SUPPLEMENTARY MATERIAL

Saracino D, *et al. J Neurol Neurosurg Psychiatry* 2021; 92:1278–1288. doi: 10.1136/jnnp-2021-326914

Appendix A1. Selection of controls

A population of 165 neurologically healthy controls was recruited in the framework of research studies (PREV-DEMALS, Predict-PGRN, and RBM 02-59). All controls had normal neurological examination and cognitive scores. None of them had personal history of neurological diseases. Seventy-seven underwent at least one brain MRI scan, whose findings resulted unremarkable. Of the total population, 114 controls were clinically followed over a mean period of 3.0±1.4 years; none developed neurological diseases in this time interval.

Appendix A2. Identification of outliers

After proper splitting of each population in discrete age classes, we looked for outliers, i.e., individuals with abnormally high plasma NfL (pNfL) levels, or abnormally fast progression, by applying the Tukey's rule (>Q3 + kIQR, where Q3 stands for third quartile, IQR for interquartile range and k a constant assuming the value of 1.5 for "minor" outliers and 3 for "major" outliers). When Tukey's rule was not applicable (too wide IQR) we considered as outliers the individuals with pNfL levels or progression above the 95th percentile for their category.

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Table A1. Clinical descriptions of four presymptomatic *C9orf72* carriers in their prodromal phase.

Individuals	First evaluation	Follow-up
Case 1 **Female** **42 years**	Baseline, 42 years: normal neurological examination, cognitive and behavioural scores. (pNfL value: **11.50 pg/mL**).	1.5 years later, 44 years: normal examination. (pNfL value 10.67 pg/mL) 3 years after baseline, 45 years: attentional deficit, perseverations and social cognition deficit (faux-pas test 21/30). Upper and lower limbs brisk reflexes. (pNfL value: **14.95 pg/mL**, ARC: **+12%**).
Case 2 **Male** **47 years**	Baseline, 47 years: normal neurological examination, cognitive and behavioural scores. CDR®+NACC-FTLD: 0. (pNfL value: **21.76 pg/mL**).	1.5 years later, 48 years: decreased reflexes. (pNfL value: **18.47 pg/mL**) 3 years after baseline, 50 years: inappropriate familiarity, joviality and mild apathy. Decline on several cognitive tests (MDRS 130/144, faux-pas test 18/30). CDR®+NACC-FTLD: 0.5. Cramps and rare fasciculations at motor evaluation. (pNfL value: **18.48 pg/mL**).
Case 3 **Male** **76 years**	Baseline, 76 years: normal neurological examination, cognitive and behavioural scores. CDR®+NACC-FTLD: 0. (pNfL value: 20.79 pg/mL).	1.5 years later, 77 years: normal examination. (pNfL value: 21.87 pg/mL) 3 years after baseline, 79 years: fasciculations, cramps in LL, decreased UL and LL reflexes, attentional and working memory deficits (direct span: 6, reverse span: 4), FAB 16/18, WCST 15/20. CDR®+NACC-FTLD: 0.5. (pNfL value: **28.70 pg/mL**, ARC: **+15%**).
Case 4 **Female** **64 years**	Baseline, 64 years: Normal neurological examination, cognitive and behavioural scores. CDR®+NACC-FTLD: 0. (pNfL value: 18.30 pg/mL).	1.5 years later, 66 years: decreased reflexes (pNfL value: 20.02 pg/mL) 3 years after baseline, 67 years: emergence of executive dysfunction, deficit in mental flexibility and perseverations (WCST 9/20, MMSE 24/30, FAB 15/18). CDR®+NACC-FTLD: 0. Motor evaluation: cramps and fasciculations. (pNfL value: **23.99 pg/mL**, ARC: **+7%**). 6 years after baseline, 70 years: spinal-onset ALS, EMG supported (amyotrophy, fasciculations, motor deficit in UL, left>right). Frontal cognitive decline (motor perseverations, emotional blunting, judgment impairment). (pNfL value : **30.40 pg/mL**)

ARC: annualised rate of change; CDR®+NACC-FTLD: Clinical Dementia Rating Instrument plus National Alzheimer's Coordinating Center Behaviour and Language Domains for Frontotemporal Lobar Degeneration; FAB: frontal assessment battery; FBI: frontal behavioural inventory; LL: lower limbs; MDRS: Mattis Dementia Rating Scale; MMSE: mini mental state examination; pNfL: plasma neurofilament light chain; UL: upper limbs; WCST: Wisconsin card sorting test. Bolded values in table are abnormal values with respect to the individual's age class.

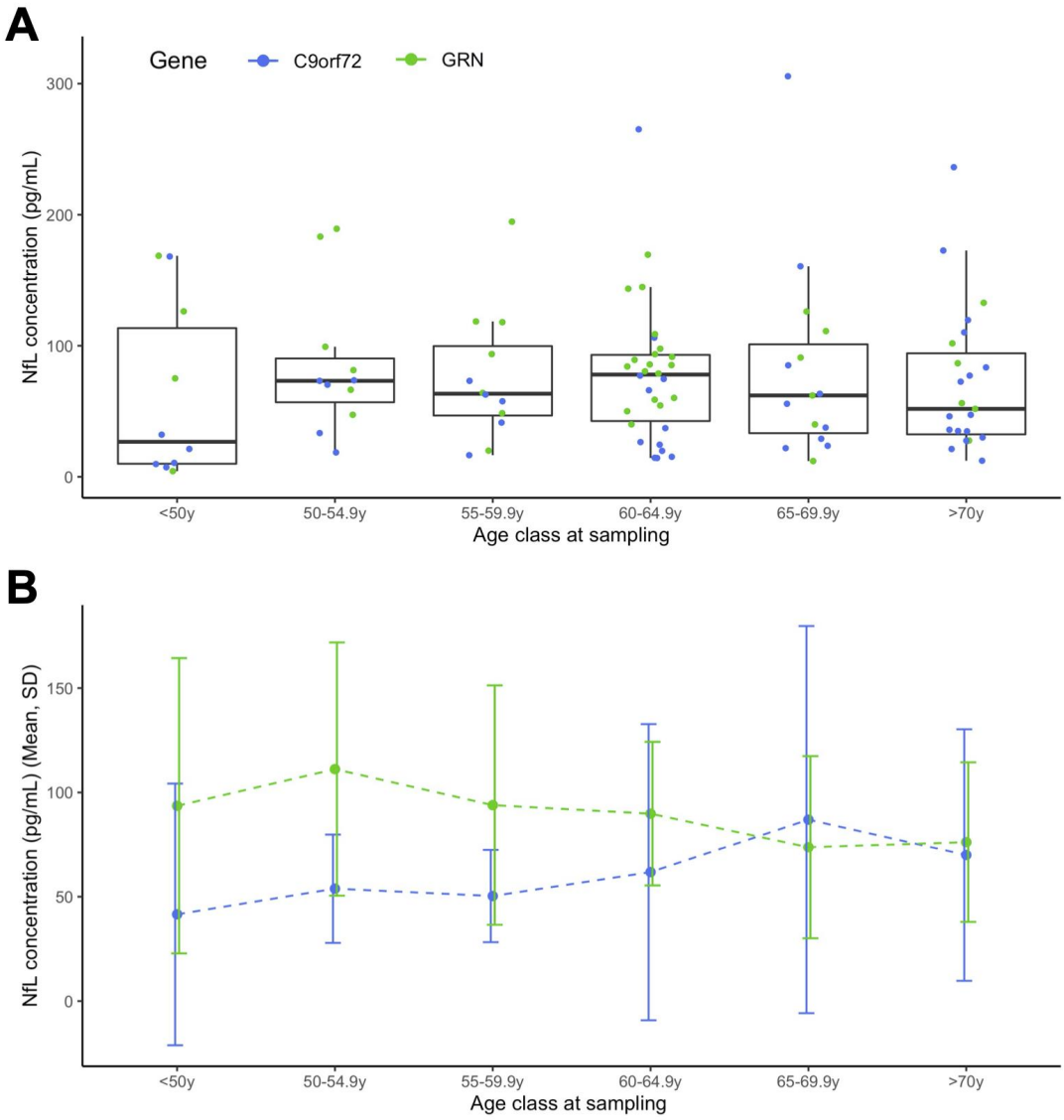
Table A2. Demographic data of 44 patients with longitudinal samplings compared with 36 controls.

	Controls	Patients		*p*-value
		C9orf72	***GRN***	
N=	36	44		-
		29	15	
Age at baseline sampling (years)	55.2 [47.8; 63.7]	62.8 [52.4; 67.2]		0.128
		63.0 [51.0; 70.2]	62.6 [58.9; 64.0]	0.304
Gender (F/M)	19 / 17	22 / 22		0.990
		15 / 14	7 / 8	0.917
Total follow-up duration (years)	1.5 [1.4; 1.9]	1.5 [1.2; 2.7]		0.438
		1.3 [1.1; 2.8]	1.6 [1.5; 2.5]	0.329

Table A3. Demographic data of 66 presymptomatic carriers with longitudinal samplings compared with 58 controls.

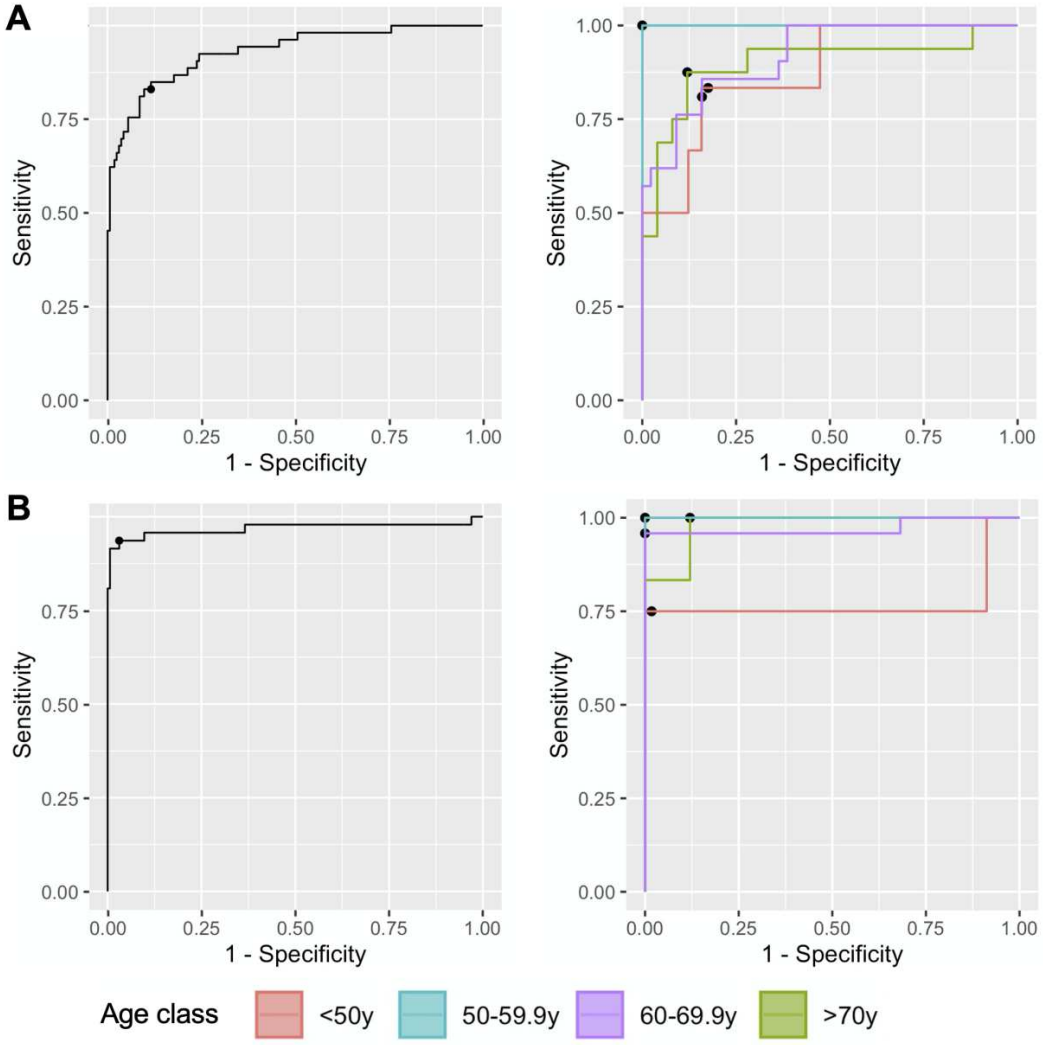
	Controls	Presymptomatic carriers		*p*-value
		C9orf72	***GRN***	
N=	58	66		-
		43	23	
Age at baseline sampling (years)	43.0 [34.4; 52.0]	41.2 [34.5; 47.3]		0.354
		42.6 [35.1; 47.3]	40.3 [33.2; 47.9]	0.624
Gender (F/M)	31 / 27	41 / 25		0.427
		27 / 16	14 / 9	0.878
Total follow-up duration (years)	2.9 [2.5; 3.1]	2.9 [2.5; 3.2]		0.956
		2.9 [2.5; 3.0]	3.3 [1.5; 4.6]	0.430

Figure A1. Distribution of plasma NfL levels according to discrete age classes in patients.



A: Dots represent individual values, box-plots show median values and quartiles, their whiskers extending to the lowest and highest values no further than 1.5*IQR. B: Mean values and SD in each age class, according to the causative gene. No significant differences were found when comparing pNfL values between the age classes (p=0.407). IQR: interquartile range; pNfL: plasma neurofilament light chain; SD: standard deviation; y: years.

Figure A2. ROC curves and optimal cut-offs that discriminate patients from controls.



A: ROC curve for pNfL values in the population of *C9orf72* patients *versus* controls, with an AUC estimated at 0.93 on the left side, and individual ROC curves for each of the age-classes on the right side (for more details see Table 3). B: The same analysis for *GRN* patients, with an overall AUC estimated at 0.97. AUC: area under curve; pNfL: plasma neurofilament light chain; ROC: Receiver-operating characteristic; y: years.