

# CSF PGRN May be Associated With Postoperative Delirium After Knee Replacement in Elderly Patients: A Prospective Nested Case-Control Study in the PNDABLE Study

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## Research

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# Abstract

**Background:** Postoperative delirium (POD) represents a serious complication following anesthesia and surgical procedures for patients undergoing surgical intervention. At present, there is no effective index to predict the occurrence of POD, so the aim of this study was to validate whether cerebrospinal fluid (CSF) PGRN could predict the occurrence of POD.

**Methods:** We conducted a prospective nested case-control and 1:1 matched (on age, diagnosis, American Society of Anesthesiologist' (ASA) physical status, duration of surgery, and intraoperative blood loss) study. A total of 600 Han Chinese patients over the age of 65~ 90 who underwent unilateral total knee arthroplasty were included in the PNDABLE study from June 2020 to November 2020. POD cases and non-POD controls were selected from using Confusion Assessment Method (CAM) on the first, second, third and seventh (or before discharge) postoperative days. Delirium severity was measured by the Memorial Delirium Assessment Scale (MDAS). In the six month, cognitive function was assessed with the modified Telephone Interview for Cognitive Status (TICS-m) and the World Health Organization Quality of Life brief version (WHOQOLBREF). CSF PGRN and core biomarkers were measured by ELISA using the microplate reader. The associations of CSF PGRN levels with POD risk and CSF core biomarkers ( $A\beta_{1-42}$ ,  $A\beta_{1-40}$ , T-tau and P-tau) were assessed. The effect of CSF PGRN on predicting POD occurrence was evaluated with the area under the receiver operating characteristic (ROC) curve (AUC).

**Results:** POD incidence was 9.7% (53/545). There were significant differences in preoperative CSF PGRN concentration between POD patients and non-POD ( $P < 0.001$ ), and CSF PGRN levels decreased with age, as demonstrated by a significantly negative correlation. CSF PGRN levels decrease with CAM scores and MDAS scores, as demonstrated by significantly negative correlations ( $r = -0.692$ ,  $P = 0.001$ ;  $r = -0.435$ ,  $P = 0.001$ ). There were positive associations of CSF PGRN with  $A\beta_{1-42}$  ( $\beta = 0.756$ ,  $P < 0.001$ ),  $A\beta_{1-40}$  ( $\beta = 0.637$ ,  $P < 0.001$ ) and negative associations of CSF PGRN with T-tau ( $\beta = -0.716$ ,  $P < 0.001$ ) and P-tau ( $\beta = -0.739$ ,  $P < 0.001$ ) in POD patients. The ROC curve analysis of PGRN showed that PGRN concentrations had high diagnostic value for POD.

**Conclusions:**  $A\beta$  pathology is associated with increasing in CSF PGRN whereas Tau pathology and neurodegeneration is associated with decreasing in CSF PGRN in POD patients. CSF PGRN can predict the occurrence of POD in elderly patients.

**Clinical Trial Registration:** [www.clinicaltrials.gov](http://www.clinicaltrials.gov), identifier ChiCTR2000033439.

## Introduction

Postoperative delirium (POD) represents a serious complication following anesthesia and surgical procedures for patients undergoing surgical intervention[1]. POD is characterized by temporary or permanent cognitive decline, memory impairment, deterioration in language comprehension and social adaptation ability, and POD particularly affects elderly people (> 65 years)[2]. POD can lead to increased mortality, prolonged hospitalization, other complications such as Alzheimer's disease, and higher

treatment costs[3]. Despite the prevalence and clinical importance of POD, its pathophysiology is poorly understood and no reliable biomarkers have been reported in previous studies.

PGRN (Progranulin), a multifunctional secretory protein, is a neurotrophic growth factor. The precursor proteins are hydrolyzed by extracellular proteases such as elastin into smaller peptide fragments called GRNs or epithelial proteins[4]. In the central nervous system, PGRN gene mainly exists in specific neurons, including microglial cells, cerebellar Purkinje cells and hippocampal pyramidal neurons, which have the functions of neurotrophs, prolongation of axons, promotion of neuron survival and the proliferation of neural stem cells [5]. Studies have shown that PGRN expression is significantly increased during neuroinflammation[6], and PGRN growth in microglia cells may play an important role in brain injury, neuroinflammation and neurodegeneration [7]. The decrease in PGRN expression in neurodegenerative diseases may be a self-protective mechanism to prevent cell damage. Some studies have found that PGRN protein is closely related to changes in cognitive function[8]. PGRN can improve memory loss in Alzheimer's disease by inhibiting amyloid beta deposition, and can also promote hippocampal nerve regeneration by inhibiting inflammatory response. Previous studies have shown that  $A\beta_{1-42}$ ,  $A\beta_{1-40}$  and Tau can predict POD. Therefore, to the best of our knowledge, no previous study has studied the association between CSF PGRN and POD.

So, the main objective of this study was to investigate the associations of preoperative CSF PGRN concentration with POD occurrence and the associations of preoperative CSF PGRN concentration CSF core biomarkers including  $A\beta_{1-42}$ ,  $A\beta_{1-40}$ , P-tau and T-tau for predicting POD.

## Materials And Methods

### PNDABLE study

The Perioperative Neurocognitive Disorder and Biomarker Lifestyle Study (PNDABLE) is intended to explore the pathogenesis, risk factors and biomarkers of perioperative neurocognitive disorders in the northern Chinese Han population. PNDABLE is aimed to identify lifestyle factors that may affect the risk of PND in the non-demented northern Chinese Han population in order to provide a basis for disease prevention and early diagnosis. This study has important scientific and practical values for establishing the standardized model of early diagnosis and prevention for PND in China. Informed consent was obtained from all the included patients before we extracted preoperative cerebrospinal fluid and blood of the patients. This study has been registered in the Chinese Clinical Trial Registry (clinical registration number ChiCTR2000033439) and approved by the Ethics Committee of Qingdao Municipal Hospital.

### Participants

This study has been registered in the Chinese Clinical Trial Registry (clinical registration number ChiCTR2000033439) and approved by the Ethics Committee of Qingdao Municipal Hospital.

The Han Chinese patients undergoing unilateral total knee arthroplasty (no gender limitations, aged 65 ~ 90, weight 50–80 kg, ASAⅠ~Ⅱ) combined with epidural anesthesia were enrolled in the PNDABLE study at Qingdao Municipal Hospital (East Hospital) from June 2020 to November 2020. The exclusion criteria include: (1) Preoperative MMSE score < 24 points; (2) A history of neurological and mental diseases such as Alzheimer's disease, Parkinson's disease, and cerebrovascular accident, etc.; (3) Drug or psychotropic substance abuse, as well as long-term use of steroid drugs and hormone drugs; (4) preoperative Ⅱ-Ⅲ hepatic encephalopathy; (5) Recent major surgery; (6) Severe visual and hearing impairments; (7) Abnormal coagulation function before surgery.

A total of 600 cognitively normal participants from PNDABLE had available information on covariates. We excluded 25 participants who had no information about MMSE, 5 participants without available CSF PGRN data, 17 participants who had no CSF biomarker data or had data outside four standard deviations (SD) of the mean, and 8 participants whose surgeries were suspended. Finally, 545 participants were included in this analysis and they were divided into two groups according to whether POD occurred or not: POD group and non-POD group. POD cases and non-POD controls were frequency-matched (1:1) on five variables using incidence density sampling. Specifically, one non-delirium control was randomly selected for each POD case from the source population according to the five matched variables, including age, diagnosis, American Society of Anesthesiologist' (ASA) physical status, duration of surgery, and intraoperative blood loss. These variables were listed in the European Society of Anesthesiology evidence-based and consensus-based guideline on POD and were considered to be risk factors for POD after hip fracture surgery. A patient recruitment flowchart is shown in Fig. 1.

The participants did not receive preoperative medications, and they were instructed not to drink for 6h and not to eat for 8h before surgery. After entering the operating room, we routinely monitored ECG, SpO<sub>2</sub> and NBP, opened vein access and extracted 3ml of whole venous blood. All patients underwent combined spinal-epidural block, with the space between lumbar 3–4 spinous processes (L3-4) as the puncture site. After successful puncture, 2ml of cerebrospinal fluid was extracted from the subarachnoid space, followed by injection of 2-2.5ml ropivacaine (0.66%) for about 30s. After anesthesia, the sensory level was controlled below the T<sub>8</sub> level. During the surgery, oxygen was inhaled via mask at 5 L/min to maintain blood pressure within +/- 20% of the baseline value. If intraoperative NBP < 90 mmHg (1mmHg = 0.133kPa) or it decreased by more than 20% of the baseline value, ephedrine 5mg was injected intravenously. If HR < 50 beats/min, atropine 0.5 mg was injected intravenously. Intravenous patient-controlled analgesia (butorphanol 0.1mg/ml + tropisetron 50 g/ml, diluted with normal saline to a total volume of 100 ml) was used in acute postoperative pain management. After the operation, the patient was sent to the anesthesia resuscitation room (PACU). If no abnormalities were found during a 30-minute observation period, then the patient could return to the ward with low-flow oxygen and continuous monitoring of vital signs.

We interviewed all the patients the day before surgery and collected their baseline data, including age, gender, body mass index (BMI), ASA physical status, years of education. Other information including comorbidities, past medical history, fracture classification, and types of anesthesia and surgery were also

collected according to the patients' medical records. All the history collection, physical evaluation and cognitive assessment related to dementia were conducted by neurologists.

## CSF core biomarker and CSF PGRN measurements

CSF samples were processed immediately within 2 h after standard lumbar puncture. Each sample was centrifuged at 2000×g for 10 min, and CSF samples were separated and stored in an enzyme-free EP (Eppendorf) tube (AXYGEN; PCR-02-C) at - 80°C for further use in the subsequent steps of this study. The samples were subjected to a maximum of two freeze-thaw cycles.

CSF PGRN and core biomarkers ( $A\beta_{1-42}$ ,  $A\beta_{1-40}$ , T-tau and P-tau) were measured by ELISA using the microplate reader (X) (Thermo Scientific Multiskan MK3). CSF PGRN measurements were done with ELISA kits (Human PGRN SimpleStep ELISA kit; BioVendor, no. RMEE103R) and CSF core biomarker measurements were done with other ELISA kits (INNOTEST; FUJIREBIO). All ELISA measurements were performed by experienced technicians in strict accordance with the manufacturer's instructions. They were blinded to the clinical information. The samples and standards were measured in duplicate, and the means of duplicates were used for the statistical analyses. All the antibodies and plates were from a single lot to exclude variability between batches. Moreover, the within-batch CV was < 5% and the inter-batch CV was < 15%.

## APOE ε4 in serum measurements

APOEε4 gene was PCR amplification primer sequence is: upstream, downstream of the 5'-ACAGAATTCGCCCCGGCCTGGTACACTGCCA - 3', 5'-TAAGCTTGGCACGGCTGTCCAAGGA - 3', PCR amplification reaction volume of 25 μL, reaction conditions for and steps for: Pre-denaturation at 95°C for 5.0 min, denaturation at 95°C for 1.0 min, annealing at 58°C for 1.0 min, extension at 72°C for 1.0 min, 32 successive cycles were carried out, and finally extension at 72°C for 5.0 min. Restriction enzymes of enzyme reaction for 20 μL volume, reaction conditions and steps for: 10 μL PCR amplification products, 5 μL 10 × BSA, Buffer, 0.2 μL 100 digest 6.0 Gh at 37 °C, on 8.0% polyacrylamide gel electrophoresis separation of restriction enzymes enzyme products, in the uv gel electrophoresis under the imager observations, reference standard and according to the length of the segments in APOEε4 sample under test.

## Neuropsychological Tests

The Mini-Mental State Examination (MMSE) was completed by neurologists 1d before surgery to assess the preoperative cognitive status and record relevant medical history. Patients whose MMSE scores < 23 points were excluded. Participants received interview preoperatively .

The assessment of delirium was performed in PACU, on the first, second, third and seventh days (or before discharge) after surgery at 9:00 am and at 17:00 pm by neurologists. We used the visual analog scale (VAS) score of 0–10 (lower scores indicating lower levels of pain) [9] to assess pain at the same time. POD was defined by the Confusion Assessment Method (CAM)[10], and POD severity was measured using the Memorial Delirium Assessment Scale (MDAS)[11]. The Chinese versions of CAM and MDAS

have been proven to have good reliability and validity in the Chinese elderly population[12–13]. Therefore, CAM-positive and MDA-positive patients postoperatively in PACU and on the first, second, third and seventh days (or before discharge) were recorded.

In the six month, cognitive function was assessed with the modified Telephone Interview for Cognitive Status (TICS-m)-a 12-item questionnaire that provides an assessment of global cognitive function by verbal communication via telephone; scores range from 0 to 50, with higher score indicating better function 23. The quality of life was assessed with the World Health Organization Quality of Life brief version (WHOQOLBREF)-a 24-item questionnaire that provides assessments of the quality of life in physical, psychological, and social relationship, and environmental domains. For each domain, the score ranges from 0 to 100, with higher score indicating better function.

## Statistical Analysis

The scheme comprises 4 biomarkers: aggregated A $\beta$  (A $\beta_{1-42}$ ), A $\beta$  (A $\beta_{1-40}$ ), aggregated P-tau and neurodegeneration (T-tau). And each biomarker is binarized based on whether they are normal or abnormal.

CSF PGRN didn't follow a normal distribution as assessed by Kolmogorov-Smirnov test ( $P < 0.001$ ) and visual inspection of the Q-Q plot (Fig. 1S). Therefore, they were log-transformed to obtain a normal distribution. All the statistical analyses described in this study are performed on the log<sub>10</sub>-transformed values. We performed the analysis after excluding outliers (defined as 4 SD below or above the group mean) in order to exclude the influence of extreme values. Two independent-samples' t tests were used for the comparisons between POD and NPOD groups. We used the Correlation analysis to explore whether CSF PGRN is related to CAM score and MDAS score. Given the different trends of PGRN at different ages in the biomarker framework, we applied a one-way ANCOVA followed by Bonferroni post hoc analyses.

We also studied the associations between CSF PGRN and the CSF core biomarkers for POD, using a multiple linear regression adjusted for age, gender, years of education, and APOE  $\epsilon 4$  carrier status. The analyses were performed in the total sample and then in subgroups stratified by age, gender, years of education and APOE  $\epsilon 4$  carrier status.

ROC curve analysis was used to evaluate the clinical diagnostic value of PGRN in POD. Statistical significance was set at  $P < 0.05$ . SPSS statistical software, version 21.0 (SPSS, Inc. Chicago, IL, USA), and GraphPad Prism software, version 6.01 (GraphPad Software, Inc., La Jolla, CA, USA), were used for data analysis.

## Results

### Participant characteristics

A total of 600 Han Chinese patients over the age of 65 ~ 90 who underwent unilateral total knee arthroplasty were included in the PNDABLE study from June 2020 to November 2020. The reasons for dropping out are shown in Fig. 1. And 545 patients (n = 545) remained for analyses. We found the incidence of POD was 9.7% (n = 53 of the 545 patients) via our postoperative assessments. A POD cases and non-POD controls were frequency matched(1:1) on five variables using incidence density sampling. Specifically, one non-delirium control was randomly selected for each POD case from the source population according to the five matched variables, including age, education level, American Society of Anesthesiologist' (ASA) physical status, duration of surgery, and intraoperative blood loss. (Fig. 1).

In this study, we found patients in the POD group had higher CAM and MDAS scores than the NPOD group. The preoperative MMSE score showed no significant difference between the POD group [28(26–29)] and the NPOD group [28(27-29.5), P = 0.330]. Postoperatively, the VAS score did not differ between patients with delirium 2(1–3) and without delirium [2(1–3), P = 0.080]. The demographic and clinical data of the participants are summarized in Table 1.

Table 1

Comparison of general condition and surgical condition, CFS biomarkers of unilateral total knee arthroplasty patients.

Variable	POD(N = 53)	Non-POD(N = 53)	P-values
Age (year) ( mean $\pm$ SD)	73.92 $\pm$ 6.84	70.64 $\pm$ 5.38	0.007
Gender (female/male)	22/31	20/33	0.421
Body mass index (kg.m <sup>-2</sup> ) ( mean $\pm$ SD)	24.8 $\pm$ 3.6	25.7 $\pm$ 3.4	0.187
Education level (year) (median and 25–75 percentile)	9(6-13.5)	12(9–14)	0.326
ASA physical status (I/II)	27/26	28/25	0.846
APOE $\epsilon$ 4 carriers (%)	7(13)	9(17)	0.587
Preoperative CFS A $\beta$ <sub>1-42</sub> (100pg·ml <sup>-1</sup> ) ( mean $\pm$ SD)	2.33 $\pm$ 1.35	3.01 $\pm$ 0.99	0.013
Preoperative CFS A $\beta$ <sub>1-40</sub> (100pg·ml <sup>-1</sup> ) ( mean $\pm$ SD)	34.37 $\pm$ 20.28	26.45 $\pm$ 9.24	0.011
Preoperative CFS T-tau (100pg·ml <sup>-1</sup> ) ( mean $\pm$ SD)	3.14 $\pm$ 2.06	1.19 $\pm$ 0.55	0.003
Preoperative CFS P-tau (pg·ml <sup>-1</sup> ) ( mean $\pm$ SD)	130.47 $\pm$ 0.51	69.02 $\pm$ 29.01	0.001
Preoperative CFS PGRN (100pg·ml <sup>-1</sup> ) ( mean $\pm$ SD)	27.17 $\pm$ 8.73	30.49 $\pm$ 10.04	0.001
Preoperative MMSE scores (median and 25–75 percentile)	28(26–29)	28(27-29.5)	0.330
Duration of anesthesia (min) ( mean $\pm$ SD)	133.97 $\pm$ 26.5	141.25 $\pm$ 30.1	0.963
Duration of surgery (min) ( mean $\pm$ SD)	125.72 $\pm$ 25.13	129.47 $\pm$ 26.32	0.455
Intraoperative blood loss (ml) ( mean $\pm$ SD)	582.44 $\pm$ 148.65	603.91 $\pm$ 152.77	0.465
Postoperative the highest CAM score ( mean $\pm$ SD)	31.81 $\pm$ 6.18	14.4 $\pm$ 2.66	0.001
Postoperative the highest MDAS score( mean $\pm$ SD)	22.75 $\pm$ 5.02	5.62 $\pm$ 2.43	0.001
Postoperative the highest VAS score (median and 25–75 percentile)	2(1–3)	2(1–3)	0.080

The categorical variables were expressed as counts. Normal data are given as **mean  $\pm$  SD**, whereas non-normal data are expressed as median and 25–75 percentile. Abbreviations: POD, postoperative delirium; MMSE, mini-mental state examination; ASA, American Society of Anesthesiologists; MDAS, memorial delirium assessment scale; VAS, Visual Analogue Scale/Score; SD, standard deviation; CSF, cerebrospinal fluid; A $\beta$ <sub>1-42</sub>, amyloid- $\beta$ 1–42; A $\beta$ 1–40, amyloid- $\beta$ 1–40; T-tau, total tau; P-tau, phosphorylated tau; PGRN, Progranulin

In this study, we found patients in the POD group, cognitive function in the six month did not differ with the NPOD group in Table 2.

Table 2  
Comparison of cognitive function of unilateral total knee arthroplasty patients in the six month.

Variable	POD(N = 53)	Non-POD(N = 53)	P-values
TICS-m score ( mean ± SD)	36.2 ± 2.5	37.32 ± 3.5	0.079
<b>WHOQOL-BREF (score)</b>			
Physical domain ( mean ± SD)	68.3 ± 3.1	69.6 ± 3.9	0.068
Psychological domain( mean ± SD)	75.1 ± 2.5	75.8 ± 2.9	0.193
Social relationships domain( mean ± SD)	67.5 ± 2.9	67.3 ± 2.8	0.789
Environment domain( mean ± SD)	83.6 ± 2.9	82.9 ± 2.5	0.205

## CSF PGRN Concentration

In this study, there were significant differences in preoperative CSF PGRN concentration between POD and NPOD groups ( $P < 0.001$ , Table 1). Besides, univariate logistic analysis (adjusted for age, gender, years of education, and APOE $\epsilon$ 4 carrier status) showed that PGRN was an independent risk factor for POD in elderly patients undergoing unilateral total knee arthroplasty (OR = 0.810, 95% CI 0.731–0.896,  $P = 0.001$ , Table 3).

Table 3  
Adjusted by gender, age, educational level and APOE  $\epsilon$ 4

	Unadjusted		Adjusted	
	Adjusted odds ratio (95% CI)	P-value	Adjusted odds ratio (95% CI)	P-value
Preoperative CFS PGRN	0.896(0.854–0.940)	0.001	0.810(0.731–0.896)	0.001
Preoperative CFS A $\beta$ <sub>1-40</sub>	1.035(1.006–1.035)	0.017	1.093(1.044–1.145)	<b>0.001</b>
Preoperative CFS A $\beta$ <sub>1-42</sub>	0.627( 0.448–0.876)	0.006	0.685( 0.459–1.022)	<b>0.461</b>
Preoperative CFS T-tau	3.438(1.915–6.170)	0.001	4.314(2.211–8.417)	<b>0.001</b>
Preoperative CFS P-tau	1.033(1.020–1.047)	0.001	1.040(1.024–1.057)	<b>0.001</b>

Since age is the main risk factor for POD, we explored whether CSF PGRN levels were related to aging. We found PGRN levels decreased with age increasing, as demonstrated by a significantly negative correlation

( $r=-0.814$ ,  $P < 0.001$ ). The results indicated that CSF PGRN decreased significantly between different age subgroups [65–70 years:  $40.55 \pm 0.73$  (100pg/ml),  $n = 51$ ,  $P < 0.001$ ; 65–70 years: $28.80 \pm 1.23$  (100pg/ml),  $n = 37$ ,  $P < 0.001$ ; >80 years:  $16.31 \pm 0.49$  (100pg/ml),  $n = 18$ ;  $P < 0.001$ ] (Fig. 2).

We found PGRN levels decreased with CAM and MDAS scores increasing, as demonstrated by significantly negative correlations in POD patients ( $r=-0.692$ ,  $P < 0.001$ ;  $r=-0.435$ ,  $P < 0.001$ ) (Fig. 3).

## Differences in CSF PGRN level between different subgroups stratified by biomarkers

In POD patients,the associations between CSF PGRN and CSF core biomarkers were tested in linear regression models adjusted for age, gender, years of education and APOE  $\epsilon 4$  carrier status. There were positive associations of CSF PGRN with  $A\beta_{1-42}$  ( $\beta = 0.756$ ,  $P < 0.001$ ),  $A\beta_{1-40}$  ( $\beta = 0.637$ ,  $P < 0.001$ ), and negative associations of CSF PGRN with T-tau ( $\beta = -0.716$ ,  $P < 0.001$ ) and P-tau ( $\beta = -0.739$ ,  $P < 0.001$ ) (Fig. 4).

There were no significant associations of CSF PGRN with  $A\beta_{1-42}$  ( $\beta = 0.187$ ,  $P = 0.178$ ), $A\beta_{1-40}$  ( $\beta = 0.148$ ,  $P = 0.288$ ),T-tau ( $\beta = -0.039$ ,  $P = 0.780$ ) and P-tau ( $\beta = -0.076$ ,  $P = 0.588$ ) in NPOD patients(Fig. 5).

## Predictive Effect of PGRN on Postoperative Delirium

Based on ROC curve analysis, the AUC was 0.821 (95%CI = 0.735, 0.889), sensitivity was 67.92, specificity was 96.23 for P- tau between POD and NPOD group(Fig. 6A).the AUC was 0.817 (95%CI = 0.730, 0.885), sensitivity was 62.26, specificity was 92.45 for T-tau between POD and NPOD group(Fig. 6B).the AUC was 0.795 (95%CI = 0.706, 0.867), sensitivity was 58.49, specificity was 94.34 for PGRN between POD and NPOD group(Fig. 6C). the AUC was 0.585 (95%CI = 0.485, 0.680), sensitivity was 33.96, specificity was 92.45for  $A\beta_{1-40}$  between POD and NPOD group(Fig. 6D). PGRN could predict POD occurrence among these study patients, suggesting that PGRN had a moderate predictive effect on postoperative delirium occurrence. The values were summarized in Table 4.

Table 4

The ROC curve analysis of PGRN showed that the concentrations of CSF's PGRN had high diagnostic value for POD

CSF's index	AUC	95%CI(L)	95%CI(U)	Youden's index	Sensitivity	Specificity
P-tau ( $\text{pg}\cdot\text{ml}^{-1}$ )	0.821	0.735	0.889	0.641	67.92	96.23
T-tau ( $100\text{pg}\cdot\text{ml}^{-1}$ )	0.817	0.730	0.885	0.5472	62.26	92.45
PGRN ( $100\text{pg}\cdot\text{ml}^{-1}$ )	0.795	0.706	0.867	0.528	58.49	94.34
$A\beta_{1-40}$ ( $100\text{pg}\cdot\text{ml}^{-1}$ )	0.585	0.485	0.680	0.264	33.96	92.45

## Discussion

The incidence of POD in our study was 9.7%, which was consistent with the previous results of 3.6–41% [14]. For example, previous studies have shown that the incidence of POD after the total knee and hip replacement under spine anesthesia is 20%[15]. There is still a great deal of controversy about the pathogenesis of POD. At present, there are several assumptions including cholinergic theory, inflammatory reaction and stress-response theory. There are many risk factors for POD, such as advanced age, preexisting cognitive decline, blood loss and blood transfusion, anesthetic medications, as well as postoperative pain, etc[16]. In recent years, people have tried to find ideal biological markers that can predict POD in order to reduce its occurrence.

CAM is an internationally recognized method for judging a delirium state, with a sensitivity of 94%-100% and a specificity of 90%-95%. MDAS is a scoring system of delirium severity[17]. It has a high degree of fit with DRS (delirium rating scale) and MMSE which are commonly used in psychiatry to judge the severity of delirium, and it is better than the two[18]. Now, MDAS has been widely used in clinical and research settings. Therefore, we used CAM and MDAS to evaluate the delirium status and the severity of delirium.

In the present study, we combined biomarker-based classification with age to assess changes in CSF PGRN (a marker for microglial activity) in POD patients. The application of this classification system enabled us to explore the associations between microglial inflammatory response and the pathophysiology of POD (including A $\beta$  pathology, tau pathology, and neurodegeneration). Our study showed that A $\beta$  pathology (defined as low CSF A $\beta_{1-42}$  and A $\beta_{1-40}$ ) increased with an increase in CSF PGRN, while tau pathology or neurodegeneration decreased with elevated CSF PGRN. This result confirmed the potential role of microglial inflammatory response in the pathogenesis of POD. Schott's evidence is that immunotherapy against amyloid can reduce downstream neurodegeneration, a process that may be mediated by changes in microglial activation[19].

In this study, we explored the associations between CSF PGRN and CSF biomarkers for POD to further provide theoretical basis for early warning and intervention of POD. Our results showed that in the entire data set and in POD, CSF PGRN was positively correlated with A $\beta_{1-42}$  and A $\beta_{1-40}$  as well as negatively correlated with T-tau and P-tau, further suggesting that POD was related to reactive microglia proliferation. The underlying mechanism of CSF PGRN remains to be investigated throughout the disease. In NPOD subjects, the correlations of PGRN with A $\beta_{1-42}$  and A $\beta_{1-40}$ , T-tau and P-tau disappeared. These findings suggest that CSF PGRN may indeed be associated with neuronal injury. It is also suggested that increased CSF PGRN in POD patients may be a protective response to mild neuronal injury.

Since age is the main risk factor for POD, we questioned whether CSF PGRN levels are related to aging. Our study showed that CSF PGRN levels to decrease with age. In recent years, the study of POD biochemical markers has become a focus issue. A $\beta$  and Tau protein, as one of the biochemical indicators, are the main markers of the pathogenesis of POD[20]. Some studies have also confirmed that the preoperative cerebrospinal fluid A $\beta$  and Tau in elderly patients are significantly correlated with the changes of postoperative cognitive function[21]. Progranulin (PGRN) is a multifunctional growth factor

expressed in a variety of tissues and involved in many physiological and pathological processes [22]. It is widely expressed in various cells of the body. Some studies have found that PGRN is highly expressed in neurons and microglia cells [23]. Little was previously known about the role of PGRN in the nervous system, but since the discovery of PGRN genetic polymorphisms, the number of studies on the role of PGRN in the brain has increased rapidly. Studies have found that PGRN can activate microglia cells and stimulate them to engulf the toxic A $\beta$  around them, which exerts neuroprotective effects [24]. Other studies have found that the content of PGRN in microglia cells which are around A $\beta$  deposits increases in mice [25]. Neurofibrillary tangles are one of the main pathological features of the neurodegenerative disease, which are closely related to two major proteins – Tau protein and CDK [26]. Tau protein is found throughout the nervous system, and its hyperphosphorylation is one of the early cytoskeletal changes during the formation of NFT [27]. Generally speaking, Tau protein is modified by 2–3 phosphate groups. The phosphorylation and dephosphorylation of tau protein maintain a dynamic balance, maintaining the stability of cytoskeleton [28]. In the pathological state of the neurodegenerative disease, Tau protein has 9–10 phosphate groups, leading to its hyperphosphorylation and the formation of NFT [29]. Hyperphosphorylated tau protein loses its original functions and cannot promote microtubule focusing and maintain cytoskeleton stability [30]. Studies have found that when PGRN is downregulated, it activates central cyclin-dependent kinase (CDK), which leads to reduced clearance of toxic A $\beta$  and oxidative stress [31]. Neurofibrillary tangles and neuronal loss caused by Tau hyperphosphorylation lead to cognitive dysfunction [32].

Evered et al found in their studies of cardiac surgery and non-cardiac surgery that preoperative low AB levels were associated with the occurrence of POD [33]. In another study, the basal level of A $\beta$ 1–40 was higher in the elderly group and more significantly in the elderly group, so it was suggested that A $\beta$ 1–40 might be one of the reasons for the higher incidence of POCD in the elderly. Tau protein is a microtubule-associated protein widely expressed in the nervous system. It binds and stabilizes the microtubule system, and performs various functions by interacting with a variety of proteins. Hyperphosphorylated Tau can cause disorders of related neurotransmitters and a large number of neuronal tangles in the brain, leading to impaired memory, learning and cognitive function. Planel et al. found that after isoflurane anesthesia in mice, Tau protein was hyperphosphorylated in the hippocampus, leading to decreased cognitive function [34]. The ROC curve analysis showed that PGRN concentrations had the great diagnostic value. Therefore, low PGRN concentrations can predict the occurrence and development of POD before surgery. Decreased CSF PGRN and its effects have been observed in the brains of the neurodegenerative disease patients. Decreased expression of PGRN in microglia cells around amyloid plaques is a self-protective mechanism to prevent cell damage, which offers prospects for the application of CSF PGRN as a biomarker for patients with cognitive dysfunction. Therefore, it is the future direction of our research to replicate our findings in animal experiments and explore the relevant mechanisms.

Our investigation had two limitations. Firstly, this is a prospective nested case-control study that limits any conclusions about disease progression. Therefore, results should be replicated in subjects with longitudinal data to analyze whether CSF PGRN levels are associated with disease progression. Second, cerebrospinal fluid collection is an invasive procedure. Monitoring the concentration of PGRN in the

plasma of patients will make clinical examination more convenient. This project will monitor the progression of the disease by measuring the changes of PGRN concentration in the peripheral blood through large-scale clinical studies.

## Conclusions And Implications

In conclusion, this study is based on a prospective nested case-control study. The results indicate that the occurrence and development of cognitive dysfunction in elderly patients after unilateral total knee arthroplasty may be related to the decreased expression of PGRN in CSF, and the concentration of PGRN in CSF decreases with age for POD patients. A $\beta$  pathology is associated with an increase in CSF PGRN in the absence of tau deposition and neurodegeneration, whereas tau pathology and neurodegeneration are associated with a decrease in CSF PGRN for POD patients. CSF PGRN can predict the occurrence of POD in elderly patients. Future studies should use CSF biomarkers to further explore the biological mechanisms underlying POD.

## Declarations

### Ethics approval and consent to participate

The present study followed the recommendations of the National Institute of Health guidelines for the care and use of laboratory animals and obtained approval from the Clinical Trial Ethics Committee of Qingdao Municipal Hospital, Qingdao, China.

### Consent for publication

Not applicable.

### Availability of data and material

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

### Competing interests

The authors declare that they have no competing interests with respect to the research, authorship, and/or publication of this article.

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### Authors' contributions

YLB conceived the current study. HT,YNL,XYD and FHL performed the experiments. RD, XL, XJS and BW analyzed data. XL ,RD and BW performed the experiments , wrote and revised the manuscript. All authors have contributed to the manuscript revising and editing critically for important intellectual content and given final approval of the version and agreed to be accountable for all aspects of the work presented here. All authors read and approved the final manuscript.

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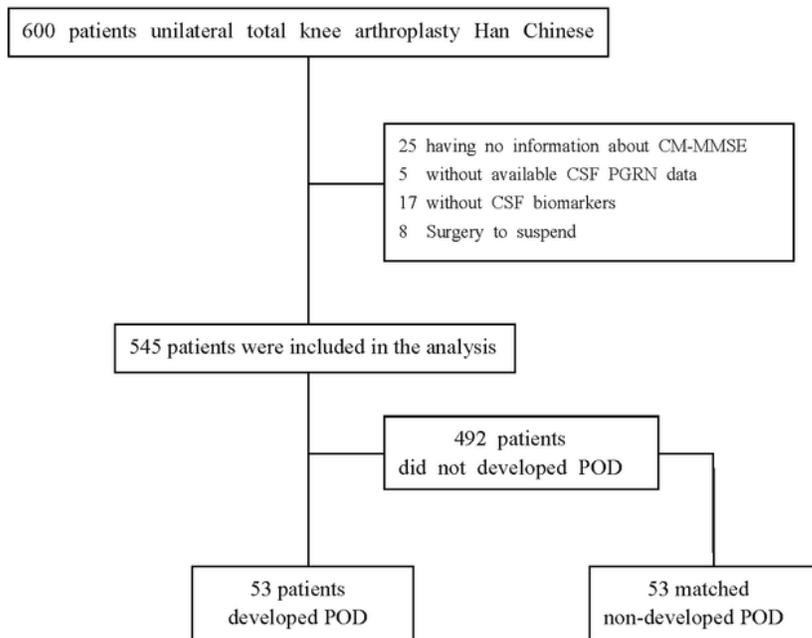
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## Figures



**Figure 1**

Flow diagram showed selection of eligible patients and the enrollment process.

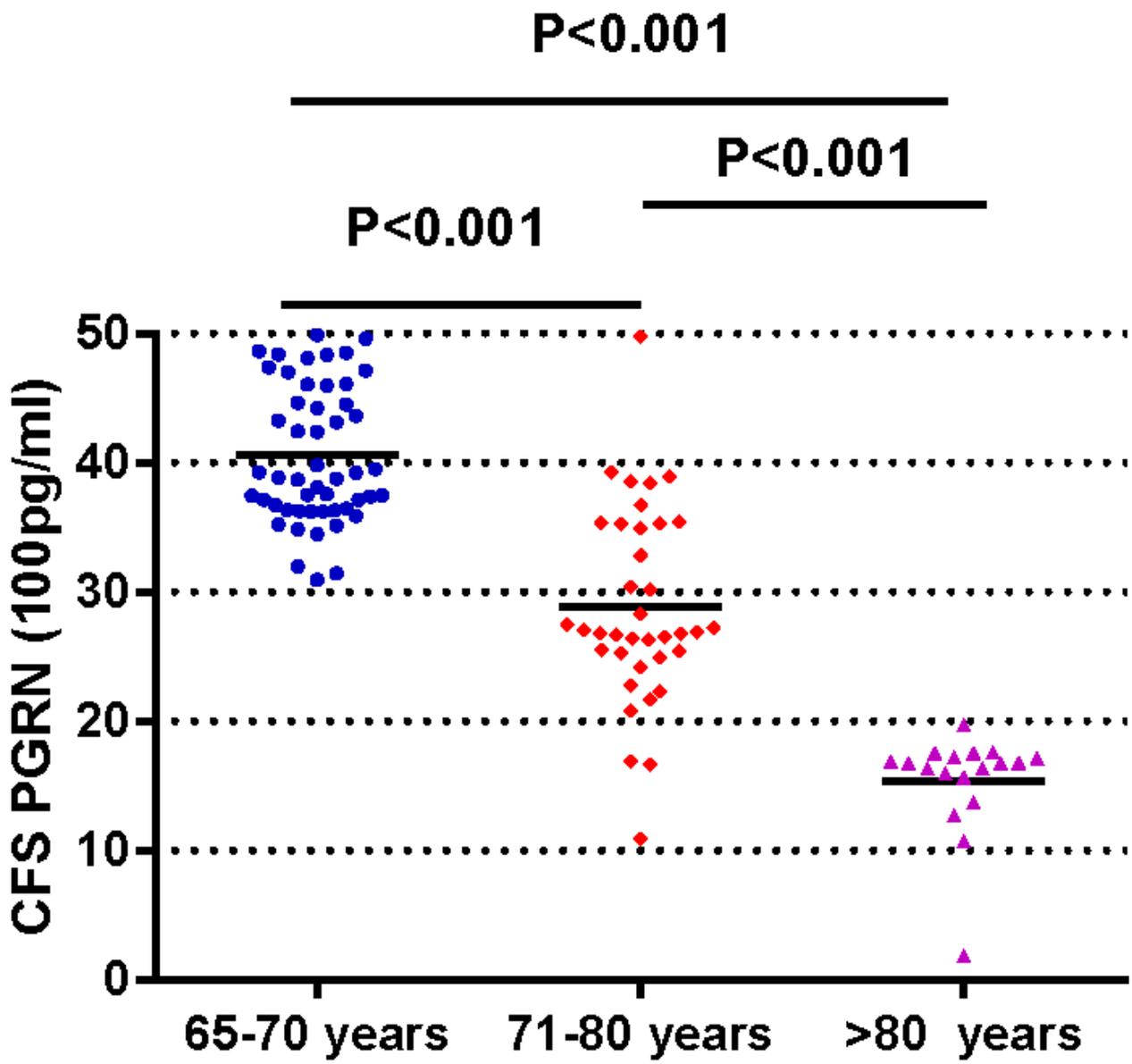


Figure 2

CSF PGRN levels are associated with different ages . P-values were assessed by student's t test.

# POD

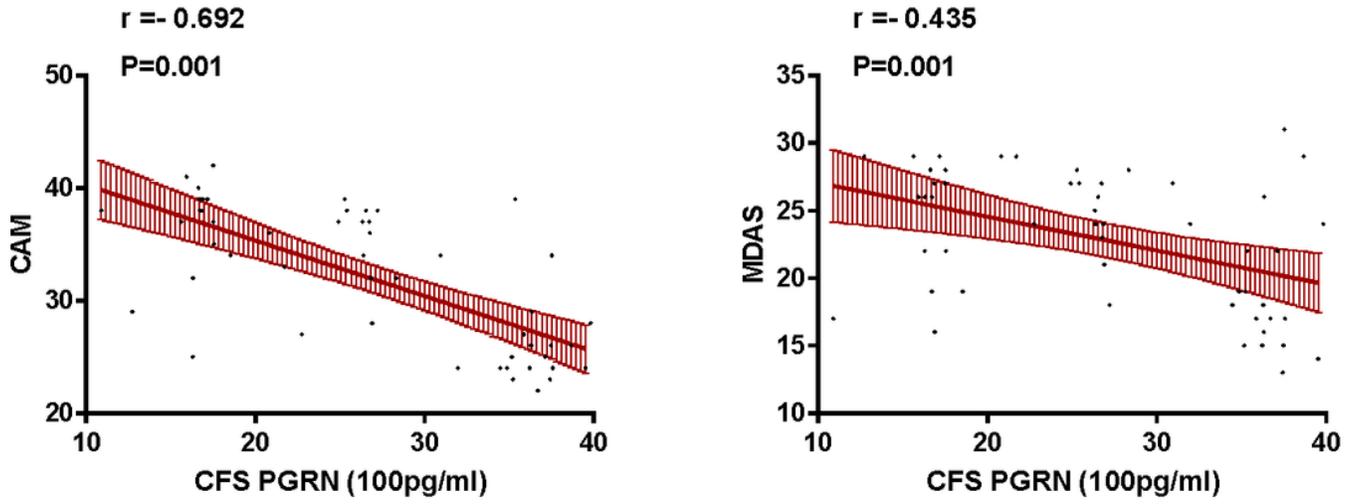


Figure 3

Associations of CSF PGRN and CAM and MDAS.

# The POD Patients

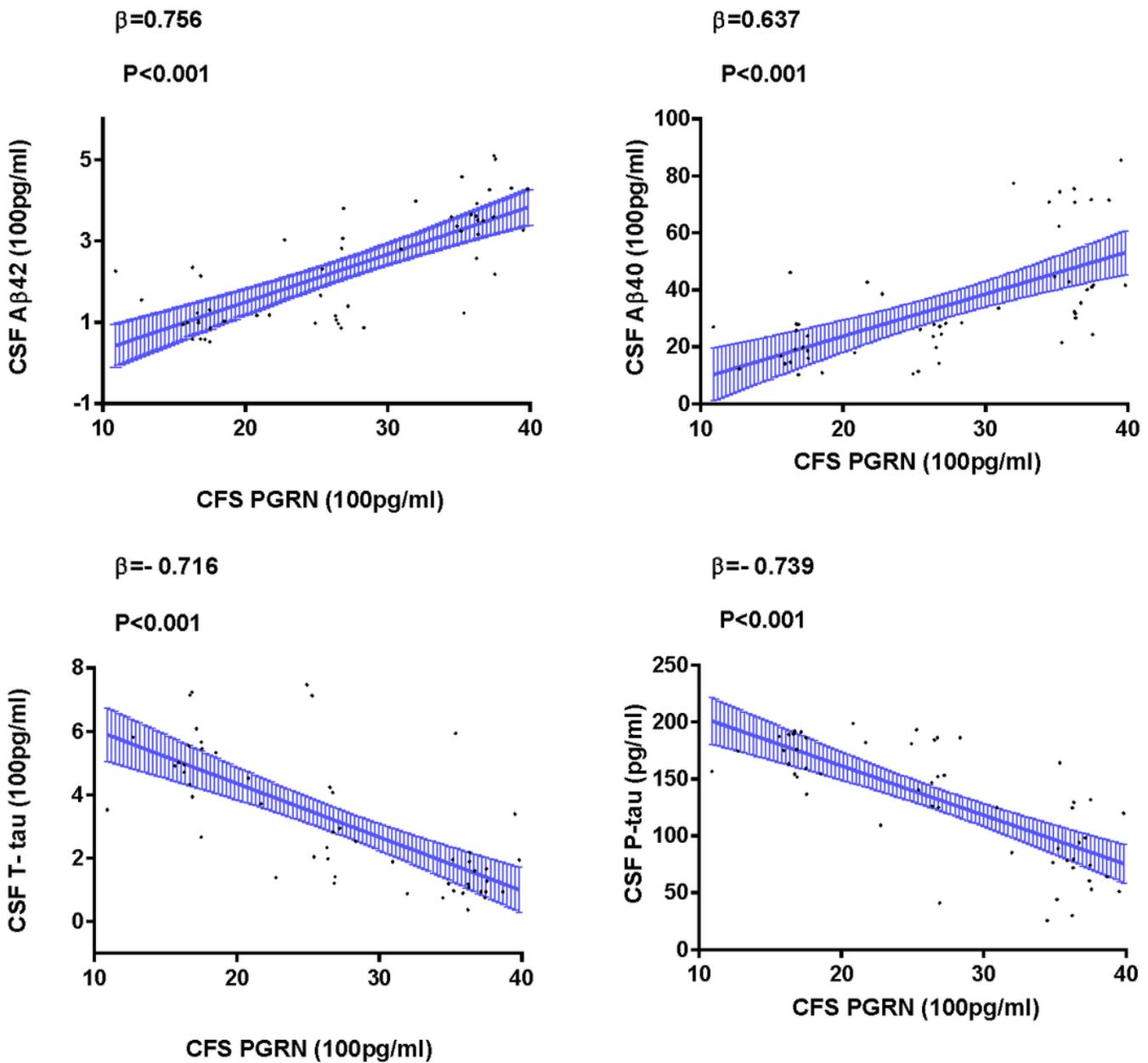


Figure 4

Associations of CSF PGRN and CSF core biomarkers. Scatter plots represent the associations of CSF PGRN with CSF core biomarkers: A $\beta$ 1–42, A $\beta$ 1–40, T-tau, P-tau, in POD groups. The normalized regression coefficients ( $\beta$ ) and P values computed by multiple linear regression after adjustment for age, gender, educational level, and APOE  $\epsilon$ 4 carrier status are shown.

# The NPOD Patients

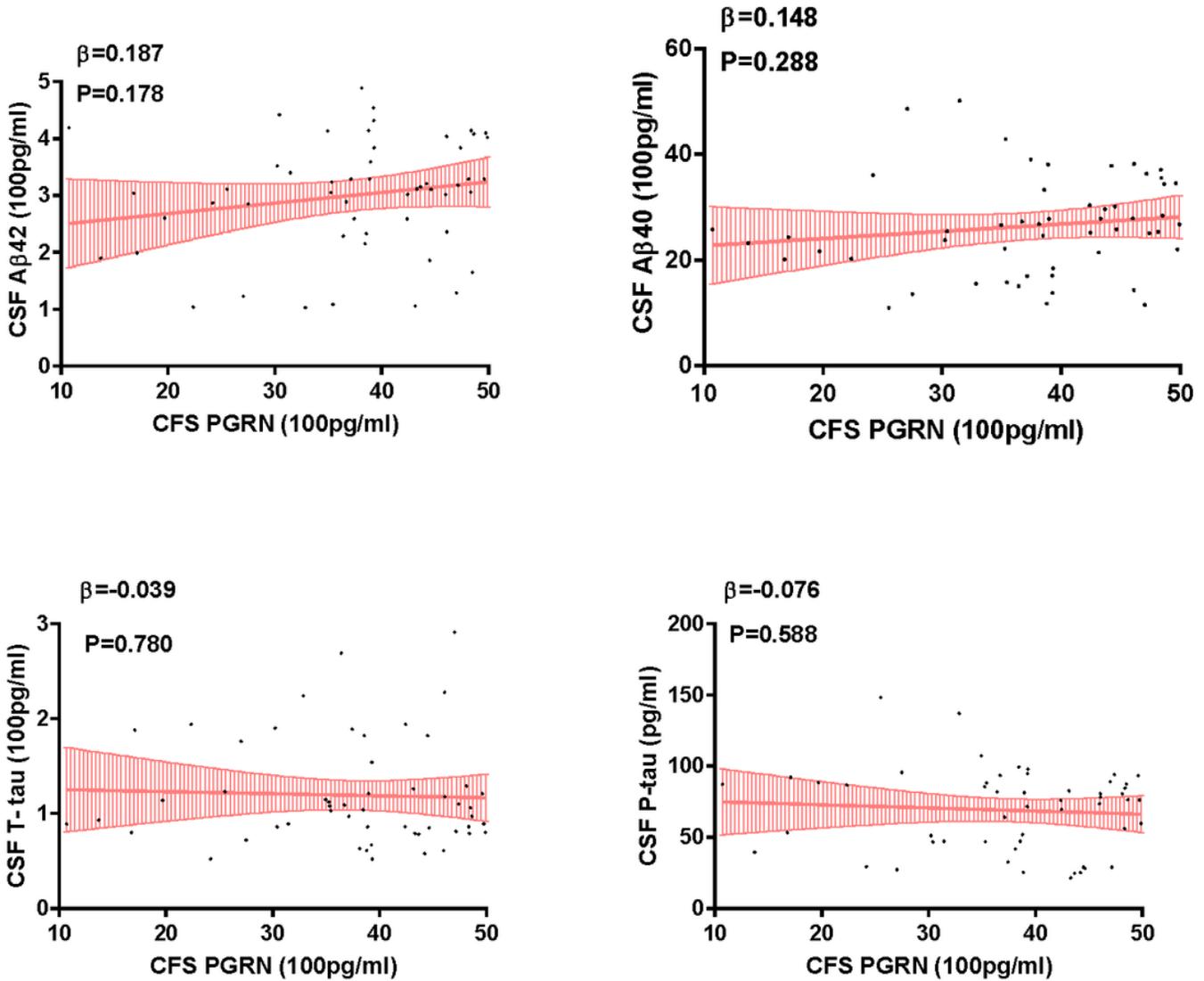
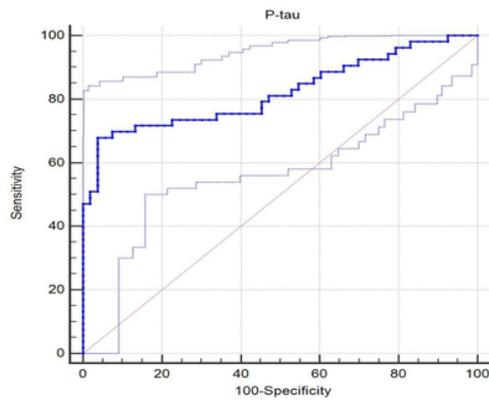
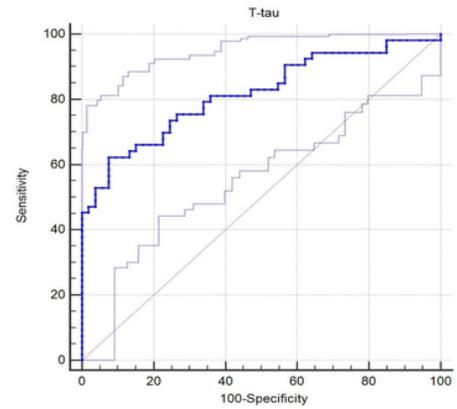
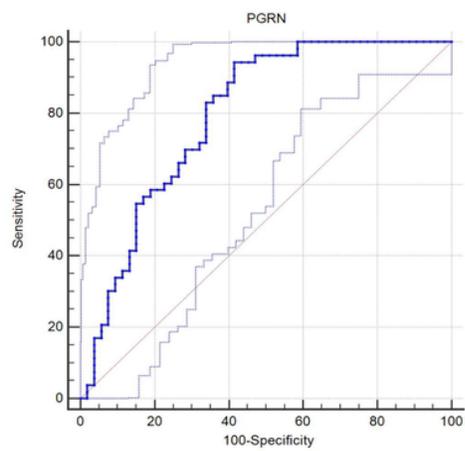
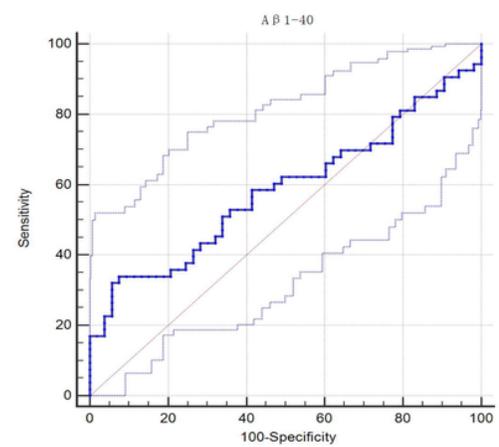


Figure 5

Associations of CSF PGRN and CSF core biomarkers. Scatter plots represent the associations of CSF PGRN with CSF core biomarkers: Aβ1–42, Aβ1–40, T-tau, P-tau in NPOD patient groups. The normalized regression coefficients ( $\beta$ ) and P values computed by multiple linear regression after adjustment for age, gender, educational level, and APOE  $\epsilon$ 4 carrier status are shown.

**A****B****C****D****Figure 6**

The ROC curve analysis of PGRN showed that the concentrations of CSF PGRN had high diagnostic value for POD

**Supplementary Files**

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- [Figure1s.pdf](#)