                       | 1   | 2  | 0      | 0   | 0  | na                            |
|         | 14 | 235                                                | 6   | 11 | 165                      | 12  | 12 | 5      | 0   | 2  | 4                             |
|         | 15 | 81                                                 | 3   | 1  | 125                      | 4   | 3  | 0      | 0   | 0  | 4                             |
|         | 16 | 60                                                 | 3   | 2  | 78                       | 7   | 5  | 1      | 0   | 0  | 4                             |
|         | 17 | 40                                                 | 6   | 0  | 34                       | 1   | 3  | 0      | 0   | 0  | 4                             |
|         | 18 | 135                                                | 2   | 6  | 171                      | 3   | 6  | 2      | 0   | 0  | 4                             |
|         | 19 | 206                                                | 16  | 21 | 230                      | 17  | 35 | 8      | 0   | 0  | 4                             |
|         | 20 | 58                                                 | 6   | 2  | 43                       | 3   | 0  | 1      | 0   | 0  | 4                             |
|         | 21 | 90                                                 | 6   | 1  | 61                       | 2   | 0  | 0      | 0   | 0  | 4                             |
|         | 22 | 59                                                 | 3   | 3  | 126                      | 4   | 0  | 0      | 0   | 0  | 3                             |
|         | 23 | 78                                                 | 6   | 0  | 82                       | 5   | 1  | 0      | 0   | 0  | 3                             |
|         | 24 | 53                                                 | 2   | 4  | 49                       | 1   | 0  | 1      | 0   | 0  | 3                             |
| FTD     | 25 | 32                                                 | 6   | 1  | 21                       | 6   | 0  | 0      | 0   | 0  | 4                             |
|         | 26 | 25                                                 | 1   | 2  | 26                       | 2   | 1  | 0      | 0   | 0  | 4                             |
|         | 27 | 20                                                 | 2   | 1  | 20                       | 0   | 0  | 0      | 0   | 0  | 3                             |
|         | 28 | 71                                                 | 7   | 4  | 53                       | 6   | 0  | 0      | 0   | 0  | na                            |
|         | 29 | 61                                                 | 1   | 2  | 87                       | 9   | 0  | 0      | 0   | 0  | 4                             |
|         | 30 | 68                                                 | 1   | 5  | 97                       | 9   | 0  | 0      | 0   | 0  | 3                             |
|         | 31 | 59                                                 | 12  | 4  | 36                       | 4   | 0  | 1      | 0   | 0  | 4                             |
|         | 32 | 77                                                 | 21  | 2  | 118                      | 16  | 0  | 1      | 0   | 0  | 4                             |
|         | 33 | 142                                                | 15  | 2  | 153                      | 14  | 0  | 0      | 0   | 0  | 4                             |
|         | 34 | 78                                                 | 10  | 9  | 129                      | 5   | 3  | 3      | 0   | 0  | 4                             |
|         | 35 | 156                                                | 9   | 1  | 117                      | 0   | 0  | 2      | 0   | 0  | 4                             |

<sup>a</sup> 5-tier grading system: 0=absent, 1=rare, 2=occasional, 3=moderate, 4=abundant

CE cerebellum, DN dystrophic neurites, FC frontal cortex, FTD frontotemporal dementia, MND motor neuron disease, na not available, NCI neuronal cytoplasmic inclusions, NII neuronal intranuclear inclusions, SC/XII spinal cord or hypoglossal nucleus.

**Supplementary table 4: Assessment of poly-GP pathology**

| Case    |    | Quantitative (inclusions per 0.95mm <sup>2</sup> ) |     |    |                          |     |    |        |     |    | Semiquantitative <sup>a</sup> |
|---------|----|----------------------------------------------------|-----|----|--------------------------|-----|----|--------|-----|----|-------------------------------|
|         |    | FC upper cortical layers                           |     |    | FC lower cortical layers |     |    | SC/XII |     |    | Cerebellum                    |
|         |    | NCI                                                | NII | DN | NCI                      | NII | DN | NCI    | NII | DN | Total                         |
| MND     | 1  | 11                                                 | 2   | 0  | 14                       | 5   | 1  | 0      | 0   | 0  | 3                             |
|         | 2  | 33                                                 | 3   | 0  | 26                       | 0   | 0  | 0      | 0   | 0  | 4                             |
|         | 3  | 24                                                 | 6   | 0  | 16                       | 2   | 0  | 0      | 0   | 0  | 3                             |
|         | 4  | 12                                                 | 4   | 0  | 8                        | 3   | 0  | 0      | 0   | 0  | 3                             |
|         | 5  | 9                                                  | 0   | 0  | 12                       | 0   | 0  | 0      | 0   | 0  | 3                             |
|         | 6  | 46                                                 | 4   | 0  | 47                       | 4   | 0  | 2      | 0   | 0  | 4                             |
|         | 7  | 40                                                 | 0   | 0  | 41                       | 0   | 0  | 0      | 0   | 0  | na                            |
|         | 8  | 6                                                  | 0   | 0  | 14                       | 2   | 0  | 0      | 0   | 0  | 2                             |
| FTD/MND | 9  | 7                                                  | 0   | 0  | 8                        | 1   | 0  | 0      | 1   | 0  | 3                             |
|         | 10 | 34                                                 | 12  | 0  | 51                       | 12  | 0  | 0      | 0   | 0  | 4                             |
|         | 11 | 17                                                 | 3   | 0  | 10                       | 3   | 0  | 0      | 0   | 0  | 4                             |
|         | 12 | 7                                                  | 3   | 0  | 23                       | 1   | 0  | 4      | 0   | 0  | 4                             |
|         | 13 | 9                                                  | 0   | 0  | 6                        | 0   | 0  | 1      | 0   | 0  | na                            |
|         | 14 | 11                                                 | 1   | 0  | 12                       | 2   | 0  | 1      | 0   | 0  | 4                             |
|         | 15 | 15                                                 | 2   | 0  | 10                       | 0   | 0  | 2      | 0   | 0  | 4                             |
|         | 16 | 14                                                 | 2   | 0  | 7                        | 0   | 0  | 1      | 0   | 0  | 3                             |
|         | 17 | 7                                                  | 1   | 0  | 6                        | 0   | 0  | 0      | 0   | 0  | 2                             |
|         | 18 | 21                                                 | 1   | 0  | 44                       | 1   | 0  | 2      | 0   | 0  | 4                             |
|         | 19 | 51                                                 | 4   | 1  | 59                       | 7   | 0  | 5      | 1   | 0  | 4                             |
|         | 20 | 21                                                 | 3   | 0  | 27                       | 2   | 0  | 1      | 0   | 0  | 3                             |
|         | 21 | 67                                                 | 1   | 0  | 30                       | 1   | 0  | 0      | 0   | 0  | 3                             |
|         | 22 | 18                                                 | 0   | 0  | 35                       | 0   | 0  | 0      | 0   | 0  | 2                             |
|         | 23 | 17                                                 | 3   | 1  | 19                       | 2   | 0  | 0      | 0   | 0  | 3                             |
|         | 24 | 7                                                  | 0   | 0  | 11                       | 1   | 0  | 0      | 0   | 0  | 3                             |
| FTD     | 25 | 7                                                  | 1   | 0  | 9                        | 2   | 0  | 0      | 0   | 0  | 4                             |
|         | 26 | 10                                                 | 1   | 0  | 12                       | 1   | 0  | 0      | 0   | 0  | 3                             |
|         | 27 | 12                                                 | 3   | 0  | 13                       | 4   | 0  | 0      | 0   | 0  | 3                             |
|         | 28 | 5                                                  | 1   | 0  | 12                       | 7   | 1  | 0      | 0   | 0  | na                            |
|         | 29 | 40                                                 | 2   | 0  | 28                       | 2   | 0  | 0      | 0   | 0  | 4                             |
|         | 30 | 7                                                  | 1   | 0  | 19                       | 2   | 0  | 0      | 0   | 0  | 3                             |
|         | 31 | 9                                                  | 2   | 0  | 15                       | 3   | 0  | 1      | 0   | 0  | 3                             |
|         | 32 | 37                                                 | 7   | 1  | 27                       | 6   | 0  | 2      | 0   | 0  | 4                             |
|         | 33 | 38                                                 | 3   | 1  | 39                       | 0   | 0  | 0      | 0   | 0  | 3                             |
|         | 34 | 35                                                 | 0   | 0  | 41                       | 4   | 0  | 4      | 0   | 0  | 4                             |
|         | 35 | 28                                                 | 0   | 0  | 35                       | 1   | 0  | 0      | 0   | 0  | 3                             |

<sup>a</sup> 5-tier grading system: 0=absent, 1=rare, 2=occasional, 3=moderate, 4=abundant

CE cerebellum, DN dystrophic neurites, FC frontal cortex, FTD frontotemporal dementia, MND motor neuron disease, na not available, NCI neuronal cytoplasmic inclusions, NII neuronal intranuclear inclusions, SC/XII spinal cord or hypoglossal nucleus.

**Supplementary table 5: Assessment of poly-GR pathology**

| Case    |    | Quantitative (inclusions per 0.95mm <sup>2</sup> ) |     |    |                          |     |    |        |     |    | Semiquantitative <sup>a</sup> |
|---------|----|----------------------------------------------------|-----|----|--------------------------|-----|----|--------|-----|----|-------------------------------|
|         |    | FC upper cortical layers                           |     |    | FC lower cortical layers |     |    | SC/XII |     |    | Cerebellum                    |
|         |    | NCI                                                | NII | DN | NCI                      | NII | DN | NCI    | NII | DN | Total                         |
| MND     | 1  | 10                                                 | 2   | 1  | 11                       | 3   | 0  | 0      | 0   | 0  | 2                             |
|         | 2  | 39                                                 | 0   | 0  | 28                       | 6   | 0  | 0      | 0   | 0  | 3                             |
|         | 3  | 11                                                 | 0   | 0  | 9                        | 1   | 0  | 0      | 0   | 0  | 4                             |
|         | 4  | 8                                                  | 1   | 0  | 12                       | 0   | 0  | 0      | 0   | 0  | 3                             |
|         | 5  | 9                                                  | 3   | 0  | 15                       | 2   | 0  | 1      | 0   | 0  | 3                             |
|         | 6  | 27                                                 | 2   | 0  | 38                       | 6   | 0  | 1      | 0   | 0  | 4                             |
|         | 7  | 10                                                 | 1   | 0  | 12                       | 2   | 0  | 0      | 0   | 0  | na                            |
|         | 8  | 3                                                  | 0   | 0  | 11                       | 1   | 0  | 0      | 0   | 0  | 0                             |
| FTD/MND | 9  | 1                                                  | 0   | 0  | 1                        | 1   | 0  | 0      | 0   | 0  | 3                             |
|         | 10 | 27                                                 | 4   | 0  | 18                       | 3   | 0  | 0      | 0   | 0  | 4                             |
|         | 11 | 17                                                 | 0   | 0  | 13                       | 1   | 0  | 0      | 0   | 0  | 3                             |
|         | 12 | 6                                                  | 1   | 0  | 6                        | 0   | 0  | 1      | 0   | 0  | 3                             |
|         | 13 | 6                                                  | 0   | 0  | 2                        | 0   | 0  | 0      | 0   | 0  | na                            |
|         | 14 | 6                                                  | 0   | 0  | 8                        | 0   | 0  | 0      | 0   | 0  | 4                             |
|         | 15 | 10                                                 | 0   | 0  | 10                       | 0   | 0  | 0      | 0   | 0  | 3                             |
|         | 16 | 8                                                  | 2   | 0  | 8                        | 0   | 0  | 0      | 0   | 0  | 4                             |
|         | 17 | 4                                                  | 0   | 0  | 3                        | 1   | 0  | 0      | 0   | 0  | 1                             |
|         | 18 | 21                                                 | 2   | 0  | 14                       | 1   | 0  | 1      | 0   | 0  | 4                             |
|         | 19 | 47                                                 | 2   | 0  | 33                       | 2   | 0  | 1      | 0   | 0  | 4                             |
|         | 20 | 9                                                  | 2   | 0  | 12                       | 1   | 0  | 0      | 0   | 0  | 3                             |
|         | 21 | 15                                                 | 2   | 0  | 10                       | 1   | 0  | 0      | 0   | 0  | 2                             |
|         | 22 | 7                                                  | 0   | 0  | 21                       | 2   | 0  | 0      | 0   | 0  | 2                             |
|         | 23 | 11                                                 | 2   | 0  | 9                        | 4   | 0  | 0      | 1   | 0  | 3                             |
|         | 24 | 18                                                 | 1   | 0  | 12                       | 4   | 0  | 0      | 0   | 0  | 2                             |
| FTD     | 25 | 14                                                 | 1   | 0  | 8                        | 0   | 0  | 0      | 0   | 0  | 4                             |
|         | 26 | 3                                                  | 0   | 0  | 4                        | 0   | 0  | 0      | 0   | 0  | 3                             |
|         | 27 | 3                                                  | 1   | 0  | 4                        | 0   | 0  | 0      | 0   | 0  | 2                             |
|         | 28 | 21                                                 | 6   | 0  | 7                        | 6   | 0  | 0      | 0   | 0  | na                            |
|         | 29 | 23                                                 | 3   | 0  | 17                       | 1   | 0  | 0      | 0   | 0  | 4                             |
|         | 30 | 7                                                  | 2   | 0  | 8                        | 0   | 0  | 0      | 0   | 0  | 3                             |
|         | 31 | 5                                                  | 1   | 0  | 3                        | 1   | 0  | 0      | 0   | 0  | 3                             |
|         | 32 | 22                                                 | 6   | 0  | 45                       | 9   | 0  | 1      | 1   | 0  | 4                             |
|         | 33 | 21                                                 | 2   | 0  | 16                       | 1   | 0  | 0      | 0   | 0  | 3                             |
|         | 34 | 21                                                 | 1   | 0  | 27                       | 3   | 0  | 1      | 0   | 0  | 2                             |
|         | 35 | 6                                                  | 1   | 0  | 9                        | 0   | 0  | 0      | 0   | 0  | 3                             |

<sup>a</sup> 5-tier grading system: 0=absent, 1=rare, 2=occasional, 3=moderate, 4=abundant

CE cerebellum, DN dystrophic neurites, FC frontal cortex, FTD frontotemporal dementia, MND motor neuron disease, na not available, NCI neuronal cytoplasmic inclusions, NII neuronal intranuclear inclusions, SC/XII spinal cord or hypoglossal nucleus.

**Supplementary table 6: Assessment of poly-PA pathology**

| Case    |    | Quantitative (inclusions per 0.95mm <sup>2</sup> ) |     |    |                          |     |    |        |     |    | Semiquantitative <sup>a</sup> |
|---------|----|----------------------------------------------------|-----|----|--------------------------|-----|----|--------|-----|----|-------------------------------|
|         |    | FC upper cortical layers                           |     |    | FC lower cortical layers |     |    | SC/XII |     |    | Cerebellum                    |
|         |    | NCI                                                | NII | DN | NCI                      | NII | DN | NCI    | NII | DN | Total                         |
| MND     | 1  | 6                                                  | 1   | 3  | 2                        | 1   | 2  | 0      | 1   | 0  | 1                             |
|         | 2  | 0                                                  | 0   | 0  | 0                        | 0   | 2  | 0      | 0   | 0  | 1                             |
|         | 3  | 3                                                  | 0   | 3  | 2                        | 0   | 2  | 1      | 0   | 0  | 0                             |
|         | 4  | 0                                                  | 0   | 0  | 1                        | 0   | 0  | 0      | 0   | 0  | 0                             |
|         | 5  | 1                                                  | 0   | 1  | 0                        | 0   | 1  | 0      | 0   | 0  | 0                             |
|         | 6  | 2                                                  | 0   | 0  | 0                        | 0   | 2  | 1      | 0   | 0  | 0                             |
|         | 7  | 0                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | na                            |
|         | 8  | 0                                                  | 0   | 2  | 2                        | 0   | 2  | 0      | 0   | 0  | 0                             |
| FTD/MND | 9  | 0                                                  | 1   | 2  | 0                        | 0   | 2  | 0      | 0   | 0  | 0                             |
|         | 10 | 1                                                  | 0   | 4  | 1                        | 0   | 1  | 0      | 0   | 0  | 0                             |
|         | 11 | 0                                                  | 0   | 4  | 1                        | 0   | 2  | 1      | 0   | 0  | 1                             |
|         | 12 | 0                                                  | 1   | 1  | 0                        | 0   | 0  | 0      | 0   | 0  | 2                             |
|         | 13 | 0                                                  | 0   | 1  | 0                        | 0   | 1  | 0      | 0   | 0  | na                            |
|         | 14 | 1                                                  | 0   | 0  | 1                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 15 | 1                                                  | 0   | 1  | 0                        | 0   | 0  | 0      | 0   | 0  | 0                             |
|         | 16 | 0                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 17 | 1                                                  | 0   | 0  | 1                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 18 | 0                                                  | 0   | 1  | 2                        | 1   | 0  | 0      | 0   | 0  | 0                             |
|         | 19 | 1                                                  | 0   | 1  | 1                        | 0   | 1  | 1      | 0   | 0  | 1                             |
|         | 20 | 1                                                  | 0   | 1  | 0                        | 0   | 2  | 0      | 0   | 0  | 0                             |
|         | 21 | 2                                                  | 0   | 1  | 1                        | 1   | 1  | 0      | 0   | 0  | 0                             |
|         | 22 | 0                                                  | 0   | 0  | 3                        | 0   | 0  | 0      | 0   | 0  | 0                             |
|         | 23 | 1                                                  | 0   | 1  | 1                        | 1   | 3  | 0      | 0   | 0  | 1                             |
|         | 24 | 1                                                  | 0   | 2  | 1                        | 0   | 3  | 0      | 0   | 0  | 1                             |
| FTD     | 25 | 4                                                  | 1   | 1  | 0                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 26 | 0                                                  | 0   | 0  | 0                        | 1   | 0  | 0      | 0   | 0  | 0                             |
|         | 27 | 1                                                  | 0   | 2  | 1                        | 0   | 4  | 0      | 0   | 0  | 1                             |
|         | 28 | 2                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | na                            |
|         | 29 | 3                                                  | 0   | 1  | 2                        | 0   | 1  | 0      | 0   | 0  | 2                             |
|         | 30 | 3                                                  | 0   | 0  | 1                        | 0   | 2  | 0      | 0   | 0  | 0                             |
|         | 31 | 0                                                  | 0   | 8  | 1                        | 2   | 3  | 1      | 0   | 0  | 0                             |
|         | 32 | 1                                                  | 1   | 4  | 1                        | 0   | 1  | 2      | 0   | 0  | 1                             |
|         | 33 | 1                                                  | 0   | 2  | 2                        | 0   | 1  | 0      | 0   | 0  | 1                             |
|         | 34 | 2                                                  | 0   | 0  | 1                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 35 | 0                                                  | 1   | 0  | 1                        | 1   | 0  | 0      | 1   | 0  | 1                             |

<sup>a</sup> 5-tier grading system: 0=absent, 1=rare, 2=occasional, 3=moderate, 4=abundant

CE cerebellum, DN dystrophic neurites, FC frontal cortex, FTD frontotemporal dementia, MND motor neuron disease, na not available, NCI neuronal cytoplasmic inclusions, NII neuronal intranuclear inclusions, SC/XII spinal cord or hypoglossal nucleus.

**Supplementary table 7: Assessment of poly-PR pathology**

| Case    |    | Quantitative (inclusions per 0.95mm <sup>2</sup> ) |     |    |                          |     |    |        |     |    | Semiquantitative <sup>a</sup> |
|---------|----|----------------------------------------------------|-----|----|--------------------------|-----|----|--------|-----|----|-------------------------------|
|         |    | FC upper cortical layers                           |     |    | FC lower cortical layers |     |    | SC/XII |     |    | Cerebellum                    |
|         |    | NCI                                                | NII | DN | NCI                      | NII | DN | NCI    | NII | DN | Total                         |
| MND     | 1  | 0                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | 0                             |
|         | 2  | 0                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | 0                             |
|         | 3  | 0                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | 0                             |
|         | 4  | 0                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 5  | 0                                                  | 0   | 0  | 1                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 6  | 2                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | 0                             |
|         | 7  | 0                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | na                            |
|         | 8  | 1                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | 0                             |
| FTD/MND | 9  | 0                                                  | 0   | 2  | 0                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 10 | 0                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 11 | 1                                                  | 1   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 12 | 1                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 1   | 0  | 0                             |
|         | 13 | 0                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | na                            |
|         | 14 | 0                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 15 | 0                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 1   | 0  | 0                             |
|         | 16 | 1                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | 0                             |
|         | 17 | 0                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 18 | 0                                                  | 1   | 0  | 0                        | 1   | 0  | 0      | 0   | 0  | 0                             |
|         | 19 | 0                                                  | 0   | 0  | 2                        | 0   | 0  | 1      | 0   | 0  | 2                             |
|         | 20 | 1                                                  | 0   | 0  | 0                        | 0   | 0  | 1      | 0   | 0  | 1                             |
|         | 21 | 0                                                  | 1   | 0  | 0                        | 1   | 0  | 0      | 0   | 0  | na                            |
|         | 22 | 0                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | na                            |
|         | 23 | 0                                                  | 1   | 0  | 1                        | 1   | 0  | 0      | 0   | 0  | 1                             |
|         | 24 | 0                                                  | 2   | 0  | 0                        | 2   | 0  | 0      | 0   | 0  | 1                             |
| FTD     | 25 | 4                                                  | 0   | 0  | 2                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 26 | 0                                                  | 0   | 2  | 0                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 27 | 0                                                  | 1   | 0  | 2                        | 0   | 0  | 0      | 1   | 0  | 1                             |
|         | 28 | 1                                                  | 1   | 0  | 1                        | 2   | 0  | 0      | 6   | 0  | na                            |
|         | 29 | 0                                                  | 0   | 0  | 1                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 30 | 1                                                  | 2   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 31 | 0                                                  | 0   | 0  | 0                        | 0   | 0  | 1      | 0   | 0  | 0                             |
|         | 32 | 0                                                  | 0   | 0  | 1                        | 0   | 0  | 0      | 0   | 0  | 1                             |
|         | 33 | 0                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | 2                             |
|         | 34 | 0                                                  | 0   | 0  | 0                        | 0   | 0  | 0      | 0   | 0  | 0                             |
|         | 35 | 0                                                  | 0   | 0  | 0                        | 2   | 0  | 0      | 0   | 0  | 0                             |

<sup>a</sup> 5-tier grading system: 0=absent, 1=rare, 2=occasional, 3=moderate, 4=abundant

CE cerebellum, DN dystrophic neurites, FC frontal cortex, FTD frontotemporal dementia, MND motor neuron disease, na not available, NCI neuronal cytoplasmic inclusions, NII neuronal intranuclear inclusions, SC/XII spinal cord or hypoglossal nucleus.

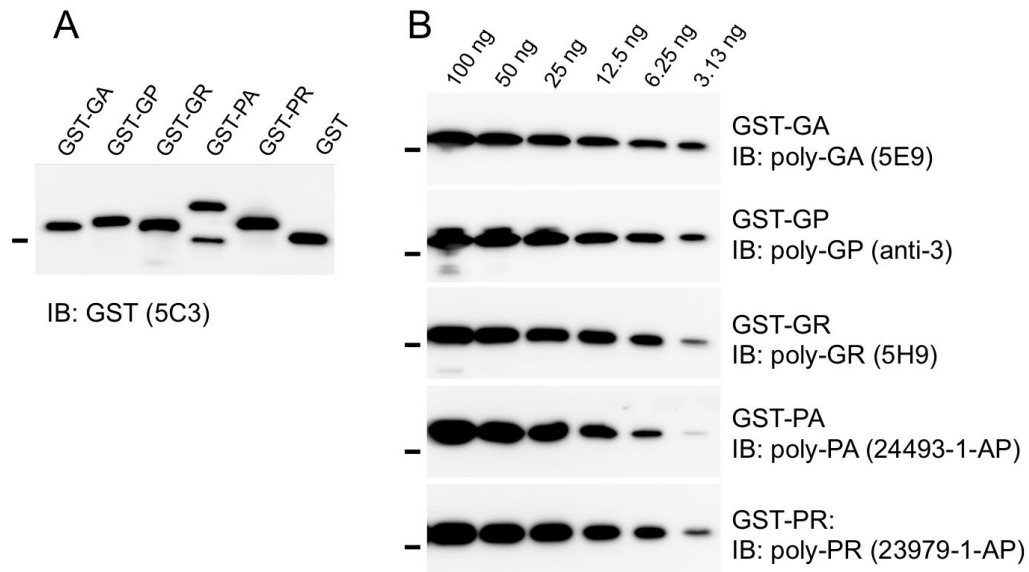
**Supplementary Table 8: Previous studies with information on relative amounts of inclusions for different dipeptide repeat proteins**

| Reference               | DPR investigated |     |     |                |                | Method of assessment | Number of cases with each phenotype |         |     | CNS regions examined                                              | Relative amounts of DPRs                                                                      |
|-------------------------|------------------|-----|-----|----------------|----------------|----------------------|-------------------------------------|---------|-----|-------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|
|                         | G A              | G P | G R | P A            | P R            |                      | FTD                                 | FTD/MND | MND |                                                                   |                                                                                               |
| Mori et al. [1]         | +                | +   | +   | -              | -              | semiquantitative     | 1                                   | 4       | 0   | hippocampus<br>cerebellum                                         | GA>GP/GR                                                                                      |
| Gendron et al. [2]      | -                | +   | -   | + <sup>a</sup> | + <sup>a</sup> | semiquantitative     | 3                                   | 3       | 3   | hippocampus<br>cerebellum                                         | GP>PR/PA                                                                                      |
| Mann et al. [3]         | +                | +   | +   | +              | +              | semiquantitative     | 9                                   | 4       | 3   | hippocampus<br>cerebellum                                         | GA>GP>GR>PA, no PR                                                                            |
| Zu et al. [4]           | +                | +   | +   | +              | +              | semiquantitative     | 2                                   | 3       | 3   | motor cortex<br>hippocampus<br>spinal cord                        | GP usually most numerous, but pattern inconsistent among cases                                |
| Liu et al. [5]          | +                | +   | +   | -              | -              | quantitative         | 5                                   | 4       | 8   | cerebellum                                                        | GA>GP>GR                                                                                      |
| Cooper-Knock et al. [6] | +                | +   | +   | +              | +              | semiquantitative     | 0                                   | 0       | 3   | cerebellum<br>spinal cord                                         | Cerebellum: GA&GR, no PA&PR<br>Spinal cord: PA&PR, no GA&GR<br>No GP inclusions in any region |
| Cooper-Knock et al. [6] | +                | -   | -   | +              | -              | quantitative         | 1                                   | 1       | 4   | cerebellum<br>spinal cord                                         | Cerebellum: GA>PA<br>spinal cord: PA>GA                                                       |
| Gomez-Deza et al. [7]   | +                | +   | +   | + <sup>a</sup> | + <sup>a</sup> | quantitative         | 0                                   | 0       | 10  | spinal cord                                                       | GA>GP>GR>PA/PR                                                                                |
| Schludi et al. [8]      | + <sup>a</sup>   | +   | +   | -              | -              | semiquantitative     | 0                                   | 3       | 2   | 36 brain regions                                                  | GA>GP>GR                                                                                      |
| Schludi et al. [8]      | + <sup>a</sup>   | -   | +   | -              | +              | quantitative         | 3                                   | 8       | 3   | frontal<br>motor cortex<br>occipital<br>hippocampus<br>cerebellum | GA>GR>PR                                                                                      |

<sup>a</sup> same antibody as used in this study;  
+, investigated; -, not investigated

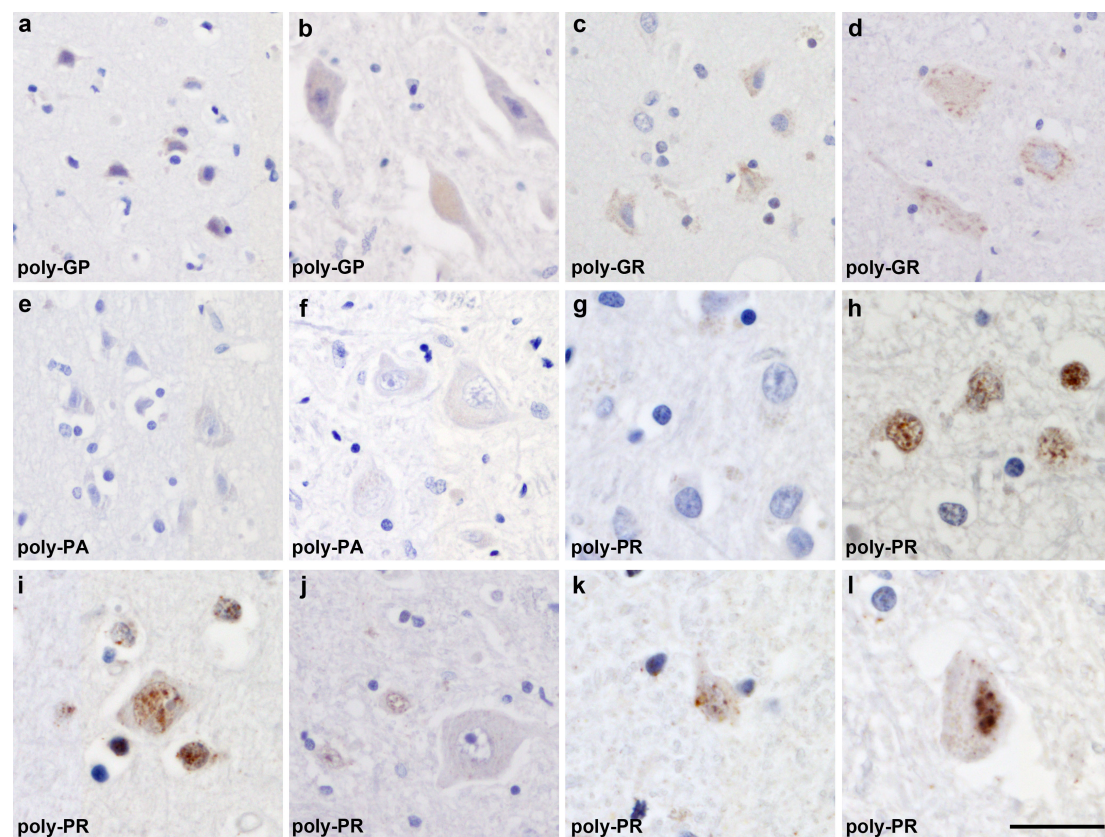
- 1 Mori K, Weng SM, Arzberger T, May S, Rentzsch K, Kremmer E, Schmid B, Kretzschmar HA, Cruts M, Van Broeckhoven C, Haass C, Edbauer D (2013) The C9orf72 GGGGCC repeat is translated into aggregating dipeptide-repeat proteins in FTL/ALS. *Science* 339: 1335-1338.
- 2 Gendron TF, Bieniek KF, Zhang YJ, Jansen-West K, Ash PE, Caulfield T, Daugherty L, Dunmore JH, Castaneda-Casey M, Chew J, Cosio DM, van Blitterswijk M, Lee WC, Rademakers R, Boylan KB, Dickson DW, Petrucelli L (2013) Antisense transcripts of the expanded C9ORF72 hexanucleotide repeat form nuclear RNA foci and undergo repeat-associated non-ATG translation in c9FTD/ALS. *Acta Neuropathol* 126: 829-844.
- 3 Mann DM, Rollinson S, Robinson A, Bennion Callister J, Thompson JC, Snowden JS, Gendron T, Petrucelli L, Masuda-Suzukake M, Hasegawa M, Davidson Y, Pickering-Brown S (2013) Dipeptide repeat proteins are present in the p62 positive inclusions in patients with frontotemporal lobar degeneration and motor neurone disease associated with expansions in C9ORF72. *Acta neuropathologica communications* 1: 68.
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- 5 Liu EY, Russ J, Wu K, Neal D, Suh E, McNally AG, Irwin DJ, Van Deerlin VM, Lee EB (2014) C9orf72 hypermethylation protects against repeat expansion-associated pathology in ALS/FTD. *Acta Neuropathol* 128: 525-541.
- 6 Cooper-Knock J, Higginbottom A, Stopford MJ, Highley JR, Ince PG, Wharton SB, Pickering-Brown S, Kirby J, Hautbergue GM, Shaw PJ (2015) Antisense RNA foci in the motor neurons of C9ORF72-ALS patients are associated with TDP-43 proteinopathy. *Acta Neuropathol* 130: 63-75.
- 7 Gomez-Deza J, Lee YB, Troakes C, Nolan M, Al-Sarraj S, Gallo JM, Shaw CE (2015) Dipeptide repeat protein inclusions are rare in the spinal cord and almost absent from motor neurons in C9ORF72 mutant amyotrophic lateral sclerosis and are unlikely to cause their degeneration. *Acta neuropathologica communications* 3: 38.
- 8 Schludi MH, May S, Grasser FA, Rentzsch K, Kremmer E, Kupper C, Klopstock T, German Consortium for Frontotemporal Lobar D, Bavarian Brain Banking A, Arzberger T, Edbauer D (2015) Distribution of dipeptide repeat proteins in cellular models and C9orf72 mutation cases suggests link to transcriptional silencing. *Acta Neuropathol*. [epub ahead of print]

**Supplementary figure 1:**



(a) Immunoblot of different recombinant 15-mer DPR proteins fused to GST (200ng) labeled with a monoclonal anti-GST antibody (clone 5C3). (b) Twofold serial dilutions of indicated recombinant GST-DPR proteins and immunoblot analysis with the respective antibody demonstrating comparable sensitivity of immunoblot assay in detecting recombinant DPR proteins.

### Supplementary figure 2:



### Spectrum of immunoreactivity with anti-DPR protein antibodies in control cases

Anti-GP immunoreactivity in control cases consists of very faint cytoplasmic labeling shown in the frontal cortex (FC) (a) and spinal cord (SC) (b) in an Alzheimer's disease (AD) case. Weak and fine granular cytoplasmic immunoreactivity is seen in a subset of neurons in control cases for poly-GR as illustrated in the FC (c) and SC (d) in an AD case. No immunoreactivity is seen in any control case with the poly-PA antibody shown for the FC (e) and SC (f) in an ALS-FTD case without *C9ORF72* mutation. With the poly-PR antibody a subset of controls shows no immunoreactivity at all (g, FC of AD case) while others show fine or coarse granular nuclear labeling and small cytoplasmic dots of variable intensity in a subset of neurons in the selected brain regions as shown in the FC of an AD case (h) and a FTLD-TDP case without *C9ORF72* mutation (i) and in the SC in AD cases (j and k) and a case with multiple system atrophy (l). Scale bar: 50  $\mu$ m (a-f, j-l), 25 $\mu$ m (g-i).