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Electronic Supplementary Material

Novel clinical associations with specific *C9ORF72* transcripts in patients with repeat expansions in *C9ORF72*

Authors: Marka van Blitterswijk, Tania F. Gendron, Matthew C. Baker, Mariely DeJesus-Hernandez, NiCole A. Finch, Patricia H. Brown, Lillian M. Daughrity, Melissa E. Murray, Michael G. Heckman, Jie Jiang, Clotilde Lagier-Tourenne, Dieter Edbauer, Don W. Cleveland, Keith A. Josephs, Joseph E. Parisi, David S. Knopman, Ronald C. Petersen, Leonard Petrucelli, Bradley F. Boeve, Neill R. Graff-Radford, Kevin B. Boylan, Dennis W. Dickson, and Rosa Rademakers

Corresponding author: Rosa Rademakers, PhD, Department of Neuroscience, Mayo Clinic, 4500 San Pablo Road, Jacksonville, FL 32224, USA. Telephone number: +1 904 953 6279. Fax number: +1 904 953 7370. Email: Rademakers.Rosa@mayo.edu

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Materials and Methods

Subjects

The Mayo Clinic Florida Brain Bank houses over 6500 specimens (4617 frozen brains), including 172 patients with frontotemporal lobar degeneration (FTLD, 143 with transactive response DNA-binding protein 43 kDa [TDP-43] pathology), 65 patients with both FTLD and motor neuron disease (FTLD/MND, 52 with TDP-43 pathology), and 98 patients with MND (81 with TDP-43 pathology). We included all subjects from this tissue bank who were identified as chromosome 9 open reading frame 72 (*C9ORF72*) expansion carriers with our previously published 2-step PCR protocol [1], for whom tissue was available from the cerebellum and/or frontal cortex, which allowed extraction of sufficient high quality RNA for our expression studies (RNA integrity number [RIN] values above seven). These subjects all demonstrated a characteristic stutter pattern on repeat-primed PCR and they showed poly(GP) pathology with immunohistochemistry.

Age at onset was estimated based on the appearance of the first disease symptoms, namely progressive cognitive dysfunction in judgment, language, or memory; or changes in behavior or personality (FTLD patients); or fasciculations, muscle weakness, falls, dysarthria, and dysphagia (MND patients). When symptoms of both FTLD and MND were noted, the earliest observation of decline was recorded for age at onset. Age at death represents the age at collection (since material was obtained from autopsy tissue). Survival after onset was defined as the interval between age at onset of disease symptoms and the age at death for all subjects. Subjects who carried expanded alleles demonstrated a characteristic stutter pattern on a repeat-primed PCR, and harbored hundreds to thousands of repeat units, as revealed with Southern blotting techniques [2]. Wild-type alleles, as opposed to expanded alleles, were visualized with a fluorescent PCR, and demonstrated a crisp band of approximately 2.3 kb on a Southern blot.

References

1. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H, Adamson J, Kouri N, Wojtas A, Sengdy P, Hsiung GY, Karydas A, Seeley WW, Josephs KA, Coppola G, Geschwind DH, Wszolek ZK, Feldman H, Knopman DS, Petersen RC, Miller BL, Dickson DW, Boylan KB, Graff-Radford NR, Rademakers R (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72 (2):245-256. doi:10.1016/j.neuron.2011.09.011
2. van Blitterswijk M, DeJesus-Hernandez M, Niemantsverdriet E, Murray ME, Heckman MG, Diehl NN, Brown PH, Baker MC, Finch NA, Bauer PO, Serrano G, Beach TG, Josephs KA, Knopman DS, Petersen RC, Boeve BF, Graff-Radford NR, Boylan KB, Petrucelli L, Dickson DW, Rademakers R (2013) Association between repeat sizes and clinical and pathological characteristics in carriers of C9ORF72 repeat expansions (Xpansize-72): a cross-sectional cohort study. The Lancet Neurology 12 (10):978-988. doi:10.1016/S1474-4422(13)70210-2

Tables

Table 1: Subject characteristics in *C9ORF72* expression cohort (NanoString; n=103)

Variable	C9Plus Cohort (n=56)	C9Minus Cohort (n=26)	Control Cohort (n=21)
Subject characteristics			
Gender (male)	32 (57%)	11 (42%)	7 (33%)
Age at onset (years)	62.0 (53.1–66.8)	..	..
Age at death (years)	66.5 (60.0–72.8)	71.0 (64.3–81.8)	88.0 (82.0–93.0)
Survival after onset (years)	5.0 (2.8–8.1)	..	..
RIN cerebellum (value)	9.4 (8.9–9.6)	9.4 (9.3–9.6)	9.3 (8.5–9.4)
RIN frontal cortex (value)	9.1 (8.6–9.7)	9.5 (9.0–9.7)	8.9 (8.8–9.2)
Repeat length cerebellum (kb)	12.2 (10.5–13.6)	..	..
Repeat length frontal cortex (kb)	25.6 (21.5–39.8)	..	..
Diagnosis			
FTLD	23 (41%)	6 (23%)	..
FTLD/MND	16 (29%)	11 (42%)	..
MND	14 (25%)	9 (35%)	..
Other	3 (5%)	..	..
Available cerebellum	54 (96%)	20 (77%)	20 (95%)
Available frontal cortex	52 (93%)	20 (77%)	20 (95%)

Data are sample median (IQR) or number (%). Information was obtained for patients with (C9Plus) and without (C9Minus) expansions in *C9ORF72*, as well as for control subjects (Control); information was unavailable regarding age at onset (n=5, C9Plus), age at death (n=1, C9Plus), survival after onset (n=5, C9Plus), and repeat length in frontal cortex (n=2, C9Plus). We used ".." when information was not available for an entire group. This *C9ORF72* expression study was performed in both the cerebellum and frontal cortex. Cerebellum was included for 54 expansion carriers (23 FTLD, 15 FTLD/MND, 14 MND, and 2 other diseases), 20 patients without *C9ORF72* repeat expansions (4 FTLD, 9 FTLD/MND, and 7 MND), and 20 control subjects without any neurological disease. We obtained frontal cortex for 52 expansion carriers (22 FTLD, 14 FTLD/MND, 13 MND, and 3 other diseases), 20 patients without *C9ORF72* repeat expansions (5 FTLD, 7 FTLD/MND, and 8 MND), and 20 control subjects without any neurological disease.

Table 2: Subject characteristics in poly(GP) and poly(GA) cohort (n=50)

Variable	C9Plus Cohort (n=50)
Subject characteristics	
Gender (male)	28 (56%)
Age at onset (years)	61.3 (52.2–66.8)
Age at death (years)	66.4 (58.8–72.7)
Survival after onset (years)	5.2 (2.7–8.1)
Repeat length cerebellum (kb)	12.0 (10.6–13.6)
Repeat length frontal cortex (kb)	25.7 (21.5–39.8)
Diagnosis	
FTLD	22 (44%)
FTLD/MND	14 (28%)
MND	14 (28%)
Other	0 (0%)
Available cerebellum	49 (98%)
Available frontal cortex	48 (96%)

Data are sample median (IQR) or number (%). Information was obtained for patients with repeat expansions in *C9ORF72* (C9Plus). This poly(GP) and poly(GA) study was performed in both the cerebellum and frontal cortex. Cerebellum was acquired for 49 expansion carriers (22 FTLD, 13 FTLD/MND, and 14 MND), and frontal cortex was obtained for 48 expansion carriers (21 FTLD, 14 FTLD/MND, and 13 MND).

Table 3: *C9ORF72* probes used for NanoString technologies

Identifier	Accession	Region	Target Sequence
Total	NM_018325.3	401–500	CTTTGATGGAAACTGGAATGGGGATCGCAGCACATATGGACTATCAATTAT ACTTCCACAGACAGAACTTAGTTTCTACCTCCCACTTCATAGAGTGTGT
Variant 1	NM_145005.4	1156–1255	GTGGAGTTTAGTACTTAAGAGTTTGTGCCCTTAAACCAGACTCCCTGGATTA ATGCTGTGTACCCGTGGGCAAGGTGCCTGAATTCTCTATACACCTATT
Variant 2	NM_018325.2	14–113	CTGCGCCCGCGGCGGCGGAGGCGCAGGCGGTGGCGAGTGGATATCTCCGG AGCATTTGGATAATGTGACAGTTGGAATGCAGTGATGTCGACTCTTTGCC
Variant 3	NM_001256054.1	31–130	GAGGTGCGTCAAACAGCGACAAGTTCCGCCCACGTAAAAGATGACGCTTG GTGTGTCAGCCGTCCCTGCTGCCCGGTTGCTTCTCTTTTGGGGGCGGGGT
Intron 1a	C9orf72_intron1	5–104	TTTGTTTTTCCCACCCTCTCTCCCCACTACTTGCTCTCACAGTACTCGCTGAG GGTGAACAAGAAAAGACCTGATAAAGATTAACCAGAAGAAAACAAGG
Intron 1b	C9orf72_intron1	918–1017	TTGGGCATCACTTGACTGATGGTAATCAGTTGTCTAAAGAAGTGCACAGAT TACATGTCCGTGTGCTCATTGGGTCTATCTGGCCGCGTTGAACACCACC

C9ORF72 probes used for digital molecular barcoding. We excluded variant 3 from our analysis because its values were too low for reliable detection.

Table 4: Expression of *C9ORF72* transcripts using TaqMan assays

TaqMan			**C9Plus versus C9Minus Cohort**			**C9Plus versus Control Cohort**			**C9Minus versus Control Cohort**		
Cerebellum	Group	P-value[a]	C9Plus	C9Minus	P-value	C9Plus	Control	P-value	C9Minus	Control	P-value
SYP + MAP2	Total	**5.65e-07**	0.67 (0.53–0.82)	0.98 (0.81–1.21)	**9.14e-06**	0.67 (0.53–0.82)	1.00 (0.86–1.18)	**2.61e-05**	0.98 (0.81–1.21)	1.00 (0.86–1.18)	0.85
	Variant 2	**1.86e-10**	0.43 (0.33–0.55)	0.94 (0.71–1.08)	**5.20e-09**	0.43 (0.33–0.55)	1.00 (0.78–1.20)	**1.26e-06**	0.94 (0.71–1.08)	1.00 (0.78–1.20)	0.54
	Variant 3	0.12	0.92 (0.63–1.25)	1.10 (0.93–1.41)	..	0.92 (0.63–1.25)	1.00 (0.76–1.30)	..	1.10 (0.93–1.41)	1.00 (0.76–1.30)	..
RPLP0 + GAPDH	Total	**6.70e-08**	0.66 (0.52–0.82)	1.03 (0.90–1.45)	**9.83e-07**	0.66 (0.52–0.82)	1.00 (0.85–1.24)	**1.57e-05**	1.03 (0.90–1.45)	1.00 (0.85–1.24)	0.67
	Variant 2	**3.08e-10**	0.45 (0.32–0.62)	1.06 (0.80–1.42)	**1.70e-08**	0.45 (0.32–0.62)	1.00 (0.82–1.50)	**6.62e-07**	1.06 (0.80–1.42)	1.00 (0.82–1.50)	0.88
	Variant 3	0.04	0.81 (0.51–1.09)	1.05 (0.79–1.46)	..	0.81 (0.51–1.09)	1.00 (0.77–1.20)	..	1.05 (0.79–1.45)	1.00 (0.77–1.20)	..
Frontal Cortex											
SYP + MAP2	Total	**1.97e-07**	0.78 (0.64–1.05)	1.30 (1.13–1.61)	**2.03e-07**	0.78 (0.64–1.05)	1.00 (0.85–1.22)	**0.02**	1.30 (1.13–1.61)	1.00 (0.85–1.22)	**0.0009**
	Variant 2	**8.53e-13**	0.58 (0.48–0.67)	1.31 (1.14–1.66)	**9.63e-12**	0.58 (0.48–0.67)	1.00 (0.73–1.28)	**1.11e-05**	1.31 (1.14–1.66)	1.00 (0.73–1.28)	**0.001**
	Variant 3	0.02	0.99 (0.74–1.41)	1.39 (0.97–2.01)	..	0.99 (0.74–1.41)	1.00 (0.80–1.54)	..	1.39 (0.97–2.01)	1.00 (0.80–1.54)	..
RPLP0 + GAPDH	Total	**3.77e-10**	0.70 (0.57–0.88)	1.34 (0.91–1.46)	**8.51e-09**	0.70 (0.57–0.88)	1.00 (0.93–1.26)	**1.95e-06**	1.34 (0.91–1.46)	1.00 (0.93–1.26)	0.26
	Variant 2	**3.87e-13**	0.51 (0.38–0.64)	1.11 (0.85–1.79)	**5.91e-11**	0.51 (0.38–0.64)	1.00 (0.87–1.11)	**2.85e-08**	1.11 (0.85–1.79)	1.00 (0.87–1.11)	0.18
	Variant 3	**0.01**	0.80 (0.58–1.13)	1.14 (0.91–1.90)	**0.004**	0.80 (0.58–1.13)	1.00 (0.72–1.83)	0.13	1.14 (0.91–1.90)	1.00 (0.72–1.83)	0.38

Data are sample median (IQR) or p-value and obtained using TaqMan assays; normalized to the geometric mean of synaptophysin (*SYP*) and microtubule-associated protein 2 (*MAP2*) or to the geometric mean of endogenous control genes ribosomal protein, large, P0 (*RPLP0*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). Information was obtained for patients with (C9Plus) and without (C9Minus) expansions in *C9ORF72*, as well as for control subjects (Control). For each brain region three tests were performed (i.e. total *C9ORF72* transcripts, variant 2 transcripts, and variant 3 transcripts), and thus, p-values below 0.017 were considered significant after Bonferroni correction. Of note, in the frontal cortex, there appears to be a significant increase in specific transcripts in patients without repeat expansions as compared to control subjects (after adjustment for neuronal loss using *SYP* and *MAP2*), which might suggest that the adjustment for neuronal loss results in an overcompensation, that the production of those transcripts is not restricted to neuronal cells, and/or that additional currently unknown factors influence the expression of those transcripts. [a] A Kruskal-Wallis rank sum test was performed to determine whether expression levels differed between groups ($p<0.017$ considered significant after Bonferroni correction); when significant differences were detected a Wilcoxon rank sum test was used for pairwise comparisons ($p<0.017$ considered significant after Bonferroni correction); when no significant differences were detected a Wilcoxon a rank sum test was not performed for pairwise comparisons (indicated with "..").

Table 5: Expression of *C9ORF72* transcripts using NanoString technologies

NanoString			C9Plus versus C9Minus Cohort			C9Plus versus Control Cohort			C9Minus versus Control Cohort		
Cerebellum	Group	P-value[a]	C9Plus	C9Minus	P-value	C9Plus	Control	P-value	C9Minus	Control	P-value
SYP + MAP2	Total	**1.18e-07**	0.58 (0.42–0.76)	0.80 (0.67–0.93)	**0.003**	0.58 (0.42–0.76)	1.00 (0.88–1.19)	**1.59e-07**	0.80 (0.67–0.93)	1.00 (0.88–1.19)	**0.008**
	Variant 1	**9.45e-06**	0.47 (0.31–0.63)	0.61 (0.55–0.75)	**0.01**	0.47 (0.31–0.63)	1.00 (0.72–1.49)	**9.04e-06**	0.61 (0.55–0.75)	1.00 (0.72–1.49)	0.02
	Variant 2	**2.19e-12**	0.31 (0.19–0.47)	0.70 (0.55–0.90)	**7.27e-07**	0.31 (0.19–0.47)	1.00 (0.87–1.13)	**5.23e-10**	0.70 (0.55–0.90)	1.00 (0.87–1.13)	**0.0006**
	Intron 1a	0.20	1.08 (0.50–1.86)	0.76 (0.26–1.40)	..	1.08 (0.50–1.86)	1.00 (0.44–1.88)	..	0.76 (0.26–1.40)	1.00 (0.44–1.88)	..
	Intron 1b	0.91	1.00 (0.66–1.59)	0.98 (0.55–1.50)	..	1.00 (0.66–1.59)	1.00 (0.66–1.57)	..	0.98 (0.55–1.50)	1.00 (0.66–1.57)	..
RPLP0 + GAPDH	Total	**0.001**	0.60 (0.36–0.93)	0.73 (0.62–1.03)	0.13	0.60 (0.36–0.93)	1.00 (0.76–1.32)	**0.0004**	0.73 (0.62–1.03)	1.00 (0.76–1.32)	0.04
	Variant 1	**0.001**	0.52 (0.28–0.83)	0.56 (0.51–0.92)	0.12	0.52 (0.28–0.83)	1.00 (0.63–1.67)	**0.0003**	0.56 (0.51–0.92)	1.00 (0.63–1.67)	0.06
	Variant 2	**1.79e-12**	0.29 (0.19–0.46)	0.65 (0.51–0.86)	**1.12e-06**	0.29 (0.19–0.46)	1.00 (0.71–1.10)	**2.62e-10**	0.65 (0.51–0.86)	1.00 (0.71–1.10)	**0.0009**
	Intron 1a	0.19	1.18 (0.49–2.38)	0.70 (0.25–1.25)	..	1.18 (0.49–2.38)	1.00 (0.55–2.09)	..	0.70 (0.25–1.25)	1.00 (0.55–2.09)	..
	Intron 1b	0.72	0.94 (0.59–1.80)	0.90 (0.51–1.25)	..	0.94 (0.59–1.80)	1.00 (0.57–1.62)	..	0.90 (0.51–1.25)	1.00 (0.57–1.62)	..
Frontal Cortex											
SYP + MAP2	Total	**3.44e-11**	0.72 (0.62–0.83)	1.13 (1.04–1.34)	**1.42e-09**	0.72 (0.62–0.83)	1.00 (0.90–1.18)	**2.35e-06**	1.13 (1.04–1.34)	1.00 (0.90–1.18)	0.03
	Variant 1	**3.86e-05**	0.69 (0.49–0.90)	1.10 (0.88–1.38)	**0.0001**	0.69 (0.49–0.90)	1.00 (0.78–1.39)	**0.001**	1.10 (0.88–1.38)	1.00 (0.78–1.39)	0.49
	Variant 2	**1.04e-12**	0.53 (0.34–0.63)	1.21 (0.94–1.43)	**1.84e-08**	0.53 (0.34–0.63)	1.00 (0.90–1.31)	**5.07e-10**	1.21 (0.94–1.43)	1.00 (0.90–1.31)	0.31
	Intron 1a	**0.0002**	2.72 (0.98–4.81)	0.00 (0.00–1.08)	**0.0005**	2.72 (0.98–4.81)	1.00 (0.00–2.34)	**0.003**	0.00 (0.00–1.08)	1.00 (0.00–2.34)	0.37
	Intron 1b	0.11	1.57 (0.96–2.36)	1.24 (0.44–2.10)	..	1.57 (0.96–2.36)	1.00 (0.37–1.74)	..	1.24 (0.44–2.10)	1.00 (0.37–1.74)	..
RPLP0 + GAPDH	Total	**4.36e-13**	0.67 (0.58–0.77)	1.12 (0.98–1.27)	**9.57e-10**	0.67 (0.58–0.77)	1.00 (0.94–1.19)	**4.85e-09**	1.12 (0.98–1.27)	1.00 (0.94–1.19)	0.15
	Variant 1	**1.76e-05**	0.68 (0.45–0.85)	0.99 (0.93–1.47)	**0.0002**	0.68 (0.45–0.85)	1.00 (0.79–1.44)	**0.0002**	0.99 (0.93–1.47)	1.00 (0.79–1.44)	0.90
	Variant 2	**3.80e-14**	0.41 (0.33–0.51)	0.97 (0.86–1.06)	**1.79e-09**	0.41 (0.33–0.51)	1.00 (0.84–1.10)	**7.71e-11**	0.97 (0.86–1.06)	1.00 (0.84–1.10)	0.76
	Intron 1a	**0.0006**	2.97 (0.80–5.43)	0.00 (0.00–1.17)	**0.0009**	2.97 (0.80–5.43)	1.00 (0.00–2.69)	**0.008**	0.00 (0.00–1.17)	1.00 (0.00–2.69)	0.35
	Intron 1b	0.20	1.55 (0.97–2.40)	1.10 (0.46–2.37)	..	1.55 (0.97–2.40)	1.00 (0.42–1.70)	..	1.10 (0.46–2.37)	1.00 (0.42–1.70)	..

Data are sample median (IQR) or p-value and obtained using NanoString technologies; normalized to the geometric mean of synaptophysin (*SYP*) and microtubule-associated protein 2 (*MAP2*) or to the geometric mean of endogenous control genes ribosomal protein, large, P0 (*RPLP0*) and glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*). Information was obtained for patients with (C9Plus) and without (C9Minus) expansions in *C9ORF72*, as well as for control subjects (Control). For each brain region five tests were performed (e.g. total *C9ORF72* transcripts, variant 1 transcripts, variant 2 transcripts, intron 1a containing transcripts, and intron 1b containing transcripts), and thus, p-values below 0.010 were considered significant after Bonferroni correction. Of note, in the cerebellum, there appears to be a significant reduction in specific transcripts in patients without repeat expansions as compared to control subjects (even after adjustment for neuronal loss using *SYP* and *MAP2*), which might suggest that the adjustment for neuronal loss is incomplete and/or that additional currently unknown factors influence the expression of those transcripts. [a] A Kruskal-Wallis rank sum test was performed to determine whether expression levels differed between groups ($p<0.010$ considered significant after Bonferroni correction); when significant differences were detected a Wilcoxon rank sum test was used for pairwise comparisons ($p<0.017$ considered significant after Bonferroni correction); when no significant differences were detected a Wilcoxon a rank sum test was not performed for pairwise comparisons (indicated with "..").

Table 6: Associations of *C9ORF72* transcripts measured by TaqMan assays and NanoString technologies

TaqMan and NanoString		**Overall Cohort**		**C9Plus Cohort**		**C9Minus Cohort**		**Control Cohort**	
Cerebellum	Group	Spearman's r (95% CI)	P-value	Spearman's r (95% CI)	P-value	Spearman's r (95% CI)	P-value	Spearman's r (95% CI)	P-value
SYP + MAP2	Total	0.84 (0.75 to 0.90)	**<2.20e-16**	0.78 (0.62 to 0.87)	**4.74e-12**	0.85 (0.62 to 0.95)	**4.65e-06**	0.74 (0.33 to 0.93)	**0.0003**
	Variant 2	0.75 (0.61 to 0.84)	**<2.20e-16**	0.64 (0.47 to 0.75)	**2.11e-07**	0.57 (0.09 to 0.88)	**0.01**	0.29 (-0.30 to 0.70)	0.23
Frontal Cortex									
SYP + MAP2	Total	0.72 (0.58 to 0.82)	**1.58e-15**	0.59 (0.33 to 0.79)	**4.91e-06**	0.90 (0.67 to 0.97)	**4.62e-07**	0.59 (0.19 to 0.83)	**0.007**
	Variant 2	0.80 (0.71 to 0.86)	**<2.20e-16**	0.51 (0.28 to 0.70)	**9.85e-05**	0.72 (0.26 to 0.95)	**0.0008**	0.70 (0.32 to 0.91)	**0.0009**

Data are Spearman's correlation coefficient *r* (95% confidence interval [CI]) or p-value and obtained using TaqMan assays and NanoString technologies; normalized to the geometric mean of synaptophysin (*SYP*) and microtubule-associated protein 2 (*MAP2*). Measurements obtained with both TaqMan assays and NanoString technologies were available for patients with (C9Plus, cerebellum n=54, frontal cortex n=52) and without (C9Minus, cerebellum n=19, frontal cortex n=18) expansions in *C9ORF72*, as well as for control subjects (Control, cerebellum n=19, frontal cortex n=19). For each brain region two tests were performed (e.g. total *C9ORF72* transcripts and variant 2 transcripts), and thus, p-values below 0.025 were considered significant after Bonferroni correction. A Spearman's test of correlation was used ($p<0.025$ considered significant after Bonferroni correction).

Table 7: Number of subjects with undetectable levels of *C9ORF72* transcripts using NanoString technologies

NanoString		**C9Plus versus C9Minus Cohort**				**C9Plus versus Control Cohort**				**C9Minus versus Control Cohort**			
Cerebellum	Group	C9Plus	C9Minus	Odds ratio (95% CI)	P-value	C9Plus	Control	Odds ratio (95% CI)	P-value	C9Minus	Control	Odds ratio (95% CI)	P-value
	Intron 1a	1 (2%)	3 (15%)	0.11 (0.002–1.48)	0.06	1 (2%)	1 (5%)	0.36 (0.004–29.61)	0.47	3 (15%)	1 (5%)	3.26 (0.24–184.90)	0.61
	Intron 1b	0 (0%)	0 (0%)	..	1	0 (0%)	0 (0%)	..	1	0 (0%)	0 (0%)	..	1
Frontal Cortex													
	Intron 1a	6 (12%)	11 (55%)	0.11 (0.03–0.42)	**0.0003**	6 (12%)	8 (40%)	0.20 (0.05–0.80)	**0.02**	11 (55%)	8 (40%)	1.81 (0.44–7.73)	0.53
	Intron 1b	3 (6%)	1 (5%)	1.16 (0.09–64.22)	1	3 (6%)	1 (5%)	1.16 (0.09–64.22)	1	1 (5%)	1 (5%)	1.00 (0.01–82.52)	1

Data are number (%), odds ratio (OR) with 95% confidence interval (CI), or p-value and obtained using NanoString technologies. Information was obtained for patients with (C9Plus) and without (C9Minus) expansions in *C9ORF72*, as well as for control subjects (Control). A Fisher's exact test was performed to determine whether the number of subjects with undetectable transcripts differed for each pairwise comparison ($p<0.017$ considered significant after Bonferroni correction). We used ".." when ORs could not be estimated.

Table 8: Associations of *C9ORF72* transcripts with disease subgroups using TaqMan assays

TaqMan			**FTLD versus FTLD/MND Cohort**			**FTLD versus MND Cohort**			**FTLD/MND versus MND Cohort**		
Cerebellum	Group	P-value[a]	FTLD	FTLD/MND	P-value	FTLD	MND	P-value	FTLD/MND	MND	P-value
SYP + MAP2	Total	0.96	0.71 (0.59–0.80)	0.63 (0.54–0.84)	..	0.71 (0.59–0.80)	0.68 (0.47–0.82)	..	0.63 (0.54–0.84)	0.68 (0.47–0.82)	..
	Variant 2	0.64	0.41 (0.33–0.50)	0.43 (0.39–0.55)	..	0.41 (0.33–0.50)	0.51 (0.23–0.63)	..	0.43 (0.39–0.55)	0.51 (0.23–0.63)	..
	Variant 3	0.26	1.01 (0.76–1.27)	0.82 (0.59–1.12)	..	1.01 (0.76–1.27)	0.81 (0.43–1.23)	..	0.82 (0.59–1.12)	0.81 (0.43–1.92)	..
Frontal Cortex											
SYP + MAP2	Total	0.55	0.84 (0.65–1.05)	0.83 (0.60–1.24)	..	0.84 (0.65–1.05)	0.70 (0.64–0.84)	..	0.83 (0.60–1.24)	0.70 (0.64–0.84)	..
	Variant 2	0.28	0.53 (0.43–0.62)	0.59 (0.49–0.67)	..	0.53 (0.43–0.62)	0.62 (0.51–0.75)	..	0.59 (0.49–0.67)	0.62 (0.51–0.75)	..
	Variant 3	0.32	0.89 (0.74–1.18)	1.15 (0.61–1.56)	..	0.89 (0.74–1.18)	1.22 (0.85–1.45)	..	1.15 (0.61–1.56)	1.22 (0.85–1.45)	..

Data are sample median (IQR) or p-value and obtained using TaqMan assays; normalized to the geometric mean of synaptophysin (*SYP*) and microtubule-associated protein 2 (*MAP2*). In total, within our cohort of *C9ORF72* expansion carriers, we examined eight different associations (i.e. disease subgroup, gender, age at onset, age at death, repeat length, and survival after onset [using three different cut-off points]) for each outcome, and thus, p-values below 0.0063 were considered significant after Bonferroni correction; in this table, only one of those eight associations is displayed (disease subgroup). [a] A Kruskal-Wallis rank sum test was performed to determine whether expression levels differed between groups ($p<0.0063$ considered significant after Bonferroni correction); when significant differences were detected a Wilcoxon rank sum test was used for pairwise comparisons ($p<0.017$ considered significant after Bonferroni correction); when no significant differences were detected a Wilcoxon a rank sum test was not performed for pairwise comparisons (indicated with "..").

Table 9: Associations of *C9ORF72* transcripts with disease subgroups using NanoString technologies

NanoString			**FTLD versus FTLD/MND Cohort**			**FTLD versus MND Cohort**			**FTLD/MND versus MND Cohort**		
Cerebellum	Group	P-value[a]	FTLD	FTLD/MND	P-value	FTLD	MND	P-value	FTLD/MND	MND	P-value
SYP + MAP2	Total	0.77	0.61 (0.43–0.74)	0.58 (0.43–0.66)	..	0.61 (0.43–0.74)	0.51 (0.41–0.73)	..	0.58 (0.43–0.66)	0.51 (0.41–0.73)	..
	Variant 1	0.85	0.55 (0.33–0.66)	0.43 (0.35–0.53)	..	0.55 (0.33–0.66)	0.46 (0.30–0.61)	..	0.43 (0.35–0.53)	0.46 (0.30–0.61)	..
	Variant 2	0.24	0.27 (0.19–0.37)	0.31 (0.18–0.46)	..	0.27 (0.19–0.37)	0.45 (0.26–0.49)	..	0.31 (0.18–0.46)	0.45 (0.26–0.49)	..
	Intron 1a	0.45	1.29 (0.66–2.09)	0.93 (0.53–1.35)	..	1.29 (0.66–2.09)	0.96 (0.25–1.53)	..	0.93 (0.53–1.35)	0.96 (0.25–1.53)	..
	Intron 1b	0.33	1.06 (0.82–1.63)	0.87 (0.68–1.33)	..	1.06 (0.82–1.63)	0.82 (0.32–1.42)	..	0.87 (0.68–1.33)	0.82 (0.32–1.42)	..
Frontal Cortex											
SYP + MAP2	Total	0.21	0.67 (0.60–0.78)	0.77 (0.65–0.84)	..	0.67 (0.60–0.78)	0.78 (0.67–0.82)	..	0.77 (0.65–0.84)	0.78 (0.67–0.82)	..
	Variant 1	0.41	0.72 (0.56–0.86)	0.77 (0.49–1.13)	..	0.72 (0.56–0.86)	0.58 (0.46–0.74)	..	0.77 (0.49–1.13)	0.58 (0.46–0.74)	..
	Variant 2	0.03	0.37 (0.28–0.57)	0.58 (0.44–0.62)	..	0.37 (0.28–0.57)	0.65 (0.47–0.75)	..	0.58 (0.44–0.62)	0.65 (0.47–0.75)	..
	Intron 1a	**0.002**	3.96 (2.10–6.25)	3.73 (1.58–4.41)	0.49	3.96 (2.10–6.25)	0.52 (0.00–1.86)	**0.001**	3.73 (1.58–4.41)	0.52 (0.00–1.86)	**0.006**
	Intron 1b	0.61	1.83 (0.97–2.51)	1.53 (1.04–2.24)	..	1.83 (0.97–2.51)	1.27 (0.55–2.30)	..	1.53 (1.04–2.24)	1.27 (0.55–2.30)	..

Data are sample median (IQR) or p-value and obtained using NanoString technologies; normalized to the geometric mean of synaptophysin (*SYP*) and microtubule-associated protein 2 (*MAP2*). In total, within our cohort of *C9ORF72* expansion carriers, we examined eight different associations (i.e. disease subgroup, gender, age at onset, age at death, repeat length, and survival after onset [using three different cut-off points]) for each outcome, and thus, p-values below 0.0063 were considered significant after Bonferroni correction; in this table, only one of those eight associations is displayed (disease subgroup). [a] A Kruskal-Wallis rank sum test was performed to determine whether expression levels differed between groups ($p<0.0063$ considered significant after Bonferroni correction); when significant differences were detected a Wilcoxon rank sum test was used for pairwise comparisons ($p<0.017$ considered significant after Bonferroni correction); when no significant differences were detected a Wilcoxon a rank sum test was not performed for pairwise comparisons (indicated with "..").

Table 10: Associations of *C9ORF72* transcripts with gender using TaqMan assays

TaqMan		**Female versus Male Cohort**		
Cerebellum	Group	Female	Male	P-value
SYP + MAP2	Total	0.63 (0.54–0.80)	0.73 (0.53–0.82)	0.39
	Variant 2	0.44 (0.25–0.55)	0.43 (0.39–0.55)	0.49
	Variant 3	0.82 (0.46–1.02)	1.12 (0.65–1.38)	0.02
Frontal Cortex				
SYP + MAP2	Total	0.70 (0.60–0.92)	0.83 (0.65–1.05)	0.33
	Variant 2	0.64 (0.52–0.74)	0.56 (0.43–0.63)	0.06
	Variant 3	0.85 (0.75–1.53)	0.99 (0.75–1.35)	0.81

Data are sample median (IQR) or p-value and obtained using TaqMan assays; normalized to the geometric mean of synaptophysin (*SYP*) and microtubule-associated protein 2 (*MAP2*). In total, within our cohort of *C9ORF72* expansion carriers, we examined eight different associations (i.e. disease subgroup, gender, age at onset, age at death, repeat length, and survival after onset [using three different cut-off points]) for each outcome, and thus, p-values below 0.0063 were considered significant after Bonferroni correction; in this table, only one of those eight associations is displayed (gender). A Wilcoxon rank sum test was used for pairwise comparisons ($p<0.0063$ considered significant after Bonferroni correction).

Table 11: Associations of *C9ORF72* transcripts with gender using NanoString technologies

NanoString		**Female versus Male Cohort**		
Cerebellum	Group	Female	Male	P-value
SYP + MAP2	Total	0.49 (0.39–0.67)	0.62 (0.44–0.78)	0.07
	Variant 1	0.44 (0.26–0.56)	0.50 (0.35–0.73)	0.12
	Variant 2	0.30 (0.19–0.47)	0.33 (0.18–0.45)	0.97
	Intron 1a	0.65 (0.34–1.35)	1.41 (0.85–2.21)	0.01
	Intron 1b	0.79 (0.59–1.14)	1.24 (0.85–1.76)	0.03
Frontal Cortex				
SYP + MAP2	Total	0.70 (0.64–0.79)	0.73 (0.62–0.83)	0.88
	Variant 1	0.59 (0.44–1.00)	0.77 (0.57–0.86)	0.53
	Variant 2	0.57 (0.44–0.65)	0.43 (0.34–0.60)	0.29
	Intron 1a	1.59 (0.23-4.44)	3.66 (1.40-4.88)	0.14
	Intron 1b	1.49 (0.90-2.30)	1.64 (1.01-2.37)	0.91

Data are sample median (IQR) or p-value and obtained using NanoString technologies; normalized to the geometric mean of synaptophysin (*SYP*) and microtubule-associated protein 2 (*MAP2*). In total, within our cohort of *C9ORF72* expansion carriers, we examined eight different associations (i.e. disease subgroup, gender, age at onset, age at death, repeat length, and survival after onset [using three different cut-off points]) for each outcome, and thus, p-values below 0.0063 were considered significant after Bonferroni correction; in this table, only one of those eight associations is displayed (gender). A Wilcoxon rank sum test was used for pairwise comparisons ($p<0.0063$ considered significant after Bonferroni correction).

Table 12: Associations of *C9ORF72* transcripts with age at onset, age at death and expansion size using TaqMan assays

TaqMan			**Overall Cohort**		**FTLD Cohort**		**FTLD/MND Cohort**		**MND Cohort**	
Cerebellum	Group	Association	Spearman's r (95% CI)	P-value	Spearman's r (95% CI)	P-value	Spearman's r (95% CI)	P-value	Spearman's r (95% CI)	P-value
SYP + MAP2	Total	Age at onset	-0.01 (-0.30 to 0.28)	0.94	-0.19 (-0.62 to 0.33)	0.41	-0.34 (-0.83 to 0.31)	0.27	0.38 (-0.15 to 0.74)	0.17
		Age at death	0.007 (-0.28 to 0.29)	0.96	-0.14 (-0.57 to 0.39)	0.52	-0.26 (-0.76 to 0.32)	0.37	0.48 (-0.007 to 0.74)	0.08
		C9ORF72 expansion size	-0.03 (-0.31 to 0.27)	0.83	0.20 (-0.21 to 0.57)	0.36	-0.43 (-0.84 to 0.22)	0.11	0.20 (-0.48 to 0.73)	0.48
	Variant 2	Age at onset	0.11 (-0.19 to 0.39)	0.46	0.08 (-0.49 to 0.56)	0.73	0.04 (-0.61 to 0.69)	0.88	0.24 (-0.27 to 0.65)	0.40
		Age at death	0.02 (-0.29 to 0.31)	0.90	0.03 (-0.46 to 0.50)	0.89	-0.09 (-0.67 to 0.56)	0.77	0.31 (-0.19 to 0.67)	0.28
		C9ORF72 expansion size	-0.09 (-0.35 to 0.19)	0.54	0.13 (-0.41 to 0.59)	0.57	-0.36 (-0.80 to 0.22)	0.19	0.18 (-0.54 to 0.74)	0.52
	Variant 3	Age at onset	-0.07 (-0.37 to 0.23)	0.61	-0.21 (-0.62 to 0.25)	0.36	-0.41 (-0.84 to 0.23)	0.17	0.13 (-0.45 to 0.63)	0.65
		Age at death	-0.03 (-0.32 to 0.25)	0.83	-0.32 (-0.70 to 0.14)	0.14	-0.29 (-0.76 to 0.35)	0.32	0.20 (-0.40 to 0.64)	0.49
		C9ORF72 expansion Size	-0.25 (-0.49 to 0.03)	0.07	-0.17 (-0.53 to 0.26)	0.44	-0.72 (-0.90 to -0.29)	**0.003**	0.17 (-0.44 to 0.69)	0.56
Frontal Cortex										
SYP + MAP2	Total	Age at onset	-0.24 (-0.50 to 0.05)	0.10	-0.50 (-0.75 to -0.12)	0.02	-0.53 (-0.96 to 0.16)	0.06	0.26 (-0.36 to 0.69)	0.38
		Age at death	-0.12 (-0.40 to 0.16)	0.41	-0.57 (-0.73 to -0.24)	**0.006**	-0.27 (-0.85 to 0.46)	0.36	0.37 (-0.23 to 0.71)	0.21
		C9ORF72 expansion size	-0.13 (-0.41 to 0.17)	0.35	-0.34 (-0.68 to 0.09)	0.12	-0.19 (-0.76 to 0.46)	0.52	0.23 (-0.50 to 0.78)	0.44
	Variant 2	Age at onset	-0.16 (-0.41 to 0.12)	0.27	-0.29 (-0.64 to 0.16)	0.22	-0.29 (-0.80 to 0.46)	0.35	0.46 (-0.21 to 0.89)	0.12
		Age at death	-0.18 (-0.42 to 0.08)	0.21	-0.19 (-0.55 to 0.30)	0.40	-0.11 (-0.68 to 0.59)	0.71	0.41 (-0.27 to 0.87)	0.17
		C9ORF72 expansion size	-0.17 (-0.45 to 0.13)	0.23	-0.11 (-0.55 to 0.36)	0.63	-0.11 (-0.70 to 0.54)	0.72	-0.33 (-0.78 to 0.30)	0.28
	Variant 3	Age at onset	-0.26 (-0.53 to 0.04)	0.07	-0.34 (-0.72 to 0.16)	0.15	-0.31 (-0.81 to 0.38)	0.30	-0.12 (-0.66 to 0.50)	0.70
		Age at death	-0.25 (-0.52 to 0.04)	0.07	-0.33 (-0.70 to 0.14)	0.13	-0.27 (-0.82 to 0.41)	0.36	-0.02 (-0.61 to 0.56)	0.96
		C9ORF72 expansion size	-0.28 (-0.55 to 0.04)	0.05	-0.25 (-0.67 to 0.24)	0.26	-0.55 (-0.93 to 0.03)	0.04	0.37 (-0.23 to 0.75)	0.21

Data are Spearman's correlation coefficient *r* (95% confidence interval [CI]) or p-value and obtained using TaqMan assays; normalized to the geometric mean of synaptophysin (*SYP*) and microtubule-associated protein 2 (*MAP2*). In total, within our cohort of *C9ORF72* expansion carriers, we examined eight different associations (i.e. disease subgroup, gender, age at onset, age at death, repeat length, and survival after onset [using three different cut-off points]) for each outcome, and thus, p-values below 0.0063 were considered significant after Bonferroni correction; in this table, only three of those eight associations are displayed (age at onset, age at death, and repeat length). A Spearman's test of correlation was used (p<0.0063 considered significant after Bonferroni correction).

Table 13: Associations of *C9ORF72* transcripts with age at onset, age at death and expansion size using NanoString technologies

NanoString			**Overall Cohort**		**FTLD Cohort**		**FTLD/MND Cohort**		**MND Cohort**	
Cerebellum	Group	Association	Spearman's r (95% CI)	P-value	Spearman's r (95% CI)	P-value	Spearman's r (95% CI)	P-value	Spearman's r (95% CI)	P-value
SYP + MAP2	Total	Age at onset	-0.12 (-0.40 to 0.18)	0.42	-0.37 (-0.76 to 0.16)	0.10	0.005 (-0.58 to 0.60)	0.98	0.02 (-0.52 to 0.52)	0.93
		Age at death	-0.06 (-0.36 to 0.22)	0.65	-0.31 (-0.71 to 0.20)	0.15	0.02 (-0.55 to 0.58)	0.94	0.12 (-0.45 to 0.56)	0.69
		C9ORF72 expansion size	-0.09 (-0.37 to 0.20)	0.51	0.01 (-0.41 to 0.42)	0.95	-0.36 (-0.93 to 0.31)	0.19	-0.12 (-0.69 to 0.49)	0.68
	Variant 1	Age at onset	-0.05 (-0.33 to 0.25)	0.75	-0.22 (-0.64 to 0.30)	0.33	0.02 (-0.59 to 0.58)	0.94	-0.06 (-0.51 to 0.46)	0.83
		Age at death	-0.002 (-0.30 to 0.28)	0.99	-0.11 (-0.53 to 0.39)	0.61	0.007 (-0.58 to 0.56)	0.98	0.05 (-0.42 to 0.52)	0.86
		C9ORF72 expansion size	-0.05 (-0.34 to 0.25)	0.74	0.09 (-0.36 to 0.52)	0.69	-0.18 (-0.79 to 0.45)	0.53	-0.41 (-0.78 to 0.18)	0.15
	Variant 2	Age at onset	0.05 (-0.24 to 0.32)	0.72	0.03 (-0.43 to 0.48)	0.90	0.21 (-0.42 to 0.75)	0.48	0.08 (-0.54 to 0.59)	0.78
		Age at death	-0.06 (-0.33 to 0.22)	0.67	-0.03 (-0.45 to 0.42)	0.90	0.17 (-0.48 to 0.77)	0.56	0.17 (-0.45 to 0.66)	0.55
		C9ORF72 expansion size	0.03 (-0.28 to 0.32)	0.83	0.19 (-0.31 to 0.61)	0.38	-0.15 (-0.76 to 0.44)	0.59	-0.16 (-0.69 to 0.46)	0.59
	Intron 1a	Age at onset	-0.24 (-0.51 to 0.05)	0.10	-0.25 (-0.70 to 0.30)	0.28	-0.41 (-0.83 to 0.24)	0.17	-0.29 (-0.79 to 0.34)	0.32
		Age at death	-0.15 (-0.42 to 0.14)	0.29	-0.27 (-0.69 to 0.23)	0.22	-0.29 (-0.78 to 0.34)	0.32	-0.27 (-0.78 to 0.36)	0.36
		C9ORF72 expansion size	-0.09 (-0.35 to 0.18)	0.50	-0.001 (-0.46 to 0.45)	1.00	-0.38 (-0.75 to 0.13)	0.17	0.09 (-0.50 to 0.61)	0.75
	Intron 1b	Age at onset	-0.25 (-0.51 to 0.03)	0.08	-0.38 (-0.76 to 0.15)	0.09	-0.41 (-0.88 to 0.33)	0.17	-0.31 (-0.81 to 0.35)	0.29
		Age at death	-0.14 (-0.40 to 0.14)	0.32	-0.30 (-0.68 to 0.18)	0.16	-0.31 (-0.77 to 0.31)	0.29	-0.30 (-0.81 to 0.34)	0.30
		C9ORF72 expansion size	-0.28 (-0.53 to -0.02)	0.04	-0.26 (-0.63 to 0.18)	0.23	-0.71 (-0.93 to -0.29)	**0.003**	0.21 (-0.46 to 0.74)	0.47
Frontal Cortex										
SYP + MAP2	Total	Age at onset	-0.25 (-0.52 to 0.06)	0.09	-0.24 (-0.57 to 0.17)	0.31	-0.44 (-0.88 to 0.23)	0.14	-0.01 (-0.63 to 0.55)	0.98
		Age at death	-0.24 (-0.49 to 0.03)	0.08	-0.22 (-0.54 to 0.17)	0.34	-0.43 (-0.85 to 0.24)	0.13	0.07 (-0.58 to 0.63)	0.82
		C9ORF72 expansion size	-0.13 (-0.40 to 0.17)	0.37	-0.14 (-0.56 to 0.31)	0.52	-0.39 (-0.87 to 0.26)	0.17	0.39 (-0.28 to 0.66)	0.19
	Variant 1	Age at onset	-0.10 (-0.40 to 0.21)	0.49	-0.14 (-0.56 to 0.32)	0.55	-0.60 (-0.93 to -0.006)	0.03	0.46 (-0.16 to 0.80)	0.11
		Age at death	-0.003 (-0.28 to 0.26)	0.99	-0.11 (-0.48 to 0.28)	0.61	-0.52 (-0.89 to 0.08)	0.06	0.54 (-0.08 to 0.84)	0.05
		C9ORF72 expansion size	0.04 (-0.23 to 0.30)	0.80	-0.10 (-0.46 to 0.27)	0.67	-0.05 (-0.70 to 0.65)	0.87	0.28 (-0.37 to 0.75)	0.34
	Variant 2	Age at onset	-0.13 (-0.41 to 0.15)	0.36	-0.09 (-0.58 to 0.40)	0.69	-0.19 (-0.76 to 0.48)	0.54	0.24 (-0.39 to 0.68)	0.43
		Age at death	-0.22 (-0.46 to 0.05)	0.12	-0.11 (-0.52 to 0.33)	0.64	-0.17 (-0.73 to 0.51)	0.57	0.27 (-0.37 to 0.73)	0.37
		C9ORF72 expansion size	-0.21 (-0.44 to 0.06)	0.13	-0.02 (-0.41 to 0.39)	0.93	-0.44 (-0.88 to 0.13)	0.12	-0.07 (-0.71 to 0.57)	0.83
	Intron 1a	Age at onset	0.09 (-0.22 to 0.36)	0.56	0.14 (-0.29 to 0.55)	0.55	-0.71 (-0.93 to -0.19)	0.007	0.15 (-0.50 to 0.69)	0.62
		Age at death	0.14 (-0.16 to 0.41)	0.31	-0.06 (-0.48 to 0.36)	0.80	-0.55 (-0.89 to 0.06)	0.04	0.22 (-0.43 to 0.73)	0.47
		C9ORF72 expansion size	0.19 (-0.10 to 0.46)	0.19	0.11 (-0.32 to 0.50)	0.63	-0.04 (-0.60 to 0.58)	0.90	0.27 (-0.39 to 0.78)	0.36
	Intron 1b	Age at onset	-0.24 (-0.50 to 0.07)	0.10	-0.44 (-0.73 to -0.02)	0.05	-0.53 (-0.90 to 0.16)	0.06	-0.03 (-0.65 to 0.62)	0.94

	Age at death	-0.10 (-0.38 to 0.18)	0.47	-0.22 (-0.56 to 0.17)	0.32	-0.45 (-0.88 to 0.20)	0.10	-0.005 (-0.63 to 0.63)	0.99
	C9ORF72 expansion size	-0.14 (-0.41 to 0.15)	0.33	-0.30 (-0.63 to 0.13)	0.17	-0.38 (-0.83 to 0.20)	0.18	0.19 (-0.48 to 0.77)	0.54

Data are Spearman's correlation coefficient *r* (95% confidence interval [CI]) or p-value and obtained using NanoString technologies; normalized to the geometric mean of synaptophysin (*SYP*) and microtubule-associated protein 2 (*MAP2*). In total, within our cohort of *C9ORF72* expansion carriers, we examined eight different associations (i.e. disease subgroup, gender, age at onset, age at death, repeat length, and survival after onset [using three different cut-off points]) for each outcome, and thus, p-values below 0.0063 were considered significant after Bonferroni correction; in this table, only three of those eight associations are displayed (age at onset, age at death, and repeat length). A Spearman's test of correlation was used ($p<0.0063$ considered significant after Bonferroni correction).

Table 14: Associations of *C9ORF72* transcripts with survival after onset using TaqMan assays

TaqMan			Overall Cohort		FTLD Cohort		FTLD/MND Cohort		MND Cohort	
Cerebellum	Group	Survival after Onset	Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
SYP + MAP2	Total	>Median	0.49 (0.26 to 0.94)	0.03	0.46 (0.17 to 1.28)	0.14	0.68 (0.18 to 2.61)	0.57	0.29 (0.08 to 1.11)	0.07
		>25th Percentile	0.56 (0.25 to 1.25)	0.16	0.10 (0.02 to 0.48)	**0.004**	2.51 (0.43 to 14.49)	0.31	0.22 (0.05 to 1.03)	0.05
		>75th Percentile	0.56 (0.28 to 1.12)	0.10	0.87 (0.31 to 2.50)	0.80	0.70 (0.18 to 2.65)	0.60	0.17 (0.03 to 0.93)	0.04
	Variant 2	>Median	1.42 (0.74 to 2.72)	0.30	0.75 (0.30 to 1.89)	0.54	5.99 (1.16 to 30.91)	0.03	0.96 (0.27 to 3.41)	0.96
		>25th Percentile	1.20 (0.57 to 2.53)	0.63	1.55 (0.41 to 5.85)	0.52	0.77 (0.18 to 3.28)	0.72	0.96 (0.27 to 3.41)	0.96
		>75th Percentile	1.53 (0.70 to 3.32)	0.28	1.53 (0.35 to 6.79)	0.58	7.28 (1.48 to 35.77)	0.01	0.61 (0.20 to 1.88)	0.39
	Variant 3	>Median	0.95 (0.51 to 1.79)	0.88	2.02 (0.64 to 6.33)	0.23	0.92 (0.28 to 3.06)	0.89	0.28 (0.07 to 1.22)	0.09
		>25th Percentile	0.50 (0.23 to 1.08)	0.08	0.20 (0.04 to 0.92)	0.04	1.16 (0.26 to 5.18)	0.84	0.31 (0.08 to 1.13)	0.07
		>75th Percentile	0.88 (0.41 to 1.85)	0.73	1.72 (0.64 to 4.62)	0.28	2.40 (0.44 to 13.18)	0.32	0.15 (0.03 to 0.83)	0.03
Frontal Cortex										
SYP + MAP2	Total	>Median	0.93 (0.49 to 1.79)	0.84	2.35 (0.78 to 7.04)	0.13	0.50 (0.16 to 1.64)	0.26	0.47 (0.12 to 1.81)	0.28
		>25th Percentile	0.32 (0.14 to 0.75)	0.009	0.08 (0.01 to 0.50)	0.007	0.10 (0.01 to 0.70)	0.02	0.48 (0.12 to 1.85)	0.29
		>75th Percentile	0.78 (0.35 to 1.71)	0.53	1.67 (0.41 to 6.78)	0.47	0.46 (0.14 to 1.58)	0.22	0.46 (0.05 to 4.07)	0.49
	Variant 2	>Median	1.13 (0.62 to 2.06)	0.70	1.03 (0.40 to 2.62)	0.96	0.67 (0.21 to 2.12)	0.49	1.92 (0.48 to 7.59)	0.36
		>25th Percentile	1.35 (0.65 to 2.80)	0.43	1.05 (0.37 to 2.95)	0.93	1.37 (0.26 to 7.16)	0.71	2.14 (0.46 to 10.04)	0.33
		>75th Percentile	0.94 (0.46 to 1.92)	0.86	0.62 (0.20 to 1.92)	0.40	0.59 (0.12 to 2.82)	0.51	3.57 (0.60 to 21.36)	0.16
	Variant 3	>Median	0.56 (0.28 to 1.14)	0.11	0.50 (0.15 to 1.61)	0.25	0.86 (0.27 to 2.74)	0.80	0.29 (0.06 to 1.38)	0.12
		>25th Percentile	0.60 (0.28 to 1.29)	0.19	0.40 (0.12 to 1.40)	0.15	0.76 (0.23 to 2.52)	0.66	0.54 (0.10 to 2.95)	0.48
		>75th Percentile	0.67 (0.31 to 1.42)	0.30	2.11 (0.42 to 10.69)	0.37	0.78 (0.23 to 2.61)	0.68	0.23 (0.04 to 1.29)	0.09

Data are hazard ratio (HR) with 95% confidence interval (CI) or p-value and obtained using TaqMan assays; normalized to the geometric mean of synaptophysin (*SYP*) and microtubule-associated protein 2 (*MAP2*). In total, within our cohort of *C9ORF72* expansion carriers, we examined eight different associations (i.e. disease subgroup, gender, age at onset, age at death, repeat length, and survival after onset [using three different cut-off points]) for each outcome, and thus, p-values below 0.0063 were considered significant after Bonferroni correction; in this table, only three of those eight associations are displayed (survival after onset with three cut-off points). A Cox proportional hazards regression model was used ($p<0.0063$ considered significant after Bonferroni correction), using a dichotomous categorical variable with three different cut-off points (median, 25th percentile, and 75th percentile) in the given subject group.

Table 15: Associations of *C9ORF72* transcripts with survival after onset using NanoString technologies

NanoString			Overall Cohort		FTLD Cohort		FTLD/MND Cohort		MND Cohort	
Cerebellum	Group	Survival after Onset	Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value	Hazard Ratio (95% CI)	P-value
SYP + MAP2	Total	>Median	0.59 (0.31 to 1.13)	0.11	1.07 (0.40 to 2.85)	0.89	0.84 (0.24 to 2.91)	0.79	..	..
		>25th Percentile	0.55 (0.26 to 1.16)	0.11	0.30 (0.09 to 1.00)	0.05	1.64 (0.37 to 7.30)	0.52	0.22 (0.05 to 1.03)	0.05
		>75th Percentile	0.62 (0.29 to 1.34)	0.22	1.03 (0.38 to 2.78)	0.96	2.09 (0.49 to 8.86)	0.32	..	..
	Variant 1	>Median	0.49 (0.26 to 0.93)	0.03	0.43 (0.16 to 1.14)	0.09	0.93 (0.29 to 2.97)	0.90	0.20 (0.04 to 1.01)	0.05
		>25th Percentile	0.31 (0.14 to 0.67)	**0.003**	0.04 (0.004 to 0.40)	0.006	0.77 (0.18 to 3.28)	0.72	0.23 (0.06 to 0.88)	0.03
		>75th Percentile	0.65 (0.31 to 1.40)	0.28	1.14 (0.43 to 3.04)	0.79	2.09 (0.49 to 8.86)	0.32	..	..
	Variant 2	>Median	2.49 (1.22 to 5.09)	0.01	2.73 (0.94 to 7.92)	0.07	3.48 (0.81 to 14.90)	0.09	0.96 (0.27 to 3.41)	0.96
		>25th Percentile	1.20 (0.57 to 2.51)	0.63	1.42 (0.45 to 4.47)	0.55	4.13 (0.74 to 23.00)	0.11	0.16 (0.03 to 0.77)	0.02
		>75th Percentile	1.38 (0.67 to 2.83)	0.38	1.66 (0.46 to 6.05)	0.44	1.52 (0.38 to 6.06)	0.55	0.86 (0.27 to 2.73)	0.80
	Intron 1a	>Median	0.67 (0.34 to 1.33)	0.26	1.03 (0.41 to 2.59)	0.95	0.80 (0.21 to 3.11)	0.75	0.10 (0.02 to 0.64)	0.02
		>25th Percentile	0.47 (0.23 to 0.98)	0.05	0.56 (0.15 to 2.02)	0.37	0.26 (0.03 to 2.27)	0.22	0.22 (0.04 to 1.07)	0.06
		>75th Percentile	0.64 (0.32 to 1.30)	0.22	0.98 (0.37 to 2.64)	0.97	0.54 (0.11 to 2.65)	0.45	0.19 (0.02 to 1.61)	0.13
	Intron 1b	>Median	0.39 (0.20 to 0.77)	0.007	0.39 (0.14 to 1.08)	0.07	0.62 (0.19 to 2.05)	0.43	0.10 (0.02 to 0.64)	0.02
		>25th Percentile	0.41 (0.17 to 1.00)	0.05	0.17 (0.03 to 0.94)	0.04	2.97 (0.27 to 32.73)	0.37	0.10 (0.02 to 0.64)	0.02
		>75th Percentile	0.66 (0.32 to 1.35)	0.25	1.07 (0.34 to 3.35)	0.91	0.77 (0.20 to 2.99)	0.70	0.16 (0.02 to 1.32)	0.09
Frontal Cortex										
SYP + MAP2	Total	>Median	0.74 (0.38 to 1.43)	0.36	1.17 (0.42 to 3.23)	0.76	0.62 (0.19 to 2.04)	0.43	0.34 (0.09 to 1.29)	0.11
		>25th Percentile	0.95 (0.48 to 1.91)	0.89	0.91 (0.35 to 2.36)	0.85	1.33 (0.35 to 5.09)	0.68	0.22 (0.03 to 1.62)	0.14
		>75th Percentile	0.57 (0.27 to 1.21)	0.14	1.36 (0.36 to 5.05)	0.65	0.66 (0.20 to 2.21)	0.50	0.23 (0.05 to 1.13)	0.07
	Variant 1	>Median	0.82 (0.44 to 1.51)	0.52	1.39 (0.52 to 3.71)	0.52	0.50 (0.16 to 1.64)	0.26	0.45 (0.12 to 1.76)	0.25
		>25th Percentile	0.23 (0.11 to 0.49)	**0.0001**	0.37 (0.12 to 1.14)	0.08	0.04 (0.004 to 0.42)	0.007	0.17 (0.04 to 0.79)	0.02
		>75th Percentile	0.90 (0.45 to 1.81)	0.77	1.07 (0.38 to 3.03)	0.89	0.97 (0.18 to 5.13)	0.97	0.27 (0.03 to 2.15)	0.21
	Variant 2	>Median	1.57 (0.81 to 3.06)	0.18	2.53 (0.90 to 7.16)	0.08	0.91 (0.28 to 2.91)	0.87	1.23 (0.30 to 4.94)	0.78
		>25th Percentile	1.11 (0.54 to 2.27)	0.78	1.38 (0.54 to 3.53)	0.51	1.19 (0.31 to 4.55)	0.80	0.21 (0.04 to 1.24)	0.08
		>75th Percentile	1.20 (0.55 to 2.61)	0.65	6.22 (1.06 to 36.47)	0.04	0.48 (0.10 to 2.32)	0.36	1.48 (0.40 to 5.38)	0.56
	Intron 1a	>Median	0.82 (0.42 to 1.59)	0.56	1.58 (0.52 to 4.78)	0.42	0.64 (0.13 to 3.24)	0.59	..	..
		>25th Percentile	0.36 (0.14 to 0.91)	0.03	0.07 (0.01 to 0.52)	0.009	0.43 (0.07 to 2.52)	0.35	..	..
		>75th Percentile	1.31 (0.59 to 2.88)	0.51	1.13 (0.42 to 3.06)	0.81	1.12 (0.22 to 5.77)	0.90	..	..
	Intron 1b	>Median	0.84 (0.44 to 1.57)	0.58	0.71 (0.27 to 1.86)	0.48	1.31 (0.35 to 4.87)	0.68	0.68 (0.19 to 2.47)	0.56

	>25th Percentile	0.51 (0.25 to 1.03)	0.06	0.13 (0.03 to 0.60)	0.009	1.16 (0.30 to 4.45)	0.83	0.51 (0.15 to 1.77)	0.29
	>75th Percentile	0.72 (0.33 to 1.56)	0.40	0.49 (0.17 to 1.43)	0.19	1.12 (0.22 to 5.77)	0.90	0.80 (0.16 to 4.03)	0.79

Data are hazard ratio (HR) with 95% confidence interval (CI) or p-value and obtained using NanoString technologies; normalized to the geometric mean of synaptophysin (*SYP*) and microtubule-associated protein 2 (*MAP2*). In total, within our cohort of *C9ORF72* expansion carriers, we examined eight different associations (i.e. disease subgroup, gender, age at onset, age at death, repeat length, and survival after onset [using three different cut-off points]) for each outcome, and thus, p-values below 0.0063 were considered significant after Bonferroni correction; in this table, only three of those eight associations are displayed (survival after onset with three cut-off points). A Cox proportional hazards regression model was used ($p<0.0063$ considered significant after Bonferroni correction), using a dichotomous categorical variable with three different cut-off points (median, 25th percentile, and 75th percentile) in the given subject group. We used ".." to indicate that the number of samples in a given group was too low to allow p-value calculation.