1   **Cerebrospinal fluid brevican and neurocan fragment patterns in human traumatic**

2   **brain injury.**

3

4   Karolina Minta[a,*], Gunnar Brinkmalm[a,b], Eric P. Thelin[c,d], Faiez Al Nimer[c], Fredrik Piehl[c],

5   Mats Tullberg[e], Anna Jeppsson[e], Erik Portelius[a,b], Henrik Zetterberg[a,b,f,g], Kaj Blennow[a,b], Ulf

6   Andreasson[a,b]

7

8   [a]Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the

9   Sahlgrenska Academy at the University of Gothenburg, Sweden [b]Clinical Neurochemistry

10   Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden [c]Department of Clinical

11   Neuroscience, Karolinska Institutet, Stockholm, Sweden [d]Department of Neurology,

12   Karolinska University Hospital, Stockholm, Sweden [e]Department of Clinical Neuroscience,

13   Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of

14   Gothenburg, Sweden [f]Department of Neurodegenerative Disease, UCL Institute of Neurology,

15   London, UK [g]UK Dementia Research Institute at UCL, London, UK

16

17   *Corresponding author:

18   Karolina Minta

19   Department of Psychiatry and Neurochemistry

20   Sahlgrenska University Hospital/Mölndal,

21   S-431 80 Mölndal, Sweden

22   e-mail: karolina.minta@neuro.gu.se

23

24

25

26   **Abstract**

27   **Background:** Altered levels of two extracellular matrix (ECM) proteoglycans, brevican and

28   neurocan, have been found in brain injury models; however, their proteolytic processing in

29   traumatic brain injury (TBI) remains unexplored. A disintegrin and metalloproteinase with

30   thrombospondin motifs (ADAMTS) is a possible contributor to ECM remodelling following

31   TBI. The aims of this study were to evaluate proteolytic brevican/neurocan patterns and

32   ADAMTS-like activity in cerebrospinal fluid (CSF) in the context of TBI.

33   **Materials and methods:** Forty-two acute TBI patients and 37 idiopathic normal pressure

34   hydrocephalus (iNPH) patients were included in the analysis of tryptic brevican and neurocan

35   peptides in CSF using parallel reaction monitoring mass spectrometry. Twenty-nine TBI and

36   36 iNPH patients were analysed for ADAMTS-like activity in CSF using a quenched

37   fluorescent substrate.

38   **Results:** The majority of CSF concentrations of brevican peptides significantly decreased in

39   TBI patients compared with the iNPH group ($p \leq 0.002$), while ADAMTS-like activity

40   increased ($p < 0.0001$). Two C-terminal brevican peptides strongly correlated with

41   unfavourable outcome of TBI patients (rho=0.85-0.93, $p \leq 0.001$).

42   **Conclusions:** The decreased CSF concentrations of brevican peptides in TBI are associated

43   with their increased degradation by ADAMTS enzymes. Furthermore, the N- and C- terminal

44   parts of brevican are differentially regulated following TBI and may serve as outcome

45   markers.

46

47   **Keywords:** brevican; cerebrospinal fluid; idiopathic normal pressure hydrocephalus;

48   neurocan; parallel reaction monitoring mass spectrometry, traumatic brain injury.

49

1. Introduction

Traumatic brain injury (TBI) is a structural and functional brain damage induced by an

external force, affecting approximately 70 million people worldwide each year [1]. TBI

involves a large spectrum of complex pathophysiological processes affecting neuronal, glial

and microvascular elements of the brain [2]. Axonal injury is considered to be a central

mechanism in TBI pathology, which in severe forms of TBI can be accompanied by other

secondary changes including blood-brain barrier (BBB) impairment, mitochondrial

dysfunction, inflammation and oxidative stress [2]. The identification of TBI severity at an

early stage is essential for the effective clinical management to limit the secondary brain

injury. TBI is clinically grouped by severity into mild, moderate and severe, commonly based

on levels of consciousness following injury (commonly using the Glasgow Coma Scale

(GCS) [3]). There are several additional classification systems for TBI severity, to which the

following belong: anatomically-based injury severity scoring (Abbreviated Injury Scale (AIS)

[4]) and computed tomography (CT) scans of the brain injury (Marshall-CT classification [5],

3

75 Rotterdam-CT score [6], Stockholm CT score [7]). In addition, the Glasgow Outcome Scale

76 (GOS) is a global assessment (5-categories) used to rate functional recovery following TBI

77 [8].

78 Cerebrospinal fluid (CSF) is a body fluid, which in terms of composition can reflect the

79 biochemical changes that occur in the brain [9]. In biomarker research, CSF is preferred over

80 other body fluids due to its proximity to the brain and decreased exposure to the confounding

81 effects of extracerebral factors. Therefore, CSF biomarkers could contain relevant markers of

82 severity and outcome following brain injury. Several CSF biomarkers show promise as tools

83 to identify and monitor brain injury, including neurofilament light (NFL) [10, 11], total tau (t-

84 tau) [10-14], S100 calcium-binding protein B (S100B) [11, 15, 16] and neuron-specific

85 enolase (NSE) [16]. These biomarkers reflect either neuronal or glial cell damage and are

86 elevated in TBI patients [10-16]. However, severe TBI involves many other neuropathological

87 changes, *e.g.*, haemorrhage, oedema and neuroendocrine complications [2].

88 Changes in the composition and function of the brain extracellular matrix (ECM) have also

89 been observed following brain injury [17] and could act as potential therapeutic targets for

90 TBI treatment. The brain's ECM is a network composed of various molecules, including

91 proteoglycans, glycoproteins and glycosaminoglycans [18]. Under physiological conditions,

92 the biochemical and biophysical properties of ECM are tightly controlled by the specific

93 composition and amount of matrix molecules, in turn supporting cellular functions [19].

94 However, during pathological conditions, such as brain injury, the ECM composition may

95 become dysregulated, which can contribute to pathology and cellular dysfunction [19]. Thus,

96 the biochemical indicators of ECM pathologies in TBI could help in understanding the role of

97 complex processes surrounding ECM production and remodelling in the aftermath of TBI.

98 Brevican and neurocan, which are CNS-specific chondroitin sulfate proteoglycans (CSPGs),

99 regulate axonal guidance and modulate synaptic connections [20]. However, following brain

injury, CSPGs are rapidly upregulated at the lesion site forming a barrier to axonal growth [20]. Thus, they might serve as candidate markers for inhibitory processes of axonal regeneration following TBI. The measurement of these proteoglycans in CSF could provide a possible route to monitor ECM pathophysiology in humans. We have previously studied brevican and neurocan in CSF following TBI, with conflicting dynamics which were shown to be assay dependent for brevican [21]. In theory, brevican might be present both as a full-length molecule and as endogenous protein fragments. These various brevican forms can indicate different pathophysiological mechanisms in TBI. It has been found that both brevican and neurocan undergo proteolytic cleavages in rodent brain [22-24]. Both proteins are substrates for matrix proteases, including a family of a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) and matrix metalloproteinases (MMPs) [25-27]. Brevican is cleaved mainly in the central region forming 53/55 kDa and 80 kDa brevican fragments [28-30]. In humans, the major MMP cleavage site is at ^{361}A/I^{362}, while ADAMTS cleaves at ^{400}E/S^{401} [26]. Although, neurocan can be present in two proteolytic forms, 130 kDa and 150 kDa, in the rat brain [23, 24], its ADAMTS and MMPs–specific proteolytic cleavage sites have not been established in human. MMPs are markedly elevated in brain tissue, CSF, and blood in patients with TBI [31, 32] and their activation attributes to the further exacerbation of the brain injury, such as the disruption of the BBB integrity by degrading tight junction proteins [33-36]. Although ADAMTS expression and activity were reported to be elevated in rodent brain following CNS injury [22, 37], its' levels or enzymatic activity in human CSF have not been studied. Since brevican and neurocan regulate neuronal plasticity, the enzymatic cleavage of these proteins may contribute to either damage or repair following brain injury [26]. However, a majority of animal studies support a role for ADAMTS in recovery following brain injury by stimulating axonal outgrowth and synaptic plasticity [26]. The proteolytic processing of brevican and neurocan in relation to human TBI is largely

125 unexplored. CSF analysis of brevican and neurocan fragmentation patterns may provide better

126 understanding of the clinical manifestations and course of TBI.

127 The aims of this study were to (1) compare the CSF concentrations of brevican and neurocan

128 tryptic peptides in TBI patients to a contrast group; (2) investigate if they are associated with

129 TBI severity as well as functional outcome following TBI; (3) evaluate their dynamics over

130 time after the brain trauma; (4) investigate ADAMTS-derived peptides in CSF; and (5)

131 explore ADAMTS-like enzymatic activity in CSF in relation to TBI.

132

133 **2. Material and methods**

134

135 *2.1. Patients*

136 Forty-two TBI patients requiring neurocritical care and intracranial pressure monitoring were

137 included in the study (Table 1). The clinical diagnostic criteria of the TBI patients have been

138 described previously [38]. CSF, collected through an external ventricular drain (EVD)

139 inserted in either one of the lateral ventricles or the third ventricle, was drawn at three time

140 points following TBI: time point 1 (1-5 days), time point 2 (4-8 days), time point 3 (7-11

141 days). Samples were centrifuged for 15 minutes at 2000 g, aliquoted and stored at -80 °C.

142 Commonly used classification systems for TBI severity indicated that the patients suffered

143 from severe trauma (Table 1). Functional outcome of patients was determined by GOS [3],

144 assessed at 12 months following TBI and dichotomized into favourable (GOS=4-5) and

145 unfavourable (GOS=1-3). The majority of the TBI patients (n=29) suffered from intracranial

146 injury, while 13 patients had extracranial complications.

147 As an alternative to a healthy control group, thirty-seven idiopathic normal pressure

148 hydrocephalus (iNPH) patients without brain trauma were included in the study as a contrast

149 group (Table 1), due to inability of collecting ventricular CSF from healthy controls. Here,

150 CSF was collected through a catheter entered into the right lateral ventricle immediately prior

151 to shunt placement.

152 To investigate ADAMTS-like activity in CSF, a subcohort consisting of 29 severe TBI and 36

153 iNPH patients was analysed (Supplementary table 1).

154 In the sample preparation and the data acquisition, TBI samples from the same individual but

155 from different time points were placed close to each other and iNPH samples were positioned

156 alternately to the TBI samples to reduce possible variability across the assay.

157

158 *2.2. Brevican/neurocan panel*

159 The panel of brevican and neurocan peptides was previously described in detail [Minta et al.

160 2020, submitted]. Briefly, 20 isotope-labelled tryptic peptides (n=9 for brevican and n=11 for

161 neurocan) (Fig. 1), labelled with both ^{13}C and ^{15}N at the C-terminal arginine or lysine were

162 used as reference peptides (JPT Peptide Technologies, Berlin, Germany). Twenty-five µL of

163 the internal standard mixture was spiked into 25 µL CSF. Reduction and alkylation, followed

164 by trypsination and sample clean-up were performed.

165 Prior to liquid chromatography-mass spectrometry (LC-MS) analysis, the samples were

166 reconstituted in 100 µL 50 mM ammonium bicarbonate (NH_4HCO_3). Each sample (90 µL)

167 was loaded onto a Hypersil Gold reversed phase HPLC C18 column (Thermo Fisher

168 Scientific) operated at a flow rate of 300 µL/min on a gradient going from 0 to 40% B over 21

169 min using a Vanquish UHPLC (Thermo Fisher Scientific). The parallel reaction monitoring

170 (PRM) MS analysis was performed using a Q Exactive hybrid quadrupole-orbitrap high

171 resolution mass spectrometer (Thermo Fisher Scientific), with electrospray ionization,

172 operated as described previously [Minta et al. 2020, submitted] [39].

173

174 *2.3. Explorative analysis of ADAMTS cleavage in CSF*

For the identification of proteolytic protein fragments generated by ADAMTS cleavage in CSF, immunoprecipitation (IP) followed by digestion by Asp-N (Sequencing Grade, Promega Corp., Madison, WI, USA) and subsequent analysis by a Dionex UltiMate 3000 nanoflow liquid chromatography system (Thermo Fisher Scientific) coupled to a Q Exactive were performed.

Two μg of monoclonal anti-brevican antibody (N-terminal B2739-70B, US Biological Life Science, Salem, MA, USA) was added to 25 μL magnetic Dynabeads M-280 Sheep Anti-Mouse IgG (Invitrogen, Carlsbad, CA, USA) and incubated for 1 h on a rocking platform at room temperature (RT). The remaining unbound antibody was washed away with phosphate-buffered saline (PBS, 10 mM Na-phosphate, 0.15 M NaCl, pH 7.4). The antibody-conjugated beads were added to 965 μL of CSF and 10 μL of Tween 20 in PBS was added to a final concentration of 0.025% (v/v) so that the total volume was 1 mL. Samples were incubated for 1 h on a rocking platform at RT. Using a KingFisher magnetic particle processor (Thermo Fisher Scientific), each sample underwent several washing steps (in 1 mL 0.025% Tween 20 in PBS (v/v), 1 mL PBS, and 1 mL 50 mM NH_4HCO_3, pH 8). Endogenous brevican protein fragments were eluted from the beads using 100 μL 0.5% formic acid in deionized water (v/v) and dried down in a vacuum centrifuge. Samples were reconstituted in 10 μL NH_4HCO_3 and shaken for 30 min. They were then reduced (30 min, 60 °C, on a shaker at 1200 rpm) with 10 μL of 10 mM dithiothreitol in 50 mM NH_4HCO_3 and next alkylated (30 min, RT, in dark, on a shaker at 600 rpm) with 5 μL of 10 mM iodoacetamide in 50 mM NH_4HCO_3. The proteolytic digestion was performed by adding 10 μL (0.05 μg) of Asp-N (15 h, 37 °C, on a shaker at 1200 rpm). Digestion was terminated by adding 5 μL of 10% formic acid in deionized water (v/v) and the samples were dried down in a vacuum centrifuge and stored at -20 °C pending analysis. The LC-MS/MS analysis was performed as previously described [Minta et al. 2020, submitted].

200

2.4. ADAMTS-like enzymatic activity assay

Fluorogenic quenched fluorescence/Förster resonance energy transfer (FRET) peptide ((Abz)-

ATESESRGAI-Lys(Dnp)-NH_2 trifluoroacetate salt) (Bachem, Bubendorf, Switzerland)

containing a sequence of brevican (aa 395-aa 405) was utilized to identify ADAMTS-like

cleaving activity.

The FRET peptide was dissolved to a concentration of 20 µM in assay buffer containing

0.01% Tween 20 (w/v), 20 mM Tris-HCl, pH 7.5, 100 mM NaCl and 10 mM $CaCl_2$. One

hundred fifty µL of 20 µM quenched FRET peptide was incubated with 150 µL of 1:2 diluted

CSF with assay buffer in a black 96-well microplate (Nunc, Roskilde, Denmark). Controls

contained either assay buffer, quenched peptide or CSF pool quality control sample, each in

separate wells. On top of the surface of the samples, 70 µL of mineral oil (Sigma-Aldrich,

Saint Louis, MO, USA) was added to prevent evaporation. The developing fluorescence was

recorded overnight at 37 °C in kinetic mode (one reading every 10 min) on a Spectramax

Gemini XPS microplate reader (Molecular Devices, San Jose, CA, USA) (excitation

wavelength 320 nm, emission wavelength 420 nm).

Control values of quenched peptide and CSF pool quality control sample were subtracted

from the ADAMTS-like activity values of the samples. The two samples with slope values ≤0

were assigned a background slope of 0. Slopes used for the analysis were calculated from

values between 15 and 750 min, including the relative fluorescence unit (RFU) range of 0-

1500 in the acquisition.

2.5. Explorative analysis of C-terminal endogenous brevican peptides in CSF

For the identification of C-terminal endogenous brevican fragments in CSF, IP and

subsequent analysis by a Dionex UltiMate 3000 nanoflow liquid chromatography system

225 coupled to a Q Exactive, were performed in the same way as for the analysis of ADAMTS

226 cleavage, with the following differences: four μg of monoclonal anti-brevican antibody (in-

227 house antibody, peptide used for immunization: [879]ALHPEEDPEGRQGRLLG[895]) was used

228 and no digestion was performed; instead the samples were analysed directly by LC-MS.

229

230 *2.6. Other markers for brain injury*

231 The assays for NFL, S100B and NSE detection in CSF have been described previously [38].

232 The CSF brevican and neurocan concentrations measured using enzyme-linked

233 immunosorbent assay (ELISA) have been reported previously [21].

234 The CSF MMP concentrations were quantified using two Milliplex MAP Human MMP

235 magnetic bead panels, HMMP1MAG-55K and HMMP2MAG-55K (EMD Millipore Corp.,

236 Billerica, MA, USA), as described previously [40].

237

238 *2.7. Validation*

239 For the brevican/neurocan PRM assay, intra- and inter-assay variabilities were determined by

240 calculating the coefficient of variation (CV) for six replicates of a CSF pool quality control

241 evenly spread out throughout the two 96-well plates. For the ADAMTS-like enzymatic

242 activity assay, two replicates of a CSF pool quality control were placed at the beginning and

243 end of the 96-well plate.

244

245 *2.8. Statistical methods*

246 As data did not show normal distribution, logarithmic transformation was applied in linear

247 regression of analysis of covariance (ANCOVA) and linear mixed model (LMM). The log-

248 transformed data followed a normal distribution. The rest of the statistical analyses were

249 performed without logarithmic transformation of the data.

250 The ANCOVA test was used to examine the differences in the ECM concentrations between

251 the two independent groups, *i.e.*, iNPH and TBI groups as well as favourable and

252 unfavourable outcome groups, taking into account the influence of age (set as covariate).

253 The LMM test was used to analyse longitudinal measurements obtained from TBI patients,

254 where the CSF concentrations of brevican and neurocan peptides were included in the model

255 as dependent variables, time point as fixed factor, individuals as random factors and age as a

256 covariate. The Akaike Information Criterion (AIC) index was used to evaluate the overall

257 model fit, where lower AIC value indicated a better fit.

258 The receiver operating characteristic (ROC) curve analysis was used to display the capacity of

259 CSF brevican and neurocan peptide concentrations to predict the unfavourable outcome

260 (GOS=1-3) for TBI patients. Areas under the curve (AUC) together with sensitivities and

261 specificities were obtained as measures of performance for the tests. Correlations were

262 investigated using Spearman's rank correlation.

263 A probability of $p \leq 0.05$ was considered statistically significant. However, in the ANCOVA

264 and LMM tests for brevican/neurocan measurements, the p-value was adjusted using

265 Bonferroni correction for multiple comparisons (n=19) and consequently a probability of

266 $p < 0.0026$ was considered statistically significant.

267 Statistical analyses were performed using GraphPad Prism, version 7.03 (GraphPad Software,

268 Inc., San Diego, CA, USA) and SPSS software, version 26 (IBM Corp., Armonk, NY, USA).

269

270

271 *2.9. Data availability*

272 The data supporting the findings in this study are available from the corresponding author,

273 upon reasonable request.

274

275 *2.10. Ethical permission*

276 Ethical approvals were provided by the Regional Ethical Board in Stockholm

277 (#2005/1526/31/2) and Gothenburg (154-05). The study was conducted in accordance with

278 the Declaration of Helsinki. Verbal or written consent was acquired from the patients or next-

279 of-kin.

280

281 **3. Results**

282 Clinical and demographic data of the patients are shown in Table 1.

283

284 *3.1. Brevican/neurocan panel*

285 The majority of the CSF concentrations of the brevican tryptic peptides located N-

286 terminally of the ADAMTS cleavage site (Set 1, see Fig. 1) ($p \leq 0.002$) and of the peptide

287 located closest to the C-terminal (B879/Set 3) ($p < 0.0001$) were significantly lower in the TBI

288 group (time point 1) when compared with the iNPH group (Fig. 2). In contrast, the two

289 peptides located C-terminally of the ADAMTS cleavage site, B741 and B834 (Set 2), did not

290 differ between the two groups (Fig. 2). The CSF concentrations of the neurocan peptides did

291 not differ significantly between the TBI and iNPH patient groups although all peptides

292 exhibited a trend toward decreased levels in the TBI group (Supplementary fig. 1).

293 All the brevican peptides significantly correlated with each other in the iNPH group

294 (rho=0.68-1.00, $p < 0.001$) (Fig. 3). In the TBI group, the N-terminal/Set 1 brevican peptides

295 highly correlated with each other (rho=0.84-0.98, $p < 0.0001$), whereas the B741 and B834

296 peptides (Set 2) did not correlate with the B879/Set 3 peptide (rho=0.12-0.23, $p > 0.05$) and

297 had a lower correlation coefficient with the N-terminal/Set 1 brevican peptides in general

298 (Fig. 3). All the neurocan peptides significantly correlated with each other in both the iNPH

299 and TBI groups (Fig. 3) (rho=0.57-0.99, $p < 0.0001$).

In outcome prediction models, the B741 and B834 peptides (Set 2) were significantly increased in TBI patients with unfavourable outcome (p≤0.001) (Fig. 4) and obtained high AUC (0.93 and 0.85, respectively) (Supplementary table 2). These two brevican peptides demonstrated comparable capacity for outcome prediction as other known brain injury markers, *i.e.*, NFL, NSE, and S100B (rho=0.78-0.89) (Supplementary table 2). There was no significant difference in CSF concentrations of the neurocan peptides between the unfavourable vs. favourable outcome groups (Supplementary fig. 2, Supplementary table 2) although most of the neurocan peptides showed a tendency to be increased in CSF in unfavourable outcome following TBI.

The CSF concentrations of N-terminal/Set 1 brevican peptides (p≤0.001) (Fig. 5) and several of the neurocan peptides (N145, N184, N194, N257, N316 and N1242) (p≤0.002) (Supplementary fig. 3) decreased over time following brain injury, from time point 1 to 3.

All of the CSF brevican peptide concentrations significantly and similarly correlated with previously analysed [21] CSF brevican concentrations from both ELISA assays in the iNPH group (rho=0.43-0.74, p<0.001) (Supplementary table 3). In the TBI group, the most C-terminally located peptide (B879/Set 3) levels did not correlate with in-house ELISA measuring full or nearly full-length brevican in CSF (rho=0.23, p>0.05), while the rest of the brevican peptides showed similar and significant association (rho=0.68-0.75, p<0.0001) (Supplementary table 3). All CSF concentrations of neurocan peptides significantly and similarly correlated with previously analysed [21] CSF concentrations of neurocan measured by ELISA assay in both the TBI and iNPH groups (rho=0.64-0.90, p<0.0001) (Supplementary table 3).

Most brevican/neurocan peptides (apart from N1195) correlated with NFL (rho=0.30-0.64, p≤0.02) (Supplementary table 4). The two C-terminal/Set 2 brevican peptides showed strong correlations with S100B and NSE (rho=0.72-0.75, p<0.0001) compared with other

brevican as well as neurocan peptides that showed no or weak correlations (Supplementary

table 4). Interestingly, there were no or weak correlations between all CSF brevican and

neurocan peptide concentrations and all severity scores (Supplementary table 4). No

significant difference was observed in CSF brevican/neurocan peptide concentrations between

mild, moderate and severe TBI patients grouped based on the GCS scale. According to the

AIS scale, 98% of the patients were severe, and thus a comparison between mild vs. more

severe cases was not deemed to be statistically feasible. There was no significant difference in

any of the CSF brevican/neurocan peptide levels between the patients that suffered from

intracranial versus extracranial trauma.

3.2. Explorative analysis of ADAMTS cleavage in CSF

Several brevican peptides proteolytically digested by Asp-N were detected in CSF IP-

purified using the N-terminally directed antibody B2739-70B. The four ADAMTS-cleaved

peptides observed were ^{375}DGLEAIVTVTETLEELQLPQEATESE^{400},

^{385}ETLEELQLPQEATESE^{400}, ^{388}EELQLPQEATESE^{400}, and ^{389}ELQLPQEATESE^{400} (Fig.

1). See Supplementary table 5 (upper part) for data on the detected peptides.

3.3. ADAMTS-like enzymatic activity assay

The ADAMTS-like activity was increased in TBI patients compared with the contrast

group (p<0.0001) (Fig. 6A). There was no significant difference in ADAMTS-like activity

between the two TBI outcome groups (Fig. 6B), although there was a trend towards higher

levels in the unfavourable outcome group. In the longitudinal measurements following TBI,

neither significant nor trend changes were observed (Fig. 6C).

The CSF slope values of ADAMTS-like activity correlated with the majority of CSF

MMP concentrations (MMP-1, -2, -3, -10) to much greater degree in the TBI group

(rho=0.78-0.87, p<0.0001) compared with iNPH group (rho=0.35-0.66, p≤0.04) (Table 2).

Additionally, the CSF slope values of ADAMTS-like activity showed significant correlation

with CSF concentrations of other biomarkers for brain injury, *i.e.*, NFL, S100B, and NSE

(rho=0.50-0.70, p<0.0001), but not with TBI severity or outcome scores (Table 2).

3.4. Explorative analysis of C-terminal endogenous brevican peptides in CSF

Twenty endogenous brevican peptides were detected in CSF IP-purified using a C-

terminally directed in-house antibody. The peptides spanned aa 879-900 (Supplementary table

5; lower part). The most abundant peptide was 879-895. No peptides located N-terminally of

aa 879 were observed. This group of peptides belong to Set 3 as indicated in Fig. 1.

3.5. Validation

Validation of the brevican/neurocan panel, showing linearity of the method and stability of

brevican/neurocan peptides during freeze-thaw cycles and at different storage conditions was

performed previously [Minta et al. 2020, submitted]. Briefly, the majority of brevican and

neurocan peptides in CSF showed analytical stability for up to five freeze-thaw cycles and

storage stability under different conditions: -80 °C for one month, -20 °C for one month, 5-8

°C for 24 h, 5-8 °C for 7 days, and RT for 24 h. In addition, the relative errors of the back-

calculated concentrations were below 20% for all calibrators, except N1195.

The variability of the brevican and neurocan peptides in quality control CSF samples had an

intra-assay CV% range of 8-20 and an inter-assay CV% range of 8-23. The B718 peptide was

excluded from the analysis due to low measured signal and consequently a large degree of

variability.

The variability of ADAMTS-like enzymatic activity measured as slopes in quality control

CSF samples had an intra-assay CV% of less than 1%.

4. Discussion

This study shows significant alterations in CSF levels of various brevican and neurocan peptides following TBI. To our knowledge, this is the first study describing the fragmentation patterns and proteolytic break-down products of brevican and neurocan in CSF in the context of TBI. Interestingly, a conspicuous discrepancy was observed for different endogenous brevican fragment groups in CSF of TBI patients, suggesting that catalysis of different parts of the brevican molecule are differentially regulated and that various brevican fragments might reflect different pathological and/or physiological processes in the brain. The N-terminal/Set 1 brevican tryptic peptides showed very similar trends of change, while the two C-terminal peptides/Set 2 did not follow this pattern. Additionally, the B879/Set 3 peptide showed a discrepancy in outcome prediction. Moreover, there was no correlation between the two C-terminal/Set 2 peptides (B741 and B834) and the B879/Set 3 peptide in the TBI group. It is known that ADAMTS cleaves brevican at ^{400}E/S^{401} (also confirmed in this study) and that the major MMP cleavage site is at ^{361}A/I^{362} [26]. This might explain the different pattern of changes between the N-terminal peptides (ranging from aa 87 to aa 330, Set 1) and C-terminal peptides (ranging from aa 718 to aa 841, Set 2). However, it does not explain the differential trend of the B879/Set 3 peptide compared with those in the Set 2 (B741 and B834). Nevertheless, the observed endogenous brevican spanning aa 879-900 show that other proteolytic cleavages occur C-terminally of aa 841. The data indicates that cleavage at ^{878}R/A^{879} is prominent. Lack of correlation between the B879/Set 3 peptide and CSF full-length (or nearly full-length) brevican measurements from in-house ELISA (previously analysed [21]) in the TBI, but not in the iNPH group, indicates that this peptide does not reflect the near full-length protein in TBI in contrast to the other brevican peptides. Altogether, this suggests that there are three separate sets of brevican proteolytic peptides

400   exhibiting different levels in CSF following TBI. The three separate CSF sets of brevican

401   fragments were previously distinguished using antibodies specific to different binding sites

402   [Minta et al. 2020, submitted].

403   Contrary to brevican, all neurocan peptides highly correlate with each other and to CSF

404   neurocan ELISA measurements in both the iNPH and TBI groups indicating similar

405   processing of neurocan in relation to TBI or iNPH. The lower CSF concentrations of brevican

406   peptides in the TBI group compared with iNPH patients could be explained by the more

407   extensive degradation of these peptides by the ADAMTS enzyme, whose activity in CSF is

408   higher in TBI. Also, the neurocan peptides showed a tendency to be lower in the TBI group.

409   Here, the brain injury-induced production of brevican/neurocan peptides might be hindered by

410   the abnormally induced ADAMTS-like activity resulting in degradation of these peptides

411   consequently leading to decreased levels in CSF. This could indicate an imbalance between

412   the proteoglycan formation and degradation in TBI, where the ADAMTS-associated

413   beneficial processes of axonal growth stimulation after injury dominantly overcome the

414   proteoglycans' inhibitory processes of axonal regeneration. Although ADAMTS-like activity

415   was different between TBI and iNPH, no change in two peptides in Set 2 (B741 and B834)

416   between these groups was seen which might indicate that the peptides are not vulnerable to

417   proteolytic degradation by ADAMTS. However, it cannot be excluded that iNPH

418   pathophysiology might also affect CSF concentrations of proteoglycans as this condition

419   group does not reflect the state of a healthy brain. In addition, the CSF biomarker profile in

420   iNPH, involving core AD biomarkers or biomarkers of axonal/microglial damage and

421   neuroinflammation, is reported to differ from healthy controls [41-44]. The similar CSF

422   concentrations of B741 and B834 for the TBI and iNPH groups together with high

423   correlations of these peptides, but not others, to the CSF S100B concentrations indicate that

424   both TBI and iNPH groups might involve similar glial cell pathophysiology. Another marker

for astroglial damage, glial fibrillary acidic protein, has previously been reported to be

increased in CSF of NPH patients compared with healthy individuals [44, 45] and it is widely

known that TBI triggers glial dysfunction [2]. Thus, CSF B741 and B834 concentrations are

potential indicators for astroglial pathophysiology. Finally, it also cannot be excluded that

TBI or iNPH treatment (for instance use of antiepileptic therapeutics [46] or diuretics/steroids

[47]) may alter the biomarker levels in CSF. However, the effects of these therapies were not

investigated in relation to CSF brevican/neurocan concentrations.

The same peptides were able to predict clinical outcome following TBI to a similar

degree as currently known biomarkers for brain injury, *i.e.*, NFL, S100B and NSE. Although,

CSF concentrations of the B741 and B834 peptides may reflect astroglial damage unspecific

to TBI, the elevated levels seen in unfavourable outcome could indicate that they represent

additional to non-glial pathological processes, unique for outcome prediction. Thus, B741 and

B834 (Set 2) might become promising novel CSF biomarker candidates for TBI assessment.

The rest of the CSF brevican peptides and all neurocan peptides could not predict the outcome

following TBI. Since ADAMTS-like activity was much higher in the TBI group compared

with the iNPH group and did not differ between the two outcome profiles, it is possible that

its active state is more specific to brain injury as an event rather than to the severity or

outcome prognosis. However, again, it cannot be excluded that very low CSF levels of

ADAMTS-like activity in the iNPH group might be a result of some other pathological

processes in this group. Overall, this study shows that controlled cleavage of brevican might

be of some biological significance. The function of the brevican proteolytic derivatives should

be further investigated, along with the mechanism of enzymatic cleavages. Low association

between the CSF brevican/neurocan peptide concentrations and severity scores might be

explained by the majority of the TBI patients suffering from severe trauma (65% based on

GCS, and 98% based on AIS classifications).

450   The decrease of CSF brevican/neurocan peptides noted over time following TBI is

451 presumably due to the clearance mechanisms of the proteoglycans after an injury-induced

452 initial increase. The above TBI-related observations are in line with the previous results from

453 our group where brevican and neurocan proteins were measured using immunoassays [21].

454   To our knowledge, this is the first study reporting an ADAMTS-derived fragment in

455 CSF. The previous study from our group [Minta et al. 2020, submitted] shows the evidence of

456 endogenous cleavage in the mid-region of brevican in human CSF. Although, the MMP-

457 derived semi-tryptic peptide ^{355}DSAQPSA^{361}, produced by MMP cleavage at ^{361}A/I^{362} and

458 tryptic cleavage at ^{354}R/D^{355}, could be potentially measurable in CSF, it was not detected in

459 the previous study [Minta et al. 2020, submitted]. In order to evaluate the ADAMTS cleavage

460 in brevican, it was not possible to use trypsin since the peptides of interest would be either too

461 short or too long. Since there were no suitable cleavages sites for trypsin for analysis of

462 ADAMTS-derived peptides (ending at aa 400), Asp-N was utilised, which cleaves

463 predominantly N-terminally of Asp but also N-terminally of Glu. Thus, to detect brevican

464 processed by ADAMTS one expected peptide would be

465 ^{375}DGLEAIVTVTETLEELQLPQEATESE^{400}, which is naturally cleaved at ^{400}E/S^{401} and by

466 Asp-N at ^{374}S/D^{375}. Here, for the first time, we report that ADAMTS cleavage at ^{400}E/S^{401} is

467 measurable in human CSF. However, we were not able to quantify them, probably due to the

468 low concentrations.

469   The correlations between ADAMTS-like activity and CSF MMP concentrations suggest

470 that these enzymes are similarly affected in TBI and less so in the iNPH group, possibly

471 because there is no single event triggering release of these enzymes in iNPH. The association

472 between ADAMTS-like activity and other biomarkers for brain injury, *i.e.*, NFL, S100B and

473 NSE, further supports that its active state might be related to axonal and glial pathology

474 following TBI.

475   The strengths of the study include a well-characterized cohort with multiple biomarker

476   and clinical data. In addition, ventricular CSF is a preferred fluid to measure the brain protein

477   content being in closer proximity to the brain compared with lumbar CSF.

478   However, there are some limitations to the study that should be acknowledged,

479   including variable numbers of samples and sampling time points in relation to trauma in TBI

480   patients, lack of ventricular CSF from healthy individuals (though practically impossible due

481   to ethical reasons), analysis of brevican/neurocan panel and ADAMTS-like activity on

482   samples from different subsets, lack of suitable semitryptic ADAMTS-specific peptide. Also,

483   it cannot be excluded that the observed enzymatic activity comes from several different

484   enzymes. Moreover, clinical limitation lies in the fact that serum samples of TBI/iNPH

485   patients were not analysed. Due to the complexity of accessing CSF, brain biomarkers of

486   tissue fate should ideally be assessed in blood to achieve a greater clinical utility.

487   In conclusion, this study demonstrates significant and clinically relevant changes in the

488   CSF concentrations of several brevican and neurocan peptides following TBI, especially the

489   B741 and B834 peptides. Further experiments are warranted to confirm these findings.

490

491   **Author contributions statement**

492   KM, GB, UA, HZ and KB created the concept of the study. FP, EPT, AJ, MT and FN

493   recruited subjects and acquired data. KM carried out the experiments, statistical analysis and

494   drafted the manuscript. KM, GB, UA, HZ, KB, EP, FP, EPT, AJ, MT and FN contributed to

495   the interpretation of the results and provided critical feedback of the manuscript. All authors

496   read and approved the final manuscript.

497

498   **Funding**

20

513

514 Conflicts of interest

515 HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed,

516 Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia

517 sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker

518 Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program.

519 KB has served as a consultant or at advisory boards for Abcam, Axon, Biogen, Lilly, MagQu,

520 Novartis and Roche Diagnostics, and is a co-founder of Brain Biomarker Solutions in

521 Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. KM, GB, EP,

522 FP, EPT, AJ, MT, FN and UA declare that they have no competing interests.

523 **Table 1:** Participant demographics.

524

525

Characteristic		TBI (n=42)	iNPH (n=37)
Sex, n (%)	Male	32 (76%)	28 (76%)
	Female	10 (24%)	9 (24%)
Age, median (interquartile interval)		58 (40-62)	67 (59-70)
Outcome (favourable/unfavourable)		40% / 60%	
Trauma severity scoring, median (interquartile interval), % severe TBI			
GCS		7 (4-10), 65%	
AIS		5 (4-5), 98%	

Abbreviations: abbreviated injury scale (AIS), Glasgow Coma Scale (GCS), idiopathic

normal pressure hydrocephalus (iNPH), traumatic brain injury (TBI)

Outcome prediction is dichotomized as unfavourable (GOS=1-3) and favourable (GOS=4-5).

A total score of 3-8 for GCS or 4-6 for AIS indicates severe TBI.

530

531

532

533

534

535

536

537

538

539

540

541

542   **Table 2.** Correlations between ADAMTS-like activity scores and other markers of brain

543   injury and characteristics in TBI and iNPH patients.

544

		TBI (n=29)	iNPH (n=36)
		ADAMTS-like activity	
age	rho	**0.522***	0.088
	p	**0.015**	0.611
MMP-1	rho	**0.800******	**0.349***
	p	**<0.0001**	**0.037**
MMP-2	rho	**0.778******	**0.544*****
	p	**<0.0001**	**0.001**
MMP-3	rho	**0.866******	**0.659******
	p	**<0.0001**	**<0.0001**
MMP-9	rho	**0.374***	0.151
	p	**0.007**	0.379
MMP-10	rho	**0.857******	**0.607******
	p	**<0.0001**	**<0.0001**
MMP-12	rho	0.258	0.223
	p	0.070	0.191
NFL	rho	**0.497******	-
	p	**<0.0001**	
S100B	rho	**0.668******	-
	p	**<0.0001**	
NSE	rho	**0.702******	-
	p	**<0.0001**	
GCS	rho	-0.266	-
	p	0.244	
AIS	rho	0.006	-
	p	0.981	
GOS	rho	-0.375	-
	p	0.094	
Stockholm CT	rho	0.326	-
	p	0.187	
Rotterdam CT	rho	0.321	-
	p	0.194	
Marshall CT	rho	0.316	-
	p	0.201	

545

546   Abbreviations: abbreviated injury scale (AIS), ADAMTS (a disintegrin and metalloproteinase

547   with thrombospondin motifs), CT (computed tomography), Glasgow Coma Scale (GCS),

548   Glasgow Outcome Scale (GOS), idiopathic normal pressure hydrocephalus (iNPH), matrix

549   metalloproteinase (MMP), neurofilament light (NFL), neuron specific enolase (NSE),

550   traumatic brain injury (TBI).

551   For age and severity or outcome scores only time point 1 was included for the correlation

552   analysis, while for MMP and brain markers all three time points were incorporated.

553   The correlation coefficients are presented as Spearman's rho.

554

555

556

557

558

559

560

561

562

563

564

565

566

567

568

569

570

571

572

573

574

575

576

577 **Figure 1:** The brevican and neurocan tryptic peptides indicated along their respective protein

578 sequences.
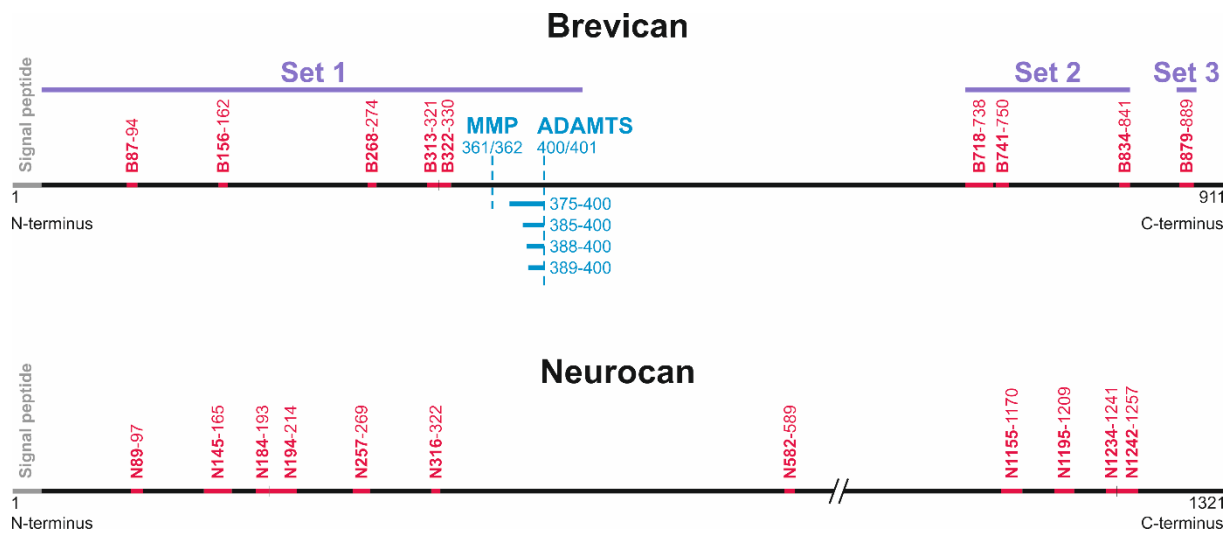
579

580 In red: the tryptic brevican and neurocan peptides measured in the MS-based panel; the

581 numbers are the aa of the peptide, where bold indicates how they are referred to in the text. In

582 blue: the major MMP cleavage site in brevican, the ADAMTS cleavage site in brevican and

583 the ADAMTS-derived peptides detected in explorative analysis of CSF using IP-MS and Asp-

584 N. In purple: indication of the three separate sets of brevican tryptic peptides quantified in

585 CSF; the division is based on data from explorative IP-MS measurements.

586

587

588

589

590

591

592

Figure 2: CSF brevican concentrations for the iNPH and TBI (time point 1; TP1) groups.
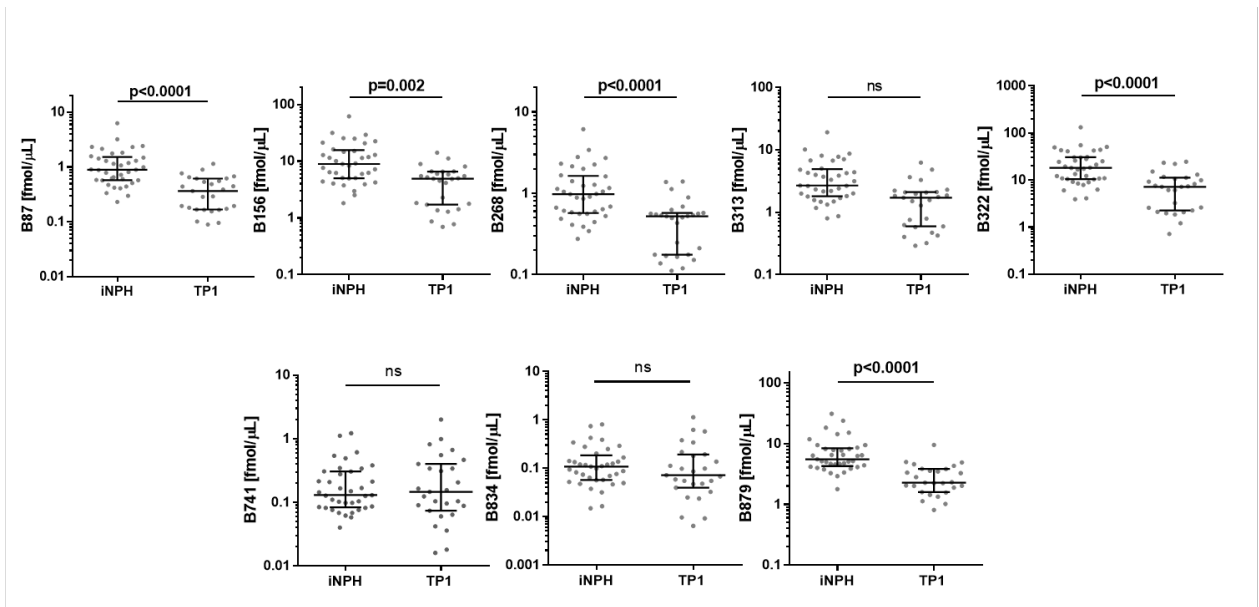
The horizontal lines represent the median and interquartile ranges.

The ANCOVA test with Bonferroni correction was used to examine the differences in log

transformed data between the two groups, accounting for the effect of age.

Number of individuals: n=37 for iNPH, n=27 for TBI (TP1).

607 **Figure 3.** Correlation matrix between CSF concentrations of brevican and neurocan peptides

608 in the iNPH group and TBI group.

iNPH → / ↓ TBI	B87	B156	B268	B313	B322	B741	B834	B879	N89	N145	N184	N194	N257	N316	N582	N1155	N1195	N1234	N1242
B87		1.0	1.0	1.0	1.0	0.8	0.8	0.9	0.9	0.9	0.9	0.9	0.9	1.0	0.9	0.9	0.9	0.9	0.9
B156	0.9		1.0	1.0	1.0	0.9	0.8	0.9	0.9	0.9	0.9	0.9	0.9	1.0	0.9	0.8	0.9	0.9	0.9
B268	0.9	1.0		1.0	1.0	0.8	0.8	0.9	0.9	0.9	0.9	0.9	0.9	1.0	0.9	0.9	0.9	0.9	0.9
B313	0.8	1.0	1.0		1.0	0.9	0.8	0.9	0.9	0.9	0.9	0.9	0.9	1.0	0.9	0.9	0.9	0.9	0.9
B322	0.9	0.9	0.9	0.9		0.9	0.8	0.9	0.9	0.9	0.9	0.9	0.9	1.0	0.9	0.9	0.9	0.9	0.9
B741	0.6	0.6	0.6	0.6	0.5		0.9	0.8	0.8	0.8	0.8	0.8	0.7	0.8	0.7	0.8	0.8	0.8	0.8
B834	0.5	0.5	0.5	0.5	0.4	0.8		0.7	0.7	0.7	0.7	0.7	0.7	0.8	0.6	0.7	0.7	0.7	0.7
B879	0.6	0.7	0.7	0.7	0.6	0.2	0.1		0.9	1.0	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
N89	0.7	0.8	0.8	0.8	0.8	0.5	0.4	0.8		0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
N145	0.7	0.9	0.9	0.9	0.8	0.6	0.4	0.8	0.9		0.9	1.0	0.9	1.0	0.9	1.0	0.9	1.0	
N184	0.7	0.8	0.9	0.9	0.8	0.5	0.4	0.8	1.0	1.0		0.9	1.0	0.9	1.0	0.9	0.9	1.0	1.0
N194	0.7	0.9	0.9	0.9	0.8	0.5	0.4	0.8	0.9	1.0	0.9		0.9	0.9	0.9	0.9	0.9	0.9	0.9
N257	0.8	0.9	0.9	0.9	0.8	0.6	0.4	0.7	0.9	1.0	1.0	0.9		0.9	1.0	0.9	1.0	1.0	1.0
N316	0.8	0.9	1.0	1.0	0.9	0.6	0.5	0.7	0.9	0.9	0.9	0.9	0.9		0.9	0.9	0.9	0.9	0.9
N582	0.5	0.6	0.7	0.7	0.6	0.3	0.2	0.9	0.8	0.8	0.9	0.8	0.8	0.7		0.9	0.9	0.9	0.9
N1155	0.6	0.7	0.8	0.8	0.6	0.6	0.4	0.8	0.8	0.9	0.9	0.9	0.9	0.8	0.9		1.0	1.0	1.0
N1195	0.6	0.7	0.7	0.7	0.8	0.2	0.2	0.5	0.7	0.8	0.8	0.7	0.8	0.7	0.6	0.6		1.0	1.0
N1234	0.6	0.8	0.8	0.8	0.7	0.4	0.4	0.7	0.9	0.9	1.0	0.9	0.9	0.8	0.8	0.9	0.8		1.0
N1242	0.7	0.8	0.8	0.8	0.8	0.3	0.2	0.6	0.8	0.9	0.9	0.8	0.9	0.8	0.7	0.7	0.9	0.9	

609

610 The correlation coefficients are presented as Spearman's rho.

611 The darker and redder the box, the closer the correlation is to positive 1. Dashed lines separate

612 the three sets of brevican peptides. Double line separates brevican and neurocan peptides.

613

614

615

616

617

618 **Figure 4.** CSF brevican peptide concentrations in relation to outcome following TBI.
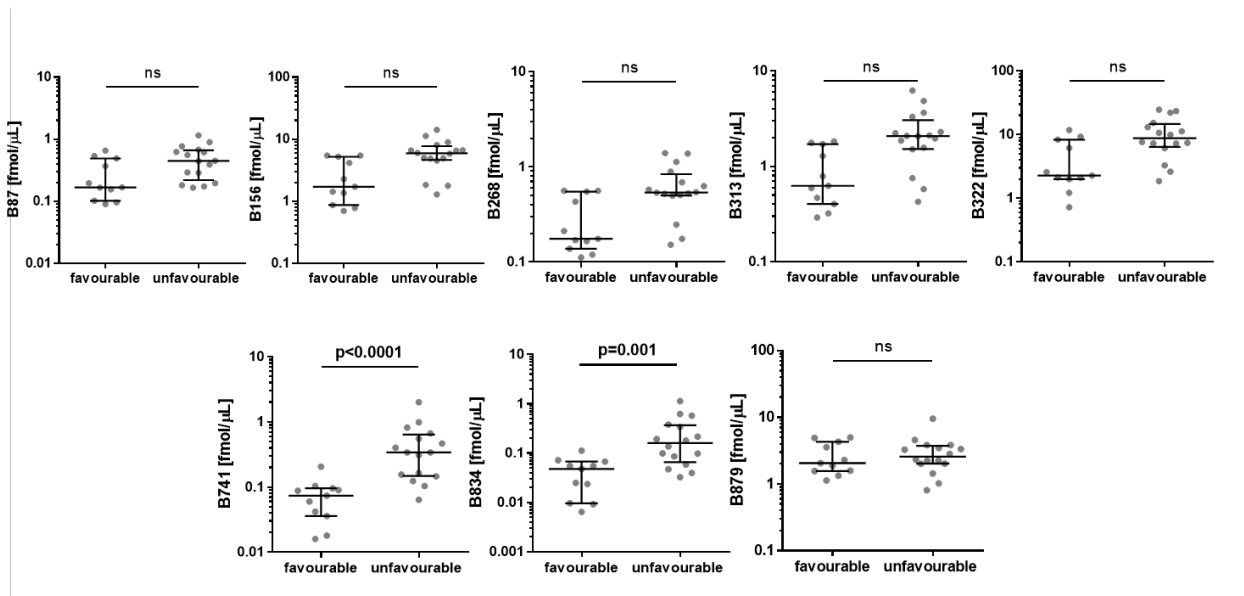
620 The horizontal lines represent the median and interquartile ranges.

621 Outcome is dichotomized into favourable (GOS=4-5) and unfavourable (GOS=1-3).

622 The ANCOVA test with Bonferroni correction was used to examine the differences in log

623 transformed data between the two groups, accounting for the effect of age.

624 Number of patients: n=11 for favourable, n=16 for unfavourable.

632   **Figure 5.** Repeated measurements of brevican peptides in TBI patients at three time points.

633   LMM with Bonferroni correction was used to examine the differences in log transformed data
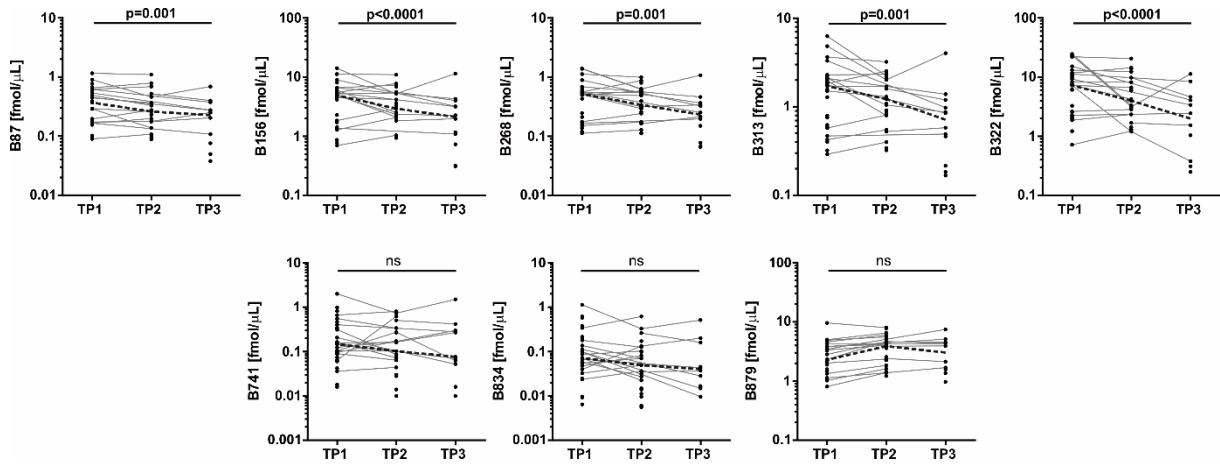
634   of repeated measures following TBI (from TP1 to TP3), accounting for the effect of age. The

635   dashed lines represent the longitudinal median changes.

636

637

638

639

640

641

642

643

644

645

646

647

648

649   **Figure 6:** ADAMTS-like activity in CSF for the iNPH and TBI (time point 1; TP1) groups

650   (A), in relation to outcome (B) and repeated measures (C) following TBI.
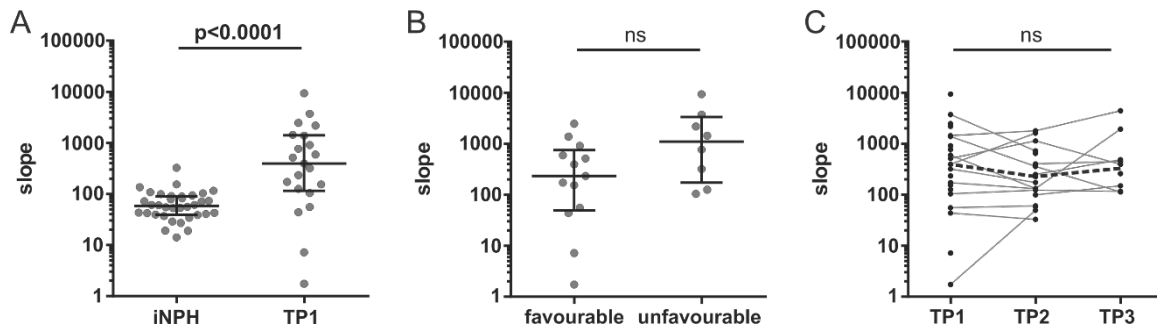
652   The horizontal lines represent the median and interquartile ranges.

653   Number of individuals: n=36 for iNPH, n=21 for TBI (TP1), n=19 for TP2, n=10 for TP3,

654   n=13 for favourable (GOS=4-5), n=8 for unfavourable (GOS=1-3) outcomes.

655   TP1 = 1-5 days, TP2 = 5-10 days, TP3 = 8-14 days.

656   Slopes used for the analysis were calculated from values between 15 and 750 min, including

657   the relative fluorescence unit (RFU) range of 0-1500 in the acquisition.

658   The ANCOVA test and LMM were used to examine the differences in log transformed data

659   between the two groups and of repeated measured following TBI, respectively, accounting for

660   the effect of age.

661

662

663

664

665

666

667

668

669

670

671 **Supplementary table 1:** Participant demographics of the subcohort used to investigate

672 ADAMTS-like activity in CSF.

Characteristic		TBI (n=29)	iNPH (n=36)
Sex, n (%)	Male	21 (72%)	28 (78%)
	Female	8 (28%)	8 (22%)
Age, median (interquartile interval)		54 (42-64)	68 (59-70)
Outcome (favourable/unfavourable)		52% / 48%	
Trauma severity scoring, median (interquartile interval), % severe TBI			
GCS		6 (3-10), 69%	
AIS		4 (4-5), 89%	

673

674 Abbreviations: abbreviated injury scale (AIS), Glasgow Coma Scale (GCS), idiopathic

675 normal pressure hydrocephalus (iNPH), traumatic brain injury (TBI)

676 Outcome prediction is dichotomized as unfavourable (GOS=1-3) and favourable (GOS=4-5).

677 A total score of 3-8 for GCS and 4-6 for AIS indicates severe TBI.

678

679

680

681

682

683

684

685

686

687

688

689 **Supplementary table 2.** ROC analysis of favourable vs. unfavourable outcome following

690 TBI of brevican/neurocan peptides and brain injury biomarkers in CSF.

peptide	AUC	Cut off [fmol/µL]	Sensitivity [%]	Specificity [%]
B87	0.744	0.171	93.8	50.0
B156	0.813	5.67	56.3	100
B268	0.778	0.465	81.3	70.0
B313	0.838	1.85	68.8	100
B322	0.775	2.60	93.8	60.0
B741	0.928	0.114	87.5	90.0
B834	0.850	0.0786	75.0	90.0
B879	0.506	1.96	81.3	40.0
N89	0.659	0.920	75.0	70.0
N145	0.725	0.945	81.3	70.0
N184	0.713	0.144	75.0	70.0
N194	0.716	0.337	81.3	70.0
N257	0.769	0.162	81.3	70.0
N316	0.788	1.46	81.3	70.0
N582	0.488	3.93	18.0	100
N1155	0.750	0.50	87.5	60.0
N1195	0.769	1.25	81.3	70.0
N1234	0.756	0.196	81.3	70.0

Biomarker	AUC	Cut off [ng/mL]	Sensitivity [%]	Specificity [%]
N1242	0.769	0.565	81.3	70.0
NFL	0.891	2.81	90.9	80.0
S100B	0.782	130	54.5	100
NSE	0.782	156	45.5	100

691

692 ROC analysis was performed to predict the unfavourable (GOS=1-3) outcome following TBI.

693 Abbreviations: area under the curve (AUC), neurofilament light (NFL), neuron-specific

694 enolase (NSE), Receiver Operating Characteristic (ROC), S100 calcium-binding protein B

695 (S100B).

696

697

698

699

700

701

702

703

704

705

706

707

708

709 **Supplementary table 3.** Correlations between brevican/neurocan peptides measured using

710 MS panel and brevican/neurocan measured using ELISA assays in TBI and iNPH patients.

		TBI			INPH		
		Brevican commercial ELISA	Brevican inhouse ELISA	Neurocan ELISA	Brevican commercial ELISA	Brevican inhouse ELISA	Neurocan ELISA
B87	rho	0.852****	0.675****	0.590****	0.565****	0.565****	0.706****
	p	0.000	0.000	0.000	0.000	0.000	0.000
B156	rho	0.902****	0.708****	0.693****	0.569****	0.616****	0.741****
	p	0.000	0.000	0.000	0.000	0.000	0.000
B268	rho	0.926****	0.712****	0.742****	0.560****	0.598****	0.711****
	p	0.000	0.000	0.000	0.000	0.000	0.000
B313	rho	0.920****	0.722****	0.723****	0.562****	0.610****	0.738****
	p	0.000	0.000	0.000	0.000	0.000	0.000
B322	rho	0.878****	0.678****	0.620****	0.584****	0.589****	0.745****
	p	0.000	0.000	0.000	0.000	0.000	0.000
B741	rho	0.532****	0.707****	0.326**	0.503**	0.742****	0.670****
	p	0.000	0.000	0.010	0.002	0.000	0.000
B834	rho	0.458****	0.754****	0.228	0.504***	0.604****	0.565****
	p	0.000	0.000	0.079	0.001	0.000	0.000
B879	rho	0.770****	0.233	0.839****	0.431**	0.544****	0.757****
	p	0.000	0.068	0.000	0.008	0.000	0.000
N89	rho	0.795****	0.434****	0.874****	0.458**	0.472**	0.746****
	p	0.000	0.000	0.000	0.004	0.003	0.000
N145	rho	0.813****	0.470****	0.869****	0.465**	0.418**	0.788****
	p	0.000	0.000	0.000	0.004	0.010	0.000
N184	rho	0.801****	0.427***	0.897****	0.466**	0.428**	0.807****
	p	0.000	0.001	0.000	0.004	0.008	0.000
N194	rho	0.810****	0.446****	0.841****	0.487**	0.435**	0.758****
	p	0.000	0.000	0.000	0.002	0.007	0.000
N257	rho	0.796****	0.459****	0.845****	0.486**	0.391*	0.769****
	p	0.000	0.000	0.000	0.002	0.017	0.000
N316	rho	0.920****	0.677****	0.785****	0.541***	0.567****	0.752****
	p	0.000	0.000	0.000	0.001	0.000	0.000
N582	rho	0.696****	0.13	0.893****	0.422**	0.369*	0.725****
	p	0.000	0.313	0.000	0.009	0.024	0.000
N1155	rho	0.710****	0.329**	0.855****	0.447**	0.415*	0.756****
	p	0.000	0.010	0.000	0.006	0.011	0.000
N1195	rho	0.588****	0.271*	0.637****	0.474**	0.426**	0.791****
	p	0.000	0.050	0.000	0.003	0.009	0.000
N1234	rho	0.718****	0.335*	0.845****	0.423**	0.432**	0.795****
	p	0.000	0.011	0.000	0.009	0.008	0.000
N1242	rho	0.693****	0.379**	0.734****	0.476**	0.430**	0.810****
	p	0.000	0.003	0.000	0.003	0.008	0.000

711

712 In TBI group, the analyte concentrations from all three time points were incorporated.

713 The correlation coefficients are presented as Spearman's rho.

Supplementary table 4. Correlations between brevican/neurocan peptides and other characteristics in TBI patients.

		age	NFL	S100B	NSE	GCS	AIS	GOS	Stockholm CT	Rotterdam CT	Marshall CT
B87	rho	0.530**	0.389**	0.323	0.365*	-0.032	-0.276	-0.348	0.120	0.019	-0.231
	p	0.004	0.002	0.063	0.037	0.879	0.164	0.076	0.551	0.924	0.247
B156	rho	0.554**	0.463****	0.385*	0.349*	-0.057	-0.364	-0.446*	0.043	-0.081	-0.349
	p	0.003	0.000	0.025	0.047	0.788	0.062	0.020	0.829	0.688	0.075
B268	rho	0.545**	0.460****	0.396*	0.431*	-0.164	-0.340	-0.405*	0.051	-0.116	-0.278
	p	0.003	0.000	0.020	0.012	0.434	0.083	0.036	0.802	0.565	0.160
B313	rho	0.601***	0.453****	0.389*	0.370*	-0.013	-0.364	-0.534**	-0.044	-0.169	-0.348
	p	0.001	0.000	0.023	0.034	0.950	0.062	0.004	0.827	0.400	0.075
B322	rho	0.575**	0.331****	0.245	0.267	-0.135	-0.414*	-0.459*	0.009	-0.171	-0.432*
	p	0.002	0.009	0.162	0.132	0.519	0.032	0.016	0.966	0.395	0.024
B741	rho	0.429*	0.536****	0.746****	0.722****	-0.264	-0.340	-0.604**	0.219	0.235	-0.083
	p	0.025	0.000	0.000	0.000	0.203	0.083	0.001	0.273	0.237	0.682
B834	rho	0.264	0.414***	0.752****	0.719****	-0.296	-0.281	-0.464*	0.373	0.384*	0.060
	p	0.184	0.001	0.000	0.000	0.151	0.156	0.015	0.055	0.048	0.766
B879	rho	0.356	0.454****	-0.028	0.052	-0.047	-0.433*	-0.122	-0.265	-0.531**	-0.417*
	p	0.069	0.000	0.877	0.774	0.822	0.024	0.545	0.181	0.004	0.031
N89	rho	0.284	0.485****	0.225	0.427*	-0.247	-0.502**	-0.194	-0.006	-0.233	-0.379
	p	0.151	0.000	0.209	0.015	0.235	0.008	0.331	0.977	0.243	0.051
N145	rho	0.419*	0.516****	0.344*	0.447**	-0.170	-0.473*	-0.327	-0.062	-0.234	-0.276
	p	0.029	0.000	0.046	0.009	0.415	0.013	0.096	0.757	0.239	0.163
N184	rho	0.315	0.556****	0.269	0.447**	-0.230	-0.522**	-0.281	-0.065	-0.215	-0.353
	p	0.110	0.000	0.124	0.009	0.269	0.005	0.155	0.749	0.282	0.071
N194	rho	0.457*	0.498****	0.323	0.376*	-0.081	-0.419*	-0.350	-0.164	-0.311	-0.373
	p	0.016	0.000	0.063	0.031	0.699	0.030	0.073	0.415	0.115	0.056
N257	rho	0.453*	0.508****	0.356*	0.466**	-0.190	-0.492**	-0.388*	-0.048	-0.243	-0.287
	p	0.018	0.000	0.039	0.006	0.363	0.009	0.045	0.813	0.222	0.147
N316	rho	0.557**	0.475****	0.327	0.421*	-0.083	-0.502**	-0.452*	-0.013	-0.213	-0.383*
	p	0.003	0.000	0.059	0.015	0.695	0.008	0.018	0.949	0.287	0.048
N582	rho	0.158	0.506****	0.011	0.196	-0.251	-0.532**	-0.003	-0.133	-0.422*	-0.235
	p	0.431	0.000	0.953	0.275	0.225	0.004	0.987	0.509	0.028	0.238
N1155	rho	0.311	0.639****	0.360*	0.491**	-0.232	-0.522**	-0.353	-0.050	-0.199	-0.253
	p	0.114	0.000	0.037	0.004	0.263	0.005	0.071	0.805	0.320	0.202
N1195	rho	0.284	0.222	-0.046	0.137	-0.252	-0.580**	-0.365	0.051	-0.217	-0.219
	p	0.159	0.111	0.811	0.486	0.235	0.002	0.067	0.805	0.286	0.283
N1234	rho	0.287	0.469****	0.035	0.291	-0.345	-0.591**	-0.381*	0.129	-0.122	-0.251
	p	0.147	0.000	0.855	0.125	0.091	0.001	0.050	0.521	0.545	0.206
N1242	rho	0.239	0.298*	0.228	0.360*	-0.196	-0.532**	-0.301	-0.037	-0.214	-0.225
		0.239	0.023	0.210	0.047	0.359	0.005	0.135	0.857	0.293	0.269

Abbreviations: abbreviated injury scale (AIS), CT (computed tomography), Glasgow Coma Scale (GCS), Glasgow Outcome Scale (GOS), neurofilament light (NFL), neuron specific enolase (NSE), traumatic brain injury (TBI).

739   For age and severity or outcome scores, only time point 1 was included for the correlation

740   analysis, while for brain markers all three time points were incorporated.

741   The correlation coefficients are presented as Spearman's rho.

742

743

744

745

746

747

748

749

750

751

752

753

754

755

756

757

758

759

760

761

762

763

764

765

766

767

768

769

770

771

772

Supplementary table 5. Detected Asp-N digested (upper part) and endogenous (lower part)

peptides using immunoprecipitation followed by mass spectrometric analysis in CSF.

Peptide	Observed m/z	Δm [ppm]	Expect value	Sequence
ADAMTS/Asp-N cleaved peptides				
23-41	698.0210	3.2	7.10E-05	DVLEGDSSEDRAFRVRIAG
28-41	526.9382	1.1	6.60E-05	DSSEDRAFRVRIAG
32-41	580.8365	0.8	2.70E-05	DRAFRVRIAG
119-130	657.3574	1.2	1.50E-07	DVSLALSELRPN
210-221	491.9325	1.3	1.10E-07	DQTVRYPIQTPR
210-223	558.6266	2.9	2.00E-08	DQTVRYPIQTPREA
227-242	877.4000	4.4	1.40E-08	DMDGFPGVRNYGVVDP
227-246	1122.5011	1.6	4.70E-05	DMDGFPGVRNYGVVDPDDLY
227-246	1130.5015	4.2	1.80E-08	DMDGFPGVRNYGVVDPDDLY
229-241	697.8400	1.9	6.60E-04	DGFPGVRNYGVVD
229-242	746.3655	0.6	5.90E-05	DGFPGVRNYGVVDP
229-246	999.4689	3.3	4.30E-07	DGFPGVRNYGVVDPDDLY
254-270	935.9971	3.9	5.40E-08	DLNGELFLGDPPEKLTL
254-271	1000.5186	3.8	2.00E-09	DLNGELFLGDPPEKLTLE
254-276	864.1076	1.8	1.00E-14	DLNGELFLGDPPEKLTLEEARAY
375-400	**1422.7034**	**3.9**	**1.10E-06**	**DGLEAIVTVTETLEELQLPQEATESE**
375-404	1072.5339	2.2	5.40E-07	DGLEAIVTVTETLEELQLPQEATESESRGA
385-400	**923.4363**	**2.9**	**1.50E-04**	**ETLEELQLPQEATESE**
388-400	**751.8470**	**0.8**	**9.80E-04**	**EELQLPQEATESE**
389-400	**687.3275**	**3.5**	**8.10E-05**	**ELQLPQEATESE**
Endogenous C-terminal peptides				
879-885	405.6837	-3.2	2.80E-02	ALHPEED
879-888	1093.4796	0.0	2.30E-05	ALHPEEDPEG
879-889	625.2920	-3.2	5.10E-06	ALHPEEDPEGR
879-890	689.3203	-4.3	2.70E-04	ALHPEEDPEGRQ
879-891	717.8332	-1.1	1.60E-06	ALHPEEDPEGRQG
879-892	795.8836	-1.3	4.70E-07	ALHPEEDPEGRQGR
879-893	568.6188	-2.5	1.20E-03	ALHPEEDPEGRQGRL
879-894	606.3135	-2.3	2.20E-06	ALHPEEDPEGRQGRLL
879-895	937.4782	-1.3	5.60E-09	ALHPEEDPEGRQGRLLG
879-896	406.8162	-0.4	3.80E-05	ALHPEEDPEGRQGRLLGR
879-897	554.7879	-1.0	2.10E-03	ALHPEEDPEGRQGRLLGRW
879-898	782.0791	-1.7	1.90E-03	ALHPEEDPEGRQGRLLGRWK
879-899	403.3827	-1.7	6.70E-05	ALHPEEDPEGRQGRLLGRWKA
879-900	506.4745	-1.8	3.80E-05	ALHPEEDPEGRQGRLLGRWKAL
880-895	451.4833	-1.7	6.90E-06	LHPEEDPEGRQGRLLG
881-895	845.4161	-3.2	8.60E-09	HPEEDPEGRQGRLLG
883-895	728.3613	-2.2	3.70E-06	EEDPEGRQGRLLG
884-895	663.8412	-0.7	3.60E-04	EDPEGRQGRLLG
886-895	541.8057	-2.2	1.20E-04	PEGRQGRLLG
889-895	400.2468	-4.3	8.30E-03	RQGRLLG

775   The four a distintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)

776   cleaved peptides are marked in bold.

777   Underlined M (<u>M</u>) indicates oxidation on methionine.

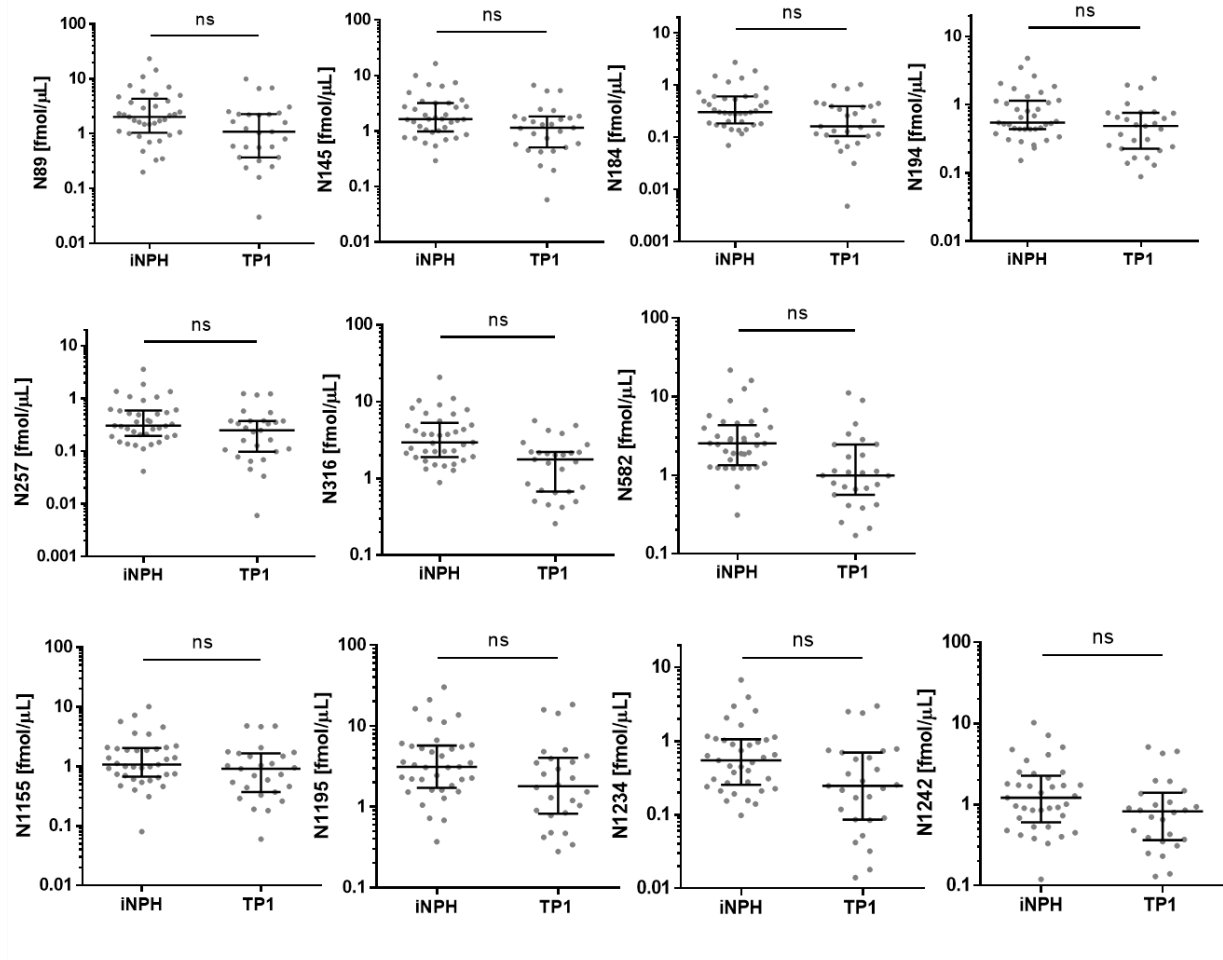778

779

780

781

782

783

784

785

786   **Supplementary figure 1:** CSF neurocan concentrations for the iNPH and TBI (time point 1;

787   TP1) groups.

788

789   The horizontal lines represent the median and interquartile ranges.

790   The ANCOVA test with Bonferroni correction was used to examine the differences in log

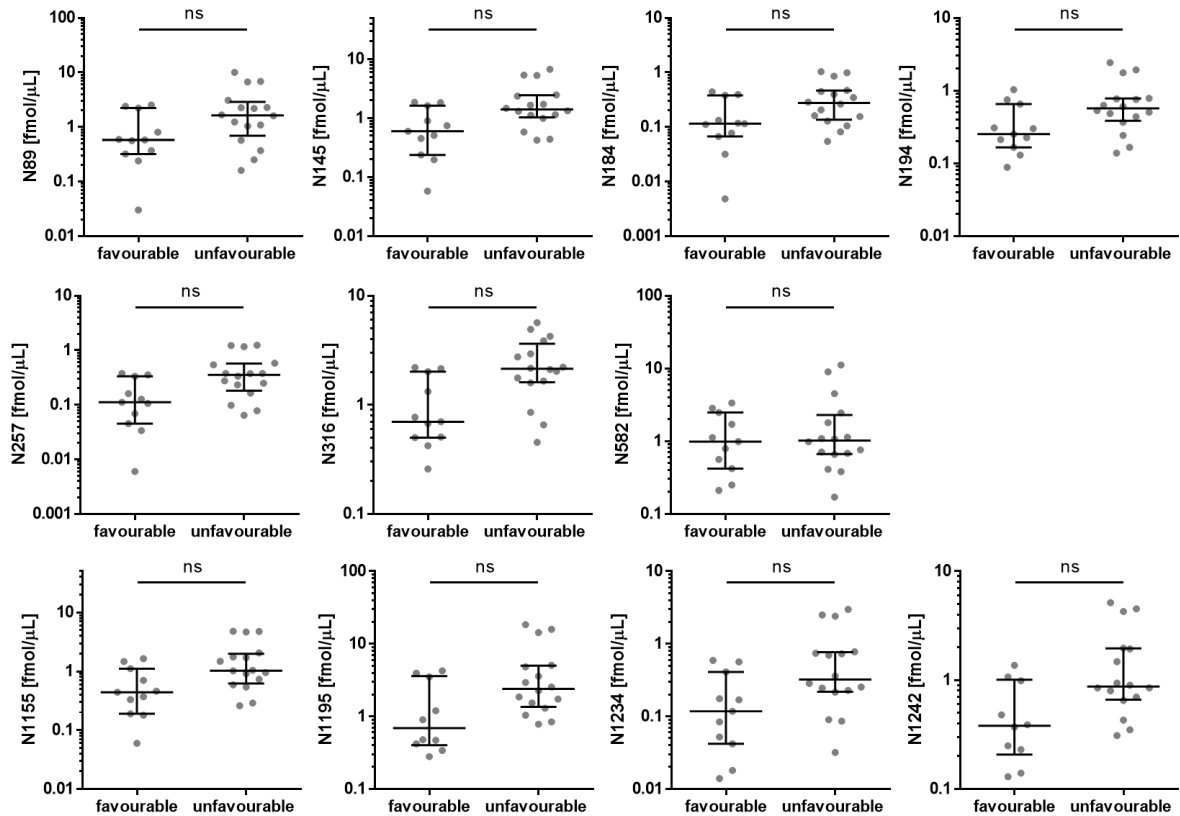791   transformed data between the two groups, accounting for the effect of age.

792   Number of individuals: n=37 for iNPH, n=27 for TBI (TP1).

793

794

795

796 **Supplementary figure 2.** CSF neurocan peptide concentrations in relation to outcome

797 following TBI.

798

799 The horizontal lines represent the median and interquartile ranges.

800 Outcome is dichotomized into favourable (GOS=4-5) and unfavourable (GOS=1-3).

801 The ANCOVA test with Bonferroni correction was used to examine the differences in log

802 transformed data between the two groups, accounting for the effect of age.

803 Number of patients: n=11 for favourable, n=16 for unfavourable.

804

805

806

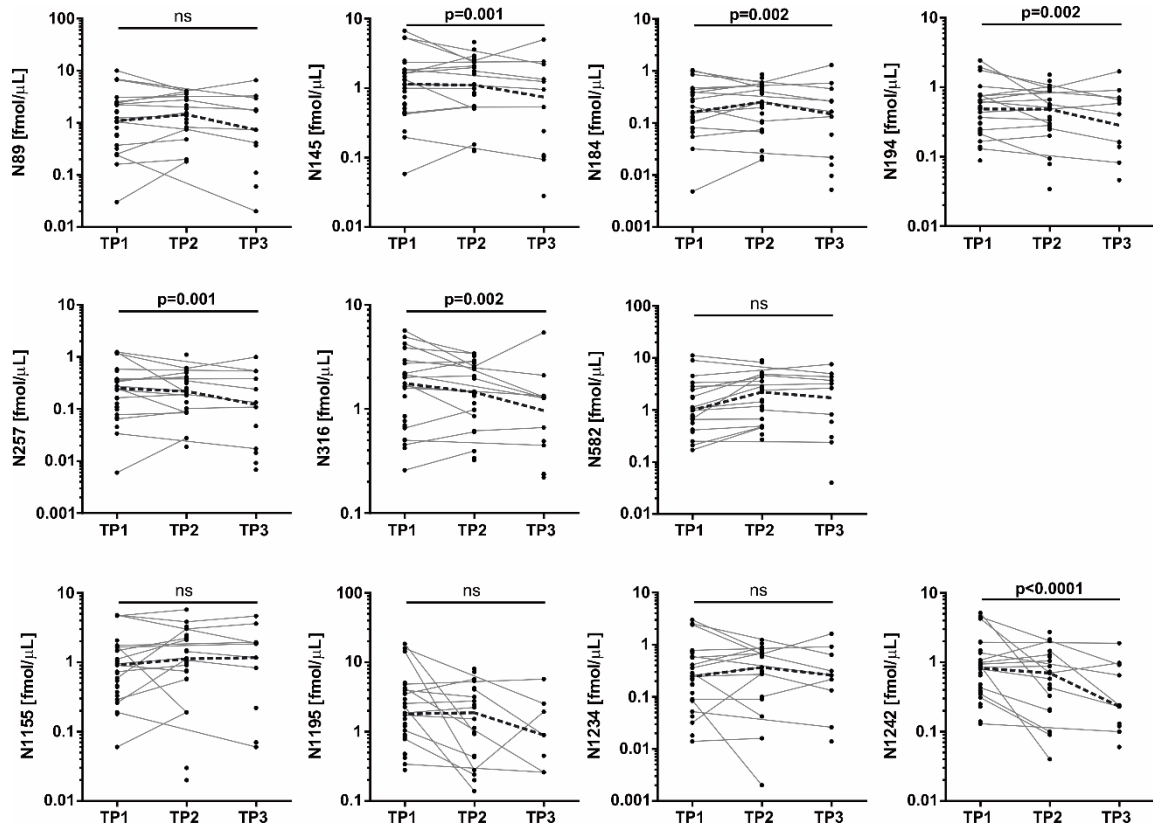807

808

809

810

811

812

813

814

815

816

817

818

819

820

821 **Supplementary figure 3.** Repeated measurements of neurocan peptides in TBI patients at

822 three time points.

834

LMM with Bonferroni correction was used to examine the differences in log transformed data

of repeated measures following TBI (from TP1 to TP3), accounting for the effect of age. The

dashed lines represent the longitudinal median changes.

838

839

840

841

842

843

844

845

846 **References:**

847   1.   Dewan MC, Rattani A, Gupta S, Baticulon RE, Hung YC, Punchak M, et al.

848   Estimating the global incidence of traumatic brain injury. J Neurosurg. 2018:1-18.

849   2.   Blennow K, Brody DL, Kochanek PM, Levin H, McKee A, Ribbers GM, et al.

850   Traumatic brain injuries. Nat Rev Dis Primers. 2016;2:16084.

851   3.   Teasdale G, Jennett B. Assessment of coma and impaired consciousness. A

852   practical scale. Lancet. 1974;2(7872):81-4.

853   4.   Gennarelli TA, Wodzin E. AIS 2005: a contemporary injury scale. Injury.

854   2006;37(12):1083-91.

855   5.   Marshall LF, Marshall SB, Klauber MR, Van Berkum Clark M, Eisenberg H,

856   Jane JA, et al. The diagnosis of head injury requires a classification based on computed axial

857   tomography. J Neurotrauma. 1992;9 Suppl 1:S287-92.

858   6.   Maas AI, Hukkelhoven CW, Marshall LF, Steyerberg EW. Prediction of

859   outcome in traumatic brain injury with computed tomographic characteristics: a comparison

860   between the computed tomographic classification and combinations of computed tomographic

861   predictors. Neurosurgery. 2005;57(6):1173-82; discussion -82.

862   7.   Nelson DW, Nystrom H, MacCallum RM, Thornquist B, Lilja A, Bellander BM,

863   et al. Extended analysis of early computed tomography scans of traumatic brain injured

864   patients and relations to outcome. J Neurotrauma. 2010;27(1):51-64.

865   8.   Jennett B, Bond M. Assessment of outcome after severe brain damage. Lancet.

866   1975;1(7905):480-4.

867   9.   Li X, Li TQ, Andreasen N, Wiberg MK, Westman E, Wahlund LO. The

868   association between biomarkers in cerebrospinal fluid and structural changes in the brain in

869   patients with Alzheimer's disease. J Intern Med. 2014;275(4):418-27.

870   10.   Zetterberg H, Hietala MA, Jonsson M, Andreasen N, Styrud E, Karlsson I, et al.

871   Neurochemical aftermath of amateur boxing. Arch Neurol. 2006;63(9):1277-80.

872   11.   Neselius S, Brisby H, Theodorsson A, Blennow K, Zetterberg H, Marcusson J.

873   CSF-biomarkers in Olympic boxing: diagnosis and effects of repetitive head trauma. PLoS

874   One. 2012;7(4):e33606.

875   12.   Franz G, Beer R, Kampfl A, Engelhardt K, Schmutzhard E, Ulmer H, et al.

876   Amyloid beta 1-42 and tau in cerebrospinal fluid after severe traumatic brain injury.

877   Neurology. 2003;60(9):1457-61.

878   13.   Zemlan FP, Jauch EC, Mulchahey JJ, Gabbita SP, Rosenberg WS, Speciale SG,

879   et al. C-tau biomarker of neuronal damage in severe brain injured patients: association with

880   elevated intracranial pressure and clinical outcome. Brain Res. 2002;947(1):131-9.

881   14.   Ost M, Nylen K, Csajbok L, Ohrfelt AO, Tullberg M, Wikkelso C, et al. Initial

882   CSF total tau correlates with 1-year outcome in patients with traumatic brain injury.

883   Neurology. 2006;67(9):1600-4.

884   15.   Goyal A, Failla MD, Niyonkuru C, Amin K, Fabio A, Berger RP, et al. S100b as

885   a prognostic biomarker in outcome prediction for patients with severe traumatic brain injury. J

886   Neurotrauma. 2013;30(11):946-57.

887   16.   Berger RP, Pierce MC, Wisniewski SR, Adelson PD, Clark RS, Ruppel RA, et

888   al. Neuron-specific enolase and S100B in cerebrospinal fluid after severe traumatic brain

889   injury in infants and children. Pediatrics. 2002;109(2):E31.

890   17.   George N, Geller HM. Extracellular matrix and traumatic brain injury. J

891   Neurosci Res. 2018;96(4):573-88.

892   18.   Zimmermann DR, Dours-Zimmermann MT. Extracellular matrix of the central

893   nervous system: from neglect to challenge. Histochem Cell Biol. 2008;130(4):635-53.

894   19.   Cox TR, Erler JT. Remodeling and homeostasis of the extracellular matrix:

895   implications for fibrotic diseases and cancer. Dis Model Mech. 2011;4(2):165-78.

896   20.   Siebert JR, Conta Steencken A, Osterhout DJ. Chondroitin sulfate proteoglycans

897   in the nervous system: inhibitors to repair. Biomed Res Int. 2014;2014:845323.

898   21.   Minta K, Cullen NC, Nimer FA, Thelin EP, Piehl F, Clarin M, et al. Dynamics

899   of extracellular matrix proteins in cerebrospinal fluid and serum and their relation to clinical

900   outcome in human traumatic brain injury. Clin Chem Lab Med. 2019;57(10):1565-73.

901   22.   Mayer J, Hamel MG, Gottschall PE. Evidence for proteolytic cleavage of

902   brevican by the ADAMTSs in the dentate gyrus after excitotoxic lesion of the mouse

903   entorhinal cortex. BMC Neurosci. 2005;6:52.

904   23.   Asher RA, Morgenstern DA, Fidler PS, Adcock KH, Oohira A, Braistead JE, et

905   al. Neurocan is upregulated in injured brain and in cytokine-treated astrocytes. J Neurosci.

906   2000;20(7):2427-38.

907   24.   Matsui F, Watanabe E, Oohira A. Immunological identification of two

908   proteoglycan fragments derived from neurocan, a brain-specific chondroitin sulfate

909   proteoglycan. Neurochem Int. 1994;25(5):425-31.

910   25.   Turk BE, Huang LL, Piro ET, Cantley LC. Determination of protease cleavage

911   site motifs using mixture-based oriented peptide libraries. Nat Biotechnol. 2001;19(7):661-7.

912   26.   Gottschall PE, Howell MD. ADAMTS expression and function in central

913   nervous system injury and disorders. Matrix Biol. 2015;44-46:70-6.

914   27.   Fontanil T, Mohamedi Y, Moncada-Pazos A, Cobo T, Vega JA, Cobo JL, et al.

915   Neurocan is a New Substrate for the ADAMTS12 Metalloprotease: Potential Implications in

916   Neuropathies. Cell Physiol Biochem. 2019;52(5):1003-16.

917   28.   Yamada H, Watanabe K, Shimonaka M, Yamaguchi Y. Molecular cloning of

918   brevican, a novel brain proteoglycan of the aggrecan/versican family. J Biol Chem.

919   1994;269(13):10119-26.

920   29.   Nakamura H, Fujii Y, Inoki I, Sugimoto K, Tanzawa K, Matsuki H, et al.

921   Brevican is degraded by matrix metalloproteinases and aggrecanase-1 (ADAMTS4) at

922   different sites. J Biol Chem. 2000;275(49):38885-90.

923   30.   Matthews RT, Gary SC, Zerillo C, Pratta M, Solomon K, Arner EC, et al. Brain-

924   enriched hyaluronan binding (BEHAB)/brevican cleavage in a glioma cell line is mediated by

925   a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) family member.

926   J Biol Chem. 2000;275(30):22695-703.

927   31.   Grossetete M, Phelps J, Arko L, Yonas H, Rosenberg GA. Elevation of matrix

928   metalloproteinases 3 and 9 in cerebrospinal fluid and blood in patients with severe traumatic

929   brain injury. Neurosurgery. 2009;65(4):702-8.

930   32.   Vilalta A, Sahuquillo J, Poca MA, De Los Rios J, Cuadrado E, Ortega-Aznar A,

931   et al. Brain contusions induce a strong local overexpression of MMP-9. Results of a pilot

932   study. Acta Neurochir Suppl. 2008;102:415-9.

933   33.   Rosenberg GA, Estrada EY, Dencoff JE. Matrix metalloproteinases and TIMPs

934   are associated with blood-brain barrier opening after reperfusion in rat brain. Stroke.

935   1998;29(10):2189-95.

936   34.   Hadass O, Tomlinson BN, Gooyit M, Chen S, Purdy JJ, Walker JM, et al.

937   Selective inhibition of matrix metalloproteinase-9 attenuates secondary damage resulting from

938   severe traumatic brain injury. PLoS One. 2013;8(10):e76904.

939   35.   Chodobski A, Zink BJ, Szmydynger-Chodobska J. Blood-brain barrier

940   pathophysiology in traumatic brain injury. Transl Stroke Res. 2011;2(4):492-516.

941   36.   Dinet V, Petry KG, Badaut J. Brain-Immune Interactions and

942   Neuroinflammation After Traumatic Brain Injury. Front Neurosci. 2019;13:1178.

943 37.   Cross AK, Haddock G, Stock CJ, Allan S, Surr J, Bunning RA, et al. ADAMTS-

944 1 and -4 are up-regulated following transient middle cerebral artery occlusion in the rat and

945 their expression is modulated by TNF in cultured astrocytes. Brain Res. 2006;1088(1):19-30.

946 38.   Al Nimer F, Thelin E, Nystrom H, Dring AM, Svenningsson A, Piehl F, et al.

947 Comparative Assessment of the Prognostic Value of Biomarkers in Traumatic Brain Injury

948 Reveals an Independent Role for Serum Levels of Neurofilament Light. PLoS One.

949 2015;10(7):e0132177.

950 39.   Brinkmalm G, Sjodin S, Simonsen AH, Hasselbalch SG, Zetterberg H,

951 Brinkmalm A, et al. A Parallel Reaction Monitoring Mass Spectrometric Method for Analysis

952 of Potential CSF Biomarkers for Alzheimer's Disease. Proteomics Clin Appl. 2018;12(1).

953 40.   Minta K, Brinkmalm G, Al Nimer F, Thelin EP, Piehl F, Tullberg M, et al.

954 Dynamics of cerebrospinal fluid levels of matrix metalloproteinases in human traumatic brain

955 injury. Sci Rep. 2020;10(1):18075.

956 41.   Jeppsson A, Zetterberg H, Blennow K, Wikkelso C. Idiopathic normal-pressure

957 hydrocephalus: pathophysiology and diagnosis by CSF biomarkers. Neurology.

958 2013;80(15):1385-92.

959 42.   Abu-Rumeileh S, Giannini G, Polischi B, Albini-Riccioli L, Milletti D, Oppi F,

960 et al. Revisiting the Cerebrospinal Fluid Biomarker Profile in Idiopathic Normal Pressure

961 Hydrocephalus: The Bologna Pro-Hydro Study. J Alzheimers Dis. 2019;68(2):723-33.

962 43.   Schirinzi T, Sancesario GM, Di Lazzaro G, D'Elia A, Imbriani P, Scalise S, et

963 al. Cerebrospinal fluid biomarkers profile of idiopathic normal pressure hydrocephalus. J

964 Neural Transm (Vienna). 2018;125(4):673-9.

965 44.   Tullberg M, Rosengren L, Blomsterwall E, Karlsson JE, Wikkelso C. CSF

966 neurofilament and glial fibrillary acidic protein in normal pressure hydrocephalus. Neurology.

967 1998;50(4):1122-7.

968   45.   Albrechtsen M, Sorensen PS, Gjerris F, Bock E. High cerebrospinal fluid

969   concentration of glial fibrillary acidic protein (GFAP) in patients with normal pressure

970   hydrocephalus. J Neurol Sci. 1985;70(3):269-74.

971   46.   Chen W, Tan Y, Ge Y, Chen Y, Liu X. The Effects of Levetiracetam on

972   Cerebrospinal Fluid and Plasma NPY and GAL, and on the Components of Stress Response

973   System, hs-CRP, and S100B Protein in Serum of Patients with Refractory Epilepsy. Cell

974   Biochem Biophys. 2015;73(2):489-94.

975   47.   Danielson M, Reinsfelt B, Westerlind A, Zetterberg H, Blennow K, Ricksten

976   SE. Effects of methylprednisolone on blood-brain barrier and cerebral inflammation in

977   cardiac surgery-a randomized trial. J Neuroinflammation. 2018;15(1):283.

978