

1 **Serum glial fibrillary acidic protein is a marker of disease severity in**
2 **frontotemporal lobar degeneration**

3
4 Alberto Benussi,¹ Thomas K. Karikari,² Nicholas Ashton,^{2,3,4,5} Stefano Gazzina,⁶ Enrico Premi,⁷
5 Luisa Benussi,⁸ Roberta Ghidoni,⁸ Juan Lantero Rodriguez,² Joel Simrén,⁹ Giuliano Binetti,¹⁰
6 Silvia Fostinelli⁸, Marcello Giunta,¹ Roberto Gasparotti,¹¹ Henrik Zetterberg,^{2,9,12,13} Kaj
7 Blennow,^{2,9} Barbara Borroni^{1*}

8
9 ¹*Neurology Unit, Department of Clinical and Experimental Sciences, University of Brescia,*
10 *Brescia, Italy*

11 ²*Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, the*
12 *Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden*

13 ³*Wallenberg Centre for Molecular and Translational Medicine, University of Gothenburg,*
14 *Mölndal, Sweden*

15 ⁴*King's College London, Institute of Psychiatry, Psychology & Neuroscience, Maurice Wohl*
16 *Clinical Neuroscience Institute, London, UK*

17 ⁵*NIHR Biomedical Research Centre for Mental Health & Biomedical Research Unit for*
18 *Dementia at South London & Maudsley NHS Foundation, London, UK*

19 ⁶*Neurophysiology Unit, ASST Spedali Civili, Brescia, Italy*

20 ⁷*Stroke Unit, ASST Spedali Civili, Brescia, Italy*

21 ⁸*Molecular Markers Laboratory, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli,*
22 *Brescia, Italy*

23 ⁹*Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden*

24 ¹⁰*MAC Memory Clinic and Molecular Markers Laboratory, IRCCS Istituto Centro San Giovanni*
25 *di Dio Fatebenefratelli, Brescia, Italy*

26 ¹¹*Neuroradiology Unit, University of Brescia, Brescia, Italy*

27 ¹²*UK Dementia Research Institute at UCL, London, United Kingdom*

28 ¹³*Department of Neurodegenerative Disease, UCL Institute of Neurology, London, United*
29 *Kingdom*

30
31 ***Corresponding author:**

32 **Barbara Borroni, MD**

33 **Clinica Neurologica, Università degli Studi di Brescia**

34 **P.le Spedali Civili 1, 25123, Brescia, Italy**

35 **Phone: 0039 0303995632**

36 **Email: bborroni@inwind.it**

37

38 **Title character count:** 113

39 **Number of references:** 40

40 **Number of tables:** 1

41 **Number of figures:** 3

42 **Abstract word count:** 231

43 **Main text word count:** 3735

44 **Search terms:** frontotemporal dementia; serum; glial fibrillary acidic protein; GFAP; biomarker;
45 survival; magnetic resonance imaging; transcranial magnetic stimulation.

46 **Abstract**

47 **Objective:** To assess the diagnostic and prognostic value of serum glial fibrillary acidic protein
48 (GFAP) in a large cohort of patients with frontotemporal lobar degeneration (FTLD).

49 **Methods:** In this retrospective study, performed on 406 participants, we measured serum GFAP
50 concentration with an ultrasensitive Single molecule array (Simoa) method in FTLD, Alzheimer's
51 disease (AD) and healthy ageing. We assessed the role of GFAP as marker of disease severity by
52 analysing the correlation with clinical variables, neurophysiological data and cross-sectional brain
53 imaging. Moreover, we evaluated the role of serum GFAP as a prognostic marker of disease
54 survival.

55 **Results:** We observed significantly higher levels of serum GFAP in patients with FTLD
56 syndromes, except progressive supranuclear palsy (PSP), compared with healthy controls, but not
57 compared with AD patients. In FTLD, serum GFAP levels correlated with measures of cognitive
58 dysfunction and disease severity, and were associated with indirect measures of GABAergic deficit.
59 Serum GFAP concentration was not a significant predictor of survival.

60 **Conclusions:** Serum GFAP is a marker of disease severity in FTLD.

61 **Introduction**

62 Frontotemporal dementia (FTD) is a genetically and pathologically heterogeneous disorder
63 characterized by personality changes, language deficits, and impairment of executive functions
64 associated with the degeneration of frontal and temporal lobes. Different phenotypes have been
65 defined on the basis of presenting clinical symptoms, i.e., the behavioural variant of FTD (bvFTD),
66 the agrammatic variant of primary progressive aphasia (avPPA), and the semantic variant of PPA
67 (svPPA) (Gorno-Tempini et al., 2011; Rascovsky et al., 2011). A significant percentage of patients
68 have associated extrapyramidal symptoms, as in progressive supranuclear palsy (PSP) (Höglinger et
69 al., 2017) and corticobasal syndrome (CBS) (Armstrong et al., 2013).

70 These clinical phenotypes share common underlying molecular and pathological substrates, and in
71 most cases, inclusions of microtubule-associated protein tau or TAR DNA-binding protein 43
72 (TDP-43) represent the pathological hallmarks of the disease (Cairns et al., 2007; Mackenzie et al.,
73 2006).

74 The heterogeneity of clinical presentations, along with unpredictable neuropathology, has
75 consistently precluded a straightforward staging of the disease. Considering the increasing
76 development of disease-modifying therapies in the spectrum of frontotemporal lobar degeneration
77 (FTLD), the demand for objective, easily accessible and low-cost biomarkers to evaluate disease
78 severity and progression has significantly increased in the last years.

79 A multitude of markers of disease severity have been recognized in the last decade, ranging from
80 neuroimaging with magnetic resonance imaging (MRI) or positron emission tomography (PET), to
81 cerebrospinal fluid (CSF) biomarkers (Borroni et al., 2018). However, the use of imaging markers
82 is prevented by the lack of common patterns across FTLD subtypes, and the helpfulness of CSF is
83 limited by the sampling method that sometimes is regarded invasive.

84 Along with recently proposed neurophysiological markers, measuring FTLD-related
85 neurotransmitter deficits non-invasively by transcranial magnetic stimulation (TMS) (Benussi et al.,
86 2020c), a giant step forward towards potentially useful biomarkers has been made with the new

87 ultrasensitive Single molecule array (Simoa) approach and the discovery of potentially useful
88 blood-based biomarkers. It has been clearly proven that concentrations of blood NfL, a marker of
89 axonal damage, are increased in FTLD and may be related to parameters of disease severity and
90 prognosis (Foiani et al., 2018; Meeter et al., 2016; Rohrer et al., 2016).

91 Moreover, recent studies have reported increased levels of glial fibrillary acidic protein (GFAP),
92 which is a marker of astrogliosis secondary to neuronal damage, in several neurodegenerative
93 disorders, including dementia with Lewy bodies, Alzheimer's disease (AD) and both sporadic and
94 genetic FTD (Abu-Rumeileh et al., 2019; Heller et al., 2020; Ishiki et al., 2016; Oeckl et al., 2019b,
95 2019a; Sudre et al., 2019). However, it has yet to be established if GFAP blood-based assays are
96 reliable in all FTLD subgroups, including CBS and PSP, and if these correlate with disease severity
97 and survival.

98 This retrospective study aimed at confirming and extending previous literature data,
99 comprehensively assessing the clinical value of serum GFAP in a large cohort of FTLD patients.

100 **Materials and Methods**

101 ***Subjects***

102 This retrospective study included 406 participants from two independent cohorts, 298 from the
103 Centre for Neurodegenerative Disorders, University of Brescia, Italy and 108 from the IRCCS
104 Istituto San Giovanni di Dio Fatebenefratelli, Brescia, Italy.

105 The cohort consisted of 282 patients meeting probable clinical criteria for a syndrome in the FTLN
106 spectrum, namely 130 bvFTD, 48 avPPA, 24 svPPA, 50 CBS and 30 PSP (Armstrong et al., 2013;
107 Gorno-Tempini et al., 2011; Höglinger et al., 2017; Rascovsky et al., 2011). Moreover, 63 patients
108 fulfilling clinical criteria for AD (McKhann et al., 2011) and 61 healthy controls (HC), recruited
109 among spouses or caregivers, were included as well.

110 Each FTLN patient underwent a neurological evaluation, routine laboratory examination and a
111 neuropsychological and behavioural assessment. In all cases, the diagnosis was supported by brain
112 structural imaging, while CSF concentrations of T-tau, P-tau₁₈₁ and A β ₁₋₄₂ were measured in a
113 subset of cases (45.0%), to rule out AD, as previously reported (Borroni et al., 2014). Furthermore,
114 in familial cases (based on the presence of at least one dementia case among first-degree relatives)
115 and early onset sporadic cases, genetic screening for *GRN*, *C9orf72* and *MAPT* P301L mutations
116 was performed; given the low frequency of *MAPT* mutations in Italy (Fostinelli et al., 2018) we
117 considered only the P301L mutation and we sequenced the entire *MAPT* gene only in selected
118 cases.

119 Each participant underwent blood collection for measurements of serum GFAP, and a subset of
120 FTLN patients underwent standardized brain Magnetic Resonance Imaging (MRI) at baseline on
121 the same scanner (n=45) to evaluate the correlation between serum biomarkers and imaging data.
122 Moreover, a subgroup of patients underwent TMS protocols (n=110) to assess the correlation
123 between serum biomarkers and neurophysiological data. For the purpose of the present study, we
124 considered TMS measures that partially and indirectly reflect the activity of several
125 neurotransmitters, including GABA_A by short interval intracortical inhibition (SICI), glutamate by

126 intracortical facilitation (ICF), GABA_B by long interval intracortical inhibition (LICI), and
127 acetylcholine by short latency afferent inhibition (SAI) (Rossini et al., 2015; Ziemann et al., 2015).
128 Full written informed consent was obtained from all subjects according to the Declaration of
129 Helsinki. The Brescia Ethics Committee approved the study protocol.

130

131 ***Clinical evaluation***

132 At baseline patients underwent a standardized neuropsychological battery which included the mini-
133 mental state examination (MMSE), the short story recall test, the Rey complex figure (copy and
134 recall), phonemic and semantic fluencies, the token test, the clock-drawing test, and trail-making
135 test (part A and part B). Disease severity was assessed with the FTLD modified clinical dementia
136 rating (FTLD-modified CDR) sum of boxes scale, while the level of functional independence was
137 assessed with the basic activities of daily living (BADL) and the instrumental activities of daily
138 living (IADL) questionnaires. Furthermore, neuropsychiatric and behavioural disturbances were
139 evaluated with the frontal behaviour inventory (FBI).
140 HCs underwent a brief standardized neuropsychological assessment (MMSE $\geq 27/30$); psychiatric or
141 other neurological illnesses were considered exclusion criteria.

142

143 ***Serum GFAP***

144 Serum was collected by venipuncture, processed and stored in aliquots at -80°C according to
145 standardised procedures. Serum GFAP was measured using a commercial....
146 The lower limits of detection for serum GFAP were 0.xxx pg/mL. Measurements were carried using
147 an HD-X analyser (Quanterix, Billerica, MA) at the same study site on consecutive days, using the
148 same batch of reagents, and the operators were blinded to all clinical information. Quality control
149 samples had a mean intra-assay and inter-assay coefficient of variation of less than 8% and 20%
150 respectively.

151

152 ***MRI acquisition, processing and analysis***

153 Brain images were collected using 3 Tesla scanner (Siemens Skyra, Erlangen, Germany) equipped
154 with a circularly polarized transmit-receive coil to obtain 3D magnetization-prepared rapid gradient
155 echo (MPRAGE) T1-weighted scans. Sequences were acquired with the following parameters:
156 repetition time 2000 ms, echo time 2.92 ms, inversion time 850 ms, slice thickness 1.1 mm, voxel
157 size 1.1×1.1×1.1, field of view 282 mm, flip angle 8°.

158 T1 scans were visually inspected and excluded from subsequent analyses if excessive motion
159 blurring or artefacts were present. Then, images were processed and analysed with the Statistical
160 Parametric Mapping software package (SPM12 v. 7771,
161 <http://www.fil.ion.ucl.ac.uk/spm/software/spm12/>), running on MATLAB 9.2 (The MathWorks,
162 Inc, Natick, MA USA). Images were spatially normalized to a reference stereotactic template
163 (Montreal Neurological Institute, MNI), and smoothed by a Gaussian kernel of 10×10×10 mm full
164 width at half maximum (FWHM). Grey matter was assessed by Voxel Based Morphometry (VBM)
165 analysis (Premi et al., 2016)

166 Moreover, we considered white matter hyperintensities burden, computed on T1-weighted and T2
167 FLAIR images using the Wisconsin White Matter Hyperintensities Segmentation Toolbox version
168 1.3 (Ithapu et al., 2014). A per-subject summary measure of total white matter hyperintensities
169 volume burden was automatically calculated on the probability map outputs, adjusting for
170 intracranial volume to account for the differences in brain sizes (Paternicò et al., 2016).

171 The association between grey matter or white matter hyperintensities and serum GFAP values was
172 considered. Age, gender and clinical phenotype were considered as confounding factors in both
173 analyses. The statistical threshold was set at 0.05 and corrected for multiple comparisons using false
174 discovery rate (FDR) at whole-brain level.

175

176 ***Transcranial Magnetic Stimulation***

177 A TMS figure-of-eight coil (each loop diameter 70 mm – D70² coil) connected to a monophasic
178 Magstim Bistim² system (Magstim Company, Oxford, UK) was employed for all TMS paradigms,
179 as previously reported (Benussi et al., 2019b). Electromyographic (EMG) recordings were
180 performed from the first dorsal interosseous muscle using 9 mm diameter, Ag-AgCl surface-cup
181 electrodes. The active electrode was placed over the muscle belly and the reference electrode over
182 the metacarpophalangeal joint of the index finger. Responses were amplified and filtered at 20 Hz
183 and 2 kHz with a sampling rate of 5 kHz.

184 Resting motor threshold (RMT) was determined on the left motor cortex as the minimum intensity
185 of the stimulator required to elicit motor evoked potentials (MEPs) with a 50 μ V amplitude in 50%
186 of 10 consecutive trials, recorded during full muscle relaxation.

187 SICI-ICF, LICI and SAI were studied using a paired-pulse technique, employing a conditioning-test
188 design. For all paradigms, the test stimulus (TS) was adjusted to evoke a MEP of approximately 1
189 mV amplitude.

190 For SICI and ICF, the conditioning stimulus (CS) was adjusted at 70% of the RMT, employing
191 multiple interstimulus intervals (ISIs), including 1, 2, 3 ms for SICI and 7, 10, 15 ms for ICF
192 (Kujirai et al., 1993; Ziemann et al., 1996). LICI was investigated by implementing two supra-
193 threshold stimuli, with the CS adjusted at 130% of the RMT, employing ISIs of 50, 100 and 150 ms
194 (Valls-Solé et al., 1992). SAI was evaluated employing a CS of single pulses (200 μ s) of electrical
195 stimulation delivered to right median nerve at the wrist, using a bipolar electrode with the cathode
196 positioned proximally, at an intensity sufficient to evoke a visible twitch of the thenar muscles
197 (Tokimura et al., 2000). Different ISIs were implemented (0, +4), which were fixed relative to the
198 N20 component latency of the somatosensory evoked potential of the median nerve.

199 For each ISI and for each protocol, ten different paired CS-TS stimuli and fourteen control TS
200 stimuli were delivered in all participants in a pseudo-randomized sequence, with an inter trial
201 interval of 5 secs (\pm 10%).

202 The conditioned MEP amplitude, evoked after delivering a paired CS-TS stimulus, was expressed
203 as percentage of the average control MEP amplitude. Average values for SICI (1, 2, 3 ms ISI), ICF
204 (7, 10, 15 ms ISI), LICI (50, 100, 150 ms ISI) and SAI (0, +4 ms ISI) were used for analysis.
205 Stimulation protocols were conducted in a randomized order. Audio-visual feedback was provided
206 to ensure muscle relaxation during the entire experiment and trials were discarded if EMG activity
207 exceeded 100 μ V in the 250 ms prior to TMS stimulus delivery. Less than 5% of trials were
208 discarded for each protocol. All of the participants were capable of following instructions and
209 reaching complete muscle relaxation; if, however the data was corrupted by patient movement, the
210 protocol was restarted and the initial recording was rejected.

211

212 ***Statistical analysis***

213 Linear regression and stepwise multiple regression analysis (including all variables with a $p < 0.100$
214 at univariate analysis) were used to characterize the relationship between serum GFAP and
215 demographic characteristics (age, age at onset, sex and mutation status).

216 Differences in clinical variables and biomarker concentrations were assessed with one-way analysis
217 of covariance (ANCOVA), corrected for age, sex and/or mutation status, with Bonferroni multiple
218 comparisons correction. Pearson's correlations were used to assess associations between serum
219 GFAP, age and education corrected clinical variables and TMS measures.

220 Survival was calculated as time from symptom onset to time of death from any cause (outcome=0)
221 or censoring date (outcome=1). Survival analysis was carried out by the Kaplan-Meier method with
222 log rank *post hoc* testing and by means of univariate stepwise Cox proportional-hazard regression
223 analysis; hazard ratios (HR) are provided with their respective 95% confidence intervals (CIs).

224 A two-sided p -value < 0.05 was considered significant and corrected for multiple comparisons using
225 false discovery rate (FDR) when appropriate. Statistical analyses were performed using SPSS (v.24;
226 SPSS, IBM).

227

228 ***Data availability***

229 All study data, including raw and analysed data, and materials will be available from the
230 corresponding author, B.B., upon reasonable request.

231 **Results**

232 ***Participant characteristics***

233 Baseline demographics, clinical variables and GFAP levels are reported in **Table 1**.

234 In the FTLD group, serum GFAP concentration correlated with age ($\beta=0.22$, $p<0.001$), age at onset
235 ($\beta=0.20$, $p=0.001$), and female sex ($\beta=0.20$, $p=0.001$), but did not correlate with the presence of a
236 pathogenic mutation ($\beta=-0.05$, $p=0.447$) at the linear regression analysis. In the stepwise multiple
237 regression model, GFAP concentration correlated with both age ($\beta=0.20$, $p=0.001$) and female sex
238 ($\beta=0.18$, $p=0.003$). Serum GFAP concentration was significantly higher in females (mean \pm SE,
239 $n=129$, 380.7 ± 22.1 pg/mL) compared with males (mean \pm SE, $n=153$, 288.2 ± 17.0 pg/mL, $p=0.001$),
240 also after correcting for age ($p=0.003$), phenotype ($p=0.001$), or both ($p=0.002$). We observed
241 comparable levels of serum GFAP in both sporadic FTLD (mean \pm SE, $n=250$, 334.2 ± 15.4) and in
242 patients with *GRN* mutations (mean \pm SE, $n=30$, 307.4 ± 26.4), while lower levels were observed in
243 *MAPT* mutation carriers (mean \pm SE, $n=2$, 202.1 ± 18.4).

244

245 ***Serum GFAP concentrations in FTLD subgroups***

246 Serum GFAP concentrations were significantly increased in most FTLD subgroups (age- and sex-
247 corrected ANCOVA, $F(8,397)=13.57$, $p<0.001$, $\eta^2=0.22$). In Bonferroni-corrected *post hoc* tests,
248 we observed significant increases in serum GFAP concentration in bvFTD, avPPA, svPPA, and
249 CBS compared with HC. Patients with avPPA had significantly higher serum GFAP concentration
250 compared with CBS and PSP. We did not observe significant differences in GFAP concentration
251 between any FTLD subgroup and AD (see **Table 1** and **Figure 1**).

252

253 ***Serum GFAP associations with disease severity in FTLD***

254 *Cognitive and behavioural assessment.* Serum GFAP concentration showed significant associations
255 with baseline BADL ($r=0.21$, $p=0.001$), IADL ($r=0.28$, $p<0.001$) and FTLD-modified CDR sum of

256 boxes ($r=0.27, p<0.001$); the higher the serum GFAP level, the greater impairment in functional
257 activities and disease severity (see **Figure 2**). Significant correlations were observed between serum
258 GFAP concentration and MMSE score ($r=-0.38, p<0.001$), phonemic ($r=-0.16, p=0.033$) and
259 semantic fluency ($r=-0.28, p<0.001$), clock drawing ($r=-0.32, p<0.001$), trail-making part A ($r=-$
260 $0.29, p<0.001$) and B ($r=-0.33, p<0.001$), and token test ($r=-0.29, p<0.001$), with higher levels of
261 serum GFAP correlating with poorer scores (see **Figure 2**).

262 No significant correlations were observed for the Rey figure copy ($r=-0.13, p=0.114$) and recall ($r=-$
263 $0.06, p=0.437$), short story ($r=-0.13, p=0.127$), and digit symbol ($r=-0.11, p=0.158$).

264 Neuropsychiatric and behavioural disturbances, evaluated with the FBI, did not correlate with
265 serum GFAP concentration ($r=0.11, p=0.087$).

266 All tests were age- and education- corrected; FDR-adjusted p -values for multiple comparisons are
267 reported for each test.

268

269 *Brain imaging.* Serum GFAP concentration correlated neither with grey matter atrophy nor with
270 white matter hyperintensities burden at the pre-established threshold ($p<0.05$, whole-brain FDR-
271 corrected).

272

273 *TMS measures.* TMS measures were performed to evaluate average SICI, ICF, LICI and SAI. In the
274 FTLD group ($n=87$), serum GFAP concentration was significantly associated with LICI ($r=0.31,$
275 $p=0.016$) (see **Figure 3**), but not with SICI, ICF or SAI.

276 Interestingly, in the AD group ($n=12$), we observed a significant association between serum GFAP
277 and average SAI ($r=0.678, p=0.015$).

278 Reported p -values are FDR-adjusted for multiple comparisons.

279

280 ***Serum GFAP associations with prognosis in FTLD***

281 Serum GFAP concentration did not predict survival in FTLD patients. At the univariate Cox
282 regression analysis there was no significant association between survival and serum GFAP
283 concentration (HR 1.00 95%CI 0.99-1.00, $p=0.866$). Patients with high serum GFAP levels (upper
284 than median values) did not have shorter survival than those with low serum GFAP levels (lower
285 than median values) according to Kaplan-Meier survival curves ($p=0.621$).

286 **Discussion**

287 In this work, we confirmed and extended previous literature showing increased serum GFAP levels
288 in most FTLD subgroups, including both the behavioural and language variants of FTD, CBS, but
289 not in PSP. Similarly, previous reports have shown that GFAP levels are increased in CSF and
290 plasma of sporadic FTD patients (Abu-Rumeileh et al., 2019; Ishiki et al., 2016; Marelli et al.,
291 2020; Oeckl et al., 2019b) and in plasma of symptomatic granulin-associated FTD (Heller et al.,
292 2020; Sudre et al., 2019). On the contrary, several reports have shown that PSP patients have only
293 slightly, non-significantly increased CSF levels of GFAP compared to healthy controls
294 (Constantinescu et al., 2010, 2009; Holmberg et al., 1998; Süssmuth et al., 2010).

295 Interestingly, we observed significantly higher levels of serum GFAP in females than in males.
296 Animal models have shown that GFAP expression is highly dependent on sex hormones, and
297 differences have been detected between males and females in the hippocampus, striatum and
298 cerebellum (Arias et al., 2009). However, to the best of our knowledge, no other human study has
299 identified sex-associated differences in GFAP expression so far.

300 We observed comparable concentrations of GFAP in sporadic FTLD and *GRN* mutation carriers,
301 while patients with *MAPT* mutations, although assessed in only few patients, showed lower levels,
302 confirming previous studies reporting raised GFAP concentration as a specific feature of *GRN*-
303 related FTD among the different pathogenic mutations.

304 GFAP concentration in FTLD was associated with disease severity and disability, and correlated
305 with deficits in several cognitive domains, in particular of executive functions and language.

306 We also observed an association between serum GFAP concentration and indirect measures of
307 GABAergic neurotransmission, which have been demonstrated to be impaired in FTLD (Benussi et
308 al., 2019a, 2018; Padovani et al., 2018), and reflect disease severity and progression (Benussi et al.,
309 2020b, 2020a). We observed that the higher the serum GFAP concentration, the greater was the
310 impairment in LICI, which is considered to reflect short-lasting postsynaptic inhibition mediated
311 through the GABA_B receptors at the level of local interneurons (Rossini et al., 2015; Ziemann et al.,

312 2015). This is in line with the existence of dynamic GABAergic-astrocyte communication, GFAP
313 being a major component of the astrocytic cytoskeleton (Mederos and Perea, 2019; Robel and
314 Sontheimer, 2016).

315 Conversely, as compared to FTD due to *GRN* mutations (Sudre et al., 2019), we failed to find an
316 association between serum GFAP and either grey matter atrophy or white matter hyperintensities
317 burden. This may be due to the more heterogeneous FTLD group herein considered in term of both
318 clinical phenotypes and underlying proteinopathies.

319 However, altogether these findings strongly support the notion that serum GFAP concentration is a
320 marker of disease intensity and severity, in a disorder where there is urgent need to find not only
321 diagnostic but also prognostic markers. Indeed, biological markers of disease severity are critical
322 for advising patients and caregivers, for evaluating potential disease modifying treatments in
323 homogeneous groups, independently of clinical phenotype, and to better understand the disease
324 pathophysiology.

325 Compared with AD, FTLD is clinically heterogeneous, with patients presenting a combination of
326 behavioural disturbances, impairment of executive functions or language deficits. Available
327 standardised neuropsychological and clinical assessments may not be ideal in detecting the effects
328 of future treatments, particularly in the early disease stages and across different FTLD subtypes. A
329 non-invasive and easy to perform peripheral biomarker may represent a practical and valuable
330 choice to assess disease severity and to categorize patients into disease subgroups.

331 Increased GFAP levels have been observed also after stroke (Dvorak et al., 2009) and brain injury
332 (Papa et al., 2014), but also in neurodegenerative processes with astrogliosis. In this context,
333 increased GFAP levels have been reported in AD (Abu-Rumeileh et al., 2019; Ishiki et al., 2016;
334 Oeckl et al., 2019a; Olsson et al., 2016), ALS (Oeckl et al., 2019b), but also in healthy ageing
335 (Vågberg et al., 2015). Indeed, GFAP concentrations have been shown to increase with age,
336 similarly to what has been observed in our study, further highlighting the importance of taking age
337 into account when interpreting plasma GFAP results.

338 Major strengths of our study are the large series of FTLD patients and the comprehensive approach
339 in correlating clinical, imaging and neurophysiological data with GFAP levels, carried out at the
340 same study site to minimize variability. A weakness of the study is the lack of autopsy
341 confirmation, which prevented correlations between serum GFAP and FTLD-related
342 proteinopathies. Secondly, longitudinal serum GFAP measurements were not available, and we
343 were not able to draw conclusions on possible changes throughout disease progression.
344 In conclusion, serum GFAP concentration is associated with disease intensity and severity in FTLD,
345 and may represent an accessible and repeatable biomarker to monitor disease progression and
346 response to disease-modifying therapies in upcoming clinical trials.

347 **Acknowledgements**

348 We thank A. Alberici for patients' recruitment, and E. Bonomi and C. Brattini for excellent
349 practical work. This study was funded by the Italian Ministry of Health (Ricerca Corrente). HZ is a
350 Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the
351 European Research Council (#681712), Swedish State Support for Clinical Research (#ALFGBG-
352 720931), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), and the
353 UK Dementia Research Institute at UCL. KB is supported by the Swedish Research Council
354 (#2017-00915), the Alzheimer Drug Discovery Foundation (ADDF), USA (#RDAPB-201809-
355 2016615), the Swedish Alzheimer Foundation (#AF-742881), Hjärnfonden, Sweden (#FO2017-
356 0243), the Swedish state under the agreement between the Swedish government and the County
357 Councils, the ALF-agreement (#ALFGBG-715986), and European Union Joint Program for
358 Neurodegenerative Disorders (JPND2019-466-236).

359

360 **Disclosures**

361 HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed and
362 CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-
363 founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-based platform company
364 at the University of Gothenburg, all unrelated to the work presented in this paper. KB has served as
365 a consultant or at advisory boards for Abcam, Axon, Biogen, Lilly, MagQu, Novartis and Roche
366 Diagnostics, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB, a GU Ventures-
367 based platform company at the University of Gothenburg, all unrelated to the work presented in this
368 paper.

369 **References**

- 370 Abu-Rumeileh, S., Steinacker, P., Polischi, B., Mammana, A., Bartoletti-Stella, A., Oeckl, P.,
371 Baiardi, S., Zenesini, C., Huss, A., Cortelli, P., Capellari, S., Otto, M., Parchi, P., 2019. CSF
372 biomarkers of neuroinflammation in distinct forms and subtypes of neurodegenerative
373 dementia. *Alzheimer's Res. Ther.* 12, 1–15. <https://doi.org/10.1186/s13195-019-0562-4>
- 374 Arias, C., Zepeda, A., Hernández-Ortega, K., Leal-Galicia, P., Lojero, C., Camacho-Arroyo, I.,
375 2009. Sex and estrous cycle-dependent differences in glial fibrillary acidic protein
376 immunoreactivity in the adult rat hippocampus. *Horm. Behav.* 55, 257–263.
377 <https://doi.org/10.1016/j.yhbeh.2008.10.016>
- 378 Armstrong, M.J., Litvan, I., Lang, A.E., Bak, T.H., Bhatia, K.P., Borroni, B., Boxer, A.L., Dickson,
379 D.W., Grossman, M., Hallett, M., Josephs, K.A., Kertesz, A., Lee, S.E., Miller, B.L., Reich,
380 S.G., Riley, D.E., Tolosa, E., Tröster, A.I., Vidailhet, M., Weiner, W.J., 2013. Criteria for the
381 diagnosis of corticobasal degeneration. *Neurology* 80, 496–503.
- 382 Benussi, A., Alberici, A., Buratti, E., Ghidoni, R., Gardoni, F., Di Luca, M., Padovani, A., Borroni,
383 B., 2019a. Toward a Glutamate Hypothesis of Frontotemporal Dementia. *Front. Neurosci.* 13,
384 304. <https://doi.org/10.3389/fnins.2019.00304>
- 385 Benussi, A., Dell'Era, V., Cantoni, V., Cotelli, M.S., Cosseddu, M., Spallazzi, M., Alberici, A.,
386 Padovani, A., Borroni, B., 2020a. Neurophysiological Correlates of Positive and Negative
387 Symptoms in Frontotemporal Dementia. *J. Alzheimers. Dis.* 73, 1133–1142.
388 <https://doi.org/10.3233/JAD-190986>
- 389 Benussi, A., Dell'Era, V., Cantoni, V., Cotelli, M.S., Cosseddu, M., Spallazzi, M., Micheli, A.,
390 Turrone, R., Alberici, A., Borroni, B., 2020b. TMS for staging and predicting functional
391 decline in frontotemporal dementia. *Brain Stimul.* 13, 386–392.
392 <https://doi.org/10.1016/j.brs.2019.11.009>
- 393 Benussi, A., Dell'Era, V., Cantoni, V., Ferrari, C., Caratozzolo, S., Rozzini, L., Alberici, A.,
394 Padovani, A., Borroni, B., 2018. Discrimination of atypical parkinsonisms with transcranial
395 magnetic stimulation. *Brain Stimul.* 11, 366–373. <https://doi.org/10.1016/j.brs.2017.11.013>
- 396 Benussi, A., Gazzina, S., Premi, E., Cosseddu, M., Archetti, S., Dell'Era, V., Cantoni, V., Cotelli,
397 M.S., Alberici, A., Micheli, A., Benussi, L., Ghidoni, R., Padovani, A., Borroni, B., 2019b.
398 Clinical and biomarker changes in presymptomatic genetic frontotemporal dementia.
399 *Neurobiol. Aging* 76, 133–140. <https://doi.org/10.1016/j.neurobiolaging.2018.12.018>
- 400 Benussi, A., Grassi, M., Palluzzi, F., Koch, G., Di Lazzaro, V., Nardone, R., Cantoni, V., Dell'Era,
401 V., Premi, E., Martorana, A., di Lorenzo, F., Bonni, S., Ranieri, F., Capone, F., Musumeci, G.,
402 Cotelli, M.S., Padovani, A., Borroni, B., 2020c. Classification Accuracy of Transcranial
403 Magnetic Stimulation for the Diagnosis of Neurodegenerative Dementias. *Ann. Neurol.* 87,
404 394–404. <https://doi.org/10.1002/ana.25677>
- 405 Borroni, B., Benussi, A., Cosseddu, M., Archetti, S., Padovani, A., 2014. Cerebrospinal fluid tau
406 levels predict prognosis in non-inherited frontotemporal dementia. *Neurodegener. Dis.* 13,
407 224–9. <https://doi.org/10.1159/000353280>
- 408 Borroni, B., Benussi, A., Premi, E., Alberici, A., Marcello, E., Gardoni, F., Di Luca, M., Padovani,
409 A., 2018. Biological, Neuroimaging, and Neurophysiological Markers in Frontotemporal
410 Dementia: Three Faces of the Same Coin. *J. Alzheimers. Dis.* 62, 1113–1123.
411 <https://doi.org/10.3233/JAD-170584>
- 412 Cairns, N.J., Bigio, E.H., Mackenzie, I.R.A., Neumann, M., Lee, V.M.Y., Hatanpaa, K.J., White III,
413 C.L., Schneider, J.A., Halliday, G., Duyckaerts, C., Holm, I.E., Okamoto, K., Munoz, D.G.,

- 414 Dickson, D.W., Ince, P.G., Trojanowski, J.Q., Mann, D.M.A., 2007. Neuropathologic
415 diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the
416 Consortium for Frontotemporal Lobar Degeneration. *Acta Neuropathol.* 114, 5–22.
- 417 Constantinescu, R., Rosengren, L., Johnels, B., Zetterberg, H., Holmberg, B., 2010. Consecutive
418 analyses of cerebrospinal fluid axonal and glial markers in Parkinson’s disease and atypical
419 parkinsonian disorders. *Park. Relat. Disord.* 16, 142–145.
420 <https://doi.org/10.1016/j.parkreldis.2009.07.007>
- 421 Constantinescu, R., Zetterberg, H., Holmberg, B., Rosengren, L., 2009. Levels of brain related
422 proteins in cerebrospinal fluid: An aid in the differential diagnosis of parkinsonian disorders.
423 *Park. Relat. Disord.* 15, 205–212.
- 424 Dvorak, F., Haberer, I., Sitzler, M., Foerch, C., 2009. Characterisation of the diagnostic window of
425 serum glial fibrillary acidic protein for the differentiation of intracerebral haemorrhage and
426 ischaemic stroke. *Cerebrovasc. Dis.* 27, 37–41. <https://doi.org/10.1159/000172632>
- 427 Foiani, M.S., Woollacott, I.O.C., Heller, C., Bocchetta, M., Heslegrave, A., Dick, K.M., Russell,
428 L.L., Marshall, C.R., Mead, S., Schott, J.M., Fox, N.C., Warren, J.D., Zetterberg, H., Rohrer,
429 J.D., 2018. Plasma tau is increased in frontotemporal dementia. *J. Neurol. Neurosurg.
430 Psychiatry* 89, 804–807. <https://doi.org/10.1136/jnnp-2017-317260>
- 431 Fostinelli, S., Ciani, M., Zanardini, R., Zanetti, O., Binetti, G., Ghidoni, R., Benussi, L., 2018. The
432 Heritability of Frontotemporal Lobar Degeneration: Validation of Pedigree Classification
433 Criteria in a Northern Italy Cohort. *J. Alzheimers. Dis.* 61, 753–760.
434 <https://doi.org/10.3233/JAD-170661>
- 435 Gorno-Tempini, M.L., Hillis, A.E., Weintraub, S., Kertesz, A., Mendez, M., Cappa, S.F., Ogar,
436 J.M., Rohrer, J.D., Black, S., Boeve, B.F., Manes, F., Dronkers, N.F., Vandenberghe, R.,
437 Rascovsky, K., Patterson, K., Miller, B.L., Knopman, D.S., Hodges, J.R., Mesulam, M.M.,
438 Grossman, M., 2011. Classification of primary progressive aphasia and its variants. *Neurology*
439 76, 1006–1014.
- 440 Heller, C., Foiani, M.S., Moore, K., Convery, R., Bocchetta, M., Neason, M., Cash, D.M., Thomas,
441 D., Greaves, C. V., Woollacott, I.O.C., Shafei, R., Van Swieten, J.C., Moreno, F., Sanchez-
442 Valle, R., Borroni, B., Laforce, R., Masellis, M., Tartaglia, M.C., Graff, C., Galimberti, D.,
443 Rowe, J.B., Finger, E., Synofzik, M., Vandenberghe, R., De Mendonca, A., Tagliavini, F.,
444 Santana, I., Ducharme, S., Butler, C.R., Gerhard, A., Levin, J., Danek, A., Frisoni, G., Sorbi,
445 S., Otto, M., Heslegrave, A.J., Zetterberg, H., Rohrer, J.D., 2020. Plasma glial fibrillary acidic
446 protein is raised in progranulin-associated frontotemporal dementia. *J. Neurol. Neurosurg.
447 Psychiatry* 263–270. <https://doi.org/10.1136/jnnp-2019-321954>
- 448 Höglinger, G.U., Respondek, G., Stamelou, M., Kurz, C., Josephs, K.A., Lang, A.E., Mollenhauer,
449 B., Müller, U., Nilsson, C., Whitwell, J.L., Arzberger, T., Englund, E., Gelpi, E., Giese, A.,
450 Irwin, D.J., Meissner, W.G., Pantelyat, A., Rajput, A., van Swieten, J.C., Troakes, C.,
451 Antonini, A., Bhatia, K.P., Bordelon, Y., Compta, Y., Corvol, J.-C., Colosimo, C., Dickson,
452 D.W., Dodel, R., Ferguson, L., Grossman, M., Kassubek, J., Krismer, F., Levin, J., Lorenzl, S.,
453 Morris, H.R., Nestor, P., Oertel, W.H., Poewe, W., Rabinovici, G., Rowe, J.B., Schellenberg,
454 G.D., Seppi, K., van Eimeren, T., Wenning, G.K., Boxer, A.L., Golbe, L.I., Litvan, I., Group,
455 for the M.D.S.P.S.P.S., 2017. Clinical diagnosis of progressive supranuclear palsy: The
456 movement disorder society criteria. *Mov. Disord.* 32, 853–864.
- 457 Holmberg, B., Rosengren, L., Karlsson, J.E., Johnels, B., 1998. Increased cerebrospinal fluid levels
458 of neurofilament protein in progressive supranuclear palsy and multiple-system atrophy
459 compared with Parkinson’s disease. *Mov. Disord.* 13, 70–77.
460 <https://doi.org/10.1002/mds.870130116>

- 461 Ishiki, A., Kamada, M., Kawamura, Y., Terao, C., Shimoda, F., Tomita, N., Arai, H., Furukawa, K.,
462 2016. Glial fibrillar acidic protein in the cerebrospinal fluid of Alzheimer's disease, dementia
463 with Lewy bodies, and frontotemporal lobar degeneration. *J. Neurochem.* 136, 258–261.
464 <https://doi.org/10.1111/jnc.13399>
- 465 Ithapu, V., Singh, V., Lindner, C., Austin, B.P., Hinrichs, C., Carlsson, C.M., Bendlin, B.B.,
466 Johnson, S.C., 2014. Extracting and summarizing white matter hyperintensities using
467 supervised segmentation methods in Alzheimer's disease risk and aging studies. *Hum. Brain*
468 *Mapp.* 35, 4219–4235. <https://doi.org/10.1002/hbm.22472>
- 469 Kujirai, T., Caramia, M.D., Rothwell, J.C., Day, B.L., Thompson, P.D., Ferbert, A., Wroe, S.,
470 Asselman, P., Marsden, C.D., 1993. Corticocortical inhibition in human motor cortex. *J.*
471 *Physiol.* 471, 501–519.
- 472 Liddelow, S.A., Barres, B.A., 2017. Reactive Astrocytes: Production, Function, and Therapeutic
473 Potential. *Immunity* 46, 957–967. <https://doi.org/10.1016/j.immuni.2017.06.006>
- 474 Mackenzie, I.R.A., Baborie, A., Pickering-Brown, S., Plessis, D. Du, Jaros, E., Perry, R.H., Neary,
475 D., Snowden, J.S., Mann, D.M.A., 2006. Heterogeneity of ubiquitin pathology in
476 frontotemporal lobar degeneration: Classification and relation to clinical phenotype. *Acta*
477 *Neuropathol.* 112, 539–549. <https://doi.org/10.1007/s00401-006-0138-9>
- 478 Marelli, C., Hourregue, C., Gutierrez, L.-A., Paquet, C., Menjot de Champfleury, N., De Verbizier,
479 D., Jacob, M., Dubois, J., Maleska, A.M., Hirtz, C., Navucet, S., Bennys, K., Dumurgier, J.,
480 Cognat, E., Berr, C., Magnin, E., Lehmann, S., Gabelle, A., 2020. Cerebrospinal Fluid and
481 Plasma Biomarkers do not Differ in the Presenile and Late-Onset Behavioral Variants of
482 Frontotemporal Dementia. *J. Alzheimer's Dis.* 1–9. <https://doi.org/10.3233/JAD-190378>
- 483 McKhann, G.M., Knopman, D.S., Chertkow, H., Hyman, B.T., Jack Clifford R, J., Kawas, C.H.,
484 Klunk, W.E., Koroshetz, W.J., Manly, J.J., Mayeux, R., Mohs, R.C., Morris, J.C., Rossor,
485 M.N., Scheltens, P., Carrillo, M.C., Thies, B., Weintraub, S., Phelps, C.H., 2011. The
486 diagnosis of dementia due to Alzheimer's disease: Recommendations from the National
487 Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for
488 Alzheimer's disease. *JALZ* 7, 263–269.
- 489 Mederos, S., Perea, G., 2019. GABAergic-astrocyte signaling: A refinement of inhibitory brain
490 networks. *Glia.* <https://doi.org/10.1002/glia.23644>
- 491 Meeter, L.H., Dopfer, E.G., Jiskoot, L.C., Sanchez-Valle, R., Graff, C., Benussi, L., Ghidoni, R.,
492 Pijnenburg, Y.A., Borroni, B., Galimberti, D., Laforce, R.J., Masellis, M., Vandenberghe, R.,
493 Ber, I. Le, Otto, M., van Minkelen, R., Papma, J.M., Rombouts, S.A., Balasa, M., Öijersted,
494 L., Jelic, V., Dick, K.M., Cash, D.M., Harding, S.R., Jorge Cardoso, M., Ourselin, S., Rossor,
495 M.N., Padovani, A., Scarpini, E., Fenoglio, C., Tartaglia, M.C., Lamari, F., Barro, C., Kuhle,
496 J., Rohrer, J.D., Teunissen, C.E., van Swieten, J.C., 2016. Neurofilament light chain: a
497 biomarker for genetic frontotemporal dementia. *Ann. Clin. Transl. Neurol.* 3, 623–636.
498 <https://doi.org/10.1002/acn3.325>
- 499 Oeckl, P., Halbgebauer, S., Anderl-Straub, S., Steinacker, P., Hussa, A.M., Neugebauer, H., Von
500 Arnim, C.A.F., Diehl-Schmid, J., Grimmer, T., Kornhuber, J., Lewczuk, P., Danek, A.,
501 Degeneration, L., Ludolph, A.C., Otto, M., 2019a. Glial fibrillary acidic protein in serum is
502 increased in Alzheimer's disease and correlates with cognitive impairment. *J. Alzheimer's Dis.*
503 67, 481–488. <https://doi.org/10.3233/JAD-180325>
- 504 Oeckl, P., Weydt, P., Steinacker, P., Anderl-Straub, S., Nordin, F., Volk, A.E., Diehl-Schmid, J.,
505 Andersen, P.M., Kornhuber, J., Danek, A., Fassbender, K., Fliessbach, K., Jahn, H., Lauer, M.,
506 Müller, K., Knehr, A., Prudlo, J., Schneider, A., Thal, D.R., Yilmazer-Hanke, D., Weishaupt,
507 J.H., Ludolph, A.C., Otto, M., 2019b. Different neuroinflammatory profile in amyotrophic

- 508 lateral sclerosis and frontotemporal dementia is linked to the clinical phase. *J. Neurol.*
509 *Neurosurg. Psychiatry* 90, 4–10. <https://doi.org/10.1136/jnnp-2018-318868>
- 510 Olsson, B., Lautner, R., Andreasson, U., Öhrfelt, A., Portelius, E., Bjerke, M., Hölttä, M., Rosén,
511 C., Olsson, C., Strobel, G., Wu, E., Dakin, K., Petzold, M., Blennow, K., Zetterberg, H., 2016.
512 CSF and blood biomarkers for the diagnosis of Alzheimer’s disease: a systematic review and
513 meta-analysis. *Lancet Neurol.* 15, 673–684.
- 514 Padovani, A., Benussi, A., Cantoni, V., Dell’Era, V., Cotelli, M.S., Caratozzolo, S., Turrone, R.,
515 Rozzini, L., Alberici, A., Altomare, D., Depari, A., Flammini, A., Frisoni, G.B., Borroni, B.,
516 2018. Diagnosis of Mild Cognitive Impairment Due to Alzheimer’s Disease with Transcranial
517 Magnetic Stimulation. *J. Alzheimers. Dis.* 65, 221–230. <https://doi.org/10.3233/JAD-180293>
- 518 Papa, L., Silvestri, S., Brophy, G.M., Giordano, P., Falk, J.L., Braga, C.F., Tan, C.N., Ameli, N.J.,
519 Demery, J.A., Dixit, N.K., Mendes, M.E., Hayes, R.L., Wang, K.K.W., Robertson, C.S., 2014.
520 GFAP out-performs S100 β in detecting traumatic intracranial lesions on computed
521 tomography in trauma patients with mild traumatic brain injury and those with extracranial
522 lesions. *J. Neurotrauma* 31, 1815–1822. <https://doi.org/10.1089/neu.2013.3245>
- 523 Paternicò, D., Premi, E., Gazzina, S., Cosseddu, M., Alberici, A., Archetti, S., Cotelli, M.S.,
524 Micheli, A., Turla, M., Gasparotti, R., Padovani, A., Borroni, B., 2016. White matter
525 hyperintensities characterize monogenic frontotemporal dementia with granulin mutations.
526 *Neurobiol. Aging* 38, 176–180.
- 527 Premi, E., Gualeni, V., Costa, P., Cosseddu, M., Gasparotti, R., Padovani, A., Borroni, B., 2016.
528 Looking for Measures of Disease Severity in the Frontotemporal Dementia Continuum. *J.*
529 *Alzheimers. Dis.* 52, 1227–1235.
- 530 Rascovsky, K., Hodges, J.R., Knopman, D., Mendez, M.F., Kramer, J.H., Neuhaus, J., van Swieten,
531 J.C., Seelaar, H., Dopper, E.G.P., Onyike, C.U., Hillis, A.E., Josephs, K. a, Boeve, B.F.,
532 Kertesz, A., Seeley, W.W., Rankin, K.P., Johnson, J.K., Gorno-Tempini, M.-L., Rosen, H.,
533 Prioleau-Latham, C.E., Lee, A., Kipps, C.M., Lillo, P., Piguet, O., Rohrer, J.D., Rossor, M.N.,
534 Warren, J.D., Fox, N.C., Galasko, D., Salmon, D.P., Black, S.E., Mesulam, M., Weintraub, S.,
535 Dickerson, B.C., Diehl-Schmid, J., Pasquier, F., Deramecourt, V., Lebert, F., Pijnenburg, Y.,
536 Chow, T.W., Manes, F., Grafman, J., Cappa, S.F., Freedman, M., Grossman, M., Miller, B.L.,
537 2011. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal
538 dementia. *Brain* 134, 2456–77. <https://doi.org/Doi 10.1093/Brain/Awr179>
- 539 Robel, S., Sontheimer, H., 2016. Glia as drivers of abnormal neuronal activity. *Nat. Neurosci.*
540 <https://doi.org/10.1038/nn.4184>
- 541 Rohrer, J.D., Woollacott, I.O.C., Dick, K.M., Brotherhood, E., Gordon, E., Fellows, A., Toombs, J.,
542 Druyeh, R., Cardoso, M.J., Ourselin, S., Nicholas, J.M., Norgren, N., Mead, S., Andreasson,
543 U., Blennow, K., Schott, J.M., Fox, N.C., Warren, J.D., Zetterberg, H., 2016. Serum
544 neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia.
545 *Neurology* 87, 1329–1336. <https://doi.org/10.1212/WNL.0000000000003154>
- 546 Rossini, P.M., Burke, D., Chen, R., Cohen, L.G., Daskalakis, Z., Di Iorio, R., Di Lazzaro, V.,
547 Ferreri, F., Fitzgerald, P.B., George, M.S., Hallett, M., Lefaucheur, J.P., Langguth, B.,
548 Matsumoto, H., Miniussi, C., Nitsche, M.A., Pascual-Leone, A., Paulus, W., Rossi, S.,
549 Rothwell, J.C., Siebner, H.R., Ugawa, Y., Walsh, V., Ziemann, U., 2015. Non-invasive
550 electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic
551 principles and procedures for routine clinical and research application. An updated report from
552 an I.F.C.N. Committee. *Clin. Neurophysiol.* 126, 1071–1107.
- 553 Sudre, C.H., Bocchetta, M., Heller, C., Convery, R., Neason, M., Moore, K.M., Cash, D.M.,
554 Thomas, D.L., Woollacott, I.O.C., Foiani, M., Heslegrave, A., Shafei, R., Greaves, C., van

555 Swieten, J., Moreno, F., Sanchez-Valle, R., Borroni, B., Laforce, R., Masellis, M., Tartaglia,
556 M.C., Graff, C., Galimberti, D., Rowe, J.B., Finger, E., Synofzik, M., Vandenberghe, R., de
557 Mendonça, A., Tagliavini, F., Santana, I., Ducharme, S., Butler, C., Gerhard, A., Levin, J.,
558 Danek, A., Frisoni, G.B., Sorbi, S., Otto, M., Zetterberg, H., Ourselin, S., Cardoso, M.J.,
559 Rohrer, J.D., 2019. White matter hyperintensities in progranulin-associated frontotemporal
560 dementia: A longitudinal GENFI study. *NeuroImage Clin.* 24, 102077.
561 <https://doi.org/10.1016/j.nicl.2019.102077>

562 Süßmuth, S.D., Uttner, I., Landwehrmeyer, B., Pinkhardt, E.H., Brettschneider, J., Petzold, A.,
563 Kramer, B., Schulz, J.B., Palm, C., Otto, M., Ludolph, A.C., Kassubek, J., Tumani, H., 2010.
564 Differential pattern of brain-specific CSF proteins tau and amyloid-beta in Parkinsonian
565 syndromes. *Mov. Disord.* 25, 1284–1288. <https://doi.org/10.1002/mds.22895>

566 Tokimura, H., Di Lazzaro, V., Tokimura, Y., Oliviero, A., Profice, P., Insola, A., Mazzone, P.,
567 Tonali, P., Rothwell, J.C., 2000. Short latency inhibition of human hand motor cortex by
568 somatosensory input from the hand. *J. Physiol.* 523 Pt 2, 503–513.

569 Vågberg, M., Norgren, N., Dring, A., Lindqvist, T., Birgander, R., Zetterberg, H., Svenningsson,
570 A., 2015. Levels and age dependency of neurofilament light and Glial Fibrillary Acidic Protein
571 in healthy individuals and their relation to the brain parenchymal fraction. *PLoS One* 10, 1–8.
572 <https://doi.org/10.1371/journal.pone.0135886>

573 Valls-Solé, J., Pascual-Leone, A., Wassermann, E.M., Hallett, M., 1992. Human motor evoked
574 responses to paired transcranial magnetic stimuli. *Electroencephalogr. Clin. Neurophysiol.* 85,
575 355–364.

576 Ziemann, U., Reis, J., Schwenkreis, P., Rosanova, M., Strafella, A., Badawy, R., Müller-Dahlhaus,
577 F., 2015. TMS and drugs revisited 2014. *Clin. Neurophysiol.* 126, 1847–68.
578 <https://doi.org/10.1016/j.clinph.2014.08.028>

579 Ziemann, U., Rothwell, J.C., Ridding, M.C., 1996. Interaction between intracortical inhibition and
580 facilitation in human motor cortex. *J. Physiol.* 496, 873–881.

581

582 **Table 1. Demographic and clinical characteristics of FTLD patients and controls**

Variable	FTLD					Controls	
	bvFTD	avPPA	svPPA	CBS	PSP	AD	HC
Number	130	48	24	50	30	63	61
Age, years	64.5±8.1	67.9±9.0	63.1±7.7	66.1±7.4	73.6±6.4	75.5±8.1	65.5±12.3
Sex, female %	58.2	43.8	59.3	52.9	51.6	31.7	20.6
Age at onset, years	61.4±7.8	64.9±8.7	60.1±7.7	63.5±7.3	69.4±6.5	74.0 ±8.3	-
Monogenic disease, %	14.9	25.0	0.0	2.3	0.0	0.0	-
Serum GFAP (pg/mL)							
mean±SE	327.6±19.4	441.4±42.4	320.8±48.9	291.9±30.4	19.1±22.8	394.8±22.2	183.1±12.0
lower-upper bound	58.4-1443.4	20.7-1397.8	67.9-973.1	57.9-1024.2	20.7-652.1	159.1-920.1	51.6-574.9

583

584 FTLD = Frontotemporal Lobar degeneration; bvFTD = behavioural variant frontotemporal dementia;
 585 avPPA = agrammatic variant of primary progressive aphasia; svPPA = semantic variant of primary
 586 progressive aphasia; CBS = corticobasal syndrome; PSP = progressive supranuclear palsy; AD =
 587 Alzheimer's disease; HC = healthy controls; GFAP = glial fibrillary acidic protein; SE = standard
 588 error.

589 Results are expressed as mean±standard deviations, unless otherwise specified. Monogenic disease:
 590 all *GRN* mutations, but 3 *MAPT* mutations (2 bvFTD and 1 CBS).

591 **Legend to Figures**

592 **Figure 1. Serum biomarkers concentrations in participants by clinical diagnosis.**

593 Serum GFAP concentrations (pg/mL) in participants by clinical diagnosis. bvFTD = behavioural
594 variant frontotemporal dementia; avPPA = agrammatic variant of primary progressive aphasia;
595 svPPA = semantic variant of primary progressive aphasia; CBS = corticobasal syndrome; PSP =
596 progressive supranuclear palsy; AD = Alzheimer's disease; HC = healthy controls. Bar graphs
597 represent mean values and error bars represent 95% confidence intervals. * $p < 0.050$; ** $p < 0.010$;
598 *** $p < 0.001$ after Bonferroni corrected *post hoc* tests.

599

600 **Figure 2. Significant association between serum GFAP and neuropsychological assessment.**

601 Association between serum GFAP concentrations (pg/mL) and (A) FTLD-CDR, (B) phonemic
602 fluencies, (C) semantic fluencies, and (D) token test.

603 GFAP = glial fibrillary acidic protein; FTLD-CDR = frontotemporal lobar degeneration-modified
604 clinical dementia rating sum of boxes; IADL = instrumental activities of daily living.

605

606 **Figure 3. Significant associations between serum biomarkers and neurophysiological**
607 **measures.**

608 Association between serum GFAP concentrations (pg/mL) and average LICI (ISI 50, 100, 150 ms
609 ISI).

610 GFAP = glial fibrillary acidic protein; SICI = short-interval intracortical inhibition; LICI = long-
611 interval intracortical inhibition; SAI = short latency afferent inhibition; ISI = interstimulus interval.