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RESEARCH ARTICLE

Galectin-3 is upregulated in frontotemporal dementia patients with subtype specificity

Sergi Borrego–Écija[1] | Agnès Pérez-Millan[1,2] | Anna Antonell[1] | Laura Fort-Aznar[1] | Elif Kaya-Tilki[3] | Alberto León-Halcón[4,3] | Albert Lladó[1,2] | Laura Molina-Porcel[1] | Mircea Balasa[1] | Jordi Juncà-Parella[1] | Javier Vitorica[4,3,5] | Jose Luis Venero[4,3] | Tomas Deierborg[6] | Antonio Boza-Serrano[1,3,4] | Raquel Sánchez-Valle[1,2]

[1]Alzheimer's disease and other cognitive disorders Unit. Service of Neurology, Fundació Recerca Clínic Barcelona-IDIBAPS, Hospital Clínic de Barcelona, Barcelona, Spain

[2]Institut of Neurosciences. Faculty of Medicine and Medical Sciences, University of Barcelona, Barcelona, Spain

[3]Departamento de Bioquímica y Biología Molecular, Facultad de Farmacia, Universidad de Sevilla, Sevilla, Spain, Sevilla, Spain

[4]Instituto de Biomedicina de Sevilla, IBiS/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Sevilla, Spain

[5]Centro de Investigacion Biomedica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain

[6]Department of Experimental Medical Sciences, Experimental Neuroinflammatory Lab, Lund University, Lund, Sweden

Correspondence
Antonio Boza-Serrano and Raquel Sánchez-Valle, Alzheimer's disease and other cognitive disorders Unit. Service of Neurology, Fundació Recerca Clínic Barcelona-IDIBAPS, Hospital Clínic de Barcelona, Barcelona, Spain.
Email: aboza@us.es and rsanchez@clinic.cat

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Abstract

INTRODUCTION: Neuroinflammation is a major contributor to the progression of frontotemporal dementia (FTD). Galectin-3 (Gal-3), a microglial activation regulator, holds promise as a therapeutic target and potential biomarker. Our study aimed to investigate Gal-3 levels in patients with FTD and assess its diagnostic potential.

METHODS: We examined Gal-3 levels in brain, serum, and cerebrospinal fluid (CSF) samples of patients with FTD and controls. Multiple linear regressions between Gal-3 levels and other FTD markers were explored.

RESULTS: Gal-3 levels were increased significantly in patients with FTD, mainly across brain tissue and CSF, compared to controls. Remarkably, Gal-3 levels were higher in cases with tau pathology than TAR-DNA Binding Protein 43 (TDP-43) pathology. Only *MAPT* mutation carriers displayed increased Gal-3 levels in CSF samples, which correlated with total tau and 14-3-3.

DISCUSSION: Our findings underscore the potential of Gal-3 as a diagnostic marker for FTD, particularly in *MAPT* cases, and highlights the relation of Gal-3 with neuronal injury markers.

KEYWORDS
C9orf72, CSF, frontotemporal dementia, galectin-3, GRN, MAPT, microglia, neuroinflammation

Sergi Borrego–Écija, Agnès Pérez-Millan, Antonio Boza-Serrano, and Raquel Sánchez-Valle contributed equally to this work.

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1 | BACKGROUND

Frontotemporal dementia (FTD) encloses a group of neurodegenerative disorders that have in common the neurodegeneration of the frontal and temporal lobes.[1] Due to its early onset, with most people presenting symptoms around the sixth decade of life, FTD is the second most common form of early-onset neurodegenerative dementia, after Alzheimer's disease (AD).[2,3] Clinically, FTD includes three different syndromes: the behavioral variant of FTD (bvFTD), the semantic variant of primary progressive aphasia (svPPA), and the non-fluent/agrammatical variant of primary progressive aphasia (nfvPPA).[4,5] FTD is a highly heritable disorder with mutations in Chromosome *9 open reading frame 72* (*C9orf72*), *Granulin* (*GRN*), and Microtubule-associated protein Tau (*MAPT*), explaining most genetic cases.[6,7] The neuropathological substrate of FTD is frontotemporal lobar degeneration (FTLD). FTD can be classified according to the abnormally deposited protein, which can be the tau protein (FTD-tau), the transactive response (TAR) DNA-binding protein 43 (FTD-TDP-43), or the FET family of proteins (FTD-FET).[1,8,9]

Along with neuronal dysfunction/death and protein accumulation, central immune system activation is a major factor in the progression of the pathology in FTD.[10] Abnormal protein conformation and accumulation activate the immune system, leading to neuroinflammation. This response involves glial activation and increased levels of pro-inflammatory factors.[10] Microglial cells are the main pool of innate immune cells in the brain, and their phenotype is crucial to understand the neuroinflammatory response. Recently, Malpetti et al., demonstrated that microglial activation measured by [11C]PK11195 in the frontal cortex could predict cognitive decline in patients with FTD.[11] Positron emission tomography (PET) studies using translocator protein (TSPO) have detected abnormal microglial activity and protein aggregation in familial cases of FTD.[12] PET analysis also revealed that microglial activation seems more prominent in frontotemporal regions.[12–14] The microglial activation pattern detected by PET analysis has also been observed in histological postmortem studies.[15,16] Neuropathological studies reveal microglial activation in cortical areas and more pronounced involvement in white matter than gray matter.[15,16]

A key molecule involved in microglial activation in neurodegenerative diseases is galectin-3 (Gal-3), a beta-galactosidase binding protein expressed mainly by microglial cells and associated with neurodegeneration.[17–19] Gal-3 is released into the extracellular space and acts in an autocrine or paracrine manner by binding to different membrane receptors, such as Toll Like Receptor 4 (TLR4) and Triggering Receptor Expressed on Myeloid Cells 2 (TREM2).[20,21]

We have demonstrated previously that Gal-3 is an important regulator microglial activity in AD[21] and in the aggregation process of α-synuclein and Lewy body formation in Parkinson's disease.[22,23] Of interest, Gal-3 was found primarily in microglia clustering around amyloid beta (Aβ) plaques and using 5xFAD model of AD lacking Gal-3 reduced the Aβ burden and improved their cognitive performance.[21] Our study also confirmed that Gal-3 acts as an endogenous TREM2 ligand, a central player in the regulation of microglial activation under disease conditions.[21] It is important to note that in AD we found Gal-3 in cerebrospinal fluid (CSF) to be strongly associated with neuroinflammation markers, synapse loss, and cognitive decline.[24] A recent study analyzing over 2000 human AD brain tissue samples identified a microglia module as a highly affected process in AD. The study found that Gal-3 ranked fifth among the top 30 microglial transcripts associated with AD, suggesting its significance in AD pathology and microglia dysfunction.[25] In support of this perspective and with regard to FTD, transgenic mice carrying FTD-related genes exhibit strong and even aberrant microgliosis and inflammatory response, especially evident in mice lacking *GRN*[26,27] and P301S *MAPT* mice.[28] Moreover, a recent study has shown that mutations in *GRN* mice led to significant microglial activation, which seems to be influenced by GPNMB (glycoprotein non-metastatic melanoma protein B) and Gal-3 in mice.[29]

In the context of traumatic injury models, it has been observed that TDP-43 induction contributes to the activation of microglial cells, leading to the upregulation of Gal-3.[30] Like in AD, the role of neuroinflammation and immune-mediated mechanisms in the development of FTD is well established.[10] Indeed, a causal role of neuroinflammation has been proposed, as evidenced by increased microglial activation in the frontal and temporal cortices, astrogliosis, and abnormal expression of pro- and anti-inflammatory factors in CSF and blood.[10,31,32]

Consequently, the present study explores Gal-3 levels and their link to FTD through brain, CSF, and serum biomarker analysis for FTD and its subtypes.

2 | METHODS

2.1 | Clinical cohort

A total of 133 participants were recruited at the Alzheimer's Disease and Other Cognitive Disorders Unit of the Hospital Clínic de Barcelona, including 115 patients fulfilling criteria for bvFTD (62 patients), svPPA (28 patients), or nfvPPA (25 patients), and 18 healthy controls (HCs) (Table 1). The clinical CSF FTD cohort has 6 *MAPT*

symptomatic mutation carriers, 5 presymptomatic *MAPT* mutation carriers, 13 *GRN* mutation carriers, and 13 *C9orf72* expansion carriers. All the participants underwent a complete clinical and neuropsychological examination. The participants included in the study had Gal-3 levels in CSF ($N = 133$ participants) and/or serum ($N = 120$ participants). All subjects were Caucasian white and did not present other neurological diseases. The study was approved by the ethics committee of the Hospital Clínic de Barcelona (HCB 2019/0105). Written informed consent was obtained from all participants.

2.2 | Human brain tissue

Frozen frontal cortices from 10 cognitively healthy controls and 37 FTD cases were obtained from the Neurological Tissue Bank, Biobanc-Hospital Clínic-IDIBAPS, Barcelona, Spain. All subjects were White. The control cases were not diagnosed with any neurological condition (other than migraine or essential tremor) during life, and the postmortem examination did not disclose findings supporting any neuropathological diagnosis, although minor vascular changes or a low grade of incidental pathologies were not exclusionary.[33] FTD cases included 18 mutation carriers (4 *MAPT*, 5 *GRN*, and 8 *C9orf72*; Table 2) and 19 sporadic FTD (10 tau and 9 TDP-43; Table 2). Written informed consent for using brain tissue and clinical data for research purpose was obtained from all patients or their next of kin following the International Declaration of Helsinki and Europe's Code of Conduct for Brain Banking. The medical ethics committee of the institutional review board of the Hospital Clínic (Barcelona) has approved the procedures for brain tissue collection.

2.3 | ELISA

Enzyme-linked immunosorbent assay (ELISA) plates from Abcam (ab269555) were used to measure the levels of Gal-3 (detection range 58.8 to 2000 pg/mL) in tissue homogenates, CSF, and serum samples. The protocol was carried out according to the manufacturer's instructions. A Biotek Synergy 2 was used to read the ELISA Gal-3 assay. All samples were run in duplicate once. Mean inter-assay Coeficients of Variances (CV) was 6.23. Samples were distributed in the plates according to the clinical group in similar proportions to avoid a bias caused by the plate. The ELISA and the analysis of the raw data were performed by different persons. CSF samples were not diluted. Tissue homogenates were diluted 1:100. Serum samples were diluted 1:20 or 1:50; the correction factor on diluted samples was applied when needed for the comparison. Our kit has been used previously to measure Gal-3 levels in brain, CSF, and serum samples[24,34]

2.4 | Protein extraction

Radioinmuno precipitation Assay Buffer (RIPA) solution was prepared with a protease inhibitor (cOmplete Protease Inhibitor Cocktail, Roche) and a phosphatase inhibitor (PhosphoStop, Roche). Frozen human tissue samples of the hippocampus and cerebral cortex were homogenized in RIPA buffer (1 mL/100 mg of tissue, Sigma-Aldrich, Germany) and sonicated briefly in ice. The pellet was ultracentrifuged subsequently at 25000 relative centrifugal force (rcf) for 25 min. The supernatant was isolated and used for analysis. Protein concentration was determined using a BCA Kit (Bio-Rad) according to the manufacturer's protocols. All the samples were normalized to the same concentration prior to the analysis.

RESEARCH IN CONTEXT

1. **Systematic review**: Inflammation is a critical component of frontotemporal dementia (FTD). We explore the literature using sources such as PubMed. We found only five publications under the term's "microglia", "cerebrospinal fluid", and "frontotemporal dementia". Therefore, the clinical practice suffers from a notable absence of reliable microglial proinflammatory markers associated with FTD.
2. **Interpretation**: We have uncovered evidence of a substantial upregulation of the microglial marker galectin-3 (Gal-3) in patients with FTD, thereby emphasizing the pivotal role of neuroinflammation in FTD pathogenesis and the utility of microglial markers as biomarkers. Notably, disparities in FTD subtypes, particularly the elevated levels of Gal-3 observed in *Microtubule-associated protein Tau (MAPT)* mutation carriers, suggest the possibility of FTD subtype-specific neuroinflammatory patterns.
3. **Future directions**: Future research must prioritize cerebrospinal fluid (CSF) longitudinal and independent cohort studies to determine the Gal-3-dependent neuroinflammatory response in the course of FTD. In addition, neuropathological investigations are needed to identify brain regions wherein microglial activation manifests most prominently.

2.5 | CSF biomarker analysis

The samples were processed within 2 h from needle-to-freezer (mean time 45 min). Both CSF and blood samples were centrifuged at 2000 × *g* for 10 min at 4°C. Then they were stored in polypropilene tubes and kept at −80°C until use: Storage tubes for CSF (eppendorf 0.5 mL Ref. 72.730.007 (Sarstedt)) and for serum (cryotubes 2 mL Ref. 363401PK (Nunc)).

Core AD biomarker concentrations were measured with INNOTEST ELISAs following the manufacturer's instructions (Fujirebio, Ghent, Belgium). CSF neurofilament light chain (NfL) concentration was measured using the ELISA kit of Uman Diagnostics distributed by IBL International (Hamburg, Germany). For γ—14-3-3 protein, we used the

TABLE 1 Group summaries are given as each measure's mean and standard deviation in brackets.

	Healthy Controls	All FTD	bvFTD	svPPA	nfvPPA	*p*-value*
N	18	115	62	28	25	–
N CSF	18	99	36	26	16	–
N Serum	13	107	50	24	20	–
Sex (male/female)	6/12	57/58	32/30	15/13	10/15	0.20
Age, years, mean (SD)	54.3 (4.8)	63.5 (9.1)	63.0 (9.2)	64.0 (8.4)	64.2 (9.9)	<0.001
Duration, years, mean (SD)	NA	3.4 (2.3)	3.8 (2.7)	2.9 (1.3)	3.0 (2.1)	–
Mean MMSE score (SD)	29.1 (1.1)	23.6 (5.8)	23.4 (6.3)	23.5 (5.9)	24.3 (4.7)	<0.001
Genetic (%)	0 (0%)	35 (30%)	25 (40%)	2 (7%)	8 (32%)	<0.001
Serum Gal-3, median [Q1, Q3]	1428.7 [1296.6, 1644.4]	1463.3 [1231.0, 1758.4]	1491.6 [1292.1, 1834.4]	1450.6 [1211.0, 1565.9]	1502.8 [1163.2, 1727.3]	0.80
CSF Gal-3, median [Q1, Q3]	459.0 [433.9, 501.6]	545.6 [437.2, 667.2]	567.4 [434.0, 701.1]	532.8 [505.0, 635.4]	484.0 [428.0, 652.6]	<0.05
CSF Aβ42, median [Q1, Q3]	901.8 [664.7, 1088.5]	761.7 [589.9, 944.5]	788.5 [550.4, 979.5]	730.5 [627.2, 896.8]	682.0 [589.9, 876.0]	0.13
CSF p-tau, median [Q1, Q3]	44.7 [37.4, 57.1]	40.5 [32.1, 55.2]	39.0 [32.0, 54.0]	37.3 [32.8, 49.0]	46.0 [35.3, 58.3]	0.30
CSF t-tau, median [Q1, Q3]	192.4 [165.5, 266.9]	276.4 [210.1, 409.5]	290.0 [214.0, 427.2]	260.5 [214.9, 327.5]	269.2 [209.4, 473.5]	<0.01
CSF YKL-40, median [Q1, Q3]	214.5 [170.5, 245.3]	289.0 [207.6, 371.5]	321.6 [193.1, 391.3]	275.8 [252.0, 317.4]	296.0 [214.5, 381.0]	<0.05
CSF NfL, median [Q1, Q3]	401.8 [358.2, 501.5]	2232.6 [1286.2, 3962.0]	2390.3 [1244.7, 4023.6]	1912.9 [1391.0, 2579.6]	2537.1 [1191.0, 4102.7]	< 0.001
CSF 14-3-3, median [Q1, Q3]	2571.9 [2231.2, 3001.4]	4075.0 [3042.0, 5301.3]	4133.4 [3042.0, 5509.7]	3539.9 [3155, 4997.2]	3835.9 [2968.1, 4782.7]	<0.001

The *p*-value is from the comparison between controls and all the FTD patients.
Abbreviations: bvFTD, behavioral variant frontotemporal dementia; nfvPPA, non-fluent variant primary progressive aphasia; svPPA, semantic variant primary progressive aphasia. *Between all FTD and controls.

TABLE 2 Group summaries of cortical samples, both genetic and sporadic, used in the study..

	Healthy Controls	*MAPT*	*GRN*	*C9orf72*	sFTD-tau	sFTD-TPD-43	All FTD	*p*-value*
N	10	4	5	8	10	9	37	
Sex (male/female)	5/5	3/1	2/3	4/5	4/6	6/3	20/18	
Age, years, mean (SD)	81.3 (12.3)	59.5 (3.1)	66.4 (4.8)	70.0 (11.9)	72.1 (5.5)	72.0 (7.9)	67.5 (2.1)	
Disease duration, mean (SD)	-	6.3 (2.1)	6.6 (2.4)	5.3 (4.08)	8 (3.5)	11 (4.24)	8.05 (3.9)	
Brain Gal-3, median [Q1 – Q3]	472 [449 – 524]	1509 [1156 – 1837]	1900 [1860 – 1947]	714 [454 – 767]	1137 [1046 – 1263]	589 [430 – 735]	974 [669 – 1286]	<0.001

Abbreviations: MAPT, microtubule-associated Protein Tau; GRN, Granulin; C9orf72, chromosome 9 open reading frame 72; sFTD, sporadic Frontotemporal dementia; TDP-43, Tar DNA binding protein 43.
* Between all FTD and controls.

ELISA kit CircuLex 14-3-3 gamma (MBL International Corporation, Woburn, MA, USA) with a CSF sample dilution of 1:5. CSF YKL-40 concentration was measured with an ELISA from QUIDEL (San Diego, CA, USA) using a CSF sample dilution of 1:2.5. The antibodies for the detection of these four biomarkers have been used by us and other authors on previous studies with CSF samples in neurodegenerative dementias. These biomarkers have been also studied individually using other technologies or antibodies.[35–38]

All analyses were performed by duplicate and experienced laboratory personnel blinded to clinical diagnosis. We are participants of the Alzheimer's Association QC program,[5] and Aβ42, total tau (t-tau), and phosphorylated tau 181 (p-tau181) levels obtained in our laboratory have been consistently within mean ± 2 SD.

2.6 | Statistical analysis

All FTD-related variables from the cortical tissue were analyzed with the Mann–Whitney test to compare independent groups. For multiple comparisons, the Kruskal–Wallis test followed by Dunn test was used as a post hoc correction to identify the pair-wise group differences. Receiver-operating characteristic (ROC) curves analysis was performed to assess the diagnostic accuracy of the Gal-3. For the statistical analysis of the CSF and serum levels of Gal-3, permutation tests with age and sex added as covariables were used. The *p*-values of these results were correct for multiple comparisons with the Benjamini & Hochberg correction. We compared the HCs, genetic FTD, and sporadic FTD patients with the same procedure. Finally, we studied in detail group differences for the different FTD groups (clinical phenotype and genetic form) and HCs with the same methodology. Multiple linear regression corrected by age and sex were applied to evaluate the association between the CSF Gal-3 and the other CSF FTD-related markers levels (Aβ42, t-tau, p-tau181, 14-3-3, YKL-40, and NfL levels) for abovementioned groups. For all the analyses, statistical significance was set at p-value < 0.05. Statistical analyses were carried out using GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA), SPSS v. 26 (IBM Corp., Armonk, NY, USA) software, and the language R in R-studio version 4.2.1 (https://www.r-project.org).

3 | RESULTS

3.1 | Demographic and clinical characteristics of participants

Demographic information of the study population and group statistics are shown in Table 1. Controls were younger than FTD patients (p-value < 0.001). Because Gal-3 levels in CSF showed a moderate correlation with age in the whole cohort (r = 0.42, p-value < 0.001), further statistical analyses were corrected for age. There were statistically significant differences in Gal-3 levels grouping by sex in the whole cohort; thus the analyses were also corrected for sex. We did not find a significant link between Gal-3 and disease duration. However, it is important

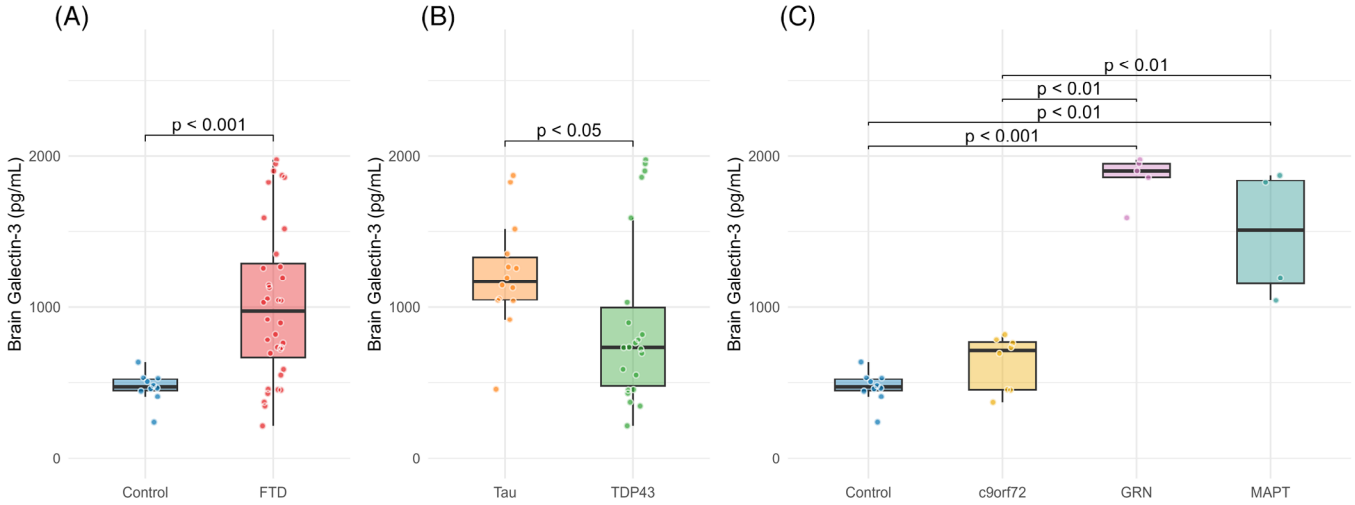

FIGURE 1 Galectin-3 (Gal-3)protein levels in brain cortex. (A) Control vs FTD cases. Gal-3 levels were significantly increased in FTD compared with controls. (B) FTD-tau vs FTD-TDP-43. FTD cases with tau pathology had higher brain levels of Gal-3 than those with TDP-43 pathology. (C) Brain Gal-3 levels in genetic carriers. *MAPT* and *GRN* carriers showed increased Gal-3 levels in brain. Cortical brain tissue was analyzed with the Mann–Whitney *U* and the Kruskal–Wallis test (multiple comparison) followed by Dunn's test used as a post hoc correction to identify the pair-wise group differences. (See Section 2.6 for further statistical analysis description.

to mention that most samples in our study were collected at the time of diagnosis, with only a few collected during the later stages of the disease.

3.2 | Galectin-3 levels are upregulated in cortical tissue from FTD

First, we evaluated Gal-3 levels in FTD brain samples. Gal-3 level was upregulated in patients with FTD as a whole compared to control samples (Figure 1A). When compared according to their neuropathological substrate, FTD-tau showed higher Gal-3 levels compared to FTD-TDP-43 cases (here we included genetic and sporadic cases with tau or TDP-43 deposition in the comparison) (Figure 1B). Finally, we evaluated Gal-3 levels in genetic cases and found increased Gal-3 levels in *MAPT* and *GRN* carriers compared to controls (Figure 1C). Both *MAPT* and *GRN* genetic cases also displayed higher Gal-3 values compared to *C9orf72* (Figure 1C). In contrast, Gal-3 levels in *C9orf72* expansion carriers did not differ from controls.

3.3 | Elevated Gal-3 levels in CSF in FTD samples

Following the brain sample analyses, we measured Gal-3 levels in CSF samples from genetic and sporadic FTD patients (Figure 2). CSF Gal-3 levels were elevated in FTD comparison to HC samples (Figure 2A). For the next analysis, we separate sporadic by clinical phenotype (bvFTD, svPPA, and nfvFTD) and genetic samples by type of mutation (*MAPT*, *GRN*, and *C9orf72*) (Figure 2B, C). The analysis of the sporadic variants of FTD resulted in higher Gal-3 levels in bvFTD compared to svPPA (Figure 2B), nfvFTD (Figure 2B), and HC samples (Figure 2B).

We observed a significant elevation of Gal-3 levels in *MAPT* carrier samples compared to *GRN* carriers (Figure 2C), *C9orf72* (Figure 2C), and HC samples (Figure 2C). No statistically significant differences were found between *GRN* and *C9orf72* groups. CSF Gal-3 levels were significantly higher for symptomatic carriers than presymptomatic *MAPT* mutation carriers (Figure 2C). In our cohort, Gal-3 CSF could be used to differentiate FTD from controls: ROC curve (area under the curve [AUC] 0.67).

3.4 | Levels in serum

Serum Gal-3 levels were also increased in FTD patients compared to controls (Figure 2D). The ROC curve, however, showed a poor performance of serum Gal-3 differentiating FTD from controls (AUC: 0.55). No significant differences were found between any of the sporadic syndromes of FTD and HCs (Figure 2E). No difference in serum Gal-3 levels were found between the different causal mutations of genetic FTD and HCs (Figure 2F).

3.5 | Association of CSF Gal-3 with other biomarkers

When studying the multiple linear regressions between different CSF biomarkers (Aβ42, t-tau, p-tau181, 14-3-3, YKL-40, and NfL) and Gal-3, we observed a significant association for FTD patients but not for the HCs (Table 3). The t-tau levels for FTD presented a moderate association with CSF Gal-3 (R = 0.43, *p*-value adjusted < 0.001) and with 14-3-3 levels (R = 0.45, *p*-value adjusted < 0.001, respectively). Gal-3 also presented a weak relationship with Aβ42 for FTD patients (R = 0.39, *p*-value adjusted < 0.001). No statistically

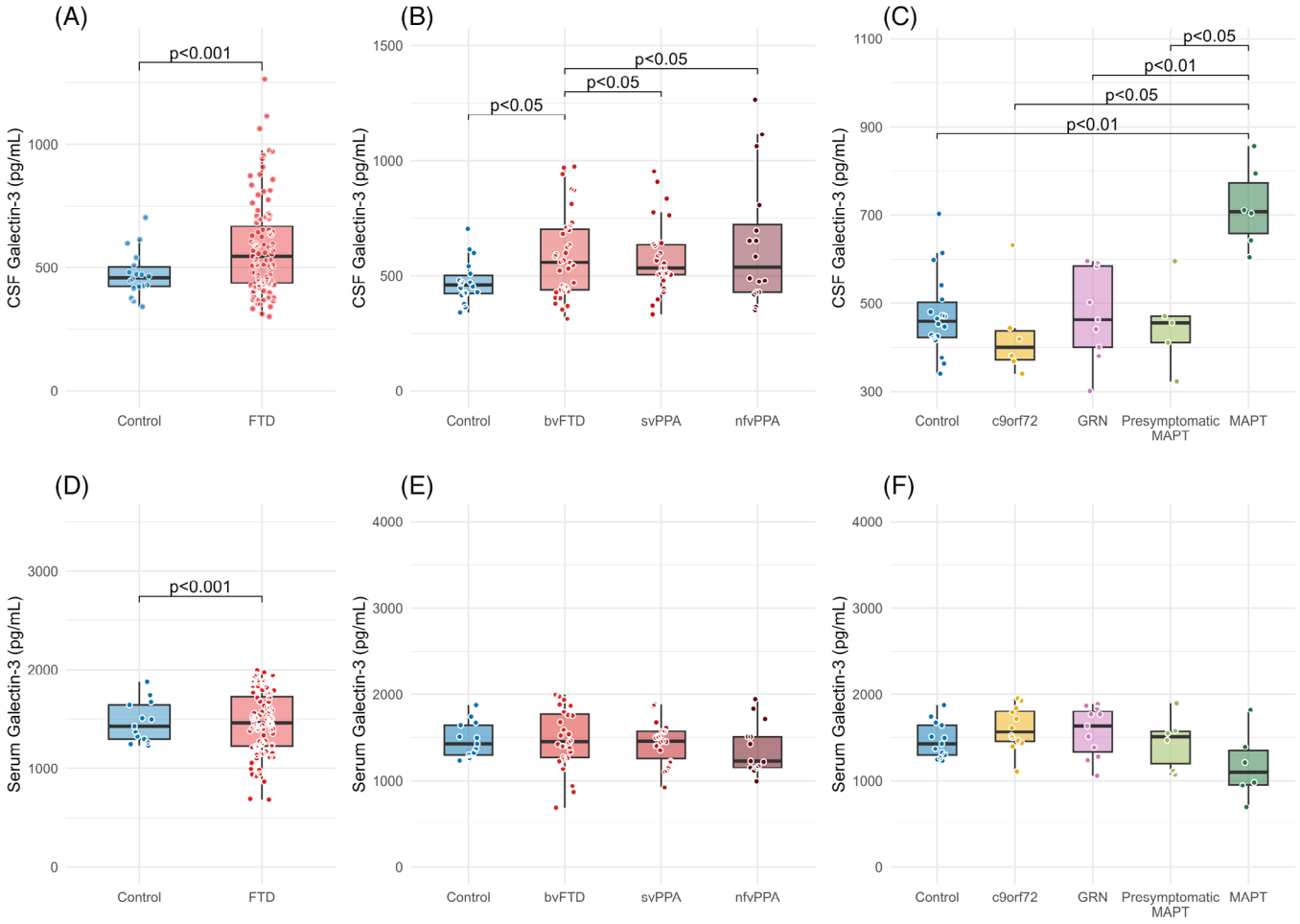

FIGURE 2 Galectin-3 levels in CSF (A, B, C) and serum (D, E, F). The *p*-values of the plots were adjusted for multiple comparisons and corrected by age and sex. (A) CSF Gal-3 levels in FTD cases are increased compared with controls. (B) Comparing FTD clinical syndromes, the bvFTD group showed higher levels of CSF Gal-3 than controls, svPPA, and nfvPPA. (C) CSF Gal-3 levels in mutation carriers revealed higher levels in *MAPT* carriers. (D) Serum levels of Gal-3 were elevated in FTD patients. (E) Serum Gal-3 levels between clinical syndromes showed higher levels in nfvPPA than in svPPA, with no differences in other comparisons. (F) No differences in serum Gal-3 levels were found between mutation carriers. (See Section 2.6 for further statistical analysis description.)

significant relationship was found between Gal-3 and p-tau181, NfL, or YKL-40. Table 3 shows the coefficients details of all the multiple linear regressions.

4 | DISCUSSION

In this study, we examined Gal-3, a microglial marker, across the FTD spectrum in neuropathological and clinical cohorts (CSF and serum levels) of both sporadic and genetic FTD patients. Our findings revealed elevated Gal-3 levels in FTD subjects' brains, CSF, and serum, thereby highlighting neuroinflammation's significance and the role of Gal-3-expressing microglia in FTD's neurodegenerative mechanism. Galectins play a crucial role in the brain's neuroinflammatory response by identifying glycan structures and sensing their modifications both intracellularly and extracellularly. Despite the importance if galectins, the regulation of their expression remains elusive.[39] Recent investigations found substantial upregulation of Gal-3 in GRN knockout Induced Pluripotent stem cells (iPSC)-derived microglia.[40] Gal-3 was detected in human studies and FTD mouse models with *GRN* gene knockout, emerging as the primary upregulated protein alongside GPNMB.[29] Other galectins such as galectin-1 (Gal-1) and galectin-9 (Gal-9) have been involved in the regulation of neuroinflammatory processes.[41,42] However, Gal-1 has been shown to deactivate microglial activation,[41] thereby reducing the associated inflammatory response. On the other hand, Gal-9 is produced mainly by astrocytes but not microglial cells and it has been shown to indirectly promote microglial activity.[42] Gal-9 CSF levels have been shown to be increased in secondary progressive multiple sclerosis[43] and have been also linked with central nervous system (CNS) immune activation and poor cognitive performance in human immunodeficiency virus (HIV) infected individuals.[44]

Due to FTD's substantial heterogeneity, significant differences emerged among clinical, genetic, and neuropathological subtypes. A relevant maker of microglial activation in disease conditions is TREM2, which is implicated in the neuroinflammatory response in AD, and has shown a strong association with FTD-tau.[45–48] We demonstrated

TABLE 3 Multiple linear regression coefficients for assessing the different CSF biomarkers trajectories by CSF Gal-3 level according to age and sex.

	Aβ42			p-Tau181			Total tau			NfL			YKL-40			14-3-3		
	β	CI 95%	*p*-value	*β*	CI 95%	*p*-value	*β*	CI 95%	*p*-value	*β*	CI 95%	*p*-value	*β*	CI 95%	*p*-value	*β*	CI 95%	*p*-value
R^2	0.1365			0.2143			0.05659			0.01183			0.1927			0.2590		
Gal3	0.6	0.3–0.9	<0.001	0.0	0.0–0.0	0.022	0.4	0.2–0.6	<0.001	1.0	-1.9–3.8	0.50	0.1	-0.1–0.3	0.40	4.7	2.5–7.0	<0.001
Age	-6.5	-12.0–-1.4	0.013	-0.1	-0.7–0.5	0.80	1.6	-1.5–4.8	0.30	12.0	-38.0–62.0	0.60	5.8	2.4–9.3	0.0010	41.0	4.1–78.0	0.030
Sex (Female vs. male)	-11.0	-107.0–85.0	0.80	-6.9	-17.0–3.1	0.20	-59.0	-117.0–0.1	0.050	16.1	-779.0–1102.0	0.70	16.0	-41.0–73.0	0.60	-527.0	-1186.0–132.0	0.12

previously that Gal-3 expressed by microglial cells can act as a TREM2 ligand.[21] In addition, we have shown a clear association of Gal-3 with tau and p-tau181 in CSF in AD and have demonstrated a clear co-localization of microglial cells expressing Gal-3 with tau protein in association of Aβ plaques.[24] Therefore, Gal-3's unique expression, pivotal role in microglial activation, and relevance in FTD progression mark it as a key molecule for future exploration, differentiating it from other galectins in understanding neuroinflammation in neurodegenerative disorders.

Microglial activation correlates strongly with FTD progression and cognitive decline.[11,12] Therefore, the reactive microgliosis observed in FTD might contribute to an upregulation of Gal-3 levels. In our study, FTD-tau exhibited higher Gal-3 levels in the brain than FTD-TDP-43. Moreover, the patients we analyzed carrying *MAPT* mutations, which cause tau pathology, showed increased Gal-3 levels in the brain. Previous neuropathological works have shown that FTD-MAPT cases present strong microglial cell activation, even more than other FTD cases.[49,50] Indeed, reactive gliosis is also prevalent in tauopathies and FTD mouse models.[27–29,51,52] Likewise, the elevation of Gal-3 in *MAPT* carriers was evident in CSF but not serum samples, indicating Gal-3 CSF's superior performance in these cases. Gal-3 serum levels did not distinguish FTD clinical forms or genetic samples, likely due to its peripheral origin (e.g., monocytes), rather than CNS microglia,[53,54] due to posttranslational modification, like phosphorylation, hampering bloodstream release[55–57] and its upregulation in other comorbidities outside the brain, such as heart disease.[58]

Regarding Gal3 levels in patients with genetic FTD, symptomatic *MAPT* carriers had higher Gal-3 levels than presymptomatic individuals, implying that Gal-3 could be a biomarker for *MAPT* carrier clinical onset or progression, pending larger longitudinal validation. In vivo evidence for presymptomatic neuroinflammation in a *MAPT* mutation carrier[59] has been found in recently. The study indicated that microglial activation is a better marker for discriminating *MAPT* mutation carriers from controls than tau protein aggregation at this pre-symptomatic disease stage of FTD.[59] This result might indicate that microglial activation in *MAPT* mutation carriers might be an early event rather than a consequence of protein dysregulation,[59] which might open up new possibilities for early anti neuroinflammatory treatments. In mouse models, Van Olst and colleagues investigated neuroinflammation in P301S MAPT mice and found that microglia changes started after neuronal p-tau deposition in the early stages of tau processing.[52] In this model, microglia adopted a p-tau-associated phenotype, morphological and functionally distinct from wild-type microglia, after neuronal p-tau accumulation was initiated. Other studies have revealed the pivotal role of microglial cells and apolipoprotein E gene (*APOE*) in driving neurodegeneration in a mouse model of tauopathy,[60] underscoring the potential critical significance of microglia in the context of FTD.

Our data demonstrated a positive association between CSF Gal-3 levels and two markers of neuronal dysfunction, 14-3-3, and t-tau.[61–63] Indeed, neuroinflammatory response has been linked to synaptic dysfunction.[64] We also demonstrated a positive relationship between CSF Gal-3 levels and t-tau in AD patients along with GAP-43 and

neurogranin, markers of synaptic dysfunction.[24] However, in this study no association was found between Gal-3 and p-tau 181, contrary to our previous study in AD patients where we observed a significant association.[24] This suggests that the mechanism of neuroinflammatory response is similar but not identical in AD compared to FTD. The timing of neuroinflammation in FTD, whether preceding neuronal dysfunction or ensuing it, is presently unclear.

Regarding *GRN* mutation, brain Gal-3 levels were also elevated in FTD subjects carrying *GRN* mutations but not in *C9orf72* expansion carriers. Other markers of inflammation, such as Glial Fibrillary Acid Protein (GFAP), have been shown to be differentially elevated in *GRN* carriers.[65] The *GRN* gene encodes the progranulin protein, which is involved in many biological processes, including inflammation, particularly in deactivating glial cells.[30] Mutations in *GRN* result in progranulin haploinsufficiency, suggesting that deficiency of progranulin in *GRN* mutation carriers may lead to pro-inflammatory glial activation and increased levels of Gal-3.[30] Indeed, recent research has highlighted the crucial role of activated microglia in *GRN* knockout mice, which drive disease progression by inducing neurodegeneration and TDP-43 protein aggregation during aging.[66] Of interest, proteomic analysis of GRN KO identified GPNMB and Gal-3 as two of the most enriched proteins in the GRN KO brain proteome, particularly in aged animals[29] and substantial upregulation of Gal-3 was found in GRN KO iPSC-derived microglia.[40] Notwithstanding, the finding of increased brain Gal-3 levels in the cortex of GRN cases was not reflected in CSF or serum in our study. This may be related to different magnitudes or dynamics of Gal-3 levels in these tissues, or due to Gal-3 upregulation occurring only in the latest stages of the disease in *GRN* carriers. More research would be needed to elucidate the role of Gal-3 in *GRN* mutation carriers.

Recent work from Woollacott and colleagues evaluated three glia-derived biomarkers in CSF: TREM2, YKL-40, and chitotriosidase in 183 participants from the Genetic FTD Initiative (GENFI), including *C9orf72*, *GRN*, and *MAPT* mutation carriers and controls. Only chitotriosidase showed increased levels in symptomatic *GRN* mutation carriers; the other group comparisons failed to show statistically significant differences.[65,67]

The differences mentioned above between neuropathological and genetic subgroups of FTD points to Gal-3 as a promising biomarker to distinguish between molecular subtypes of FTD. Although our clinical cohort did not include cases with confirmed neuropathology, our results indicated that clinical phenotypes usually associated with FTD-tau (i.e., nfvPPA) showed increased levels of CSF Gal-3 compared with clinical phenotypes usually associated with FTD-TDP-43 (i.e., svPPA). When determining the diagnostic significance of Gal-3 in distinguishing between FTD patients and controls, our analysis of ROC curves revealed that both serum and CSF Gal-3 levels displayed less accuracy compared to more established biomarkers such as NfL for this comparison.[68–71]

We note several limitations in our work. First, despite the considerable sample size of our cohort of patients with FTD, the heterogeneity of this disease makes smaller clinical or genetic subgroups leading to a lack of statistical power needed to explore subtle differences between subgroups. Second, even though we included a neuropathological cohort where we found differences in brain Gal-3 levels, the participants included in the clinical cohort lack neuropathological confirmation. Acknowledging the need for validation through a replication cohort, we are aware of limitations in obtaining CSF genetic samples from FTD individuals. In addition, we recognize the constrained sensitivity of the applied Gal-3 ELISA, as more advanced/validated platforms like single molecule array (Simoa, Quanterix) or Mesoscale Discovery platform (Mesoscale Diagnostics) lack specific Gal-3 assays. In addition, there was a significant age difference between FTD patients and controls, which may have been a confounding factor, although the analysis was adjusted for age difference. Although we did not find any significant correlation between Gal-3 and disease duration, it is important to mention that most samples in our study were collected at the time of the clinical diagnosis, with few samples collected in the later stages of the disease. A longitudinal approach would be needed to determine the association between Gal-3 level and the progression of the pathology. Finally, the presence of other co-pathologies might also induce Gal-3 elevation. However, the individuals in our cohort underwent measurements of AD-related biomarkers, and their values indicated the absence of AD pathology.

To sum up, our study robustly establishes heightened Gal-3 levels in patients with FTD, underscoring its pivotal role in neuroinflammation and potentially driving the disease pathogenesis. This deepens our comprehension of FTD's mechanisms, highlighting microglial markers as valuable biomarkers. Notably, FTD subtype variations, particularly a unique Gal-3 increase in *MAPT* mutation carriers, signifying subtype-specific neuroinflammation. Our findings align with preclinical models, accentuating neuroinflammation's acceleration of FTD progression. This accentuates potential immunomodulatory therapies and suggests evaluating microglial activation for refined clinical trial participant selection.

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CONFLICT OF INTEREST STATEMENT

R.S.V. has served in advisory boards meetings for Wave Life Sciences, Ionis, UCB, Prevail, Pfizer, and Novo Nordisk and has received personal fees for participating in educational activities from Roche Diagnostics and Neuroxpharma. The other authors declare no conflicts of interest; they declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest. Conflicts of Interest Author disclosures are available in the Supporting Information.

CONSENT STATEMENT

All the subjects provided informed consent and the study was approved by the Hospital Clínic de Barcelona Ethics Committee (HCB 2019/0105).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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