

## RESEARCH ARTICLE

# CSF placental growth factor – a novel candidate biomarker of frontotemporal dementia

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## Abstract

**Objective:** Diagnosis of frontotemporal dementia (FTD) is complicated by the overlap of clinical symptoms with other dementia disorders. Development of robust fluid biomarkers is critical to improve the diagnostic work-up of FTD. **Methods:** CSF concentrations of placental growth factor (PIGF) were measured in the discovery cohort including patients with FTD ( $n = 27$ ), Alzheimer disease (AD) dementia ( $n = 75$ ), DLB or PDD ( $n = 47$ ), subcortical vascular dementia (VaD,  $n = 33$ ), mild cognitive impairment that later converted to AD (MCI-AD,  $n = 34$ ), stable MCI (sMCI,  $n = 62$ ), and 50 cognitively healthy controls from the Swedish BioFINDER study. For validation, CSF PIGF was measured in additional independent cohort of FTD patients ( $n = 22$ ) and controls ( $n = 18$ ) from the Netherlands. **Results:** In the discovery cohort, MCI, MCI-AD, AD dementia, DLB-PDD, VaD, and FTD patients all showed increased CSF levels of PIGF compared with controls (sMCI  $P = 0.019$ ; MCI-AD  $P = 0.005$ ; AD dementia, DLB-PDD, VaD, and FTD all  $P < 0.001$ ). PIGF levels were 1.8–2.1-fold higher in FTD than in AD, DLB-PDD and VaD (all  $P < 0.001$ ). PIGF distinguished with high accuracy FTD from controls and sMCI performing better than tau/A $\beta$ 42 (AUC 0.954–0.996 versus 0.564–0.754,  $P < 0.001$ ). A combination of PIGF, tau, and A $\beta$ 42 (tau/A $\beta$ 42/PIGF) was more accurate than tau/A $\beta$ 42 when differentiating FTD from a group of other dementias (AUC 0.972 vs. 0.932,  $P < 0.01$ ). Increased CSF levels of PIGF in FTD compared with controls were corroborated in the validation cohort. **Interpretation:** CSF PIGF is increased in FTD compared with other dementia disorders, MCI, and healthy controls and might be useful as a diagnostic biomarker of FTD.

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## Introduction

Frontotemporal dementia (FTD) is one of the most common early-onset dementia with a reported prevalence rate of 3–26% in demented people with disease onset before 65 years of age.<sup>1</sup> The core features of FTD are progressive deterioration in behavior, executive function or language caused by neuronal loss in frontal and anterior temporal cortices.<sup>2,3</sup> Based on clinical presentation FTD is broadly classified into behavioral-variant FTD (bvFTD), semantic-variant primary progressive aphasia (svPPA or semantic dementia (SD)) and nonfluent variant primary progressive aphasia (nfvPPA or PNFA).<sup>4,5</sup> Neuropathologically, FTD is characterized by either intraneuronal inclusions containing tau, TAR DNA-binding protein with molecular weight 43 kDa (TDP-43), or fused-in-sarcoma (FUS) proteins.<sup>1,6</sup> Approximately 10–20% of all FTD cases show autosomal dominant inheritance.<sup>7</sup> The majority of genetic FTD is due to mutations in *MAPT*,<sup>8–10</sup> *GRN*,<sup>11,12</sup> or *C9orf72*<sup>13–15</sup> genes, which are associated with accumulation of tau (in *MAPT* mutation carriers) or TDP-43 (in *GRN* and *C9orf72* mutation carriers) inclusions. Diagnosis of FTD is challenging because of the heterogeneity of clinical presentations, symptomatic overlap with other neurodegenerative disorders and difficulties to distinguish bvFTD, particularly in early stages, from primary psychiatric conditions which leads to long periods of diagnostic delay.<sup>16</sup> Although progression of symptoms and imaging biomarkers may provide important diagnostic clues, there is a need for more cost-efficient and less time-consuming fluid biomarkers that could improve differential diagnosis of FTD.<sup>17</sup> In this study, we identified placental growth factor (PIGF) as a new candidate biomarker of FTD. PIGF is a member of the vascular endothelial growth factor (VEGF) family, originally described in placenta but later found to be expressed in other organs.<sup>18</sup> In addition to its regulatory role in pregnancy, accumulating evidence point to the biological effects of PIGF in pathological inflammation and angiogenesis associated with ischemia, hematologic diseases, and cancer.<sup>19</sup> Several studies have implicated PIGF in central nervous system disorders. Upregulation of PIGF mRNA and protein in the brain has been shown in mouse models of ischemia.<sup>20,21</sup> Furthermore, we have demonstrated elevated CSF levels of PIGF in Parkinson's disease (PD), Parkinson's disease dementia (PDD) and dementia with Lewy bodies (DLB).<sup>41</sup> Here, we measured cerebrospinal fluid (CSF) levels of PIGF in FTD and four major forms of neurodegenerative disorders with dementia. The discovery cohort

included a total of 278 patients with FTD, AD, DLB, PDD, and VaD as well as stable MCI (sMCI), MCI that progressed to AD (MCI-AD) and 50 cognitively healthy controls. We validated findings in the discovery cohort in additional independent cohort of FTD patients ( $n = 22$ ) and controls ( $n = 18$ ) from the Netherlands. Finally, in the discovery cohort, we assessed the performance of PIGF as a biomarker distinguishing FTD from controls or patients with other dementias.

## Subjects and Methods

### Study participants

Discovery cohort: Seventy-five patients with AD, 47 patients with DLB-PDD, 33 patients with VaD, 27 patients with FTD (25 bvFTD, 2 SD) and 50 healthy controls were recruited at the Memory Clinic of Skåne University Hospital in Malmö, Sweden. This cohort also included 96 individuals (recruited from the same clinic) with a baseline diagnosis of MCI. After an average clinical follow-up period of 5.7 years (3.0–9.6), 34 of those had converted to AD (MCI-AD), whereas 62 remained cognitively stable (sMCI). All study participants were assessed by medical doctors with extensive experience in cognitive disorders. All patients with a clinical syndrome of dementia met the DSM-III-R criteria for dementia<sup>22</sup> combined with the NINCDS-ADRDA criteria for AD,<sup>23</sup> the NINDS-AIREN criteria for VaD<sup>24</sup> or criteria of probable DLB according to the 2005 consensus criteria.<sup>25</sup> FTD patients were diagnosed according to Rascovsky (bvFTD)<sup>26</sup> or Neary (SD) criteria.<sup>27</sup> The FTD patients were recruited either from clinical practice or from a longitudinal FTD research study.<sup>28</sup> All patients had minimum cerebral computed tomography (most often MRI) as imaging modality, and CSF analysis of AD biomarkers were used as exclusion criteria with in-house cutoffs for clinical routine practice established at the Clinical Neurochemistry Laboratory, University of Gothenburg, Sweden following strict procedures for quality control to assure long-term stability of biomarker levels.<sup>29</sup> Of the 27 bvFTD patients, 18 were probable bvFTD, and 3 possible bvFTD, 4 definite bvFTD (3 by confirmation of TDP-43 pathology postmortem and one *C9orf72* repeat expansion carrier), and 2 SD. Patients with MCI at baseline had to fulfill the criteria advocated by Petersen.<sup>30</sup> The healthy participants were not allowed to have any cognitive complaints or any significant neurological or psychiatric illness and they needed to have a well-preserved general cognitive functioning. A careful clinical

interview, together with an assessment of global function (Mini-Mental State Examination, MMSE), delayed recall (Alzheimer's Disease Assessment Scale Cognitive Subscale, ADAS Cog, 10 word list delayed recall), attention (a quick test of cognitive speed, AQT) and visuospatial and executive function (cube-drawing test and clock test), was done to rule out mild cognitive impairment. AD biomarkers were not considered in the diagnostic process. The characteristics of the cohort are given in Table 1.

**Validation cohort:** This independent cohort included 18 cognitively healthy controls, 22 patients with FTD (14 bvFTD, 6 SD, 2 PNFA) and 5 presymptomatic individuals with a *GRN* mutation that were recruited at the memory clinic of the Erasmus Medical Center. FTD patients were diagnosed according to Rascovsky (bvFTD)<sup>26</sup> or Gorno-Tempini (SD and PNFA) criteria.<sup>31</sup> Healthy controls and presymptomatic *GRN* mutation carriers were ascertained in our longitudinal FTD-RisC cohort in which asymptomatic first-degree relatives (at-risk individuals) of patients with autosomal dominant FTD are followed.<sup>32</sup> Screening of the familial mutation is performed to divide at-risk individuals into presymptomatic mutation carriers and healthy controls, and investigators remain blinded to individual mutation status. The characteristics of the cohort are given in Table 2.

The design of this study has been approved by the Local Ethics Committee of Lund University, Sweden and by the Local Ethics Committee of Erasmus Medical Center, the Netherlands and the study procedure was conducted in accordance with the Helsinki Declaration. All

**Table 2.** Validation cohort, demographic data, clinical characteristics, and CSF levels of PIGF.

	Control (n = 18)	FTD* (n = 22)
Age	54.0 (9.2)	62.4 (7.6) <sup>a</sup>
Sex, (% female)	61%	54%
APOE	N/A	N/A
1 or 2 ε4 alleles		
MMSE**	29.6 (0.7)	23.5 (5.2) <sup>a</sup>
PIGF, pg/mL	42.2 (19.9)	59.7 (23.9) <sup>a</sup>

F, female; FTD, frontotemporal dementia; MMSE, Mini Mental State Examinations; PIGF, placental growth factor.

\*FTD group included 14 bvFTD, 6 SD, and 2 PNFA cases (2 patients with *GRN* mutation and 1 patient FTD with motor neuron disease).

\*\*MMSE was available from 18 controls and 15 FTD patients.

Data are shown as mean (SD, n) unless otherwise specified. Differences between the groups were compared using Student's *t*- and chi-square tests; <sup>a</sup>*P* < 0.05 compared with controls.

study participants (or legal representatives) gave their written informed consent to research.

### CSF sampling and biological assays

For all patients and controls, CSF samples were drawn with the patients nonfasting. CSF was collected in polypropylene tubes and mixed gently to avoid gradient effects. All samples were centrifuged within 30 min at +4°C at 2000g for 10 min to remove cells and debris. Samples were stored in aliquots at –80°C pending biochemical analysis. CSF PIGF was measured using electrochemiluminescence immunoassay as per the

**Table 1.** Discovery cohort, demographic data, clinical characteristics, and CSF levels of PIGF.

	Control (n = 50)	sMCI (n = 62)	MCI-AD (n = 34)	AD (n = 75)	DLB-PDD (n = 47)	VaD (n = 33)	FTD* (n = 27)
Age	74.2 (5.1)	69.2 (7.5) <sup>a</sup>	74.9 (7.7) <sup>b</sup>	76.4 (7.4) <sup>b</sup>	74.5 (6.3) <sup>b</sup>	75.9 (7.9) <sup>b</sup>	70.1 (6.6) <sup>a,c,d,e,f</sup>
Sex, (% female)	72%	56%	65%	68%	40% <sup>a,c,d</sup>	46% <sup>a</sup>	44% <sup>a,d</sup>
APOE	27%	47% <sup>a</sup>	82% <sup>a,b</sup>	65% <sup>a,b</sup>	54% <sup>a,c</sup>	25% <sup>b,c,d,e</sup>	27% <sup>c,d</sup>
1 or 2 ε4 alleles							
MMSE	29.0 (1.0)	28.2 (1.2)	26.4 (1.7) <sup>a,b</sup>	19.5 (3.3) <sup>a,b,c</sup>	21.9 (5.1) <sup>a,b,c,d</sup>	21.7 (4.4) <sup>a,b,c,d</sup>	22.8 (6.3) <sup>a,b,c,d</sup>
Aβ42, pg/mL	695 (282)	486 (201) <sup>a,b</sup>	317 (78) <sup>a,b</sup>	260 (105) <sup>a,b</sup>	340 (173) <sup>a,d</sup>	396 (190) <sup>a,b,d</sup>	709 (295) <sup>b,c,d,e,f</sup>
Aβ40, pg/mL	5206 (1545)	3821 (1377) <sup>a</sup>	4232 (1345) <sup>a</sup>	3899 (1376) <sup>a</sup>	3170 (1137) <sup>a,b,c,d</sup>	3238 (1285) <sup>a,c,d</sup>	4509 (1660) <sup>a,b,e,f</sup>
tau, pg/mL	443 (165)	437 (175)	645 (227) <sup>a,b</sup>	766 (266) <sup>a,b,c</sup>	472 (171) <sup>c,d</sup>	441 (192) <sup>c,d</sup>	385 (214) <sup>c,d</sup>
PIGF, pg/mL	54.8 (15.8)	64.1 (31.8) <sup>a</sup>	70.5 (20.8) <sup>a</sup>	79.5 (33.6) <sup>a</sup>	89.5 (41.4) <sup>a,b</sup>	94.2 (40.5) <sup>a,b,c</sup>	166.7 (63.4) <sup>a,c,d,e,f</sup>

AD, Alzheimer disease; DLB-PDD, dementia with Lewy bodies or Parkinson's disease with dementia; F, female; FTD, frontotemporal dementia; sMCI, mild cognitive impairment; MCI-AD, MCI that progressed to AD; MMSE, Mini Mental State Examinations; PIGF, placental growth factor; VaD, vascular dementia.

\*FTD group included 25 bvFTD (1 patient with *C9orf72* mutations and 3 patients with TDP-43 positivity neuropathologically) and 2 SD cases. *APOE* data were only available from 11 FTD patients.

Data are shown as mean (SD, n) unless otherwise specified. Demographic factors and clinical characteristics were compared using one-way ANOVA and chi-square tests. PIGF was analyzed with univariate general linear models controlling for age and sex. <sup>a</sup>*P* < 0.05 compared with controls, <sup>b</sup>*P* < 0.05 compared with sMCI, <sup>c</sup>*P* < 0.05 compared with MCI-AD, <sup>d</sup>*P* < 0.05 compared with AD, <sup>e</sup>*P* < 0.05 compared with DLB-PDD, <sup>f</sup>*P* < 0.05 compared with VaD.

manufacturer's protocol (Meso Scale Discovery, Gaithersburg, MA) with some modifications. Briefly, 10% bovine serum albumin was added to the blocking buffer and the samples were incubated overnight at +4°C. All samples were measured in duplicates. Samples from the validation and discovery cohorts were analyzed on separate occasions using different PIGF assay lots. Detection limits in the validation and discovery cohorts were 2.7 pg/mL and 3.1 pg/mL, respectively. Mean intraplate and interplate coefficients of variance (CV) were 4.5% and 7.5% in the discovery cohort and 5.2% and 3.3% in the validation cohort. Intraplate CV was below 20% for all samples except one with CV of 23%. This sample did not affect the results and was therefore included in statistical analysis. Samples were randomized according to diagnosis across plates/runs to minimize the effects of run-to-run variation. Our previous study has shown that PIGF levels do not correlated with CSF storage time (unpublished data). CSF amyloid  $\beta$  ( $A\beta$ ) 42,  $A\beta$ 40, and tau (total) were analyzed with Euroimmun immunoassay (EUROIMMUN AG, Lübeck, Germany). CSF neurofilament light chain (NfL) was analyzed as previously described.<sup>33</sup>

## Statistical analysis

SPSS version 22 (IBM, Armonk, NY) and R version 3.3.1<sup>34</sup> was used for statistical analysis. CSF PIGF levels were not normally distributed and therefore ln-transformed before the analysis. The effects of age, sex, and *APOE* genotype were tested with Pearson's correlation analysis and Student's *t*-tests. Group differences were assessed using Student's *t*-tests, one-way ANOVA and univariate general linear models (GLM). Linear regressions were used to investigate associations with CSF  $A\beta$  and tau and clinical characteristics. Age and sex were included in all regression models to control the confounding effects of these factors. Because of the relatively small sample size we did not adjust statistical analysis for age and sex in the validation cohort. Diagnostic accuracies of CSF biomarkers were assessed using receiver operating characteristic (ROC) curve analysis. Area under the curve (AUC) of two ROC curves were compared using a bootstrap procedure ( $n = 2000$  iterations). Alpha-level of significance was set at  $P < 0.05$ .

## Results

### Discovery cohort

#### Associations with demographic and clinical characteristics

CSF levels of PIGF correlated positively with age in controls ( $r = 0.284$ ,  $P = 0.045$ ) and in sMCI ( $r = 0.529$ ,

$P < 0.001$ ), AD ( $r = 0.231$ ,  $P = 0.046$ ), and FTD ( $r = 0.550$ ,  $P = 0.003$ ) patients and were higher in men than women in controls ( $P = 0.004$ ) and in patients with sMCI ( $P = 0.011$ ), DLB-PDD ( $P = 0.032$ ), and VaD ( $P = 0.011$ ). We did not find any differences in CSF PIGF concentrations between *APOE*  $\epsilon$ 4 allele carriers and non-carriers. CSF PIGF did not correlate with MMSE scores in any of the diagnostic groups or with disease duration in FTD group.

### CSF levels of PIGF in diagnostic groups

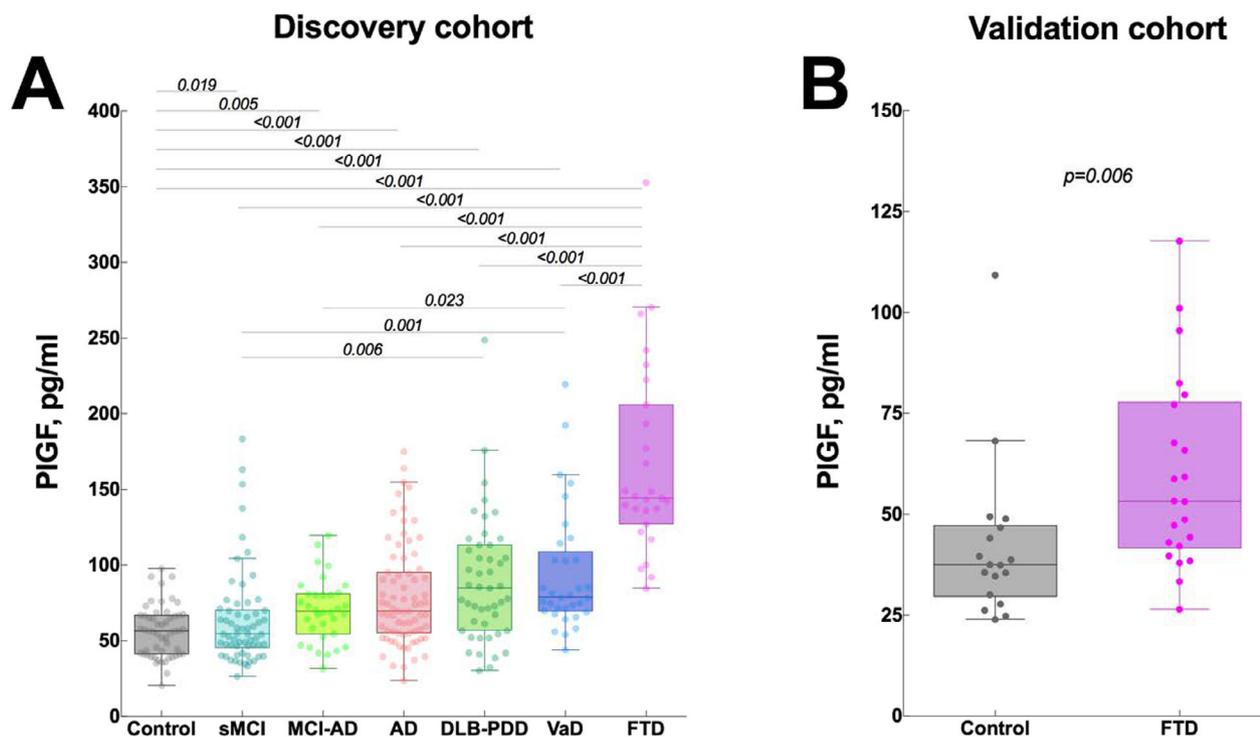
We next compared PIGF levels between different diagnostic groups using GLM adjusted for age and sex. CSF levels of PIGF were elevated in sMCI ( $P = 0.019$ ), MCI-AD ( $P = 0.005$ ), AD dementia ( $P < 0.001$ ), DLB-PDD ( $P < 0.001$ ), VaD ( $P < 0.001$ ), and FTD ( $P < 0.001$ ) compared with cognitively healthy controls (Fig. 1A and Table 1). Notably, FTD patients showed 1.8- to 2.1-fold higher PIGF levels compared to other dementias: AD, DLB-PDD, and VaD (all  $P < 0.001$ , Fig. 1A and Table 1). PIGF concentrations were also increased in FTD compared to sMCI and MCI-AD (both  $P < 0.001$ , Fig. 1A and Table 1). The results were very similar when two patients with SD were excluded from the analysis (data not shown).

In addition, we measured CSF levels of PIGF in another group of 14 cognitively healthy controls and 8 patients with FTD (Supplementary Methods and Table S1) on a separate occasion and using different PIGF assay lot. Similarly, we found increased levels of PIGF in FTD patients compared to controls ( $P < 0.001$ , Fig. S1).

### CSF PIGF as biomarkers of FTD

Previous studies have suggested that the CSF tau/ $A\beta$ 42 ratio can accurately distinguish FTD from AD dementia (AUC 0.86–0.93).<sup>35–37</sup> Here we compared the accuracy of tau/ $A\beta$ 42, PIGF, tau/PIGF, and tau/ $A\beta$ 42/PIGF in separating FTD patients from other diagnostic groups (Table 3 and Table S2). PIGF alone showed very high accuracies, sensitivities, and specificities when differentiating FTD from both controls (AUC 0.996, sensitivity 100%, specificity 96%; Fig. 2A) and sMCI (AUC 0.954, sensitivity 100%, specificity 84%) performing significantly better than tau/ $A\beta$ 42 (AUC 0.954–0.996 vs. 0.564–0.754,  $P < 0.01$ ). We did not observe any further improvement in AUCs for tau/PIGF and tau/ $A\beta$ 42/PIGF.

We then studied whether PIGF could improve the differential diagnosis of FTD versus prodromal AD (MCI-AD), AD dementia, and other dementia types, that is, DLB-PDD and VaD. The performance of tau/ $A\beta$ 42/PIGF



**Figure 1.** CSF levels of PIGF in dementia disorders. (A) Discovery cohort, CSF levels of PIGF in patients with AD, sMCI, MCI-AD, AD, DLB-PDD, VaD, FTD (25 bvFTD and 2 SD) and cognitively healthy controls. (B) Validation cohort, CSF levels of PIGF in patients with FTD (14 bvFTD, 6 SD, 2 PNFA) and cognitively healthy controls. AD, Alzheimer's disease; DLB-PDD dementia with Lewy bodies or Parkinson's disease with dementia; FTD, frontotemporal dementia; bvFTD, behavioral variant FTD; sMCI, stable mild cognitive impairment; MCI-AD, MCI that progressed to AD; SD, semantic dementia; VaD, vascular dementia.

**Table 3.** Discovery cohort, Receiver Operating Characteristic (ROC) analysis of PIGF as a biomarker of FTD.

	tau/A $\beta$ 42	PIGF	tau/PIGF	tau/A $\beta$ 42/PIGF
FTD versus controls*	0.564 (0.425–0.704)	0.996 <sup>a</sup> (0.988–1.000)	0.984 <sup>a</sup> (0.962–1.000)	0.946 <sup>a</sup> (0.889–1.000)
FTD versus sMCI	0.754 (0.638–0.870)	0.954 <sup>b</sup> (0.913–0.995)	0.962 <sup>a</sup> (0.927–0.996)	0.967 <sup>a</sup> (0.934–1.000)
FTD versus other dementia	0.932 (0.892–0.972)	0.905 (0.856–0.955)	0.934 (0.894–0.975)	0.972 <sup>b,c,d</sup> (0.949–0.996)
FTD versus MCI-AD	0.983 (0.957–1.000)	0.981 (0.955–1.000)	0.991 (0.976–1.000)	0.999 (0.995–1.000)
FTD versus AD	0.990 (0.977–1.000)	0.925 <sup>e</sup> (0.875–0.975)	0.991 <sup>c</sup> (0.978–1.000)	0.997 <sup>c</sup> (0.992–1.000)
FTD versus DLB-PDD	0.897 (0.828–0.966)	0.895 (0.822–0.969)	0.882 (0.806–0.958)	0.954 <sup>d,e,f</sup> (0.912–0.996)
FTD versus VaD	0.850 (0.754–0.945)	0.875 (0.786–0.964)	0.881 (0.795–0.967)	0.941 <sup>e</sup> (0.883–0.998)

AD, Alzheimer disease; AUC, area under the ROC curve; CI, confidence interval; DLB-PDD, dementia with Lewy bodies or Parkinson's disease with dementia; FTD, frontotemporal dementia; sMCI, mild cognitive impairment; MCI-AD, MCI that progressed to AD; PIGF, placental growth factor; VaD, vascular dementia.

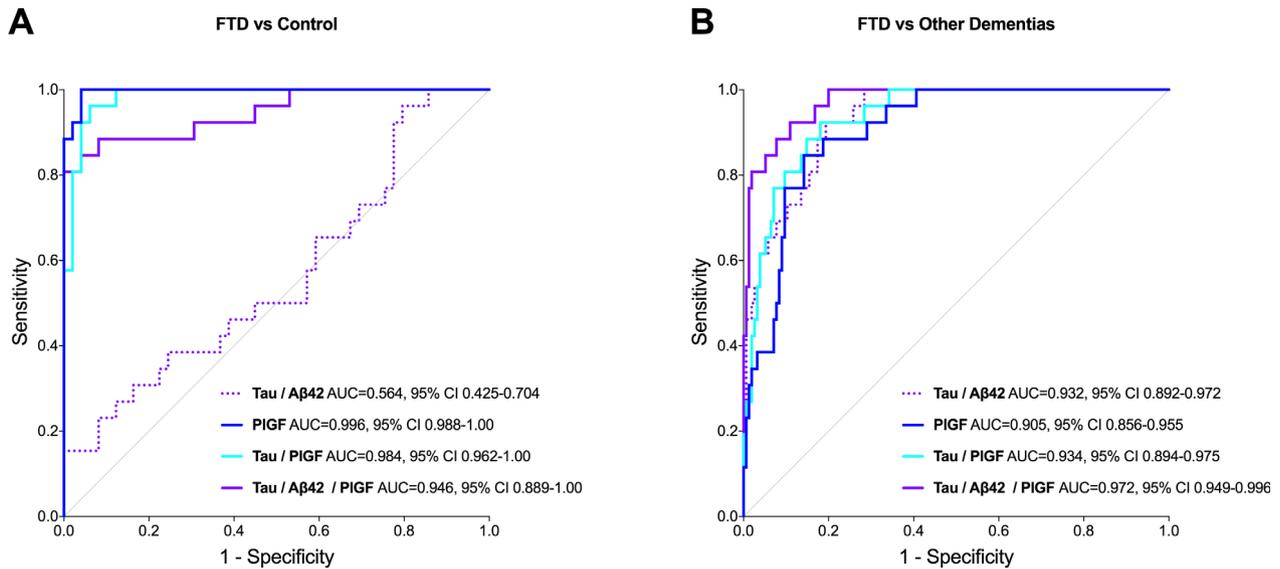
Data are shown as AUC (95%CI). \*tau data were missing from three individuals (1 control, 1 sMCI, and 1 FTD) and these individuals were excluded from all ROC analysis.

<sup>a</sup> $P < 0.001$  compared with tau/A $\beta$ 42; <sup>b</sup> $P < 0.01$  compared with tau/A $\beta$ 42; <sup>c</sup> $P < 0.01$  compared with PIGF; <sup>d</sup> $P < 0.05$  compared with tau/PIGF; <sup>e</sup> $P < 0.05$  compared with tau/A $\beta$ 42; <sup>f</sup> $P < 0.05$  compared with PIGF.

was significantly better compared to tau/A $\beta$ 42 when distinguishing FTD from the group of other dementias (AUC 0.972 vs. 0.932,  $P < 0.01$ , Fig. 2B), FTD from DLB-PDD (AUC 0.954 vs. 0.897,  $P < 0.05$ ), and FTD from VaD (AUC 0.941 vs. 0.850,  $P < 0.05$ ). In addition, tau/A $\beta$ 42/PIGF showed higher sensitivities and/or

specificities compared with tau/A $\beta$ 42 for differentiating FTD from other dementias (Table S2).

We also compared PIGF with neurofilament light chain (NFL), another promising biomarker of neuronal damage in FTD.<sup>38–40</sup> In a subcohort of 267 individuals, PIGF, tau/PIGF and/or tau/A $\beta$ 42/PIGF showed higher accuracies



**Figure 2.** ROC curve analysis in the discovery cohort. ROC curve analysis of CSF biomarkers for distinguishing FTD from controls (A) and FTD from other dementias (B). AUC, area under the ROC curve; CI, confidence interval.

than NfL when differentiating FTD from other dementia groups including AD (Table S3).

### Associations with CSF A $\beta$ and tau

CSF PIGF was positively associated with A $\beta$ 40 in FTD patients ( $\beta = 0.501$ ,  $P = 0.020$ ; adjusted for age and sex). In contrast, we found a negative correlation between PIGF and A $\beta$ 42 in the controls ( $\beta = -0.354$ ,  $P = 0.034$ ; adjusted for age and sex) but not in other groups. There were no significant associations between CSF PIGF and tau.

### Validation cohort

To confirm our findings in the discovery cohort, we measured CSF levels of PIGF in the validation cohort from the Netherlands. Similar to the discovery cohort, we found increased levels of PIGF in FTD patients (not including the 5 presymptomatic individuals with GRN mutations) compared to controls ( $P = 0.006$ , Fig. 1B, Table 2). Notably, the differences in PIGF levels between controls and FTD were more pronounced in the discovery cohort. The range of CSF concentration of PIGF also differed between the two cohorts. Possible explanations for these results might be differences in preanalytical sample handling and lot-to-lot variation in the performance of the PIGF kits.

The validation cohort comprised 14 bvFTD and 6 SD patients. We found that CSF PIGF levels were increased in bvFTD but not in SD ( $P = 0.006$  and  $P = 0.200$ , Fig. S2).

Finally, we measured PIGF in five presymptomatic individuals with GRN mutation. The mean PIGF concentration in this presymptomatic GRN group was almost as high as in bvFTD ( $60.3 \pm 37.9$  pg/mL and  $63.4 \pm 25.4$  pg/mL), however, the difference in the levels between the controls and presymptomatic GRN did not reach statistical significance ( $P = 0.156$ ) most likely due to the small sample size.

### Discussion

In the discovery cohort, we demonstrated that CSF levels of PIGF were increased in different dementia subtypes and particularly in FTD compared to cognitively healthy controls, with FTD patients showing 1.8- to 2.1-fold higher PIGF concentration than individuals with AD, DLB-PDD, and VaD. We corroborated our findings of elevated CSF PIGF in another group of controls and FTD patients from the same clinical center in Sweden and in the validation cohort from the Netherlands. Furthermore, we report that when combined with tau and A $\beta$ 42, PIGF performed better than tau/A $\beta$ 42 alone in distinguishing FTD from DLB-PDD, VaD, and all other dementias grouped together. Finally, PIGF showed higher accuracy than tau/A $\beta$ 42 in differentiating FTD from controls and sMCI.

These findings are in agreement with, and extend, our previous data on increased CSF levels of PIGF in PDD, DLB, and PD patients compared to control individuals.<sup>41</sup> Studies investigating the role of PIGF in neurodegenerative diseases are sparse and it is at present

unclear how PIGF could be linked to the core pathological features of the FTD or other dementia disorders. Hypoxia and reactive oxygen species are strong inducers of VEGF family members, including PIGF.<sup>42–44</sup> Expression of PIGF is increased in mouse astrocytes and endothelial cells following cerebral ischemia.<sup>20,21</sup> Interestingly, astrogliosis in frontal and temporal regions is one of the core histopathological hallmarks of FTD.<sup>6</sup> Furthermore, frontotemporal lobar degeneration has been shown to be accompanied by oxidative damage that targets primarily astrocytes.<sup>45</sup> Thus, it is possible that in FTD, PIGF is increased in response to astrogliosis and oxidative stress.

In contrast to CSF A $\beta$ 42 and tau, PIGF showed very high accuracy when discriminating FTD patients from controls and even sMCI patients (AUCs > 0.95) with the performance similar to CSF NfL (Table S3).<sup>38–40</sup> Although CSF NfL is a promising biomarker of neuronal damage in neurodegenerative disorders and disease severity in FTD,<sup>39,40,46,47</sup> it does not provide significant added value to CSF A $\beta$ 42 and tau for differential diagnosis of FTD because CSF levels of NfL are also elevated in many other dementias, for example, progressive supranuclear palsy (PSP) and VaD.<sup>48,49</sup> Postmortem investigations previously indicated that 10–30% of patients clinically diagnosed with FTD, had Alzheimer disease (AD).<sup>50–53</sup> However, it was later found that FTD and AD dementia differ in CSF levels of the core AD biomarkers, A $\beta$ 42 and tau: FTD patients have consistently shown higher A $\beta$ 42 and lower tau levels compared to AD dementia patients.<sup>54–56</sup> Furthermore, several studies including one in an autopsy-proven cohort, have reported that the tau/A $\beta$ 42 (or A $\beta$ 42/tau) ratio discriminated with high sensitivity (70–86%) and specificity (82–94%) between FTD and AD cases.<sup>35–37</sup> Nevertheless, there is a lack of biomarkers that could differentiate FTD from other forms of dementia such as, for example, DLB-PDD or VaD both of which may share clinical symptoms with FTD.<sup>57,58</sup> In this study, we demonstrated that PIGF combined with tau and A $\beta$ 42 (tau/A $\beta$ 42/PIGF) distinguished with high accuracy (AUCs > 0.94) FTD from DLB-PDD, VaD, and from all other types of dementia (DLB-PDD, VaD, and AD) grouped together performing significantly better than tau/A $\beta$ 42. While PIGF did not differentiate FTD from AD any better than tau/A $\beta$ 42, its accuracy was very high with AUC over 0.92. Furthermore, PIGF and/or its ratios, performed better than NfL when distinguishing FTD from other dementia groups including AD. Of note, although the diagnosis of FTD was in the first hand based on assessment of clinical symptoms and neuroimaging findings, treating physicians had access to CSF A $\beta$ 42 and tau data. Consequently, it is possible that the diagnostic performance of PIGF in comparison with A $\beta$ 42 and tau was

underestimated given the availability of CSF AD biomarkers (but not PIGF) in the diagnostic process.

One limitation of this study is that we did not measure p-tau. Recent data have indicated that p-tau/A $\beta$ 42 performs better than t-tau/A $\beta$ 42 when differentiating autopsy-confirmed frontotemporal lobar degeneration from AD.<sup>59</sup> Thus, future studies are needed to establish if PIGF could further improve the accuracy of p-tau/A $\beta$ 42 in distinguishing FTD from other dementias. Another limitation is that because FTD is a rare disease the samples size was small with only few cases had autopsy-confirmed diagnosis. Future studies in larger cohorts of neuropathologically confirmed cases should investigate PIGF levels across different dementia disorders and different clinical, pathological, and genetic FTD subtypes.

We demonstrate that CSF PIGF is increased in FTD compared with sMCI, MCI-AD, DLB-PDD, VaD, and control groups and that PIGF in combination with A $\beta$ 42 and tau accurately differentiates FTD from other dementia disorders, stable MCI patients, and cognitively healthy controls. These results suggest that PIGF offers significant promise as diagnostic biomarker of FTD and merit further studies in larger clinical cohorts.

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## Author Contributions

OH, AFS, LHM, KN, MLW, CN, KB, JCS, and SJ collected the data and reviewed the manuscript for intellectual content. OH and SJ designed the study, analyzed,

and interpreted the data, prepared figures, and cowrote the manuscript. All authors read and approved the final manuscript.

## Conflict of Interest

Santillo, Meeter, Landqvist Waldö, Nilsson, van Swieten, Janelidze report no disclosures. Blennow has served as a consultant or at advisory boards for Alector, Alzheon, CogRx, Biogen, Lilly, Novartis, and Roche Diagnostics, and is a cofounder of Brain Biomarker Solutions in Gothenburg AB, a GU Venture-based platform company at the University of Gothenburg, all unrelated to the work presented in this paper. Dr Hansson has acquired research support (for the institution) from Roche, GE Healthcare, Biogen, AVID Radiopharmaceuticals, Fujirebio, and Euroimmun. In the past 2 years, he has received consultancy/speaker fees (paid to the institution) from Lilly, Roche, and Fujirebio.

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## Supporting Information

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

**Table S1.** Demographic data, clinical characteristics, and CSF levels of PIGF in another group of 14 cognitively

healthy controls and 8 patients with FTD where CSF levels of PIGF were measured on a separate occasion and using different PIGF assay.

**Table S2.** Sensitivities, specificities, and maximized Youden index for PIGF as FTD biomarker in the discovery cohort.

**Table S3.** Receiver Operating Characteristic (ROC) analysis of PIGF and NfL as biomarkers of FTD in the discovery cohort.

**Figure S1.** CSF levels of PIGF in cognitively healthy controls and FTD patients in another group of 14 cognitively healthy controls and eight patients with FTD where CSF levels of PIGF were measured on a separate occasion and using different PIGF assay.

**Figure S2.** CSF levels of PIGF in FTD subtypes.