

Appendix A. Biomarkers

Biomarker selection

For biomarker selection, we extensively searched for relevant literature about presymptomatic FTD-*GRN* in Pubmed. We reviewed all empirical studies that included at least a presymptomatic *GRN* mutation carrier group. Next, we determined which biomarkers were frequently reported as abnormal in previous empirical studies and included these biomarkers accordingly, restricted to fluid biomarkers, grey matter brain regions, white matter tracts, and cognition. The selected biomarkers were: serum NfL [1-3], MMSE [4-6], cognitive domains of language, attention and processing speed, executive functioning, and social cognition [5, 7-9]; left and right volumes of the insula, frontal lobe, parietal lobe and the temporal lobe [4, 6, 10-16] [17-19] [20, 21]; white matter tracts: left and right fractional anisotropy of anterior thalamic radiation, superior longitudinal fasciculus, uncinate fasciculus, and forceps minor [10, 17-19, 22, 23]. Although the *GRN* mutation affects plasma progranulin protein levels, these levels were not selected as biomarker, as research has shown that these remain stable in both the presymptomatic and symptomatic stage [6, 24].

MRI processing and ROI calculation

An overview of MRI acquisition parameters is presented in Appendix Table 1. The standard voxel-based morphometry pipeline from FSL [25-27] was used to process T1-weighted images. In brief, the brain was extracted from the images, and we carefully checked the brain extraction for missing brain tissue and areas of non-brain tissue, and adjusted the image accordingly. We corrected RF inhomogeneities by bias field correction with a Markov random field model and subsequently segmented the brain in grey matter, white matter, and cerebrospinal fluid images [28]. A study specific grey matter template was created in standard space using a balanced set of subjects, and all grey matter segmentations were registered to this template with non-linear registration,

and then corrected for any local expansion or contraction by modulation of the Jacobian warp field [26]. Last, an isotropic Gaussian kernel with a sigma of 3mm was applied for smoothing of the grey matter images. Total intracranial volume (TIV) was calculated as the sum of the volumes from grey matter, white matter and cerebrospinal fluid in standard space. The structures from the MNI-atlas were used as grey matter ROIs. We extracted volumetric measurements from the ROIs by registering the structural MNI-atlas [29] to the grey matter images in standard space, and multiplying the grey matter density of the ROI with the total volume of the ROI, resulting in the grey matter volume within the ROI. Left and right regions were considered separately.

Diffusion tensor images were corrected for motion artefacts and eddy currents by alignment to the $b=0$ image, and subsequently, the tensor was fitted at each voxel to create fractional anisotropy (FA) images. The FA images were processed with the tract-based spatial statistics (TBSS) pipeline as implemented in FSL [30]. Using non-linear registration, the images were aligned to the FMRIB58_FA template and then averaged into a mean FA image. The mean FA image was thresholded at 0.2 and thinned into a white matter skeleton. All individual FA images were projected onto this skeleton, resulting in skeletonized FA data for each participant. The probabilistic tracts from the Johns Hopkins University atlas [31] were applied as white matter ROIs to the skeleton mask, and the masked ROIs were used to extract FA values from the individual tracts. Left and right tracts were considered separately.

Table A.1. MRI acquisition protocols

	Rotterdam 1	Rotterdam 2	Brescia	Barcelona
N (s/p/nc)	3/22/24	5/9/6	7/17/0	1/6/1
Scanner	Philips Achieva 3T	Philips Achieva 3T	Siemens Skyra	Siemens Trio Tim
Head Coil	8 channel SENSE	32 channel SENSE	32 channel	64 channel
T1 weighted imaging				
TR	9.8 ms	6.8 ms	2000 ms	2000 ms
TE	4.6 ms	3.1 ms	2.9 ms	2.9 ms
FOV	224x168 mm	256x256 mm	282x282 mm	282x282 mm
Voxel size	0.88x0.88x1.2 mm	1.1mm ³	1.1 mm ³	1.1mm ³
Flip angle	8°	8°	8°	8°
Slices	140	207	208	208
Diffusion tensor imaging				
TR	8250 ms	7000 ms	7300 ms	7300 ms
TE	80 ms	69 ms	90 ms	90 ms
FOV	256x256 mm	240x240 mm	240x240 mm	240x240 mm
Voxel size	2x2x2mm	2.5x2.5x2.5mm	2.5x2.5x2.5mm	2.5x2.5x2.5mm
Slices	70	59	59	59
Directions	60	68	68	68
B-values	0/1000 s/mm ²	0/1000 s/mm ²	0/1000 s/mm ²	0/1000 s/mm ²

Numbers are subjects included after quality check. Abbreviations: s = symptomatic, p = presymptomatic, nc = non-carrier, TR = repetition time, TE = echo time, FOV = field of view.

Cognitive assessment

The following cognitive tests were performed, depending on the protocol from the local site. For language, the Boston Naming Task [32] and semantic fluency (animals) [33] were used. Tests concerning attention and processing speed were the Trail making test part A [34], Stroop part 1 and 2 [35], symbol substitution [36], letter digit substitution task [37], and forward digit span [36]. For executive functioning, we used Trail making test part B [34], Stroop task part 3 [35], phonological fluency [33] and digit span backwards [36]. Tests for social cognition were the Ekman faces test [38], emotion recognition from the mini social cognition and emotional assessment (MINI-SEA) [39], and Happé cartoon task [40]. Raw scores from tests in which a higher score indicates worse performance were reversed (i.e. Trail making test, Stroop). We transformed all raw test scores to z-scores, based on the mean and standard deviation of the non-carriers. Subsequently, cognitive domains were composed as the mean z-score of all available tests within that domain per individual, disregarding missing tests.

Table A.2. Availability and characteristics of cognitive data

	Symptomatic						Presymptomatic	
	Total (n=35)*		bvFTD (n=17)		nfvPPA (n=16)		N=56	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
MMSE	29	-3.07 ± 1.50	15	-3.26 ± 1.69	14	-2.87 ± 1.28	55	0.22 ± 1.01
Language	32	-2.86 ± 1.37	16	-2.69 ± 1.58	14	-3.00 ± 1.24	55	0.28 ± 1.09
<i>Boston naming test</i>	25	-1.97 ± 1.32	13	-1.75 ± 1.44	12	-2.21 ± 1.19	55	0.57 ± 1.37
<i>Semantic fluency</i>	31	-3.28 ± 1.38	15	-3.14 ± 1.57	14	-3.45 ± 1.30	55	0.00 ± 1.31
Attention, concentration and mental processing speed	33	-2.35 ± 1.17	16	-2.43 ± 1.32	15	-2.26 ± 1.12	55	-0.05 ± 0.75
<i>TMT-A</i>	32	-2.65 ± 1.62	16	-2.91 ± 1.63	14	-2.23 ± 1.66	55	-0.01 ± 0.92
<i>Stroop card 1&2</i>	17	-3.05 ± 2.18	10	-2.96 ± 2.20	7	-3.18 ± 2.32	55	0.14 ± 1.02
<i>LDST</i>	4	-2.06 ± 1.59	2	-2.09 ± 1.99	2	-2.03 ± 1.90	17	0.22 ± 0.70
<i>Symbol substitution</i>	17	-2.35 ± 1.41	7	-3.01 ± 1.13	10	-1.89 ± 1.45	22	0.00 ± 1.29
<i>Digit span forward</i>	31	-1.66 ± 1.05	15	-1.41 ± 1.29	14	-1.95 ± 0.74	55	-0.26 ± 0.95
Executive functioning	32	-2.33 ± 0.97	15	-2.23 ± 1.19	15	-2.37 ± 0.79	55	-0.03 ± 0.75
<i>TMT-B</i>	28	-2.63 ± 0.97	13	-2.50 ± 1.11	13	-2.69 ± 0.89	55	0.03 ± 0.79

<i>Stroop card 3</i>	14	-3.84 ± 2.29	8	-3.73 ± 2.50	6	-3.98 ± 2.20	55	-0.36 ± 1.05
<i>Phonological fluency</i>	29	-2.12 ± 0.95	14	-1.92 ± 1.02	15	-2.30 ± 0.87	55	0.30 ± 1.36
<i>Digit span backwards</i>	30	-1.65 ± 1.14	15	-1.61 ± 1.44	13	-1.61 ± 0.75	55	-0.08 ± 1.11
Social cognition	15	-1.87 ± 0.76	7	-2.15 ± 0.92	8	-1.62 ± 0.52	51	-0.10 ± 1.02
<i>Ekman faces</i>	3	-0.70 ± 0.60	2	-0.37 ± 0.18	1	-1.36 ± N/A	26	0.14 ± 0.89
<i>Mini-SEA Emotion Recognition</i>	10	-1.98 ± 0.83	3	-2.65 ± 1.13	7	-1.69 ± 0.52	22	-0.62 ± 0.98
<i>HappeTOM</i>	5	-2.07 ± 0.86	4	-2.32 ± 0.75	1	-1.05 ± N/A	28	0.42 ± 0.77
<i>Happe non TOM</i>	5	-1.65 ± 0.81	4	-1.81 ± 0.84	1	-1.03 ± N/A	28	0.34 ± 1.20

Abbreviations: bvFTD = behavioural variant frontotemporal dementia, nfvPPA = non-fluent variant primary progressive aphasia, MMSE = mini mental state examination, TMT = trail making test, LDST = letter digit substitution task, mini-SEA = mini social cognition and emotional assessment, TOM = theory of mind. Values are mean z-scores ± standard deviation based on non-carriers, uncorrected for confounding factors. * The two remaining participants presented with cortico-basal degeneration.

Biomarker statistics

Before modelling, we checked skewed distributions in the biomarkers with the following graphs and tests: histograms, q-q plots, skewness and kurtosis values (values between 2 and -2 indicate normality), Kolmogorov-Smirnov and Shapiro-Wilk's tests (values above 0.05 indicate normality). When three or more tests indicated skewness, the distributions were adjusted using log-transformations (log10), i.e. neurofilament light chain levels, MMSE, BNT, Trail Making Test, Stroop, facial emotion recognition. In the case of cognitive tests, log-transformation was performed before transforming raw scores to z-scores.

Biomarker characteristics and statistical differences between groups are presented in Table A.3. Symptomatic mutation carriers had higher NfL levels, lower grey matter volumes, impaired white matter microstructure, and worse cognitive functions than both presymptomatic mutation carriers and non-carriers in all selected biomarkers. Post-hoc analysis revealed that these differences in biomarkers were specifically driven by the bvFTD patients. For nfvPPA patients, we found higher NfL levels and worse cognitive performance than both presymptomatic mutation carriers and non-carriers. NfvPPA patients showed smaller grey matter volumes than both presymptomatic mutation carriers and non-carriers, especially in left-sided ROIs, and lower fractional anisotropy levels in the left anterior thalamic radiation, left uncinate fasciculus, and the forceps minor. The volume of the right frontal lobe was smaller in nfvPPA patients compared with presymptomatic mutation carriers. Furthermore, bvFTD patients had smaller volumes of the right frontal and temporal lobe than nfvPPA patients, and lower fractional anisotropy values in the forceps minor, left superior longitudinal fasciculus and right uncinate fasciculus. There were no differences in any of the selected biomarkers between presymptomatic mutation carriers and non-carriers.

Table A.3. Biomarker characteristics after correction for confounding factors

		Symptomatic			Presymptomatic
		Total	bvFTD	nfvPPA	
<i>Neurofilament light chain</i>		1.90 ± 0.25*	1.89 ± 0.23*	1.91 ± 0.28†	1.10 ± 0.22
<i>GM volume</i>	<i>Left frontal lobe</i>	-2.75 ± 1.8*	-3.42 ± 2.06*	-2.46 ± 1.40†	0.30 ± 0.65
	<i>Right frontal lobe</i>	-1.72 ± 1.79*	-2.76 ± 1.43*‡	-0.93 ± 1.79§	0.30 ± 0.65
	<i>Left insula</i>	-2.32 ± 1.56*	-2.45 ± 1.79*	-2.35 ± 1.51†	-0.32 ± 0.95
	<i>Right insula</i>	-1.02 ± 1.13*	-1.47 ± 1.26*	-0.74 ± 0.98	-0.08 ± 0.84
	<i>Left parietal lobe</i>	-1.87 ± 1.11*	-2.18 ± 1.39*	-1.74 ± 0.84†	-0.03 ± 1.02
	<i>Right parietal lobe</i>	-1.19 ± 2.00*	-1.42 ± 2.08*	-0.89 ± 2.15	-0.06 ± 0.96
	<i>Left temporal lobe</i>	-2.97 ± 2.42*	-3.21 ± 2.59*	-2.98 ± 2.51†	-0.19 ± 0.96
	<i>Right temporal lobe</i>	-1.14 ± 2.66*	-2.22 ± 3.40*‡	-0.12 ± 1.69	-0.08 ± 0.94
<i>FA</i>	<i>Left anterior thalamic radiation</i>	-2.28 ± 1.34*	-2.73 ± 1.60*	-1.77 ± 0.98†	-0.33 ± 0.95
	<i>Right anterior thalamic radiation</i>	-1.24 ± 1.23*	-1.78 ± 1.51*	-0.66 ± 0.66	-0.27 ± 0.77
	<i>Forceps Minor</i>	-3.00 ± 1.52*	-4.01 ± 1.52*‡	-2.08 ± 0.96†	0.46 ± 0.93
	<i>Left superior longitudinal fasciculus</i>	-1.50 ± 1.39*	-2.42 ± 1.28*‡	-0.61 ± 0.96	0.02 ± 0.88
	<i>Right superior longitudinal fasciculus</i>	-1.14 ± 1.12*	-1.47 ± 1.14*	-0.74 ± 1.06	-0.11 ± 0.60
	<i>Left uncinate fasciculus</i>	-2.63 ± 1.15*	-3.00 ± 1.43*	-2.29 ± 0.88†	-0.35 ± 0.86
	<i>Right uncinate fasciculus</i>	-1.92 ± 2.16*	-3.19 ± 2.07*‡	-0.77 ± 1.74	-0.51 ± 1.12
<i>MMSE</i>	-2.71 ± 1.19*	-2.71 ± 1.28*	-2.71 ± 1.14†	0.06 ± 0.91	
<i>Attention and processing speed</i>	-2.06 ± 1.09*	-2.11 ± 1.15*	-2.05 ± 1.12†	-0.22 ± 0.65	

<i>Executive functioning</i>	-2.12 ± 0.88*	-2.00 ± 0.99*	-2.24 ± 0.82 [†]	-0.14 ± 0.72
<i>Language</i>	-2.54 ± 1.23*	-2.35 ± 1.33*	-2.84 ± 1.17 [†]	0.13 ± 0.97
<i>Social cognition</i>	-1.89 ± 0.64*	-2.13 ± 0.74*	-1.52 ± 0.42 [†]	-0.19 ± 0.96

Abbreviations: bvFTD = behavioural variant frontotemporal dementia, nfvPPA = non-fluent variant primary progressive aphasia, GM volume = grey matter volume, FA = fractional anisotropy, MMSE = Mini Mental State Examination. Values are mean z-score (based on non-carriers) ± standard deviation, after correction for confounding factors of age, gender, MRI protocol, and years of education.

* Both the entire group of symptomatic mutation carriers and only bvFTD patients significantly differed from presymptomatic mutation carriers as well as non-carriers ($p < 0.05$, Bonferroni corrected)

[†] Significant difference between nfvPPA patients and presymptomatic mutation carriers as well as non-carriers ($p < 0.05$, Bonferroni corrected)

[‡] Significant difference between bvFTD patients and nfvPPA patients ($p < 0.05$, Bonferroni corrected)

[¶] Significant difference between nfvPPA patients and presymptomatic mutation carriers ($p < 0.05$, Bonferroni corrected)

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