Differential Impairment Of Cerebrospinal Fluid Synaptic Biomarkers In The Genetic Forms Of Frontotemporal Dementia

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Abstract

Background: Approximately a third of frontotemporal dementia (FTD) is genetic with mutations in three genes accounting for most of the inheritance: *C9orf72*, *GRN* and *MAPT*. Impaired synaptic health is a common mechanism in all three genetic variants and so developing fluid biomarkers of this process could be useful as a readout of cellular dysfunction within therapeutic trials.

Methods: A total of 193 cerebrospinal fluid (CSF) samples from the GENetic FTD Initiative including 77 presymptomatic (31 *C9orf72*, 23 *GRN*, 23 *MAPT*) and 55 symptomatic (26 *C9orf72*, 17 *GRN*, 12 *MAPT*) mutation carriers as well as 61 mutation-negative controls were measured using a microflow LC PRM-MS set-up targeting 15 synaptic proteins: AP-2 complex subunit beta, complexin-2, beta-synuclein, gamma-synuclein, 14-3-3 proteins (eta, epsilon, zeta/delta), neurogranin, Rab GDP dissociation inhibitor alpha (Rab GDI alpha), syntaxin-1B, syntaxin-7, phosphatidylethanolamine-binding protein 1 (PEBP-1), neuronal pentraxin receptor (NPTXR), neuronal pentraxin 1 (NPTX1), and neuronal pentraxin 2 (NPTX2). Mutation carrier groups were compared to each other and to controls using a bootstrapped linear regression model, adjusting for age and sex.

Results: CSF levels of eight proteins were increased only in symptomatic *MAPT* mutation carriers (compared with controls) and not in symptomatic *C9orf72* or *GRN* mutation carriers: beta-synuclein, gamma-synuclein, 14-3-3-eta, neurogranin, Rab GDI alpha, syntaxin-1B, syntaxin-7, and PEBP-1, with three other proteins increased in *MAPT* mutation carriers compared with the other genetic groups (AP-2 complex subunit beta, complexin-2, and 14-3-3 proteins zeta/delta). In contrast, CSF NPTX1 and NPTX2 levels were affected in all three genetic groups (decreased compared with controls), with NPTXR concentrations being affected in *C9orf72* and *GRN* mutation carriers only (decreased compared with controls). No changes were seen in the CSF levels of these proteins in presymptomatic mutation carriers. Concentrations of the neuronal pentraxins were correlated with brain volumes in the presymptomatic period for *C9orf72* and *GRN* groups, suggesting that they become abnormal in proximity to symptom onset.

Conclusions: Differential synaptic impairment is seen in the genetic forms of FTD, with abnormalities in multiple measures in those with *MAPT* mutations, but only changes in neuronal pentraxins within the *GRN* and *C9orf72* mutation groups. Such markers may be useful in future trials as measures of synaptic dysfunction, but further work is needed to understand how these markers change throughout the course of the disease.

Background

Frontotemporal dementia (FTD) is the most common cause of dementia affecting people under the age of 60. Clinically, it presents heterogeneously, manifesting as a behavioural variant (bvFTD), language impairment (primary progressive aphasia, PPA), or with a motor presentation (either amyotrophic lateral sclerosis, FTD-ALS, or an atypical parkinsonian disorder). The FTD spectrum is characteristically associated with neuronal dysfunction and loss in the frontal and temporal lobes, but more widespread cortical, subcortical, cerebellar and brainstem involvement is now also recognized(1). Around a third of people with FTD have a genetic cause, with the most common mutations occurring in three genes: *GRN* (progranulin), *C9orf72* (chromosome 9 open reading frame 72) and *MAPT* (microtubule-associated protein tau)(2,3). Lastly, the underlying pathology of FTD can be one of three forms: cellular inclusions containing abnormal forms of tau, TAR DNA-binding protein 43 (TDP-43) or FET proteins (*FUS* (*Fused in sarcoma*), *EWS* (*Ewing sarcoma*) and *TAF15* (*TATA-binding associated factor 15*))(4).

The interaction between clinical phenotype, neuroanatomical features, genotype, and pathology is complex and means that FTD can be hard to diagnose (particularly its specific pathological form during life) and difficult to track over time. To further examine some of these outstanding issues in the FTD field, researchers have aimed to develop fluid biomarkers, measured typically in cerebrospinal fluid (CSF), serum or plasma using a variety of different techniques. Biomarkers can provide an insight into the underlying pathophysiology of FTD, and in the context of clinical trials could offer a direct experimental medicine approach to understanding molecular mechanisms through measurement of biofluids pre-and post-intervention(5).

Whilst some pathways are specific to certain pathogenetic forms of FTD, studies in recent years have particularly highlighted the importance of synaptic health (6–10) as one of the major pathophysiological mechanisms across the FTD spectrum. Progressive synaptic dysfunction and loss have been shown to occur in FTD, raising the hypothesis that any changes in synaptic proteins in brain tissue may also be reflected in their concentrations within the CSF (and potentially the blood) of people with FTD. In this study, we investigated a panel of CSF synaptic markers in presymptomatic and symptomatic people with genetic FTD from the GENetic Frontotemporal dementia Initiative (GENFI), hypothesizing that we would find differential abnormalities across *MAPT*, *GRN* and *C9orf72* mutation carriers.

Methods

Participants and sample collection

Participants were recruited from the GENFI study, which follows patients with FTD to a pathogenic mutation in *MAPT*, *GRN*, or *C9orf72* (symptomatic mutation carriers) and healthy at-risk first-degree relatives (either presymptomatic mutation carriers or non-carriers) (11). We included 77 presymptomatic mutation carriers (31 *C9orf72*, 23 *GRN*, 23 *MAPT*), 55 symptomatic mutation carriers (26 *C9orf72*, 17 *GRN*, 12 *MAPT*) and 61 non-carriers. Age at time of CSF sample collection was not statistically different within each group and a similar percentage of males and females were included (Table 1). Participants were assessed using a standardized history and examination and classified as symptomatic if they met consensus diagnostic criteria(12,13). The CDR Dementia Staging Instrument with National Alzheimer Coordinating Centre Frontotemporal Lobar Degeneration component (CDR® plus NACC FTLD) was used to assess disease severity. Local ethics committees at each site approved the study and all participants provided written informed consent.

Table 1
Demographics of participants in the study. N, number of participants. Values are shown as mean (standard deviation).

	non-carriers	Presymptomatic carriers			Symptomatic carriers		
		C9orf72	GRN	MAPT	C9orf72	GRN	MAPT
N	61	31	23	23	26	17	12
Age at CSF sampling	44.9(13.2)	42.2(11.1)	45.3(13.8)	40.3(10.6)	63.3(9.2)	63.1(7.5)	61.4(7.4)
Sex (% females)	62.3	51.6	47.8	65.2	38.5	52.9	50
CDR plus NACC FTLD sum of boxes	0.2(0.5)	0.3(0.6)	0.3(1)	0.6(0.7)	10.5(5.6)	9.4(5.4)	8.3(4.5)

CSF collection and LC-MS/MS analysis

CSF was collected in polypropylene tubes through a lumbar puncture and centrifuged to remove insoluble material and cells. Supernatants were aliquoted and stored at -80°C within 2 hours after withdrawal. For the mass spectrometry analysis, sample preparation was performed as described previously (14). LC-MS/MS analysis was performed using a microflow HPLC, equipped with a Hypersil Gold reversed-phase column (100 × 2.1 mm, particle size 1.9 µm, Thermo Fisher Scientific), and a Triple Quadrupole mass spectrometer (6495 Triple Quadrupole LC/MS system, Agilent Technologies). LC-MS settings are shown in Appendix 2. To monitor the performance of the assay over time, quality control (QC) sample replicates were injected at regular intervals during runs. The panel of synaptic markers included (Fig. 1): AP-2 complex subunit beta, complexin-2, beta-synuclein, gamma-synuclein, 14-3-3 proteins (eta, epsilon, zeta/delta), neurogranin, Rab GDP dissociation inhibitor alpha (Rab GDI alpha), syntaxin-1B, syntaxin-7, phosphatidylethanolamine-binding protein 1 (PEBP-1), neuronal pentraxin receptor (NPTXR), neuronal pentraxin 1 (NPTX1), and neuronal pentraxin 2 (NPTX2). Table 2 shows the proteins and their respective proteotypic peptides targeted in the multiple reaction monitoring mass spectrometry analysis (14).

Table 2
The 15 synaptic proteins and their respective peptides included in the panel measured.

Protein	Accession	Sequence	Position
AP-2 complex subunit beta	P63010	NVEGQDMLYQSLK	[880–892]
		IQPGNPNYTLCLK	[905–917]
Complexin-2	Q6PUV4	AALEQPCGSLTRPK	[84–98]
Beta-synuclein	Q16143	EGVVQGVASVAEK	[46–58]
Gamma-synuclein	O76070	ENVVQSVTSVAEK	[46–58]
14-3-3 protein eta	Q04917	AVTELNEPLSNEDR	[29–42]
14-3-3 protein epsilon	P62258	LISSIEQK	[62–69]
14-3-3 protein zeta/delta	P63104	VVSSIEQK	[61–68]
Neurogranin	Q92686	KGPGPGGPGGAGVAR	[54–68]
Rab GDI alpha	P31150	QLICDPSYIPDR	[279–290]
Syntaxin-1B	P61266	QHSAILAAPNPDEK	[56–69]
Syntaxin-7	O15400	EFGSLPTTPSEQR	[72–84]
PEBP-1	P30086	LYEQLSGK	[180–187]
		NRPTSISWDGLDSGK	[48–62]
Neuronal pentraxin receptor	O95502	NNYMYAR	[302–308]
		LVEAFGGATK	[479–488]
Neuronal pentraxin-1	Q15818	LENLEQYSR	[144–152]
		CESQSTLDPGAGEAR	[89–103]
Neuronal pentraxin-2	P47972	VAELEDEK	[177–184]
		WPVETCEER	[419–428]

Other biomarkers

Participants underwent volumetric T1-weighted magnetic resonance imaging according to the harmonized GENFI protocol on a 3T scanner. All images underwent a quality control check and scans with movement or artefacts were removed from the analysis. Only scans from mutation carriers were included in the correlative analysis: of the 132 participants, 111 scans were available for the analysis: 49 *C9orf72*, 34 *GRN* and 28 *MAPT* mutation carriers. Neuroanatomical regions of interest were generated as previously described using an automated atlas segmentation propagation and label fusion strategy called Geodesic Information Flow (11). Specifically, total brain volume and volumes of the frontal, temporal and parietal cortices were calculated and expressed as a percentage of total intracranial volume, computed with SPM12 (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, London, UK) running under Matlab R2014b.

Participants also had plasma samples collected as part of the GENFI protocol. Plasma was collected, processed, and stored in aliquots at -80°C according to standardised procedures. Plasma neurofilament light chain (NfL) levels were correlated with synaptic proteins with only measures from mutation carriers included: of 132 participants, 108 plasma NfL values were available for the analysis: 47 *C9orf72*, 34 *GRN* and 27 *MAPT* mutation carriers. Plasma NfL concentration was measured with Single molecule array (Simoa) technology using the Neurology 4-Plex A kit (Quanterix, Billerica, USA) on an HD-X Analyzer following the manufacturer's instructions (Quanterix, Billerica, USA). Measurements were completed in duplicate (all CVs below 15%) over a total of 3 batches, each with an 8-point calibration curve tested in triplicate and 2 controls tested in duplicate, as reported before (15).

Data processing and statistical analysis

Mass spectrometer data processing was performed in Skyline 20.1 (MacCoss Lab Software). All peaks were visually inspected and adjusted if required for optimal peak area calculation. The relative peptide concentration (fmol/μL) was obtained by the ratio of the total area for each peptide against the total area of the corresponding internal standard (IS) multiplied by the amount of IS added per volume of CSF.

All statistical analyses were performed in STATA (v.16) and RStudio (R version 4.0.2). The Shapiro-Wilk test was performed to determine the normality of distribution of each synaptic marker in each group. The levels of each synaptic protein were compared between groups using a linear regression model adjusting for age at CSF sample collection and sex; bootstrapping with 2000 repetitions was used if the synaptic measures were not normally distributed.

Spearman correlation coefficients were assessed for the synaptic markers between their values and other biomarker data including normalized volumes of total brain, frontal cortex, temporal cortex, and parietal cortex, plasma NfL and the CDR plus NACC FTLD sum of boxes score.

Results

Concentrations of synaptic markers by genotype

Significant increases in CSF levels of several synaptic proteins were seen in symptomatic *MAPT* mutation carriers compared with controls (Fig. 2, Supplementary Table 1): beta-synuclein, gamma-synuclein, 14-3-3 eta, neurogranin, Rab GDI alpha, syntaxin 1B, syntaxin-7 and PEBP-1 (both peptides). CSF levels of all of these proteins except 14-3-3 eta, were increased in the symptomatic *MAPT* group compared with the symptomatic *C9orf72* group (as was one of the AP-2 complex subunit beta peptides). Similarly, levels of all of these proteins except 14-3-3 eta and both beta and gamma-synuclein were increased in the symptomatic *MAPT* group compared with the symptomatic *GRN* group (as was complexin-2). Furthermore, 14-3-3 zeta/delta was additionally increased in the symptomatic *MAPT* group compared with both *C9orf72* and *GRN* symptomatic mutation carriers. CSF levels of all of these proteins except syntaxin-7 were increased in the symptomatic *MAPT* mutation carriers compared with the presymptomatic *MAPT* mutation carriers (Fig. 2).

In contrast, CSF concentrations of the neuronal pentraxins were found to be decreased in most of the mutation carrier groups compared with controls. At least one of the peptides measured for NPTXR, NPTX1 and NPTX2 were decreased in symptomatic *C9orf72* and *GRN* mutation carriers compared to controls and to their respective presymptomatic group. One NPTX1 and one NPTX2 peptide were also decreased in the symptomatic *MAPT* group compared to controls and the presymptomatic *MAPT* mutation carriers (Fig. 2).

Correlations of synaptic markers with other biomarkers

For the synaptic markers that had increased CSF concentrations in *MAPT* mutation carriers, no significant correlations were seen with brain volumes, NfL or CDR plus NACC FTLD.

However, a number of significant correlations were seen with the CSF levels of neuronal pentraxins across the genetic groups (Table 3). In presymptomatic *C9orf72* mutation carriers, there were significant positive correlations of total brain volume with almost all the measures ($r = 0.36$ to 0.53). Additionally, there were significant positive correlations in this group with temporal cortex volume for NPTXR (NNYMYAR: $r = 0.50$, $p = 0.006$; LVEAFGGATK: 0.42 , 0.023) and NPTX2 (WPVETCEER: 0.46 , 0.013 ; VAELEDEK: 0.49 , 0.007), and with parietal cortex volume for NPTX1 (CESQSTLDPGAGEAR: 0.41 , 0.029 ; LENLEQYSR: 0.39 , 0.038). Two of the neuronal pentraxins (NPTXR NNYMYAR and NPTX2 VAELEDEK) were significantly negatively correlated with CDR plus NACC FTLD. In the presymptomatic *GRN* group there were significant positive correlations with the frontal lobe ($r = 0.41$ to 0.63) and parietal lobe ($r = 0.40$ to 0.59) for almost all of the measures. There were no correlations with any of the imaging measures in the presymptomatic *MAPT* group but there was a significant correlation with NfL for NPTX1 CESQSTLDPGAGEAR ($r = 0.46$, $p = 0.040$).

Table 3

Correlations of each neuronal pentraxin peptide with brain volumes derived from structural T1-weighted MR imaging (total brain as well as frontal, temporal and parietal cortical volumes), NfL (plasma neurofilament light chain concentration), and CDR plus NACC FTL D sum of boxes score.

		Presymptomatic <i>C9orf72</i>		Symptomatic <i>C9orf72</i>		Presymptomatic <i>GRN</i>		Symptomatic <i>GRN</i>		Presymptomatic <i>MAPT</i>		Symptomatic <i>MAPT</i>	
		rho	p.value	rho	p.value	rho	p.value	rho	p.value	rho	p.value	rho	p.value
NPTXR NNYMYAR	total brain	0.42	0.025	0.23	0.365	0.14	0.532	0.48	0.161	0.24	0.303	0.66	0.157
	frontal	0.19	0.321	0.41	0.090	0.53	0.011	0.73	0.017	0.15	0.522	0.81	0.053
	temporal	0.50	0.006	0.14	0.588	0.30	0.182	0.77	0.009	-0.06	0.810	0.30	0.564
	parietal	0.11	0.571	0.10	0.704	0.52	0.014	0.51	0.131	0.17	0.483	0.64	0.170
	NfL	0.00	0.994	-0.68	0.005	0.02	0.925	0.08	0.821	-0.14	0.572	0.94	0.016
	CDR plus NACC FTL D	-0.38	0.044	-0.29	0.248	0.00	0.997	-0.28	0.425	0.19	0.422	-0.21	0.697
NPTXR LVEAFGGATK	total brain	0.49	0.007	0.08	0.761	0.26	0.248	0.60	0.067	0.27	0.253	0.70	0.125
	frontal lobe	0.36	0.056	0.25	0.309	0.63	0.002	0.52	0.122	0.12	0.625	0.81	0.052
	temporal lobe	0.42	0.023	0.02	0.933	0.25	0.258	0.71	0.022	-0.25	0.297	0.41	0.420
	parietal lobe	0.31	0.097	-0.05	0.840	0.47	0.028	0.75	0.013	0.12	0.624	0.61	0.198
	NfL	-0.06	0.736	-0.65	0.009	0.08	0.711	-0.17	0.647	-0.20	0.402	0.96	0.011
	CDR plus NACC FTL D	-0.20	0.302	-0.06	0.818	0.13	0.579	-0.03	0.933	0.09	0.705	-0.07	0.891
NPTX1 CESQSTLDPGAGEAR	total brain	0.36	0.052	0.11	0.654	0.34	0.118	0.17	0.633	0.23	0.327	0.40	0.427
	frontal lobe	0.25	0.183	0.06	0.812	0.52	0.012	0.42	0.225	0.00	0.996	0.54	0.264
	temporal lobe	0.20	0.300	-0.05	0.833	0.41	0.058	0.40	0.255	-0.09	0.711	0.01	0.984
	parietal lobe	0.41	0.029	-0.24	0.345	0.59	0.004	0.44	0.205	0.08	0.740	0.50	0.315
	NfL	0.04	0.856	-0.51	0.051	0.07	0.757	-0.22	0.539	-0.46	0.040	0.54	0.348
	CDR plus NACC FTL D	0.10	0.601	-0.07	0.770	-0.12	0.593	0.06	0.870	-0.01	0.961	-0.45	0.373
NPTX1 LENLEQYSR	total brain	0.53	0.003	0.11	0.651	0.27	0.231	0.59	0.071	0.23	0.338	0.87	0.026
	frontal lobe	0.30	0.110	0.36	0.145	0.54	0.010	0.62	0.056	0.08	0.740	0.69	0.126
	temporal lobe	0.36	0.056	0.11	0.657	0.27	0.227	0.57	0.083	-0.21	0.366	0.73	0.100
	parietal lobe	0.39	0.038	0.10	0.699	0.40	0.067	0.71	0.022	0.01	0.953	0.48	0.340
	NfL	-0.09	0.634	-0.62	0.014	-0.09	0.697	-0.28	0.434	-0.32	0.176	0.69	0.198
	CDR plus NACC FTL D	-0.15	0.445	-0.07	0.770	0.08	0.722	0.05	0.902	0.16	0.511	-0.35	0.502
NPTX2 WPVETCEER	total brain	0.37	0.047	0.32	0.193	0.28	0.209	0.60	0.070	0.22	0.344	0.86	0.028

		Presymptomatic <i>C9orf72</i>		Symptomatic <i>C9orf72</i>		Presymptomatic <i>GRN</i>		Symptomatic <i>GRN</i>		Presymptomatic <i>MAPT</i>		Symptomatic <i>MAPT</i>	
	frontal lobe	0.16	0.410	0.51	0.031	0.52	0.013	0.80	0.005	0.11	0.639	0.55	0.260
	temporal lobe	0.46	0.013	0.27	0.273	0.21	0.350	0.58	0.078	0.04	0.862	0.83	0.043
	parietal lobe	0.11	0.581	0.21	0.401	0.52	0.012	0.62	0.054	0.02	0.941	0.33	0.530
	NfL	0.07	0.723	-0.53	0.043	0.12	0.590	-0.25	0.485	-0.10	0.661	0.45	0.450
	CDR plus NACC FTL D	-0.34	0.068	-0.44	0.066	0.03	0.911	-0.09	0.796	0.21	0.381	-0.50	0.309
NPTX2 VAELEDEK	total brain	0.38	0.045	0.38	0.120	0.25	0.257	0.50	0.145	0.31	0.189	0.95	0.004
	frontal lobe	0.21	0.277	0.60	0.008	0.41	0.059	0.80	0.006	0.16	0.489	0.80	0.054
	temporal lobe	0.49	0.007	0.42	0.080	0.20	0.377	0.65	0.043	0.12	0.623	0.75	0.085
	parietal lobe	0.18	0.343	0.27	0.274	0.45	0.035	0.50	0.141	0.11	0.657	0.67	0.148
	NfL	0.05	0.786	-0.48	0.073	0.06	0.781	-0.08	0.837	-0.03	0.886	0.67	0.215
	CDR plus NACC FTL D	-0.37	0.048	-0.49	0.041	0.00	0.989	-0.24	0.497	0.18	0.436	-0.60	0.204

In the symptomatic *C9orf72* group, the majority of the synaptic markers were significantly negatively correlated with NfL concentration ($r=-0.48$ to -0.68), whilst in the symptomatic *GRN* group, most of the measures positively correlated with either frontal ($r = 0.42$ to 0.80), or parietal ($r = 0.44$ to 0.75), cortical volumes. In the *MAPT* mutation carriers there was a significant positive correlation with both NPTX2 peptides ($r = 0.86$, $p = 0.028$; $r = 0.95$, $p = 0.004$) and one of the NPTX1 peptides with total brain volume ($r = 0.87$, $p = 0.026$).

Discussion

In this study we showed an increased CSF concentration of multiple synaptic markers in symptomatic *MAPT* mutation carriers (11 of 15 proteins tested). In contrast, concentrations of the neuronal pentraxins were decreased in all three symptomatic genetic groups. Although no group-wise differences in CSF levels were seen presymptomatically, correlations with brain volumes in the *C9orf72* and *GRN* groups suggests that the neuronal pentraxins change in the lead up to symptom onset as the brain volume starts to decrease.

For the proteins found to have abnormal CSF levels in *MAPT* mutations, little is known previously about their involvement in the pathophysiology of FTD. Beta- and gamma-synucleins are present in the proteinaceous aggregates characteristic of the alpha-synucleinopathies (16) although their normal function is still unclear. Previous studies have shown an increase in these markers in the CSF of people with Alzheimer's disease (AD) (14,17,18) but in one prior study of beta-synuclein in the CSF of people with undifferentiated FTD, the levels were normal (17). The results in this study therefore represent a novel association with *MAPT* mutations.

14-3-3 proteins are highly expressed in the brain, particularly enriched in the presynaptic site and are implicated in synaptic plasticity by acting as modulators of neurotransmission (19). Although they are established biomarkers for Creutzfeldt-Jakob disease, they have also been genetically linked to AD and found to colocalize with tau in the neurofibrillary tangles as well as in Lewy bodies in Parkinson's disease (20). Furthermore, increased levels of 14-3-3 protein have previously been reported in CSF from people with FTD (not differentiated into a specific form) in a single study, as well as in people with AD (14,21). Here we show increased levels of 14-3-3 eta protein in the symptomatic *MAPT* mutation carriers when compared with the non-carrier group, and for 14-3-3 zeta/delta, when compared with the other symptomatic groups. These results could potentially indicate a specific relationship with tau pathology, related to the deposition in neurofibrillary tangles of 14-3-3 proteins.

Neurogranin has been well-studied in the AD field as a fluid biomarker over recent years (22,23). It is a postsynaptic molecule involved in long-term potentiation and synaptic plasticity mediated by Ca^{2+} and calmodulin signalling pathways (24,25). In CSF, neurogranin shows an increase in people with AD compared with controls (14,26–28). Furthermore, increased concentrations of neurogranin in CSF predict cognitive decline from mild cognitive impairment (MCI) to AD (29,30). In a previous study of FTD levels of neurogranin were not significantly different to controls (31), although when stratified into those with tau and TDP-43 pathology, there was a trend for an increase in the tau group. A further study has also shown that neurogranin was significantly decreased in comparison to controls in plasma exosomes from people with FTD (32). In this study, we show an increase in neurogranin levels in symptomatic *MAPT* mutation carriers, again suggesting a specific relationship with tau pathology.

AP-2 complex subunit beta, the syntaxins, Rab GDI alpha and PEBP-1 are all implicated in the process of synapse vesicle exocytosis and neurotransmitter release at the synaptic cleft and their CSF levels have previously been shown to be abnormal in AD (14,33–36). Syntaxins participate in the formation of the soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) complex, where they participate in synapse vesicle exocytosis together with complexin-2, which modulates the function of the SNARE complex (33,37). The levels of syntaxin 1B have been shown to be increased at an early preclinical stage in the CSF of people likely to develop AD, even before core CSF biomarkers for neurodegeneration (34). None of these proteins have been previously studied in FTD, but given their increase also in AD, it may be that these are all tau-specific markers of synaptic dysfunction, and further study in other primary tauopathies would be important.

Finally, we showed changes across all three genetic groups in the neuronal pentraxins. Pentraxins are multifunctional proteins divided in different groups according to their length. They are not exclusively localized in the central nervous system (CNS) and are involved in the inflammatory response as well as synaptic plasticity among other functions (35). The sub-family of neuronal pentraxins includes the soluble neuronal pentraxins 1 (NPTX1) and 2 (NPTX2) and the transmembrane neuronal pentraxin receptor (NPTXR). NPTXs are implicated in synaptic plasticity, synapse formation and remodelling (36). Both the two secreted NPTX1 and NPTX2 and the transmembrane receptor NPTXR have been found in several studies to be decreased in CSF in AD compared with controls(14,38–41) and appear to be markers of disease progression in AD (42–44). Recent proteomic studies have shown decreased levels of NPTXR in symptomatic genetic FTD, in all three genetic groups (45). A further study additionally showed that NPTX2 was decreased in symptomatic mutation carriers in all three groups compared with controls whilst NPTX1 was decreased in *C9orf72* and *MAPT* mutation carriers (46). In our study, NPTX1 and NPTX2 were significantly decreased in all symptomatic groups but NPTXR was only decreased in *C9orf72* and *GRN* mutation carriers. In one prior study levels of NPTX2 in CSF correlated with disease progression, with the suggestion also that NPTX2 levels change just prior to symptom onset (46,47). The correlations of the neuronal pentraxins with brain volumes in the presymptomatic *C9orf72* and *GRN* mutation carriers suggests that for these two groups NPTXR, NPTX1 and NPTX2 change in proximity to symptom onset as brain volumes start to decrease. In the symptomatic *C9orf72* expansion carriers the neuronal pentraxin levels correlated with NfL, which can be variable in concentration in this group (48). However, it suggests that at least in *C9orf72* expansion carriers the neuronal pentraxins may be a measure of disease intensity and speed of progression. In contrast, in the *GRN* and *MAPT* mutation carriers, neuronal pentraxin concentrations correlated with brain volumes, suggesting that here they may be a measure of disease severity rather than intensity.

Limitations

Limitations of the study include the limited number of CSF samples in each group after stratification. However, this is the largest study so far of synaptic biomarkers in this uncommon disease and replicates prior work on neuronal pentraxins. Further work to replicate the findings in other *MAPT* mutation cohorts (and other primary tauopathies) as well as longitudinal analysis within the GENFI cohort will be important. Lastly, the specific synaptic markers panel used here was designed specifically to target AD pathology based on prior research and selected from a broad proteomic study in an AD cohort (49). It may therefore be that this panel was more likely to find abnormalities in tauopathies and that other synaptic proteins not included in the panel might be better biomarkers for assessing synaptic dysfunction in FTD, particularly in those with TDP-43 pathology.

Conclusion

In this study we show differential involvement of synaptic proteins in the three main genetic groups accounting for familial FTD. Our results suggest that different pathways may be related to synaptic health in relation to the underlying proteinopathy found in each mutation. Future studies will focus on replication of these findings, longitudinal analyses of these measures, and a broader proteomic study to better customise a synaptic biomarker panel targeted to different forms of FTD.

List Of Abbreviations

FTD: frontotemporal dementia

GENFI: GENetic Frontotemporal dementia Initiative

bvFTD: behavioural variant FTD

PPA: primary progressive aphasia

FTD-ALS: FTD with amyotrophic lateral sclerosis

GRN: progranulin gene

C9orf72: chromosome 9 open reading frame 72 gene

MAPT: microtubule-associated protein tau gene

TDP-43: TAR DNA-binding protein 43

FET: FUS, EWS and TAF15 proteins

FUS: Fused in sarcoma

EWS Ewing sarcoma

TAF15: TATA-binding associated factor 15

CSF: cerebrospinal fluid

CDR® plus NACC FTLD: the CDR Dementia Staging Instrument with National Alzheimer Coordinating Centre Frontotemporal Lobar Degeneration component

LC-MS/MS: Liquid chromatography-mass spectrometry/mass spectrometry

HPLC: High pressure liquid chromatography

Rab GDI alpha: Rab GDP dissociation inhibitor alpha

PEBP-1: phosphatidylethanolamine-binding protein 1

NPTXR: neuronal pentraxin receptor

NPTX1: neuronal pentraxin 1

NPTX2: neuronal pentraxin 2

SPM12: Statistical Parametric Mapping

NfL: neurofilament light chain

IS: internal standard

AD: Alzheimer's disease

MCI: mild cognitive impairment

SNARE: soluble N-ethylmaleimide-sensitive factor attachment receptor

CNS: central nervous system

Declarations

Ethics approval and consent to participate

The study was reviewed and approved by all countries' respective Ethics committees and all participants signed an informed consent to take part in the research.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, BioArctic, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Pharmatrophix, Prothena, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program, outside the work presented in this paper. HZ has served at scientific advisory boards and/or as a consultant for Abbvie, Alektor, Annexon, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. JDR has served as a consultant or on an advisory board for Alektor, Prevail Therapeutics, Denali, Arkuda Therapeutics, Takeda, UCB, Wave Life Sciences, Novartis. The other authors declare no conflicts of interest.

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Authors' contributions

AS-E and JN have contributed equally to this paper. AS-E and JN have contributed to the experimental part of the work and the data generation. IS has contributed to the sample processing and database managements. AS-E and JDR have contributed to the writing of the manuscript. JN, AB, HZ and KB have contributed to the review of the manuscript. The rest of authors part of the GENFI cohort have contributed with samples for the study and have reviewed the manuscript. All authors read and approved the final manuscript.

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Authors' information

Not applicable.

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Figures

Figure 1
Diagrammatic representation of the synapse and the role of the different synaptic proteins included within the mass spectrometry panel. Adapted from Nilsson *et al.* (14)

Figure 2
Cerebrospinal fluid (CSF) concentrations of the synaptic panel proteins (all peptides measured) in the GENFI cohort including 23 presymptomatic *MAPT* (PS MAPT), 31 *C9orf72* (PS C9) and 23 *GRN* (PS GRN) mutation carriers, 12 symptomatic *MAPT* (S MAPT), 26 *C9orf72* (S C9) and 17 *GRN* (S GRN) mutation carriers, and 61 non-carriers. Linear regression model adjusting for age at CSF sample collection and sex; bootstrapping with 2000 repetitions was used if the synaptic measures were not normally distributed. Results shown in fmol/μL. p-values: * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, and **** p ≤ 0.0001. The bars indicate the median. Specific means, IC and p-values indicated in Supplementary Table 1.

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